Electrochemical Inactivation of *Bacillus* Spores in Drinking Water Using a Quaternary Oxide Electrode

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Abstract

Bacillus spores are resistant to disinfection methods and their deliberate introduction to water systems is a potential threat that requires improved methods to ensure water safety. *Bacillus thuringiensis* (BT) and *B. anthracis* Sterne (BA) spores were used to investigate the effectiveness of electrochemical (EC) disinfection processes using different solution and operational conditions. We tested several anode materials for EC disinfection and found that the most efficient material was the quaternary metal oxide (TiO_2-Sb_2O_5-SnO_2-RuO_2). An open free gassing cell was used in batch and flow configurations to test the quaternary oxide anodes for spore inactivation. The flow configurations had the advantage of a more stable operation compared to the batch method that resulted in gradual heat buildup and electrolyte pH decrease. The solution composition and current density were varied to achieve the optimal spore inactivation conditions. The presence of chloride ions at low concentrations was found to be critical for the effective inactivation of BT spores. Active chorine was produced *in-situ* by anodic oxidation of chloride content in the solutions. Moreover, soluble active chlorine species, produced *in situ* electrochemically resulted in an active chlorine residual that continued to kill spores after passage through the EC cell. Chloride-free electrolytes (phosphate buffers) had a distinctly lower BT spore inactivation efficiency when used in the flow configuration. Local tap water was used as a realistic test solution to measure disinfection of the BT spores. The local tap water was found to contain average chloride concentrations of 1.2 mM (range of 0.7 mM to 2.2 mM) resulting in active chlorine concentrations in the range of 0.35 mM to 0.5 mM (25 mg/L to 35 mg/L). Scanning electron images of the EC treated spores indicate damage to the outer membranes resulting in disruption and leakage of the spore contents. EC water disinfection

processes using inexpensive electrode materials (mixed metal oxides) and drinking water with either low levels of naturally occurring or introduced chloride ions could serve as a viable alternative technology to chlorination or ozonation, especially against difficult to disinfect biological threats such as *Bacillus* spores.

Keywords: *Bacillus thuringiensis*; *Bacillus anthracis;* spores; electrochemical; drinking water system; inactivation; disinfection

1 Introduction

Ensuring the safety of our water systems requires effective disinfection technologies against biological threats, including those that are naturally occurring or deliberately introduced. Bacterial endospores, such as those produced by *Bacillus* bacteria, can persist in the environment for long periods and are highly resistant to inactivation by heat, radiation, and chemical disinfectants including active chlorine (Dychdala 2001; Young and Setlow 2003; Young and Setlow 2004; Rice et al. 2005; Rose et al. 2005; Setlow 2006). Therefore, the inactivation of bacterial spores represents a significant challenge for established water treatments including chlorination, ozonation and UV irradiation.

Typically, water systems are disinfected by the addition of chlorine gas or hypochlorite solutions (bleach), representing potential dangers during the transportation, storage, and use of large amounts of these toxic materials. Alternatives to traditional chlorine are actively being investigated including electrochemical (EC) methods (Martinez-Huitle and Brillas 2008). EC water disinfection methods have been known since the 1950's and have recently gained renewed interest for the inactivation of viruses and bacteria (Drees et al. 2003). On site generation of chlorine using EC methods has the advantages of improved operator safety, higher quality chlorine, less environmental impact, and potential for lower operating costs (Boal 2009). However, before these advantages can be realized, additional research and development need to be done on EC methods, in particular, their effectiveness against the more resistant organisms such as bacterial spores.

Depending on the particular electrolysis cell configuration, the EC treatment can subject water to strong reducing or oxidizing environments, thus avoiding the introduction of external

chemicals. Water itself or dissolved ions could serve as precursors for the formation of various strong oxidants during the anodic reactions. The EC process termed electrochlorination generates active chlorine from dissolved chloride ions, either through the direct contact of the electrodes with the water system, or as a separate step where the electrodes are contacted with a concentrated chloride solution (a brine) to generate a high concentration of active chlorine that is then added to the water system (Martinez-Huitle and Brillas 2008). In addition to chlorine generation, the anodic water oxidation reactions also produce a wide range of reactive oxygen species (Jeong et al. 2006) with disinfecting power, comparable to ozone (Diao et al. 2004). Boron doped diamond (BDD) electrode materials increasingly are a material of choice for EC waste water treatment. They have the widest potential window in aqueous media and are stable at harsh operating conditions. Anodic polarizations $E > 3V$ (SHE) are routinely achieved on BDD electrodes due to high oxygen evolution overvoltage thus producing a mix of powerful persistent oxidants (Polcaro et al. 2010). However, their use for drinking water disinfection is rather problematic as significant quantities of perchlorate are formed in the presence of trace chloride in a variety of anodic reaction at high potentials (Bergman et al. 2009; Bergman 2010) Perchlorate is a potent endocrine disruptor and its concentration in drinking water is limited to 15 ppb by the Environmental Protection Agency (Bergman 2010). Recently Bergman (Bergman et al. 2009) compared perchlorate formation in two continuous technical bipolar electrochemical cells equipped with BDD and mixed oxide electrodes. Perchlorate concentrations in water were three orders of magnitude higher in a cell using BDD electrodes compared to a cell using mixed oxide electrodes at similar current densities (Bergman et al. 2009). Importantly, it was concluded that mixed oxide electrodes would produce no perchlorate in most practical applications when used at low current densities, such as used in this study.

The mixed oxidants obtained from the EC treatment of brine (sodium chloride solutions) have been used to measure the disinfection efficiency of oocysts from *Crytosporidium parvum* and *Clostridium perfringens* spores (Venczel et al. 1997; Casteel et al. 2000; Venczel et al. 2004). Venczel et al. (Venczel et al. 2004) found that the mixed oxidants from brine were more effective than chlorine at inactivating MS2 bacteriophage, *E. coli* bacteria, *Vibrio cholerae* bacteria, and *Cl. perfringens* spores, however the mixed oxidants did not reliably inactivate the *C. parvum* oocysts (Venczel et al. 2004). Clevenger et al. (Clevenger et al. 2007) compared commercial electrochlorination devices to conventional chlorination and found that the mixed oxidants from the EC devices produced similar results for the disinfection of the bacteriophage MS2 and *B. subtilis* spores. A study by Son et al. (Son et al. 2004) found that the mixed oxidants generated from brine using a commercial EC device were 20% to 50% more effective for the disinfection of *E. coli* and *B. subtilis* spores at pH 8.2, but at acidic (pH 5.2) and neutral condition the two processes demonstrated similar efficiency. In addition to inactivation by *in situ* produced chlorine, the direct contact of microorganisms with electrodes in the EC system has been shown to result in cellular membrane damage due to the strong electric fields within the double layer (Drees et al. 2003).

In this study, we used *B. thuringiensis* (BT) spores as a safe simulant and confirmed the results with *B. anthracis* (BA), Sterne (vaccine) strain. Previous studies have indicated that the chlorine inactivation kinetics of BA and BT are comparable (Rice et al. 2005; Rose et al. 2005). We also measured similar disinfection kinetics for BT and BA (Sterne strain) in a previous study (Morrow et al. 2008). In addition to equivalent inactivation kinetics using active chlorine, BT is also closely related to BA based on their DNA sequences (Radnedge et al. 2003) and the

exosporium (outermost spore layer) composition of BA and BT is also similar (Matz et al. 2001). All of these factors justify the use of BT as a safe simulant for BA.

The goals of this study were to investigate the effect of the solution conditions and electrode materials on the disinfection of *Bacillus* spores, a challenging biological threat. Several anode materials were tested and found to have a strong influence on the effectiveness of the disinfection process. We analyzed spore electrolytic inactivation in chlorine-free phosphate buffers, low concentrations of sodium chloride, and local tap water using measurements of active chlorine formed during the process. The EC process was studied in batch and single pass flow configurations.

2 Materials and methods

2.1 Spore Preparations and Measurement

A commercial preparation of BT (Thuricide, Bonide Insecticide, Oriskany, NY)[1] was prepared as previously described (Morrow et al. 2008; Morrow and Cole 2009). BA spores were obtained from U.S. Army Dugway Proving Ground (Dugway, UT) and characterized as previously described (Almeida et al. 2008). Local water from the laboratory cold water tap (Gaithersburg, MD) was used for over approximately 18 months during the course of these experiments. The average pH of the tap water was 7.5 (standard deviation = 0.3, N=6) and the average conductivity was 0.4 mS/cm (standard deviation = 0.1, N=6). A typical concentration of chloride in the local tap water was 42 mg/L (1.2 mM) with a range from 26.4 mg/L to 77 mg/L (Washington Suburban Sanitary Comission 2009). Free chlorine in the tap water was generally at

[1] Certain commercial equipment, instruments, or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology or the U.S. government, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose."

very low or undetectable concentrations using N, N-diethyl-p-phenylenediamine (DPD) analytical assay (Hach Company, Loveland, CO). To ensure the complete removal of any residual chlorine and inactivation of any bacteria present in the tap water, it was either filtered through a 0.45 μm nylon filter and allowed to set overnight before use or autoclaved (121 °C for 45 min), followed by cooling to room temperature before use. Free and total chlorine concentrations were measured using the DPD reaction and calibrated using active chlorine standards (Hach Company, Loveland, CO).

Plating on Luria-Bertani (LB) agar plates was used to determine the concentration of the spore stocks, the starting concentrations, and the EC treated samples (Morrow et al. 2008). The spore samples were diluted using phosphate buffered saline (0.01 M phosphate, 0.138 M NaCl, 0.0027 M KCl, pH 7.4, Sigma Chemical Co., St. Louis, MO) containing 0.01% (vol./vol.) Triton X-100 (PBS-Triton X100). The spore stocks, diluted samples and treated samples were vortexed (30 sec) immediately before use to achieve uniform dispersion in the electrolyte. The samples were diluted and spread on LB agar plates and incubated at 35 °C overnight. Colonies were counted and used to calculate the colony forming units per mL (CFU/mL).

2.2 BT Spore Disinfection in Solution using Active Chlorine

The disinfection of the BT spores using active chlorine in solution was also measured for comparison purposes at ambient temperature (21 °C to 22 C°). A solution of active chlorine (10 mg/L) was prepared by dilution of household bleach in phosphate buffer (0.1 M sodium phosphate pH 7.8). The solution was stirred for 30 min before active chlorine concentration measurement. BT spores were suspended in the chlorine-phosphate buffer solution at a concentration of approximately 5×10^5 CFU/mL in a borosilicate glass vial. The glass vial was placed on a rocker to gently suspend the spores during the inactivation process. Samples were

taken at 10 min time intervals by vortexing the vial (5 sec) and removing a sample. The spores were diluted using PBS-Triton X100 dilution buffer and the concentration of viable spores determined as described above. The concentration of viable spores was measured by dilution and plating on LB agar plates, as described above.

2.3 Assembly of the EC cell

Spore inactivation was performed in a single compartment open rectangular acrylic cell (inner dimensions 6.5 mm thick, 45 mm wide, and 65 mm high, Fig. 1) The cell contained two 50 mesh anodes (38 mm wide and 70 mm high), positioned on both sides of a Ni-Cr –Ti alloy grid (20 mesh) cathode, connected to Model 263A Potentiostat/Galvanostat (EG&G, Princeton, NJ). Ti grid (50 mesh, 42 mm by 85 mm Johnson –Matthey, West Deptford , NJ) was coated with quaternary oxide layer according to Houck et al. (Houk et al. 1998) and used as the anode. Briefly, the metal oxide films were formed using a thermal procedure in which Ti or Pt substrates were alternately painted with the solution of the four metal salts: 0.4M $SnCl_2$. $2H_2O$, 0.03M $SbCl_3$, 0.08 M $RuCl_3$ and 0.02M $TiCl_4$ in a 1:1 mixture of 12M HCl and i-propanol followed by heating above the flame of a Bunsen burner for about 15 s. After ten wetting and heating cycles, the electrode was annealed in a muffle furnace for 1 h at 600 °C. The geometric surface area for the anodes was estimated at 67 cm^2 and 120 cm^2 for the cathode. Mesh nylon wire grids (#10 from Small Parts Inc., Miami Lakes, FL) were used to separate the anodes from the cathode (Figure 1). The cell was assembled by placing the cathode in the middle and the anodes at the sides using the spacers to separate anode/cathode (Figure 1). A Ag/AgCl (3M KCl) reference electrode (Microelectrodes, Inc. Bedford, NH) was used for potentiostatic experiments. The electrochemical cell could process approximately 11 ml of spore solution in a batch mode.

2.4 Inactivation of spores using the EC cell

Solutions for EC inactivation were prepared by adding BT or BA spore stock to the test solution to a concentration of approximately 0.5 - 1 x 10^5 CFU/mL. The volume of solution placed in the cell was 11 mL for the batch experiments. Electrolyte samples were removed periodically at different time intervals to determine the viable spore concentration as described above. In some experiments a solution of sodium thiosulfate (0.1 M , 0.2 μm filter sterilized) was added to a final concentration of 1 mM concentration to quench the residual active chlorine.

Experiments with BA (Sterne) were performed using the batch mode in a biological safety level 2 laboratory. The EC chamber containing BA spores was placed inside a vented (0.2 μm filter) plastic box and placed inside an operating biosafety cabinet during the time the current was applied. Samples were removed from the electrolysis cell and the concentrations of viable spores in the solutions were determined as described above.

2.5 Flow Disinfection Experiments

Continuous electrolyte flow through the cell was established with a two-channel peristaltic pump (Minipuls 3, Gilson, Middleton, WI, Fig. 1) using 1.5 mm (internal dimension) polyvinyl chloride (PVC) pump tubing. Polytetrafluoroethylene (PTFE) tubing 0.8 mm (internal dimension) was connected to the peristaltic pump tubing. One pump channel removed solution from sample reservoir (a glass beaker gently stirred with a PTFE-coated stir bar) and delivered it to the top section of the cell. The second pump channel removed sample from the bottom of the cell and used to collect the treated sample after passage through the EC cell (single pass configuration, Fig. 1). The cell was filled with 6 mL or 11 mL of the test solution and flow established at 2 mL/min for most experiments, except where noted.

2.6 Spore imaging using a scanning electron microscope

Scanning electron micrographs (SEM) were obtained on dried spores using a Zeiss Ultra 60 microscope. Following electrolysis treatment, the spore suspension in 5mM NaCl was concentrated by centrifugation and washed several times with water and ethanol. The suspension in anhydrous ethanol was deposited on a graphite coated aluminum stub and allowed to dry overnight in a desiccator. The immobilized spores were sputter-coated with a 5 nm layer of gold-palladium prior to imaging to provide a conductive surface and reduce surface charging.

3 Results and Discussion

3.1 Solution inactivation of Bacillus spores using active chlorine

We measured the CT value (chlorine concentration multiplied by the time) required to achieve a 2 $\log_{10}$ reduction in viability of BT spores using active chlorine. This was done to compare the results obtained with the EC processes to conventional chlorination processes. The CT value to achieve a 2 $\log_{10}$ inactivation of BT spores in 0.1 M sodium phosphate buffer (pH 7.8) at ambient temperature (21 °C to 22 C°) using 10 mg/L chlorine was 150 min·mg/L (standard deviation = 60, N= 3). This value for BT inactivation is comparable to literature values measured in a potassium phosphate buffer (measured at pH 8 and 23 °C) for BT spores (Rice et al. 2005).

3.2 Comparison of anode electrode materials for EC inactivation of BT spores

Given the importance of the electrode material for the microorganism inactivation efficiency using the EC process (Panizza 2010), we screened several candidate anode materials, including indium tin oxide film, platinum sheet, single crystal silicon carbide and quaternary

metal oxides (TiO_2-Sb_2O_5-SnO_2-RuO_2). All of the investigated materials were dimensionally stable under our experimental conditions at current densities up to 50 mA/cm^2 and anode potential up to +3.5V vs. Ag/AgCl, with the exception of silicon carbide. Silicon carbide was found unsuitable for water electrolysis due to significant corrosion when polarized at E> +1.2 V.

We compared the results of the various anode materials using a chloride-free 0.1 M sodium phosphate buffer (p7.8) in the batch configuration for the disinfection of BT spores. BT spores (1 x 10^5 cfu/mL) in 11 mL of buffer was added to the cell and then current (200 mA) applied. The solution was sampled after 30 min and viable spores measured. Indium tin oxide anode equipped cell showed up to 2 log decrease of viable BT spores at E=3.5 V in 30 min., while Pt sheet only 0.5 log at comparable polarization. The most efficient anode material was the quaternary metal oxide (TiO_2-Sb_2O_5-SnO_2-RuO_2) that demonstrated more than a 4 log spore inactivation in 30 min at similar conditions. Given their well documented stability against corrosion in aggressive electrolytes for production of chlorine, and negligible perchlorate generation (Bergman et al. 2009), quaternary oxide anodes were investigated further for spore inactivation experiments under a range of conditions.

3.3 Effect of the electrolyte composition

The inactivation of BT and BA spores was initially studied using the EC cell in the batch mode. The cell was filled with 11 mL of test solution containing the spores. Local tap water, NaCl solutions, and sodium phosphate buffers were used to test inactivation of BT and BA spores in the batch mode. Figure 2 shows the typical inactivation of BA spores during electrolysis in autoclaved tap water. BA spores (4.5 x10^4 CFU/mL) were placed in the cell for 10 min in the open circuit conditions and sampled for viability, followed by the application of 50 mA for 5 min, resulting in no detectable spores when analyzed.

When used in the batch mode the pH of the solution decreased and the temperature gradually increased upon extended current flow. As expected, the pH of the sodium phosphate buffers were more stable, however, the pH eventually decreased at higher current densities. Sodium phosphate buffers could be used for inactivation of BT spores in the batch mode, but it was not as effective as NaCl solutions (1 and 5 mM) or tap water with naturally occurring chloride content. Figure 3 shows the oxidation of 5 mM sodium chloride in the batch mode plotted together with the stationary polarization curve. The cell production of the active chlorine follows the rise in the anodic current and indicates that oxygen evolution does not interfere with the chloride oxidation reaction.

We have explored the EC cell with flow configurations to reduce temperature variation and pH drift. We have investigated chloride free phosphate buffers, sodium chloride solutions, and a local tap water as a realistic water system. Initially a loop configuration was used, where the sample was added to the top of cell and removed from the bottom and returned to the sample reservoir. In this loop configuration there was no measureable solution temperature rise and the changes in pH were greatly reduced. The loop method resulted in effective disinfection of the spores using tap water and sodium chloride solutions. Determining disinfection efficiency was more difficult with the loop configuration because of treated solution being mixed with untreated solution in the sample reservoir. Active chlorine produced in the cell would continue to disinfect the spores in the reservoir, but this complicates the comparison of different conditions for disinfection efficiency measurements. In order to determine efficiency of different solution conditions we used single pass-through measurements. The single pass-through experiments would also be a more realistic model of a continuous EC process using a drinking water input.

Table 1 shows the EC operating conditions and the resulting pH and the resulting chloride concentrations (determined by the DPD reaction) in the single pass configuration. The local tap water contains sufficient amounts of chloride to produce active chlorine in the 25 to 35 mg/L range in our experiments (Table 1). The average pH of the tap water was 7.5, but we found that autoclaving the water increased the pH readings to over 8, possibly due to the removal of dissolved gasses such as CO_2. The pH of tap water that was filtered and allowed to equilibrate overnight in contact with the air did not significantly change from the initial pH 7.5 value. The pH of autoclaved tap water and 5 mM NaCl tended to increase after EC treatment and the filtered tap water tended to decrease (Table 1). The cause of this observation was not investigated.

The disinfection of *Bacillus* spores by chlorine is strongly influenced by the properties of the solution including the pH, temperature, and the possible presence of other ions in the solution, such as organic matter, ammonia, and other halogens (Dychdala 2001; Rice et al. 2005; Rose et al. 2005). Chlorine gas dissolved in water forms hypochlorous acid (HOCl). Water can also be disinfected by adding sodium (or calcium) hypochlorite to the water. Disinfection of microorganisms is significantly enhanced at pH below 7.5 where the HOCl form predominates and far less effective above 7.5 where the hypochlorite (OCl^-)ion predominates (Dychdala 2001).

Table 2 contains data on the inactivation of BT spores using the single-pass configuration. Sodium thiosulfate was added immediately to one set of samples to quench the activity of the active chlorine formed in the cell. Another set of samples were allowed to remain in the residual active chlorine before adding the sodium thiosulfate (addition time shown in Table 2). The samples with the delayed addition of sodium thiosulfate were prepared to determine the effect of the residual chlorine for increased disinfection of the BT spores.

The data in Table 2 shows the importance of pH on the spore disinfection. Tap water that was filtered was more efficient at disinfection of BT compared to autoclaved tap water (Table 1). These results are presumably due to the increased effectiveness of the higher concentrations of active chlorine produced that were more active at around pH 7 in the filtered tap water. In a similar fashion, the autoclaved tap water samples had residual active chlorine that continued to react with the BT spores (Table 2). The autoclaved 5 mM NaCl also had a high pH after EC (Table 1) and the initial sample had lower disinfection, but had high residual chlorine concentration that continued to disinfect the BT spores (Table 2). The initial disinfection increased when a low concentration of sodium chloride (1 mM) was buffered with 10 mM sodium phosphate pH 7.1 (Table 2). Sodium phosphate (pH 7.1) alone in the absence of dissolved chloride did not show any significant disinfection in the single pass configuration (Table 2). However, in the batch mode spore inactivation was measured using sodium phosphate buffer after 30 min, possibly due to the production of short lived oxidants, such as the hydroxyl radicals.

3.4 Variables in production of active chlorine

Figure 3 shows the direct effect of the anode potential on the oxidation of 5 mM NaCl to chlorine in the EC in the potentiostatic batch mode. When the cell is operated in a flow mode the residence time of the solution will affect the degree of chloride oxidation. Table 3 shows the effect of flow rate on the production of chlorine in tap water. This data shows that the higher flow rates result in lower chlorine production. These results indicate that the residence time at high flow is insufficient to achieve full oxidation of the available chloride under these conditions.

3.5 SEM images of EC treated BT spores

An ellipsoidal shape is typical for untreated viable spores, as shown in Fig. 4. Image analysis of 30 intact spores has shown that they were 1.4 μm ± 0.1 μm micrometer long and 0.8 μm ±0.1 μm micrometer wide with a clearly visible surface texture of the intact spore coat. Dynamic light scattering measurements of the spore colloidal suspension show a narrow size distribution around 1μm in diameter (data not shown).

Spore electrolysis for 5 min in 5 mM NaCl at 200 mA in the batch mode shows a rather dramatic morphology change (Fig. 5) when compared to the intact spores (Fig. 4). A relatively short electrolytic treatment in the chloride containing solution causes considerable spore shrinking. Spores loose their ellipsoidal shape collapse, becoming pseudospherical with an average diameter of 1.1 μm ±0.1 μm (n=30), and a visible increase in the external membrane roughness. This suggests that the strong oxidative treatment during electrolysis can produce pores in the outer spore coating, thus allowing spore internal contents to leak under SEM vacuum (Fig. 5).

Electron microscopy images of the spore suspension, subjected to the 30 min electrolytic treatment only contain the spore remnants that have irregular shape in the size range from 0.3 μm to 0.7 μm and some larger aggregates (Fig. 6). Apparently, the spore coat becomes severely damaged during the prolonged electrolysis resulting in fragments of the spore structure. A similar inactivation mechanism was proposed for spore treatment with ozonated water by Cross et al. 2003 (Cross et al. 2003).

4 Conclusions

The use of EC processes for treating drinking water is shown to be effective for inactivation of *Bacillus* spores, a challenge for conventional disinfection processes. Mixed metal oxide electrodes effectively produce active chlorine from water containing low concentration of chloride ions. This study shows the importance of carefully controlling the process variables in the including the pH, chloride content of the water, residence time, and voltage (and current) applied to the cell. The role of reactive oxygen species in EC processes has been investigated for the disinfection of *E coli* (Jeong et al. 2006). They found that under their conditions using a boron-doped electrode in chloride-free buffers that the hydroxyl free radical ($OH\cdot$) was the main oxidative species responsible for inactivation of the *E coli* bacteria. A study using a β-$Pb0_2$ electrode fluoride resin also demonstrated the effectiveness of EC generated hydroxyl free radicals for inactivation of coliform bacteria (Cong et al. 2008). The short life time of the hydroxyl radical (Sies 1993) may explain the lack of spore inactivation in the current study using chloride-free buffers and the single pass flow configuration, where the spore residence time in the EC cell is insufficient to encounter the short-lived oxidants.

Although BDD electrodes can effectively produce mixed oxidants, their use for treating drinking water is problematic because of the production of perchlorate, while mixed metal oxide electrodes did not produce significant concentrations under the same conditions (Bergman et al. 2009). The mixed metal oxide electrodes used to produce active chloride *in situ* also have the advantages of producing an active chlorine residual, but without the expense and danger of storing and using large amount of chemicals.

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Table 1 Solutes, EC Conditions, and Chlorine Production in Single Pass Configuration

Solution	Voltage Range (V)	Chlorine (mg/L)	pH Range After EC
Tap water- autoclaved	7.2-7.9	25.1 (4.1)	8.6-9.8
Tap water- filtered	9.9-11.5	35.0 (1.2)	6.3-7.0
1 mM NaCl, 10 mM sodium phosphate buffer, pH 7.1- Filtered	7.1-7.8	18.4 (4.0)	7.1-7.3
5 mM NaCl- autoclaved	7.0-7.1	51.8 (5.9)	9.1-10.8
10 mM Sodium phosphate buffer, pH 7.1- filtered	8.7-12.8	None detected	7.1-7.4

Flow rate was 2 mL/min. In all cases the current was 200 mA. Measurements are the means of at least three measurements. A single standard deviation of the mean is shown in parenthesis.

Table 2 EC Disinfection of BT Spores in Single Pass Configuration.

Solution	Initial log reduction, $-\log_{10}$ (Cf/Ci)	Log reduction in residual, $-\log_{10}$ (Cf/Ci), time in residual
Tap Water- autoclaved	1.1 (0.1)	3.1 (0.4) plus 1 h
Tap Water- filtered	4.4 (0.2)	>4.5, plus 2.5 h
1 mM NaCl, 10 mM sodium phosphate, pH 7.1- filtered	3.8 (0.1)	>4.5, plus 2 h
5 mM NaCl- autoclaved	1.7 (0.1)	2.4 (0.4) plus 1 h
10 mM sodium phosphate, pH 7.1- filtered	0.2 (0.1)	0 (0.1) plus 1 h

Flow rate was 2 mL/min and current was 200 mA. BT spores (approximately 50,000-100,000 CFU/ml) were suspended in the solutions and subjected to EC. Samples were collected and sodium thiosulfate was added to 1 mM final concentration to samples for the initial disinfection. Other samples were allowed to remain in the solution for the indicated time in the generated residual chlorine before analysis. The reduction was calculated by the $\log_{10}$ of the final concentration (Cf) divided by the initial concentration (Ci). Samples with no detectable spores were noted as < -4.5, an estimate of the lower limit of detection using our plating method for viability measurement. Measurements are the means of at least three measurements. A single standard deviation of the mean is shown in parenthesis.

Table 3 Effect of Flow Rate on the Production of Chlorine in Autoclaved Tap Water.

Flow rate	Chlorine (mg/L)
2 mL/min	29.8 (1.5)
4 mL/min	23.4 (0.6)
8 ml/min	15.7 (0.3)

Autoclaved tap water was pumped at the indicated flow rates with a current of 200 mA. Cell volume was 11 mL. Measurements are means of at least three measurements. A single standard deviation of the mean is shown in parenthesis.

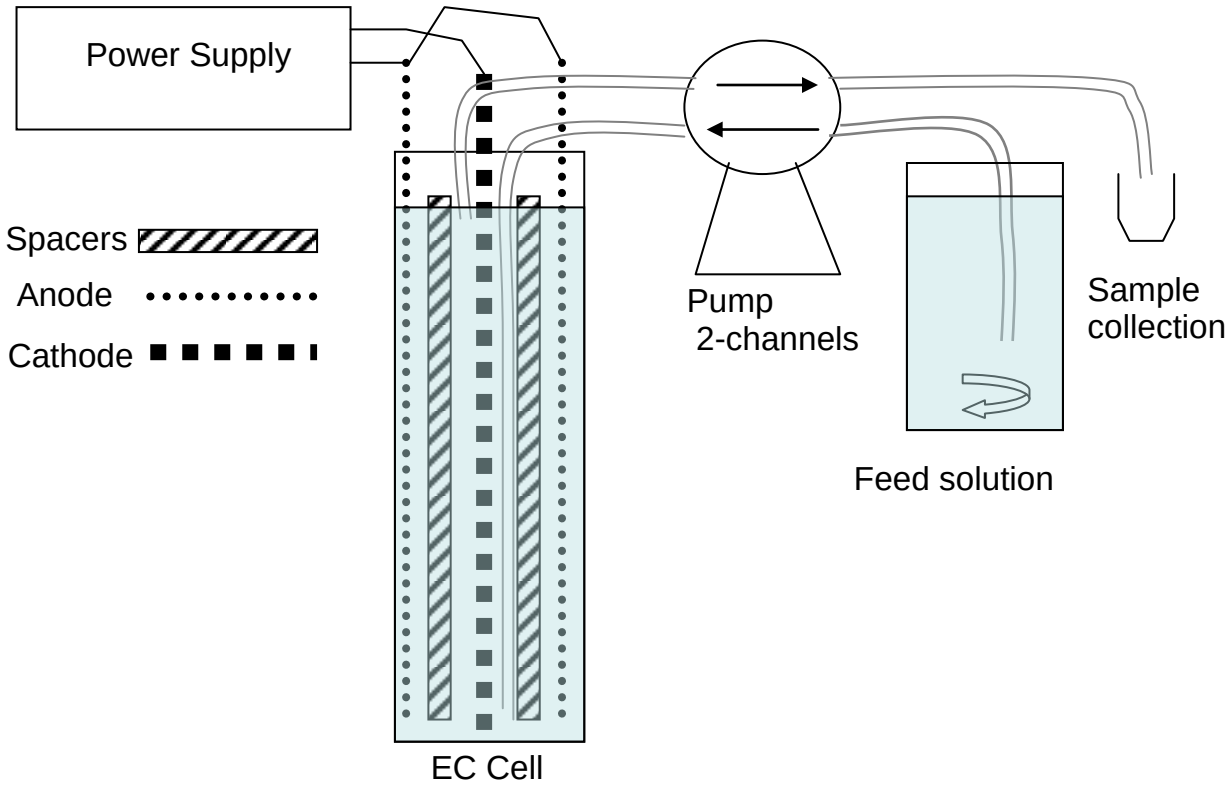


Figure. 1- Diagram of EC cell.

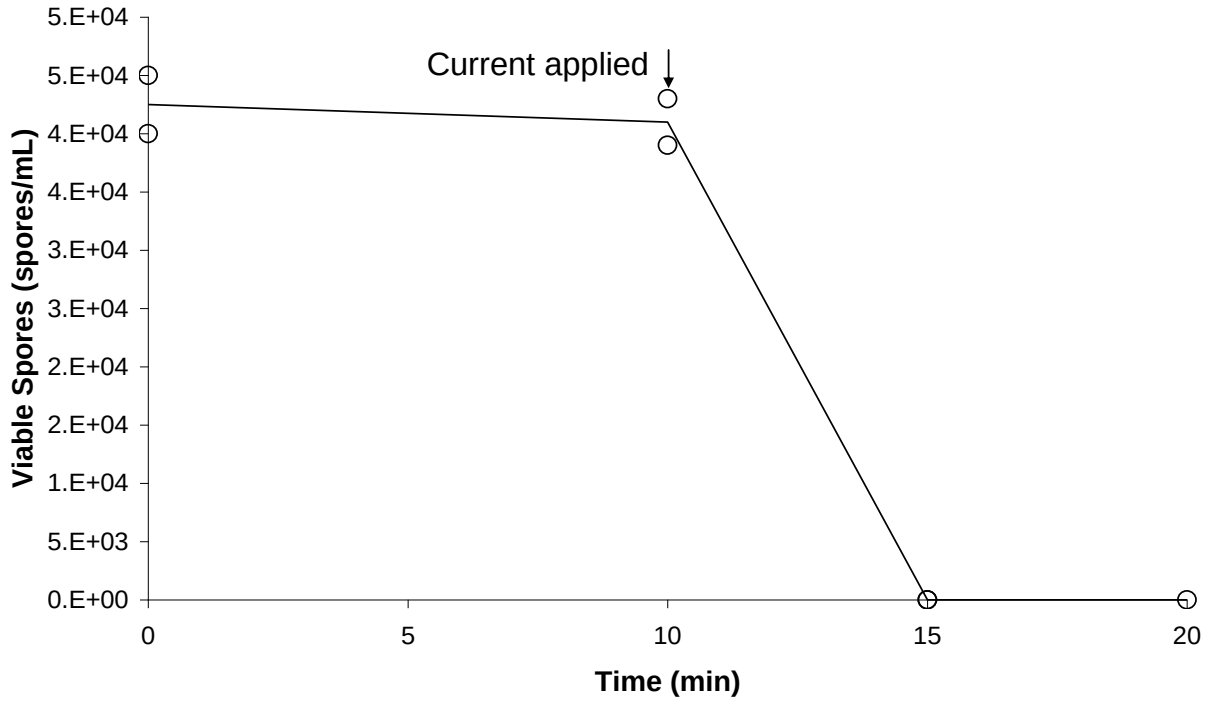


Figure. 2- Inactivation of BA spores in autoclaved tap water in the batch configuration. BA spores were placed in the cell at time zero and after 10 min a current (50 mA) was applied as indicated.

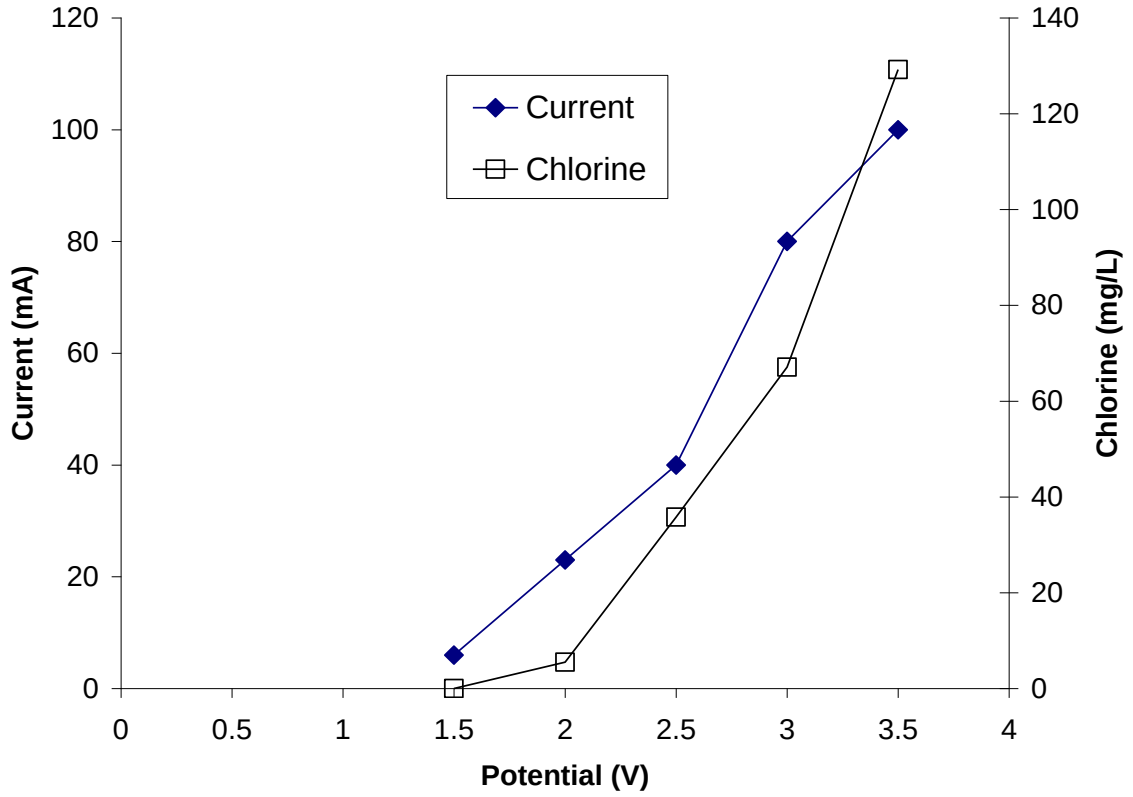
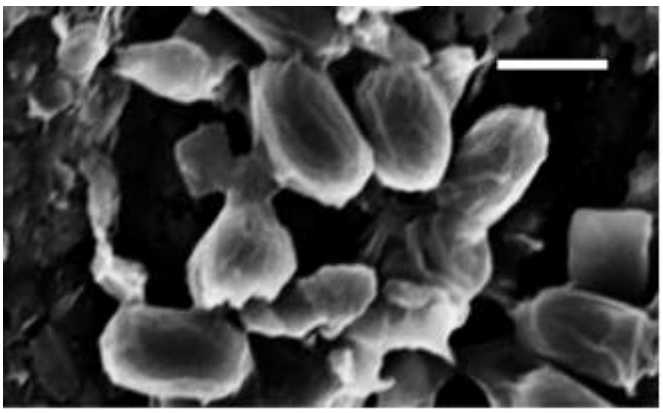
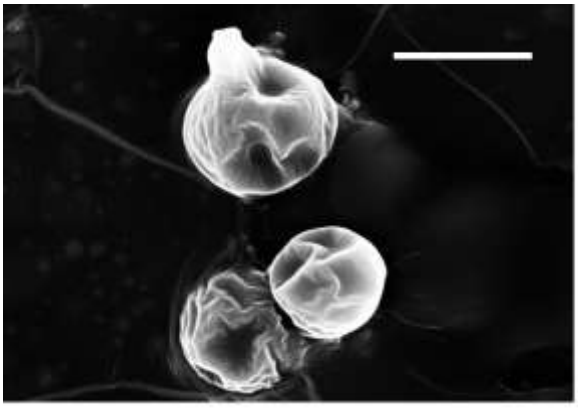


Figure. 3- EC oxidation of 5 mM NaCl in the potentiostatic batch mode. The cell was filled with 11 mL of 5 mM NaCl and the indicated potential was applied and the current measured. After 5 min the active chlorine concentrations in the solution were measured using the DPD reaction. Data is average of two measurements.

A.



B.



C.

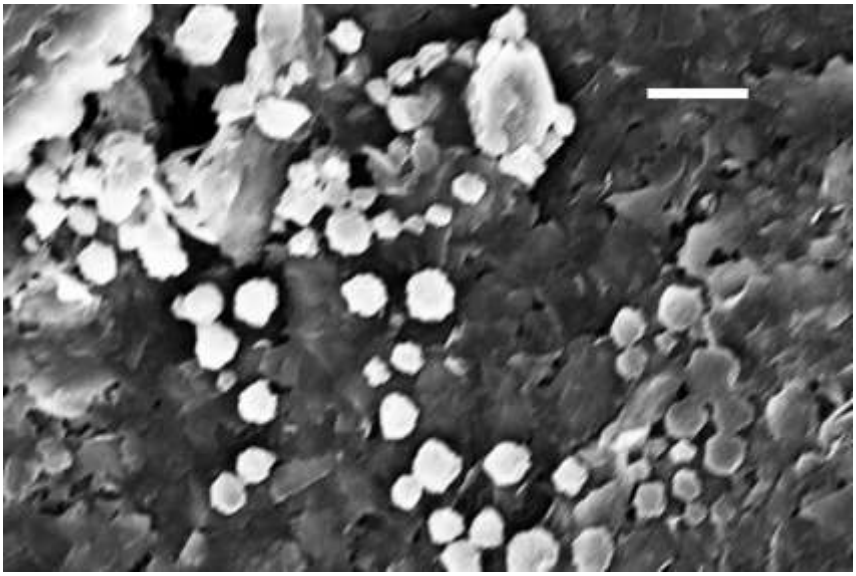


Fig. 4- SEM of dehydrated viable BT spores. A drop of ethanol solution of spores was placed on a graphite paste film coated aluminum coupon and allowed to dry overnight. All pictures include a white 1 micrometer dimension bar. A, Image of spores prior to EC treatment. B, SEM Image of BT Spores in 5 mM NaCl After EC Treatment at 200 mA for 5

min. C, SEM image of BT Spores in 5 mM NaCl After EC Treatment at 200 mA for 30 min.