Utilizing silver and copper ions for bacterial disinfection and subsequent removal of ions to supply safe drinking water

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Abstract

Inadequate access to safe drinking water is a critical challenge facing millions of people in third world countries. Ionic silver and copper both have proven biocidal capacities and are used in various water treatment applications such as cooling water and swimming pool disinfection. However, these methods of bacterial inactivation have not been expanded to include drinking water treatment due to the inherent health risks associated with consuming high concentrations of the metals which can accumulate in the systems, and therefore also the treated water. The goal of this research was to utilize copper and silver ions to inactivate bacteria in order to provide a method of drinking water treatment that does not require the use of toxic chemicals or large amounts of electricity, as under resourced communities do not have reliable access to these resources. This research also examined methods of removing or recovering residual metal from treated water. The ability to reduce the residual metal concentration not only allows the effluent to meet drinking water guidelines, it also allows for the concentration of metal used during disinfection to be significantly larger than used in previous research. By using a metal concentration that is one or two orders of magnitude higher than typical silver or copper ionization the disinfection is able to be carried out faster and to a fuller extent, all the while not increasing the risk to the consumer as the metal concentration can be decreased to within an acceptable range post disinfection. The lab scale, proof of concept methods used in this research show strong potential as prospective techniques to provide safe drinking water to people in third world nations.

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Table of Contents

List of Figures

List of Tables

Nomenclature

2-DE- Two-dimensional electrophoresis

ABNC- Active but nonculturable

ATP- Adenosine triphosphate

CFU- Coliform-forming units

DNA- Deoxyribonucleic acid

E_{ap}- Applied voltage

EFTEM- Energy-filtering transmission electron microscopy

FAA- Flame atomic adsorption

i- Electric current.

ICP-OES- Inductive coupled plasma-optical emission spectrometry

LRV- log removal value

MALDI-TOF MS- Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry

MBC- Minimum bactericidal concentration

MFC- Microbial fuel cell

MIC- Minimum inhibitory concentration

OCC- Open circuit control

SEM- Scanning electron microscopy

SHE- Standard hydrogen electrode

TEM-EDS- Transmission-electron microscopy-energy-dispersive spectroscopy

W- Energy consumed during disinfection

1. Introduction

1.1 Global drinking water issues

A critical day-to-day challenge facing people in under resourced communities and remote societies is insufficient access to safe drinking water (Shannon et al., 2008). Water-related diseases caused by inadequate drinking water, hygiene, and sanitation are responsible for an estimated 3.6 million deaths per annum (Prüss- Üstün et al., 2008). Conventional drinking water disinfection methods, such as chlorination, ozonation and ultraviolet light disinfection can successfully inactivate harmful microorganisms; however, these methods also require industrialized infrastructure for electric energy and chemicals that are often not available in under resourced communities on a reliable basis. The overall objective of the research conducted was to develop drinking water disinfection methods that can be constructed at low costs and operated without grid electricity or toxic chemicals so that under resourced communities can have access to safe drinking water.

1.2 Electrochemical cells and redox reactions

Electrochemical cells are an emerging technology that have a wide range of applications including medical, technological, and water treatment applications. These cells consist of two electrodes connected into a circuit. If a small voltage is applied between the two electrodes a current is induced in the system and a redox (reduction/oxidation) reaction occurs in the cell (e.g. Eq. 1-1 & 1-2). The oxidation reaction (loss of an electron) occurs at the anode (e.g. reverse

reaction of Eq. 1-1) while the reduction reaction (gain of an electron) occurs at the cathode (e.g. forward reaction of Eq. 1-2)

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad E° = 1.23 \text{ V vs. SHE (standard hydrogen electrode)} \quad (1\text{-}1)$$

$$4H^+ + 4e^- \rightarrow 2H_2 \quad E° = 0.00 \text{ V vs. SHE} \quad (1\text{-}2)$$

Depending on the composition of the electrodes, various ions can be released into the system from the anode. For example, if an anode is constructed out of silver, Ag^+ ions will be released via oxidation, also creating an electron in the process (reverse reaction of Eq. 1-3). An electron will then react with an Ag^+ ion at the cathode, reducing the ion back into metallic silver (forward reaction of Eq. 1-3). Over time an increase in silver concentration will be seen within the cell, as the reduction reaction at the cathode is rate limiting.

$$Ag^+ + e^- \leftrightarrow Ag_{(s)} \quad E° = 0.80 \text{ V vs. SHE} \quad (1\text{-}3)$$

1.3 Research Objectives

The primary goal of this research is to develop and test new drinking water disinfection mechanisms involving ionic silver and copper. This will involve determining the extent of inactivation that occurs when bacteria are exposed to the metal ions as well as ensuring the resulting water has a concentration of metal such that it meets drinking water regulations and guidelines. During the first study a voltage was applied between two silver electrodes to release silver ions and inactivate *E. coli* RS2gFP. This bacteria strain was chosen as it is a commonly used indicator bacteria and is resistant to certain antibiotics which can be incorporated into the system to minimize contamination. The electrochemical cell was run in a batch setup and

samples were taken periodically to determine both the *E. coli* concentration as well as the silver concentration resulting from the redox reactions. Another important aspect of this experiment was the recovery of the silver ions that was necessary in order to reduce health risks of drinking the effluent and conserve an expensive metal. The goals of this experiment were to:

1) Demonstrate effective microbial inactivation using the electrochemical silver disinfection method;

2) Establish a correlation between the electric current and resulting silver concentration for the estimation of disinfection performance;

3) Demonstrate further recovery of silver from treated water;

4) Quantify the energy requirement for this disinfection method.

The second study focussed on utilizing copper ions as the disinfection mechanism for drinking water and subsequently removing the metal from the solution. Similarly to the silver experiment, both the extent of microbial inactivation and the resulting metal concentration were critical aspects of this research as both adequate bacteria death and sufficient copper removal were required to ensure the water was safe to consume. Copper removal, not recovery, was prioritized as the price of copper is low enough that retaining the metal was not necessary. This experiment aimed to:

1) Exhibit microbial inactivation via the electrochemical copper disinfection method;
2) Demonstrate removal of copper from effluent water containing sufficiently high concentrations of copper
3) Determine the energy necessary to perform the microbial inactivation

1.4 Layout of Thesis

Chapter 2 of this thesis is a literature review of past research pertaining to silver and copper as biocidal agents, silver and copper ionization, and silver and copper nanoparticles. Chapter 3 focuses on the silver aspect of the conducted experiments, mainly a proof-of-concept experiment to determine the biocidal capacity of silver ions and a method of silver recovery within an electrolysis cell. Chapter 4 concentrates on the findings of the copper disinfection study performed as well as the copper removal portion of the experiment. Chapter 5 is the overall conclusions drawn from the conducted research as well as suggestions for future work.

2. Literature Review

2.1 Silver

2.1.1 Introduction

The biocidal capacity of ionic silver has been demonstrated in a number of modern scientific studies. In a 2008 study a research group found that silver nanoparticles (average size = 14 ± 6 nm) and silver ions had detrimental effects on both nitrifying organisms and *E. coli* PHL628-gfp (Choi et al., 2008). At a concentration of 1 mg L^{-1} silver, nanoparticles were found to inhibit the growth of nitrifiers by 86% while ions only hindered the growth by 42%. However, at a concentration of approximately 0.45 mg L^{-1} silver, the silver ions reduced the growth of *E. coli* by 100% while the nanoparticles only impeded it by 55%. This lends credence to the conclusion that nanoparticles and ions, while both disinfection agents, use different mechanisms

to inactivate bacteria.

Another group found that silver ions could be introduced to a system by applying a current between 2 silver electrodes, effectively linking silver ionization with electrochemical cells (Spadaro et al., 1974). They also saw an increase in the amount of silver released when a larger current was present, and the corresponding biocidal impact on *E. coli* and *S. aureus* showed a similar increase with a larger silver concentration. This group focussed their sampling on the areas directly adjacent to the electrodes and found that disinfection of bacteria only occurred at the anode, while the cathode displayed continuing growth of bacteria even when a current was being applied. This helps confirm that the silver ions, which are the medium of disinfection, are created at the anode while they are recovered at the cathode, effectively limiting the amount of bacterial inactivation that can occur in its immediate vicinity.

2.1.2 Silver ionization

The exact method that silver ions use to inactivate microorganisms is not yet confirmed as multiple impacts of silver have been observed when used to disinfect microbes. Some research has found that silver ions may disrupt the electron transfer chain of bacteria, rendering them unable to produce ATP (Adenosine triphosphate) and effectively inactivated (Yamanaka et al., 2005). Through energy-filtering transmission electron microscopy (EFTEM), two-dimensional electrophoresis (2-DE), and matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) it was found that silver penetrates the cell and interacts with the ribosomes, inhibiting the expression of various proteins and enzymes necessary for the production of ATP. However, in 2000, another group used combined electron microscopy and X-

ray microanalysis to view the impact that silver ions have on *E. coli* and *S. aureus* (Feng et al., 2000). They found granules containing silver both on the cell wall as well as within the cell body. Additionally, the cell membrane appeared to become unattached from the cell wall, and the DNA lost its replicative ability. In 2008 a research group found that a silver ion concentration of 0.2 ppm was able to achieve a log removal value (LRV) of 5 for both *E. coli and S. aureus* (Jung et al., 2008). This group used both a conventional plate count method as well as flow cytometric analysis. There were discrepancies between the plate count and cytometric results, with the microbial inactivation rate being far greater in the plate count method. These differences are thought to be attributed to bacteria being in an ABNC (active but nonculturable) state after being treated with the silver solution. TEM was also used to determine if there were any visible effects on silver on the bacteria. Similarly to another study, the cell membrane appeared to become detached from the cell wall which could lead to death of the microorganism (Feng et al., 2000).

Silver ions have also been shown to become collected or absorbed into bacteria and biofilms (Silvestry-Rodriguez, N., et al. 2008). While some biofilms are unhindered by this until a threshold concentration is reached (Silvestry-Rodriguez, N., et al. 2008), it allows for an interesting method of inactivation to occur in bacteria, as some bacteria can act as a reservoir of silver, effectively inactivating live bacteria they come into contact with post silver treatment. Due to Le-Chatelier's Principle (also called the equilibrium law), bacteria that had been inactivated with silver ions are able to be used as a disinfection instrument themselves as they have stored silver within their membrane (Wakshlak et al. 2015). When bacteria that had been inactivated with silver comes into contact with active bacteria that have not contacted silver some of the silver from the inactivated cells is transferred from the dead cell to the live one,

effectively inactivating the live cell. Initially, *Pseudomonas aeruginosa* PAO1 was inactivated using silver nitrate, and then the solution was centrifuged to remove the bacteria from the solution. Fresh live bacteria (that hadn't been exposed to silver up to this point) were then exposed to either the silver nitrate supernatant or the dead bacteria killed by the silver and significant bacteria inactivation was observed by both methods. Bacteria killed by low silver concentrations (1, 2 ppm) had a higher bactericidal affect than the supernatant that killed it, while higher silver concentrations (5, 10, 15, 20 ppm) resulted in similar LRV of both the bacteria and the supernatant. To ensure the bacteria were not being killed by some other mechanism, *Pseudomonas aeruginosa* was killed using heat and then fresh bacteria were exposed to these dead microbes and no noticeable inactivation occurred. Therefore, it is the presence of silver that allowed inactivation to occur, using inactivated microorganisms as a medium of interaction with new bacteria.

2.1.3 Silver nanoparticles

The largest advantage that silver nanoparticles have over other methods of disinfection is their extremely large surface areas (310 to 31000 nm^2 with diameters from 10 to 100 nm), which allow for a higher level of contact with microorganisms to be obtained (Rai et al., 2009). An experiment conducted in 2004 used SEM and TEM to determine that silver nanoparticles penetrate through cell membranes of *E. coli*, leaving holes in the protective layer, and accumulate within the cell membrane (Sondi & Salopek-Sondi, 2004). These holes in the cell wall significantly increase the overall permeability of the cell, eventually leading to cell death. Morones et al. conducted research that resulted in similar findings (Morones et al. 2005). However, they also observed a difference in inactivation mechanism based on the size of the

nanoparticles. When nanoparticles are within the 1-10 nm range they are able to attach to the cell membrane and disrupt its normal function, including respiration and permeability. As the nanoparticles become larger (10-100 nm) they appear to penetrate deeper into the cell, interacting with sulphur and phosphorus containing compounds within the cell such as DNA.

2.1.4 Impact on current research

There are numerous published works on the biocidal ability of silver and the various applications it is used for; however, drinking water applications have not yet been fully investigated. Also, there are very few studies that examine processes to remove, or more specifically, recover silver from treated water, as most applications are not based on being the primary disinfection mechanism for drinking water. Silver ionization usually involves using low silver concentrations, typically in the 0.1-0.4 mg L^{-1} range or below. Therefore, if a recovery method were to be addressed, using a higher concentration of silver could theoretically increase both the speed and extent of bacterial disinfection. Thus a proof of concept technique was introduced that uses higher silver concentrations than other research, as well as a recovery step to avoid unnecessary losses of valuable materials, all the while requiring minimal energy as to be provided by solar panels or bicycle generators.

2.2 Copper

2.2.1 Introduction

Copper (II) inactivates microorganisms and has been used to control microbial growth in various applications such as toxic algal bloom management and hospital applications (McKnight

et al. 1983). Metallic copper itself is able to disinfect bacteria a certain degree, as shown by a study done on various hospital surfaces (Mikolay et al., 2010). By replacing various surfaces in a hospital (light switches, push plates, and door knobs) with an alloy containing copper, researchers were able to compare the amount of coliform-forming units (CFU) present on each surface compared to the control subjects (made of the original material). Statistically significant differences were observed between the different materials, with the copper containing fewer CFU by up to 33%.

Copper removal from solution has been accomplished in previous research. Using a MFC (microbial fuel cell), an experiment was done that successfully removed over 99.88% of copper from a solution, all the while creating electricity (Heijne et al., 2010). The copper was deposited onto the cathode and no impurities (CuO, Cu_2O) were observed. While this method may have been effective, it is impractical as no significant removal was accomplished before 3-4 days elapsed and it took between 5-7 days for the concentrations to reach their minimum values. Similarly, other conducted research found that the copper removal on the cathode was dependent on multiple criteria, including initial copper concentration and inter-electrode distance (Tao et al., 2011). As the electrodes were moved closer together the output voltage increased, which increased the current intensity, leading to a larger amount of copper being deposited onto the cathode. Again, the time required for the reaction to occur is too large to be applicable in everyday use, needing over 480 hr. to remove just 92% of the initial copper.

2.2.2 Copper ionization

Copper ionization is a potential disinfection process, which is currently used primarily to disinfect non-potable water containing pathogens such as *Legionella pneumophilia*, and is

ordinarily used in combination with another form of disinfection (Cachafeiro et al., 2007). One drawback to copper ionization, when compared to other metal ionization methods, such as silver, is that copper has a weaker biocidal capacity than many other metals. This means that more copper ions or a longer time period is required to achieve the same disinfection as accomplished by other methods. Due to this, copper ionization has very few applications on its own, without being paired with silver ionization or another form of disinfection (chlorine, etc…).

There are multiple benefits to using copper ionization alongside silver ionization. First of all, copper is less expensive than silver, so if a certain amount of silver can be replaced with copper and still meet disinfection guidelines, it typically makes economic sense to do so. Secondly, certain microorganisms, such as *B. subtilis*, are more detrimentally affected by copper compared to silver (Rupareilia et al., 2008).

2.2.3 Copper nanoparticles

Copper nanoparticle applications have been developed and investigated for water treatment and microbial control (Ruparelia et al., 2008, Yoon et al., 2007). The main advantage of copper nanoparticles is their substantially large surface areas and thus their application amplifies the interaction between pathogens and copper materials (Ren et al., 2009). Similarly to copper ionization, copper nanoparticles can be paired with other disinfection mechanisms in order to increase the overall bacterial inactivation. Using disk diffusion tests, MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) it was determined that *B. subtilis* was more negatively affected by copper nanoparticles than silver (Ruparelia et al., 2008). It was also observed that the copper nanoparticles had an oxide layer form over their surface, which may play a role in the decreased biocidal capacity. In another study, it was found

that the susceptibility constant of *B. subtilis* was higher for copper nanoparticles than silver, again concluding that copper is a more effective biocide for these bacteria (Yoon, 2007). This research also investigated the impact of nanoparticle size on susceptibility, and while trends were observed, further research was required before any conclusions could be drawn.

Copper nanoparticles can cause serious health effects when residual nanoparticles are present in treated drinking water because they can become accumulated in human bodies (World Health Organization, 2008, Chen et al., 2006). Also, if the water is released to natural water systems, copper nanoparticles can be concentrated along the food chain in aquatic ecosystems as they are insoluble in water (Lee et al., 2008). By analyzing mice infected with copper nanoparticles, Chen et al. observed that the kidneys, liver, and spleen were the organs most affected by the metal (Chen et al., 2006). Also, male specimens were more harshly impacted and showed more severe symptoms than their female counterparts. Copper nanoparticles are not just detrimental to animal life, but also negatively impact the growth and lifespan of plants (Lee et al., 2008). TEM-EDS (transmission-electron microscopy-energy-dispersive spectroscopy) was used to observe the impact of both copper nanoparticles as well as ions on *P. radiates* (mung bean) and *T. aestivum* (wheat). It was found that it was the increasing nanoparticle concentration that impeded plant growth and life, while increasing the copper ion concentration did not impact the plant life detrimentally.

2.2.4 Impact on current research

While copper ionization is a confirmed method of disinfection, determining uses for it when it isn't coupled with another technology is an issue, due to its sub-par biocidal capacity at typical ionization concentrations. Also, current copper removal methods, such as microbial fuel

cell recovery, take upwards of 7 days to successfully remove copper from solution (Heijne et al., 2010). There is an opportunity to investigate the inactivation ability of copper ions at a much higher concentration than typically used in ionization. However, if a higher concentration of copper is going to be used, an effective and rapid method of copper removal must also be examined in order to make the treated water non-toxic. Therefore, a proof of concept technique was introduced that releases a higher copper concentration into solution than typically used, and a subsequent copper removal step was implemented after the disinfection, with minimal electrical energy requirements so solar panels and bicycle generators can provide sufficient energy.

2.3 Copper-silver ionization

Pairing silver ionization with copper ionization has become standard practise in many applications such as cooling water systems, swimming pools, air conditioning units, and hospital drinking water systems (Martinez et al., 2004, Landeen et al., 1989, Stout et al., 2003). Stout et al. (2003) investigated the effectiveness of copper-silver ionization systems used in hospitals to prevent legionnaires' disease. Preventing the disease was accomplished by controlling the amount of Legionella in the hospital's water system. In this research 16 hospitals were evaluated that had each previously reported cases of hospital-acquired legionnaires' disease. Before the ionization systems were installed, 47% of the hospitals reported that almost one third of their water sources contained Legionella. After 5-11 years of production (based on initial installation time), 43% of the hospitals reported no water sources contained Legionella, and more importantly, no hospital-acquired legionnaires' cases had occurred at any hospital with the ionization system.

Landeen et al. evaluated the impact of copper-silver disinfection on swimming pools by exposing bacteria known to inhabit pools to copper and silver ions (Landeen et al., 1989). They also examined the combination of copper-silver disinfection with free chlorine, one of the most common methods of swimming pool disinfection. Their findings show that combining copper-silver ionization with a low dose of free chlorine is more effective than either silver-copper ionization or a high dose of free chlorine on their own. They were able to achieve a *L. pneumophila* LRV of 5 with copper-silver ionization and 0.2 mg L^{-1} free chlorine in only 7 minutes of exposure, compared to a LRV of 2 achieved from free chlorine without ionization. Similarly, *E. coli* concentrations decreased by 99.99% (log 4) in just one minute when exposed to the combination, compared to log 3 removal using just free chlorine. A similar study was performed by Martinez et al., with their focus being on using copper-silver ionization to treat cooling water (Martinez et al., 2004). Their findings supported that a combination of copper-silver ionization and chlorine was extremely effective on many pathogens, including iron-related bacteria, sulphate-reducing bacteria, and slime-forming bacteria. Their treatment was able to inactivate over 99% of all of these pathogens, resulting in a significantly reduced rate or corrosion and biofilm formation.

3. Proof-of-concept study regarding silver ion disinfection and recovery

Abstract

Limited access to disinfected drinking water is a critical challenge in third world countries. Ionic silver has biocidal capabilities and silver ionization is an established method for cooling water disinfection. However, silver ionization cannot be used in drinking water treatment because of its inherent human health risks. In this proof-of-concept study, a laboratory scale electrochemical reactor was developed where ionic silver is released via oxidation of metallic silver and subsequently recovered through cathodic reduction. Complete inactivation of *Escherichia coli* was demonstrated in 30 min at an application of 1.2 V. A high silver concentration (>1 mg L^{-1}) was achieved in less than two minutes of water residence time within the disinfection reactor. The effluent silver concentration was linearly proportional to the electric current, allowing for reliable estimation of silver concentration and corresponding disinfection performance to be accomplished by measuring electric current using an electrical multimeter. The disinfected water was further treated in a separate silver recovery cell. The silver concentration dropped down to safe levels for continued human consumption (0.1 mg L^{-1}) via spontaneous cathodic silver reduction coupled with anodic oxidation of metallic aluminum. The estimated energy requirement was 6.1 kJ to treat 1 m^3 of water; thus, solar panels or bicycle generators can provide sufficient energy for disinfection. The electrochemical disinfection and subsequent silver recovery methods have a strong potential as a prospective method to supply safe drinking water in under resourced and remote societies.

3.1 Introduction

Limited access to safe drinking water is a serious and insistent challenge in underdeveloped and remote societies (Shannon et al, 2008, Sobsey et al., 2008, Enger et al., 2012). Water-related diseases by contaminated drinking water, hygiene, and sanitation cause an estimated 3.6 million deaths per annum (Prüss-Üstün, et al., 2008). Water treatment using various disinfection methods, such as chlorination, ozonation, and ultraviolet light radiation, can effectively inactivate microorganisms including human pathogens; however, people in under resourced societies cannot afford sufficient energy or infrastructure to produce the disinfection chemicals and operate the reactors for drinking water treatment (Sedlak & von Gunten, 2011). Thus, the main goal of this study is to develop an affordable disinfection method for people in under resourced societies.

Silver was used for water disinfection and antimicrobial therapy in ancient civilizations and the biocidal capacity of ionic silver has been demonstrated in a number of modern scientific studies (Lemire et al., 2013, Thurman et al., 1989, Spadaro et al., 1974, Russel & Hygo, 1994, Dankovich et al., 2015, Choi et al., 2008). With the strong biocidal capacity, silver ionization has been used to control *Legionella* pathogens in cooling water systems and hospitals (Stout & Yu, 2003, Stout et al., 1998, Liu et al., 1994). However, silver applications in drinking water treatment have been limited by potential health risks of continued silver consumption and high costs of silver. Silver concentration should be 0.1 mg L^{-1} or lower for continued human consumption to avoid potential adverse health risks (Gleick, 2002, US EPA, 2013). However, silver concentration at 0.1 mg L^{-1} or lower can substantially decrease the disinfection efficiency, indicating that silver concentration should be sufficiently high (e.g., 1 mg L^{-1} or higher) for

reliable and rapid inactivation of microorganisms (Silvestry-Rodriguez et al., 2008). In addition, the high concentration requirement for effective disinfection needs a large amount of silver, making the method expensive and economically infeasible. Applications of nano-sized silver particles have been extensively investigated as an alternative method for water disinfection and treatment (Dankovich et al., 2015, Choi et al., 2008, Loo et al., 2015, Schoen et al., 2010). Such nano-sized particles dramatically increase the effective surface area and thus improve the biocidal capacity of silver. However, silver nanoparticles can cause potential human health risks and they can accumulate in organisms along the food chain if they are released into natural water systems (Tang et al., 2009, Kim et al., 2009, Khan et al., 2015). Thus, this study focused on using millimetre-sized silver granules as the source of silver ions for water disinfection.

Here we have suggested and examined an innovative disinfection method where silver ions are released and simultaneously recovered via two electrode reactions (Fig 3-1A): one discharges silver ions into the solution through the oxidation of metallic silver at the anode (forward reaction of Eq. 3-1) while the other retrieves the dissolved silver by the reduction reaction at the cathode (reverse reaction of Eq. 3-1). It should be emphasized that during conventional silver ionization silver ions are dispersed into untreated water with no means of recovery. The cathodic reduction reaction differentiates this system from typical silver ionization techniques.

$$Ag_{(s)} \leftrightarrow Ag^{+} + e^{-} \qquad (3\text{-}1)$$

As silver ions travel from the anode to the cathode, the space between the two electrodes becomes an active disinfection zone where microorganisms are inactivated. Since the silver ions

are recovered at the cathode as metallic silver, the disinfected water is expected to have a low silver concentration. In addition, the residual silver ions in the disinfected water can be further recovered by coupling the silver reduction reaction (reverse reaction of Eq. 3-1) with aluminum oxidation reaction (forward reaction of Eq. 3-2), allowing safe human consumption of the treated water.

$$Al_{(s)} \leftrightarrow Al^{3+} + 3e^{-} \qquad (3\text{-}2)$$

Aluminum is a commonly used chemical in drinking water treatment for flocculation of suspended particles in water and excessive Al^{3+} will be naturally removed by aluminum hydroxide precipitation (Benjamin & Lawler, 2013). Also, the electric energy requirement is expected to be low since the first redox couple (forward and backward reactions of Eq. 3-1) is thermodynamically neutral and the second set of the redox reactions (backward reaction of Eq. 3-1 and forward reaction of Eq. 3-2) is driven spontaneously (Bard & Faulkner, 2001). Compared to currently available disinfection methods (e.g., chlorination, ozonation, and ultraviolet radiation), this electrochemical silver disinfection is expected to require an almost negligible amount of electric energy for microbial inactivation. Thus, a large amount of water can be treated using photovoltaic solar panels or bicycle generators, providing safe drinking water to people in under resourced societies. The specific objectives of this study are to: (1) demonstrate effective microbial inactivation using the electrochemical silver disinfection method; (2) establish a correlation between the electric current and resulting silver concentration for the estimation of disinfection performance; (3) demonstrate further recovery of silver from the treated water using the second set of the electrode reactions; and (4) quantify the energy requirement for this disinfection method.

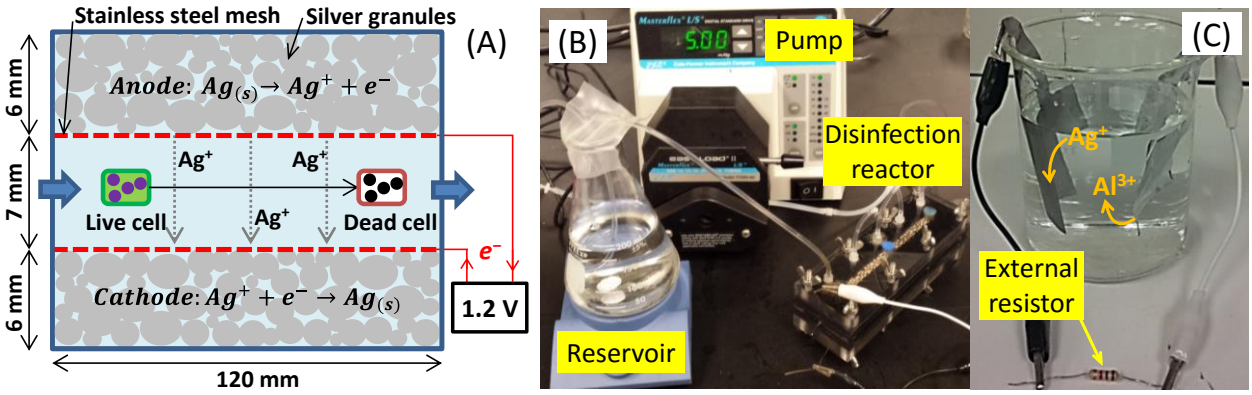

Figure 3-1. (A) Reactions expected in the electrochemical disinfection reactor. (B) Experimental setup for the disinfection experiment. (C) Silver recovery cell using aluminum foil.

3.2 Experimental methods

3.2.1 Electrochemical disinfection reactor

The disinfection reactor was constructed using 3 thin low-density polyethylene (LDPE) blocks covered with polyacrylic plates (McMaster Carr, USA). Each block was drilled to make a narrow inner chamber (width 0.8 cm, length 12 cm). The middle chamber (0.7 cm thick) was used for water flow and the upper and lower chambers (0.6 cm thick) were filled with silver granules (Silver Casting Grain, 99.9% purity, granule size between 1.6 and 6.4 mm, Alfa Aesar, USA) to be used as the anode and cathode (Fig 3-1A & B). Approximately 34.2 g of the silver granules were sandwiched between a polyacrylic end plate and a piece of stainless steel mesh (SS-306, 200 × 200 mesh, McMaster Carr, USA). The upper layer of the silver granules was used as the anode and the lower layer was the cathode. The silver granules were tightly sandwiched so that the whole layer behaved as an electrical continuum and the stainless steel mesh was used as the current collector. In a separate experiment, corrosion of the stainless steel

mesh was not observed (1.2 V application for 24 hr.). The effective reactor volume was approximately 11.2 mL while the empty volume was 17.7 mL without the silver granules.

3.2.2 *Escherichia coli* preparation

Escherichia coli RS2gFP was used to examine the disinfection capacity. The microbial strain was enriched in a nutrient broth at 37ºC to an approximate concentration of 10^8 CFU mL^{-1}. The nutrient broth was prepared with 10 g HiVeg lysate, 5 g yeast extract, 10 g sodium chloride, 10 mg rifampicin, and 100 mg kanamycin in 1 L of deionized water (Passmore et al., 2010). The bacterial cells were separated from the nutrient solution in a centrifuge at 8500 rpm for 10 min (Allegra 25R, Beckman Coulter, Germany). The centrifuged cells were then suspended in autoclaved 1 mM phosphate buffer solution (0.15 mM KH_2PO_4; 0.85 mM Na_2HPO_4). These centrifuge and suspension steps were repeated twice for further removal of nutrients. The suspended solution was diluted to ~10^5 colony forming units (CFU) mL^{-1} using 1 mM phosphate buffer solution before it was used in the silver disinfection experiment.

3.2.3 Reactor operation for disinfection test

Prior to the disinfection test, 70% (v/v) ethyl alcohol was pumped at 5 mL min^{-1} (Masterflex LS, Cole-Parmer Instrument Company, USA) through the reactor for 20 min to control microbial contamination (Rutala & Weber, 2008). The ethyl alcohol was replaced by deionized water which was pumped for another 30 min to remove residual ethanol from the reactor. After this cleaning process, the reactor was emptied and the prepared *E. coli* solution (~10^5 CFU mL^{-1}) was pumped at 5 mL min^{-1} for 15 min and the effluent was discarded. This

rinsing step with the *E. coli* solution is necessary to minimize the bacterial loss by adsorption on silver granules and reactor surfaces during the disinfection experiment. The main part of the disinfection experiment was performed for 30 min by circulating 200 mL of the solution between a reservoir and the disinfection reactor at 5 mL min^{-1} (Fig 3-1B). The reservoir was gently stirred using a magnetic stirrer.

During the disinfection experiment, two different voltage conditions (0.6 and 1.2 V) were applied and the resulting electric current was recorded every 30 sec using potentiostat (MGP-2, BioLogic, France). In addition to the two applied voltage conditions, two control experiments were conducted. In one control test, the *E. coli* solution was circulated between the reservoir and reactor without any voltage applications (Open Circuit). In another control experiment, the *E. coli* solution was kept in the reservoir without the circulation (No silver). All experiments were conducted in an air-conditioned laboratory at 22.1 ± 0.7°C.

3.2.4 Plate counting

Agar plates were prepared with 15 g agar, 5 g yeast extract, 10 mg rifampicin, 100 mg kanamycin, 10 g HiVeg lysate, and 10 g sodium chloride in 1 L of deionized water (Passmore et al., 2010). The added antibiotics (rifampicin and kanamycin) are necessary to selectively culture *E. coli* RS2gFP.

During the silver disinfection experiment, a sample of 0.2 mL was taken from the reservoir at time 0, 10, 20, and 30 min. The sample was serially diluted depending on the expected amount of viable cells. For instance, samples at 0 min were diluted by a factor of 10 and 100 while samples at 30 min with 1.2 V application were not diluted. The diluted or

undiluted samples (0.1 mL) were seeded on the agar plates and the seeded plates were incubated at 37ºC for 24 hr.

3.2.5 Reactor operation for current-silver correlation

The continuous-flow experiment was conducted using 1 mM phosphate buffer solution without the *E. coli* strain to correlate the electric current and concentration of ionic silver present in the effluent from the electrochemical disinfection reactor. The applied voltage increased stepwise every 22 min from open circuit, 0.3, 0.6, 0.9 and 1.2 V while 1 mM phosphate buffer solution was continuously pumped through the electrochemical disinfection reactor at 5 mL min^{-1} (no recirculation). At the end of each applied voltage condition, the effluent was taken and acidified using nitric acid (1% v/v) to be analyzed for silver in inductive coupled plasma-optical emission spectrometry (ICP-OES) (Vista Pro, Varian Inc., Australia). The effluent samples were also analyzed for pH and conductivity (Seven Multi, Mettler Toledo Group, Switzerland). There were no discernable changes in pH and conductivity from the influent as pH ranged between 7.2 and 7.4 while the conductivity was also stationary between 222 and 237 µS cm^{-1}.

In a separate continuous-flow operation, samples in the water flow chamber of the reactor were taken through predrilled holes located at 3, 6, and 9 cm from the inlet point. Taken samples were acidified and analyzed in ICP-OES to determine the silver concentration within the reactor.

3.2.6 Silver recovery test using aluminum foil

The silver recovery cell was prepared using a glass beaker with a 5 × 4 cm^2 piece of household aluminum foil as the anode and a 5 × 4 cm^2 piece of stainless steel mesh (SS-306, 200

× 200 mesh, McMaster Carr, USA) as the cathode (Fig 3-1C). The prepared electrodes were located a few centimeters from each other in the silver recovery cell without mixing conditions. The two electrodes were externally connected through a resistor (1000, 4700, or 9700 Ω). The potential drop across the external resistor was monitored every 30 sec using potentiostat (MGP-2, BioLogic, France) to determine the electric current generated in the silver recovery cell using Ohm's law. The silver recovery cell was filled with 200 mL of silver solution. The silver solution was collected from the electrochemical silver reactor (applied voltage = 1.2 V; flow rate = 5 mL min^{-1}) and stored in a glass container for 16 hr. prior to the silver recovery experiment. During the silver recovery experiment, a 4 mL sample was taken every 3 hr. for the first 12 hr. and then another sample was collected at 24 hr. The samples were acidified and analyzed for silver concentration in ICP-OES (Vista Pro, Varian Inc., Australia).

3.2.7 Silver recovery efficiency

Coulombic efficiency is the charge-based ratio between the amount of silver recovered and total charge transferred via the electrode reactions in the silver recovery cell as:

$$\text{Coulombic efficiency} = \frac{FVz\Delta c}{\int i\, dt} \qquad (3\text{-}3)$$

F is the Faraday constant, *V* is the volume of solution (0.2 L), *z* is the charge of ionic silver (+1), Δ*c* is the change in ionic silver concentration during the silver recovery test (mol L^{-1}), and *i* is the electric current (A).

3.3 Results and discussion

3.3.1 Disinfection effectiveness

Escherichia coli RS2gFP was successfully inactivated in the electrochemical system and the disinfection performance was substantially enhanced with an applied voltage. When 1.2 V was applied, viable *E. coli* colonies were not detected after 30 min, indicating complete inactivation of the microorganism (Table 3-1). When the applied voltage was decreased to 0.6 V, the electrochemical disinfection system showed 99.6% and 99.8% removal after the 30-min treatment (Table 3-1). For the open circuit condition (no electric current), a partial inactivation of *E. coli* was demonstrated with the observed 60.3% and 79.5% removal in 30 min (Table 3-1). This partial removal without electric current can be explained by silver ions becoming dissolved via non-electrode reactions because no distinct decreases in CFU were observed during the no-silver control experiment (Table 3-1). It should be noted that the total surface area of the silver granules was substantially large (~63 cm^2), allowing active silver dissolution via non-electrode reactions.

Table 3-1. Amount of viable cells (CFU mL^{-1}) during electrochemical disinfection. (Water volume = 200 mL; recirculation flow rate = 5 mL min^{-1}; 22.1 ± 0.7°C; N = 2)

	1.2 V Application		0.6 V Application		Open Circuit		No Silver	
0 min	675000	208000	120000	178000	78200	428000	340000	348000
10 min	7670	5700	13630	19930	26100	620000	564000	209000
20 min	10	330	6680	19910	84400	522000	256000	348000
30 min	0*	0*	480	300	16000	170000	552000	459000

* No detection of viable colonies

3.3.2 Ionic silver concentration

Ionic silver (Ag^{+}) released from the electrode reactions was the active disinfectant in the electrochemical system. In the abiotic experiment under the continuous flow condition, the silver concentration from the reactor increased as the applied voltage increased (Fig 3-2A). The open circuit condition resulted in 0.15 mg L^{-1} of silver in the effluent, indicating that silver is naturally dissolved from the silver granules without electrode reactions, explaining the partial inactivation of the bacteria under open circuit condition (Table 3-1). There was no statistically significant change in the effluent silver concentration for the increased applied voltage up to 0.6 V (Fig 3-2A). The relatively low silver concentration (0.2 mg L^{-1}) at the 0.6 V application explains the incomplete disinfection capacity (99.6% and 99.8%). It should be noted that the drinking water treatment regulations in developed countries commonly require at least log 3 removal of bacteria (i.e., 99.9% inactivation); therefore, the 0.6 V application does not provide adequate removal of bacteria for drinking water standards (Government of Ontario, 2006). When the applied voltage was increased above 0.6 V to 1.2 V, the silver concentration in the effluent increased sharply to 1.15 mg L^{-1} (Fig 3-2A). As a result, the 1.2 V application achieved complete inactivation of the *E. coli* strain in 30 min (Table 3-1). The comparison between the inactivation results (Table 3-1) and silver concentration (Fig 3-2A) indicates that the silver concentration should be 1 mg L^{-1} or higher for effective disinfection of *E. coli* (i.e. log 3 or higher removal in 30 min). When the

silver concentration at 1.2 V application (1.15 mg L^{-1}) is normalized by the total surface area of the silver granules, 0.012 mg L^{-1} of Ag^{+} was produced per cm^2 of the silver granule surface (average silver granule diameter = 4 mm; total mass of silver granules in the reactor = 68.4 g; metallic silver density = 10.49 g cm^{-3}).

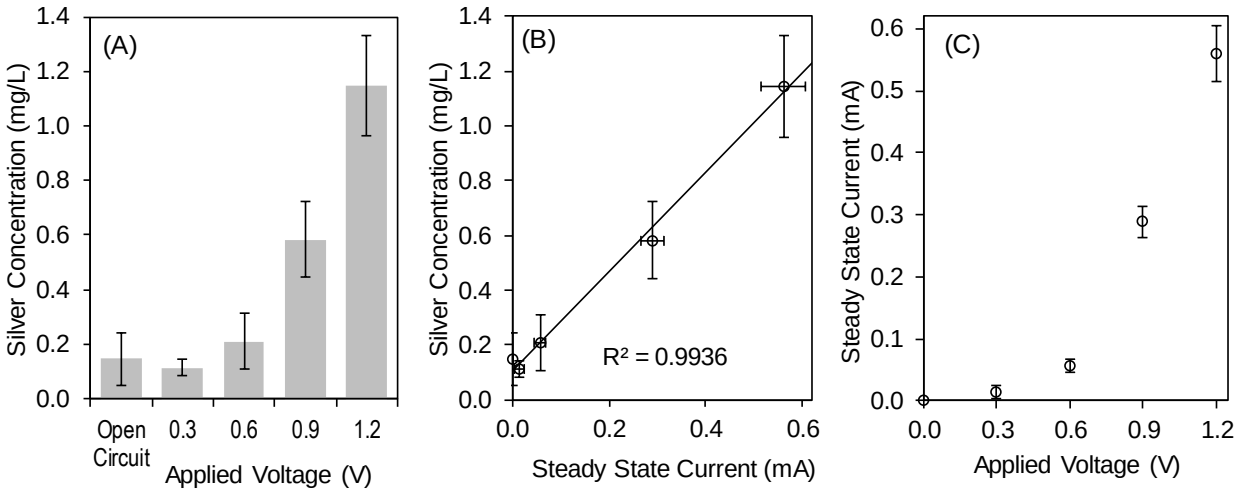

Figure 3-2. (A) Effluent silver concentration from the electrochemical disinfection reactor. (B) Linear correlation between the electric current and silver concentration. (C) Correlation between the electric current and applied voltage. (The steady-state current was obtained by averaging measured current values over the last 10 min of 22 min continuous-flow operation.) (Error bar = 2 × SD; N = 3)

The silver concentration in the effluent was linearly proportional to the electric current based on the R^2 value obtained (Fig 3-2B). This clear linear trend indicates that the effluent silver concentration can be estimated by measuring electric current in the electrochemical disinfection reactor. Measurement of silver concentration needs expensive analytical instruments (e.g., inductively coupled plasma, atomic adsorption, or photo colorimetry) and various

chemicals for sample preparation, making it practically impossible to measure silver concentration in under resourced communities (Eaton & Franson, 2005). However, silver concentration needs to be determined to estimate the disinfection efficiency in the Chick-Watson model (Benjamin & Lawler, 2013):

$$\frac{dX}{dt} = -kc^n \qquad (3\text{-}4)$$

X is the viable microbial population, *k* is the rate constant for disinfection, *c* is the disinfectant concentration, and *n* is the coefficient of dilution. The linear trend between the silver concentration and electric current will allow reliable estimation of the silver concentration and resulting disinfection effectiveness by measuring current using an electric multimeter.

If the applied potential exceeds 1.23 V, the water electrolysis reactions start contributing to the electric current and result in an overestimation of silver concentration as well as disinfection effectiveness. Therefore, we recommend that the electrochemical silver reactor be operated below the threshold voltage for water electrolysis (1.23 V).

3.3.3 Energy losses

The electric current in the silver reactor showed an exponential increase with the increasing applied voltage (Fig 3-2C). In an electrolytic cell, the applied voltage is equal to the sum of the equilibrium potential, overpotential loss, and resistive potential loss. For the highest voltage application of 1.2 V, the ohmic resistance accounted for ~0.17 V of the energy loss (inter electrode distance = 0.7 cm; solution conductivity = 230 µS cm^{-1}; *I* = 0.56 mA; cross sectional

area = 9.6 cm^2). This estimation indicates that the majority of the applied voltage was consumed for the electrode overpotential and ohmic losses. The electrode overpotential loss can be reduced by increasing the surface area of the electrode. While relatively coarse silver granules (1.6 - 6.4 mm in diameter) were used in this study, finer granules or other types of silver materials can be used to increase the electrode surface area.

The overall electrode reaction is thermodynamically neutral; that is, metallic silver at the anode is oxidized to ionic silver, which is then reduced to metallic silver at the cathode. Therefore, the equilibrium potential loss was created solely by the difference in ionic silver concentration between the anode and cathode surfaces; thus, this loss can be denoted as the concentration overpotential loss. The local concentration of ionic silver at the anode surface is higher than that at the cathode surface because ionic silver is created at the anode while it is removed at the cathode. This concentration difference can be minimized by inducing sufficient mixing conditions in the reactor. In this proof-of-concept study, the electrode silver granules were separated by a relatively fine stainless steel mesh (200 × 200 mesh) from the 0.7-cm wide inter-electrode space where the water flows easily without any obstacles. The estimated Reynolds number of 121 indicates laminar flow in the reactor (flow velocity = 0.0009 m s^{-1}; water viscosity at 22°C = 8.9 $\times 10^{-4}$ Pa·s), lacking hydrodynamic mixing (Finnemore & Franzini, 2006). Based on this discussion, it is suggested that, in future studies and practical applications, the reactor be designed to induce mixing conditions using lumpy spacers or receive water directly to the anode region and discharge it from the cathode side. These suggested design changes will help reduce the overpotential and equilibrium potential losses in the silver disinfection reactor.

3.3.4 Residence time requirement

In a separate set of the continuous-flow experiment, the silver concentration in the electrochemical silver reactor increased linearly in the water flow direction and reached its maximum value of 1.54 mg L^{-1} at 9 cm from the inlet point (Fig 3-3). The water flow path in the reactor was 12-cm long and the mean hydraulic residence time was 2.2 min during the experiment (effective volume = 11.2 mL; flow rate = 5 mL min^{-1}). Thus, less than 1.7 min of hydraulic residence time was actually required to attain the maximum silver concentration. This result indicates that the silver release reaction is achieved rapidly (less than 2 min) and thus the electrochemical silver reactor can be built in a compact size and readily transported to provide safe drinking water to people in remote communities.

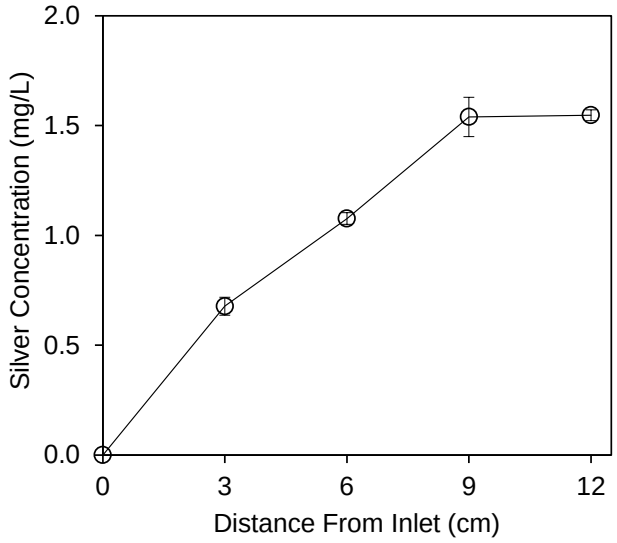

Figure 3-3. Silver concentration in the electrochemical disinfection reactor during continuous flow operation. (Applied voltage = 1.2 V; flow rate = 5 mL min^{-1}; length of the flow path in the reactor = 12 cm; error bar = 2 × SD; N = 3)

3.3.5 Silver recovery using metallic aluminum

A guideline on drinking water quality by the World Health Organization (WHO) states that silver concentration in drinking water should be 0.1 mg L^{-1} or lower for prolonged human consumption without potential adverse health risks (WHO, 2008). This guideline indicates that the water treated at 1.2 V is unsafe due to its high silver concentration above 1 mg L^{-1}. During the silver recovery experiment using the aluminum galvanic cell (Fig 3-1C), ionic silver concentration dropped to 0.1 mg L^{-1} in 12 hr. and the rate of silver recovery was not affected by the external resistance between 1000 and 9700 Ω (Fig 3-4A). It should be noted that the solution used in this recovery experiment was the effluent from the electrochemical silver reactor (applied potential = 1.2 V; flow rate = 5 mL min^{-1}) and kept in a glass container for approximately 16 hr. prior to this silver recovery test. The resulting aluminum release does not pose a health concern to humans. Aluminum salts (e.g., aluminum sulfate and poly aluminum chloride) are commonly added in drinking water treatment as a coagulant. In water, Al^{3+} is rapidly converted to $Al(OH)_3$ since it has a very low solubility limit (~10^{-5} M) under neutral pH ranges (US EPA, 2013). Thus, the aluminum oxidation reaction in the silver recovery cell does not pose human health risks. It should also be noted that the silver recovery cell was operated without mixing and the inter-electrode distance was approximately 2 cm. The hydrostatic condition and relatively distanced electrodes imply that the slow recovery (requiring ~12 hr.) can be accelerated by providing mixing conditions or minimizing the inter-electrode distance.

In the silver recovery cell, the theoretical equilibrium potential at the aluminum anode ranges from −1.77 to −1.79 V vs. SHE by the Nernst equation ($\{Al^{3+}\}$ = 0.01-0.1 mg L^{-1}; 22°C) while the cathode potential is between 0.45 and 0.52 V vs. SHE ($\{Ag^{+}\}$ = 0.1-1.5 mg L^{-1}; 22°C),

creating the open circuit potential of 2.22 to 2.31 V. This open circuit potential is large enough to drive the expected electrode reactions (backward reaction of Eq. 3-1 and forward reaction of Eq. 3-2) without any expensive catalysts or external power supplies to recover silver.

The electric current gradually decreased with time and the increasing external resistance from 1000 to 9700 Ω (Fig 3-4B). The coulombic efficiency values calculated using Eq. 3-3 were 29% (1000 Ω), 36% (4700 Ω), and 102% (9700 Ω), increasing with the increasing external resistance. This result indicates that the majority of the cathode reaction was driven by the reduction of ionic silver with the large external resistance (9700 Ω); however, the smaller external resistances resulted in other reduction reactions at the stainless steel cathode, such as reduction of dissolved oxygen into water.

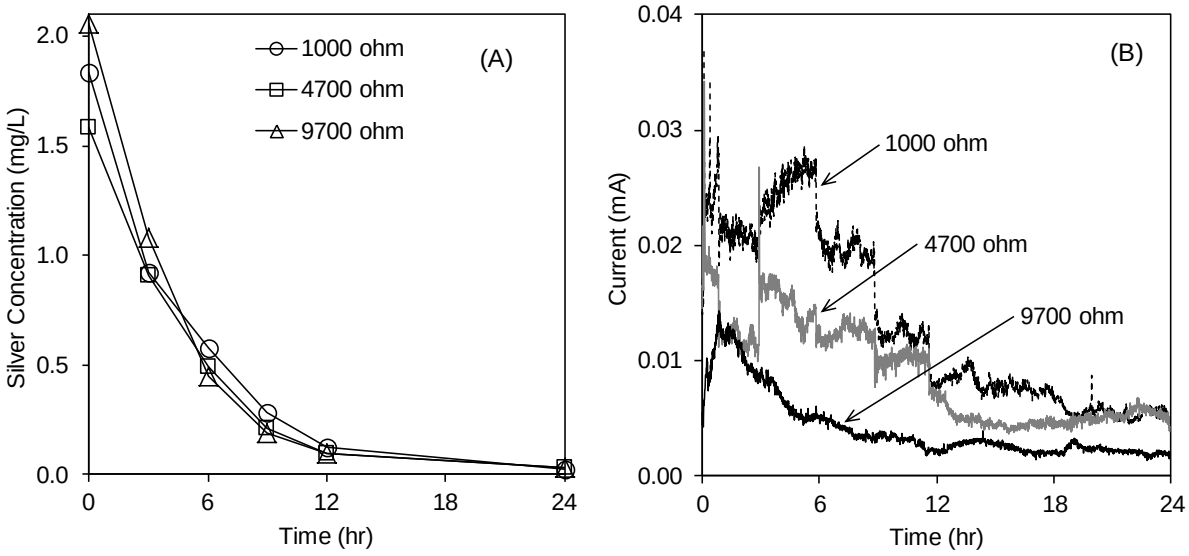

Figure 3-4. Silver recovery from 200 mL silver solution using aluminum foil with various external resistors. (A) Silver concentration in the silver recovery cell. (B) Electric current during the silver recovery experiment. (The initial silver solution was the effluent from the electrochemical disinfection reactor at 1.2 V and 5 mL min^{-1}.)

3.3.6 Energy requirement

The energy requirement for the electrochemical disinfection is small enough to be operated by solar panels or bicycle generators. During the experiment, 1.22 J of electric energy was consumed for the treatment of 0.2 L of the *E. coli* solution (1.2 V; 0.56 mA; 30 min), resulting in the energy requirement of 6.1 kJ per treatment of 1-m^3 water. Commercial photovoltaic solar panels can produce electric power between 35 and 103 kJ h^{-1} per 1-m^2 solar panel (total solar power assumed to be 411 kJ h^{-1}; solar panel efficiency between 8.5 and 25%) (Schiermeier et al., 2008, Green et al., 2010). This calculation indicates that a 1-m^2 photovoltaic solar panel can provide enough energy to disinfect 5.7 to 16.9 m^3 of water in 1 hr. A bicycle generator is known to create electric power up to 1080 kJ h^{-1}, which can be used to disinfect 177.2 m^3 of water per hour (MNS Power Generating Systems, 2015). Since the reactor in this study was prepared as a proof-of-concept design, there is a room to further reduce energy consumption in practical applications. For instance, the inter-electrode distance (0.6 cm in this study) can be decreased and the electrode surface area can be increased by decreasing the silver granule size (1.6 - 6.4 mm in this study).

3.4 Conclusions

Limited access to clean and safe drinking water is a global water challenge that millions of people currently face in third world countries. The disinfection system proposed and examined in this proof-of-concept study was targeted to prove that this system has a potential to provide safe drinking water to under resourced societies without industrial infrastructure for centralized

water treatment. In the lab-scale experiments, we demonstrated that the electrochemical reactor achieved complete inactivation of *E. coli* from $>10^5$ CFU mL^{-1} to zero viable cells in 30 min when 1.2 V was applied to the system. The rapid inactivation of *E. coli* was achieved by a substantially high residual silver concentration (above 1 mg L^{-1}) and the high silver concentration was attained in less than 2 min of water residence within the reactor. A clear linear relationship between the silver concentration and electric current was established. Such a relationship will be useful to estimate disinfection effectiveness by measuring electric current, not silver concentration. Note that silver analyses require expensive analytical instruments and sample preparation chemicals. In the silver recovery cell with aluminum oxidation, the high silver concentration was reduced in the silver recovery cell to the safe level for long-term human consumption (0.1 mg L^{-1}). This silver recovery reaction was driven simultaneously without any electric energy demand and the energy requirement for the disinfection was substantially small so that a bicycle pedal generator or photovoltaic solar panel can provide sufficient energy for drinking water treatment. These conclusions support that the demonstrated disinfection and recovery system has the potential to provide safe drinking water to people in under resourced communities around the world. The examined system can supply clean water for a long time with only a small amount of metallic silver. It should be noted that the disinfection experiment was performed separately from the silver recovery experiment to avoid potential removal of *E. coli* cells by aluminum precipitants. In practical applications, the aluminum electrode can be incorporated in the disinfection reactor so that ionic silver can be recovered directly onto the silver electrodes, allowing an extended use of the silver electrodes for water purification.

3.5 Acknowledgements

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4. Proof-of-concept study regarding copper ion disinfection and removal

Abstract

Inadequate access to drinking water is a crucial problem faced by people in third world countries. Copper ions have biocidal capacities but are also toxic if ingested in high enough concentrations. In this proof of concept study we have developed an electrochemical disinfection reactor where metallic copper releases copper ions via electrically induced oxidation and subsequently recovers the ions via reduction. The copper ions act as a biocidal agent to inactivate *E. coli* and a LRV of up to 2.81 was attained after 2 hr. of application at an applied voltage of 1.5 V. Residual copper was removed from the solution via pH alteration, lowering the copper concentration from over 16.8 mg L^{-1} to as low as 0.74 mg L^{-1}, well within World Health Organization guidelines for safe drinking water. The estimated electrical energy needed to disinfect 1 m^3 of water is 45.5 kJ. This amount of energy is easily produced by readily available photovoltaic solar panels or portable bicycle generators. This disinfection method and corresponding copper removal show a large potential to supply drinking water to people with inadequate access.

4.1 Introduction

A critical day-to-day challenge facing people in under resourced communities is insufficient access to safe drinking water. Conventional drinking water disinfection methods, such as chlorination, ozonation and ultraviolet light disinfection, require industrialized infrastructure for electric energy and chemicals that are often not available in under resourced communities. The main objective of this proof of concept study was to develop a drinking water disinfection method that can be constructed at low costs and operated without grid electricity or toxic chemicals.

Copper inactivates microorganisms and has been used to control microbial growth in various applications such as toxic algal bloom control, air conditioning systems and swimming pools (McKnight et al., 1983, Martinez et al., 2004, Landeen et al., 1989). An established disinfection process is copper ionization, which is used mostly to disinfect non-potable water containing pathogens, such as *Legionella pneumophilia* (Liu et al., 1994, Lin et al., 1996, Miuetzner et al., 1997, Cachafeiro et al., 2007, Landeen et al., 1989). In addition to copper ionization, copper nanoparticle applications have been developed and investigated for water treatment and microbial control (Ruparelia et al., 2008, Esteban-Cubilo et al., 2006, Yoon et al., 2007). Copper nanoparticles provide substantially large surface areas and thus their application amplifies the interaction between pathogens and copper materials (Ren et al., 2009). However, copper nanoparticles pose a serious health risk as residual nanoparticles that are present in treated water can accumulate in human bodies (World Health Organization, 2008, Chen et al., 2006). Also, if the water is released to natural water systems, copper nanoparticles can be

concentrated along the food chain in aquatic ecosystems (Lee et al., 2008). Thus, we focused on utilizing Cu^{2+} ions as the active disinfectant in this study.

In this experimental work, we integrated electrochemical copper ionization with an efficient copper removal process so that the disinfected water can be safely supplied as drinking water. In electrochemical copper ionization a small voltage is applied between two copper electrodes. Copper is then oxidized to Cu^{2+} ions at the anode (backward reaction of Eq. 4-1) and the Cu^{2+} ions migrate to the cathode where they are reduced to metallic copper (forward reaction of Eq. 4-1).

$$Cu^{2+} + 2e^{-} \leftrightarrow Cu_{(s)} \qquad E° = 0.337 \text{ vs. SHE} \qquad (4\text{-}1)$$

While the copper ions are migrating from the anode and cathode, they inactivate microorganisms present in the water (Fig 4-1A). After electrochemical copper ionization, a large amount of residual copper ions remains present in the ionized water due to the rate limiting reduction reaction at the cathode. Even though this copper ionization technique is commonly used for non-potable water treatment, such as cooling water disinfection and swimming pool cleaning, this method cannot be applied in drinking water disinfection because high concentration of residual copper ions is toxic for human health (Matrinez et al., 2004, Landeen et al., 1989). In this study, we induced precipitation of copper oxide (Eq. 4-2) (Stumm and Morgan, 2012) by increasing pH to remove residual ionic copper using commonly available chemicals (e.g., caustic soda, lime stone) so that this method can be broadly applied in under resourced communities.

$$Cu^{2+} + 2OH^{-} \rightarrow CuO_{(s)} + H_2O \qquad (4\text{-}2)$$

Another challenge in using copper ions as a disinfectant is their relatively weak biocidal capacity. As a result, copper ionization is often paired with silver ionization and chlorine-based chemicals to improve microbial inactivation (Stout et al., 1998, Rohr et al., 1999, Liu et al., 1994, Blanc et al., 2005). However, one major benefit of copper ionization over silver ionization is the substantially lower cost of the metal. Thus, in this study, we focused on maximizing the disinfection capacity by increasing copper ion concentration to establish this method as a stand-alone technology for water disinfection. The copper ion concentration (>10 mg L^{-1}) also make our method distinct from conventional copper ionization where ionic copper levels range from 29 µg L^{-1} up to 1 mg L^{-1}, and typically fall into the 0.1-0.4 mg L^{-1} range (Cachafeiro et al., 2007, Landeen et al., 1989, Lin et al., 1996). Based on lab experiment results, we evaluated the feasibility of this method for broad applications in remote and under resourced communities by estimating electric energy consumption and alkalinity chemical requirements.

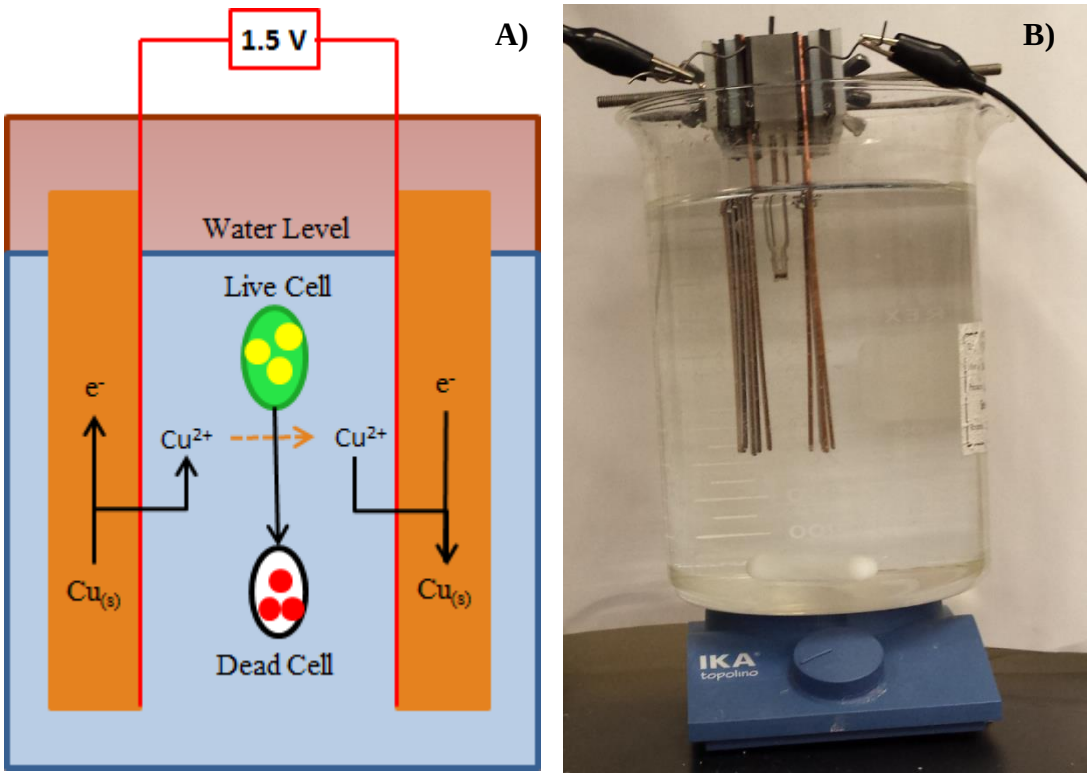

Figure 4-1: Copper electrochemical disinfection of *E. coli*. A) Representation of electrochemical reactions and disinfection. B) Experimental setup for disinfection tests.

4.2 Experimental methods

4.2.1 Electrochemical disinfection cell construction

The electrochemical cell consists of multiple 12.5 cm-long copper plates (1.0 cm wide and 0.1 cm thick) while 7.8 cm was immersed in water during experiment (Fig 4-1B). The anode consists of 5 copper plates and the cathode was 2 copper plates. This electrode configuration gives an effective surface area of 86.3 cm^2 for the anode and 34.52 cm^2 for the cathode, allowing more rapid dissolution of copper ions at the anode than copper reduction at the cathode to favour the ion release and subsequent disinfection step over the reduction recovery portion of the

process. The inter-electrode distance was 1.2 cm and a Ag/AgCl reference electrode was placed in this area during disinfection tests (Fig 4-1B). During disinfection experiments, the electrochemical cell contained 1 L of solution (Fig 4-1B). Prior to each disinfection test the copper plates were cleaned using sand paper.

4.2.2 *Escherichia coli solution* preparation

Escherichia coli RS2gFP was used in the disinfection experiment. The *E. coli* strain was cultivated in a nutrient solution at 37°C for 15-18 hr. to reach an approximate initial concentration of 10^8 CFU mL^{-1}. The nutrient solution was prepared using a ratio of 10 g HiVeg lysate, 5 g yeast extract, 10 g sodium chloride, 100 mg kanamycin and 10 mg rifampicin per 1 L of deionized water. Note that *E. coli* RS2gFP can grow with the antibacterial chemicals (kanamycin and rifampicin) (Passmore et al., 2010). After incubation, *E. coli* was separated from the nutrient solution via centrifugation at 8500 rpm for 10 min (Allegra 25R, Beckman Coulter, Germany). The cells were then suspended in 1 mM phosphate buffer solution (0.15 mM KH_2PO_4; 0.85 mM Na_2HPO_4). The centrifugation step was conducted three times to ensure that all nutrient composites were properly removed. After centrifugation, the *E. coli* cells were dispersed in the 1 mM phosphate buffer solution at an estimated concentration of 10^9 CFU mL^{-1}. For the disinfection experiment, the prepared *E. coli* concentration was diluted to approximately 10^5 CFU mL^{-1} using 1 mM phosphate buffer solution.

4.2.3 Disinfection tests

For the disinfection test, the *E. coli* solution (10^5 CFU mL^{-1}) was adjusted to pH of 4.9, 6.0, or 7.4 by adding 57, 46, or 0 µL of 1 M nitric acid, respectively. The designated voltage (1.0, 1.5, or 2.0 V) was applied and resulting electric current was monitored and recorded every 10 seconds using a potentiostat (MGP-2, BioLogic, France). Aside from the tests with applied voltages, two control experiments were conducted: one with the copper electrodes in the electrochemical cell without electric current (Open circuit control); and the other without the copper electrode (No copper control). All disinfection experiments were performed in duplicate at 22.9 ± 2.1°C.

During the disinfection test, 0.1 mL samples were taken every 30 min for plate counting. If necessary, each disinfection sample was immediately diluted by 10 or 100 times using 1 mM phosphate buffer solution depending on expected microbial concentration. The sample was plated on an agar plate which was then incubated at 37°C for 24 hr. for microbial colony growth. The agar was prepared using 15 g agar, 5 g yeast extract, 10 g Hiveg lysate, 10 g sodium chloride, 100 mg kanamycin, and 10 g rifampicin in 1 L deionized water and then poured into sterile petri dishes.

An additional 8-mL sample was taken every 30 min during the disinfection test and the sample was acidified using 2 mL 1% (v/v) nitric acid. The acidified sample was analyzed for copper ions in FAA (Flame atomic adsorption) spectrometry (Varian, SpectrAA220, Varian Ag, 327.4 nm).

4.2.4 Energy requirement estimation

The electric energy requirement for electrochemical disinfection was calculated using:

$$W = E_{ap} \int i dt \qquad (4\text{-}3)$$

W is the energy consumed during disinfection, E_{ap} is the applied voltage to the electrochemical cell, and *i* is the electric current.

4.2.5 Copper removal tests

The copper removal test was performed separately from the disinfection test using 1 mM $NaHCO_3$ with a pH adjustment to approximately 4 using hydrochloric acid. In the electrochemical cell, 1 L of the $NaHCO_3$ solution was ionized at 1.5 V for 4 hr. The copper ionized solution was split into five 0.2-L glass containers. The copper ionized solutions were respectively dosed with: NaOH (0.1, 0.5, and 1 mM); 1-M HCl (0.2 mL); and no pH change as control. The solutions were rapidly mixed for 5 min followed by 10-min of gentle mixing for flocculation. The solution containers were then covered and left with no mixing thereafter. An 8-mL sample was taken at 1, 2, 3, and 22.5 hr. after the NaOH or HCl addition, Nitric acid (2 mL 1% v/v) was added to each sample for FAA analysis to determine the copper concentration. The samples were also analyzed for pH and conductivity (Seven Multi, Mettler Toledo Group, Switzerland). The copper removal experiment was performed in an air conditioned laboratory at 22.9 ± 2.1°C.

4.3 Results and discussion

4.3.1 Effect of applied voltage on disinfection

E. coli inactivation was most effective (2.8 log inactivation or 99.8% inactivation at 120 min) at 1.5 V application while 2.0 V application resulted in a poor inactivation result (1.6 log-removal at 120 min) (Fig 4-2A). The ionic copper concentration in the electrochemical disinfection reactor increased with time and was the highest (11.6 mg L^{-1} at 2 hr) at an applied voltage of 1.5 V (Fig 4-2B). Note that the ionic copper concentration was smaller at 2.0 V (9.0 mg L^{-1}). This result is consistent with the log removal result as 1.5 V application showed better inactivation than 2.0 V (Fig 4-2A). The comparison between the log inactivation result and copper concentration confirms that the disinfection performance is proportional to ionic copper concentration. It should be emphasized that the substantially high residual copper concentration (11.6 mg L^{-1} at 1.5 V), compared to 0.1-0.4 mg L^{-1} in most conventional copper ionization treatments, makes this method unique and allows for more rapid disinfection (Cachafeiro et al., 2007, Landeen et al., 1989, Lin et al., 1996). In the open circuit control experiment, a log-removal of 0.9 (i.e., 87% inactivation) was achieved at 2 hr (Fig 4-2A) while ionic copper was not detected until the 2 hr point (Fig 4-2B). These results imply that *E. coli* cells were partially inactivated by physical contacts with metallic copper surface.

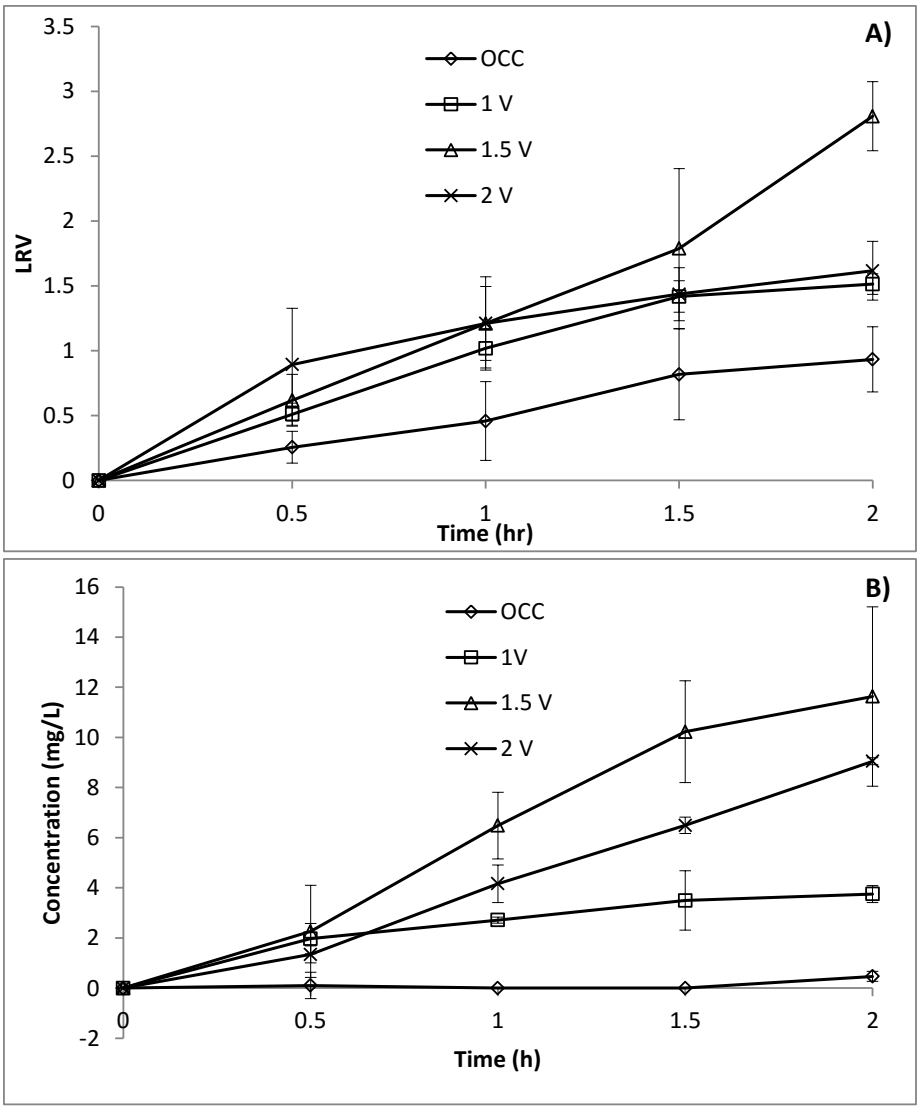

Figure 4-2: LRV and resulting copper concentration from the disinfection test over a range of applied voltages. A) Log removal value vs. time. B) Copper concentration vs. time. (pH = 6; water volume = 1 L; temperature = 22.9 ± 2.1 °C) (Error bar = 2 × SD; N = 4)

4.3.2 Competing reaction at the anode

The electric current and individual electrode potential were stationary during the disinfection experiment (Fig 4-3). A greater applied voltage led to a higher electric current (Fig 4-3A) as well as a more positive anode potential and more negative cathode potential (Fig 4-3B).

The higher current indicates more rapid dissolution of copper at the anode; thus, the copper concentration is expected to be higher. However, the copper concentration decreased (Fig 4-2B) with the increased electric current at 2.0 V application compared up to an applied voltage of 1.5 V (Fig 4-3A). This result indicates that there is an anode reaction competing against the copper dissolution at the 2.0 V application. When the applied voltage was 2.0 V, the resulting anode potential ranged between 0.877 and 0.908 V vs. SHE, which is more positive than that for the oxygen evolution reaction by water electrolysis at neutral pH conditions as:

$$O_2 + 4H^+ + 4e^- \leftrightarrow 2H_2O \quad \text{E = 0.816 and 0.875 V vs. SHE (pH 7 and 6, respectively)} \quad (4\text{-}4)$$

As a result, the oxygen evolution reaction competed against the copper dissolution reaction at the anode under 2.0 V application, leading to reduced copper ion concentration even with the increased electric current. It should be noted that gas bubble formation was observed on the surface of the anode for 2.0 V application. Note that the anode potential at 1.5 V application was 0.716 to 0.788 V vs. SHE and lower than the oxygen evolution threshold. Thus, there was only copper oxidation at the anode, resulting in the highest copper concentration. This finding indicates that the anode potential should be carefully monitored to avoid oxygen evolution reaction to keep ionic copper concentration high (i.e., > 10 mg L^{-1}) and thus accelerate water disinfection.

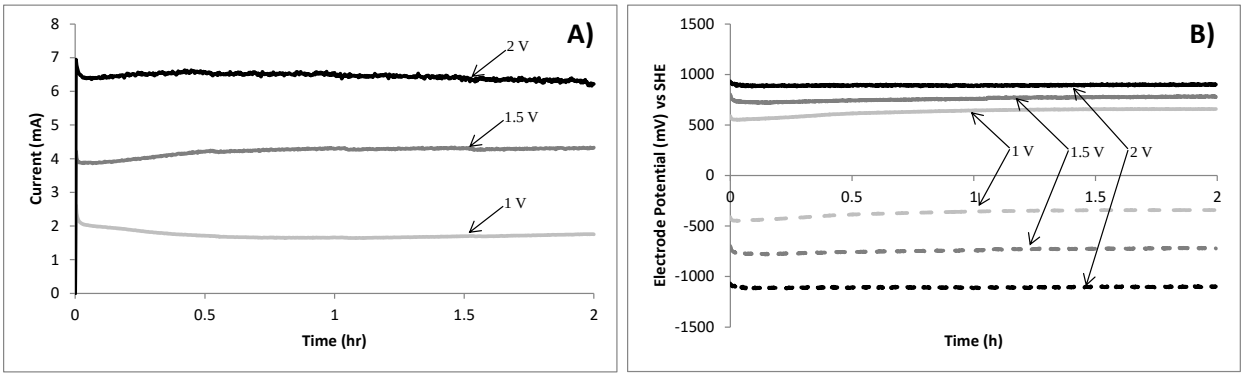

Figure 4-3: Induced electric current and electrode potential during the disinfection test over a range of applied voltages. A) Electric current vs. time. B) Anode (more positive) and cathode (more negative) potential vs. time. (pH = 6; water volume = 1 L; temperature = 22.9 ± 2.1 °C)

Both the Canadian drinking water guidelines and the EPA National Primary Drinking Water Regulations say a level of 0 detectable bacteria per 100 mL of water must be maintained to meet drinking water regulations (Health Canada, 2014, Table of Regulated Drinking Water Contaminants, 2016). In this proof-of-concept study, the electrode surface area was relatively small (86.3 cm^2 of anode and 35.42 cm^2 of cathode) per liter of reactor volume. As a result, the copper concentration increased gradually over 2 hr. (Fig 4-2B). By using thin and long copper wires or copper granules as the electrode material (rather than copper plates), the electrode area to volume ratio can be dramatically increased so that high copper concentration can be rapidly attained during disinfection, achieving more effective log-inactivation of microorganisms (Parr and Kim, 2016).

4.3.3 Energy requirement

For the 1.5 V application, the energy requirement for disinfection was 45.5 J using Eq. 3. Readily available photovoltaic solar panels are capable of producing between 35 and 103 kJ h^{-1} (assuming an efficiency between 8.5-25%) for every m^2 of surface area; thus, a 1 m^2 solar panel can treat between 0.8 and 2.3 m^3 of water every hr. (Schiermeier et al., 2008, Green et al., 2010). A portable bicycle generator can provide energy to disinfect 1.6 m^3 every hr. while a larger, less portable bike generator can treat 23.7 m^3 every hr. (MNS Power Generating Systems, 2015).

4.3.4 Effect of pH on disinfection

At pH 5 and 6 the log removal increased linearly with contact time and ranged between 2 and 2.8 (99 to 99.8% inactivation) at 2 hr. (Fig 4-4A). However, when the initial pH was 7, the log inactivation was dropped down to 0.4 (60% inactivation) at 2 hr. (Fig 4-4A). This result shows that electrochemical copper disinfection is very sensitive to pH conditions. Note that the solution pH did not affect the bacterial viability in separate control experiments where the *E. coli* solutions of pH 5, 6, and 7 were left in a glass container without copper for 2 hr. (Table S1 in Supplementary Information).

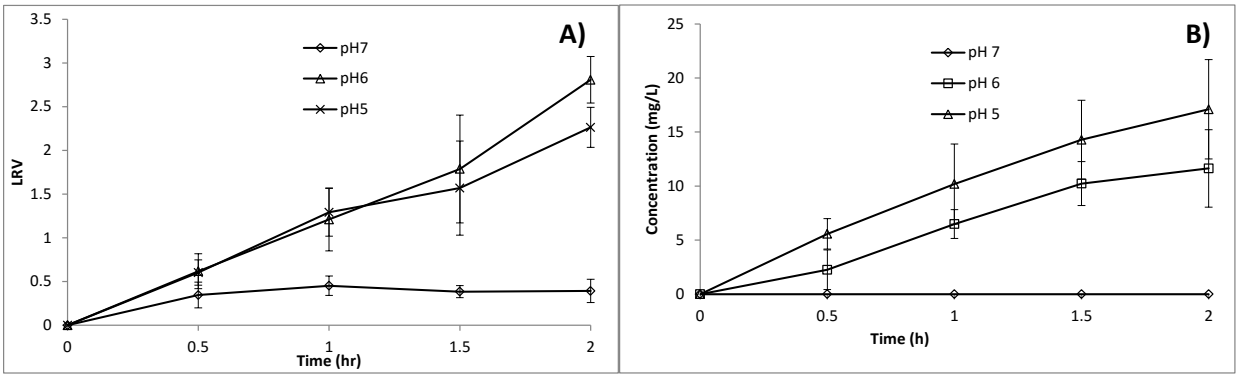

Figure 4-4: LRV and resulting copper concentration from the disinfection test over a range of pH values. A) Log removal value vs. time. B) Copper concentration vs. time. (Applied voltage = 1.5 V; water volume = 1 L; temperature =22.9 ± 2.1 °C) (Error bar = 2 × SD; N = 4)

With a pH of 7 the copper concentration during the disinfection experiment was negligible, below the detection limit (Fig 4-4B), explaining the limited disinfection (log 0.4 inactivation) at 2 hr. (Fig 4-4A). At pH 5 and 6, sufficiently high copper concentrations was attained above 10 mg L^{-1} at 2 hr. (Fig 4-4B), explaining the meaningful log inactivation (Fig 4-

4A). Therefore, the slightly acidic pH condition (pH of 6 or lower) is thought to be required to provide optimal conditions for oxidative copper dissolution at the anode and thus microbial inactivation

4.3.5 Precipitation on anode surfaces at pH7

For pH of 7, the electric current was initially high at 3.8 mA but it rapidly dropped in 5 min (Fig 4-5A). Consequently the anode potential increased sharply from 924 mV to 1139 mV in the first 5 min, (Fig 4-5B), indicating that the anode overpotential (energy required to drive the electrode reaction) was increased substantially. It should be emphasized that the electric current and anode potential were initially similar for the 3 pH conditions at time zero (Fig4- 5). During the disinfection experiment at pH 7, a substantial amount of green to blue precipitant was created on the anode surface while such precipitation was not visible or observed for the experiment at pH 5 and 6. The anode precipitant is thought to decrease the effective surface area of the anode and thus increased the overpotential for copper oxidation; as a result, the anode potential increased sharply (Fig 4-5B) and thus decreased the electric current (Fig 4-5A).

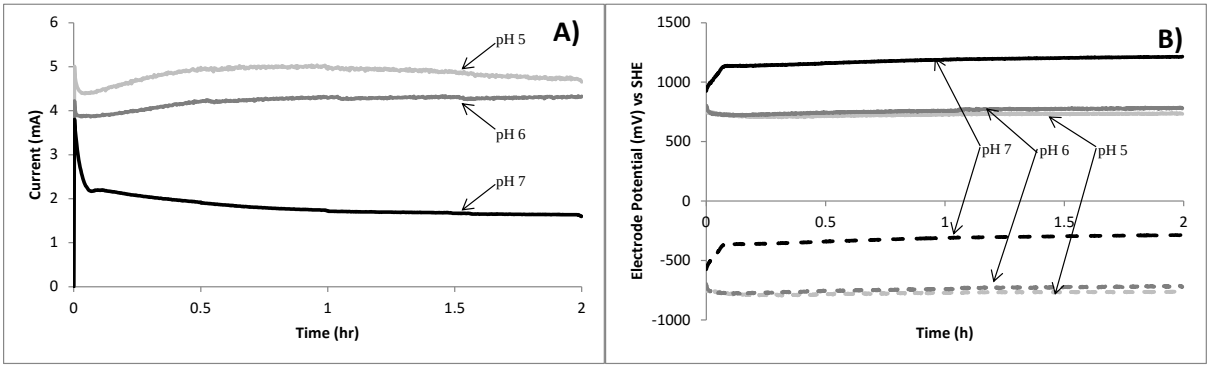

Figure 4-5: Induced electric current and electrode potential during the disinfection test over a range of pH values A) Electric current vs. time. B) Anode (more positive) and cathode (more negative) potential vs. time. (Applied voltage = 1.5 V; water volume = 1 L; temperature = 22.9 ± 2.1 °C)

4.3.6 Copper removal after disinfection

By increasing pH of the copper ionized solution, ionic copper was successfully removed by driving copper oxide precipitation (Eq. 4-2). Over the 22.5 hr. sampling period the samples from the acidified control solution displayed an increase in copper concentration while samples from the control and 0.1 mM NaOH dose showed very little to moderate copper decrease (Table 4-1). The 0.5 and 1 mM NaOH dose samples had the greatest removal of copper, with each approaching or reaching the WHO guideline limit for copper. The 0.5 mM dose resulted in the greatest copper removal, with up to 88% of the copper being removed within the first 3 hours and up to 95% being removed over 22.5 hr.

Table 4-2 Resulting copper concentrations over 24 hr as a product of pH change via HCl or NaOH dosing.

	Acidified Control				Control				0.1 mM NaOH				0.5 mM NaOH				1 mM NaOH			
	Trial 1		Trial 2		Trial 1		Trial 2		Trial 1		Trial 2		Trial 1		Trial 2		Trial 1		Trial 2	
Time (h)	pH	CC	pH	CC	pH	CC	pH	CC	pH	CC	pH	CC	pH	CC	pH	CC	pH	CC	pH	CC
0.0	6.9	16.8	6.2	16.9	6.9	16.8	6.2	16.9	6.9	16.8	6.2	16.9	6.9	16.8	6.2	16.9	6.9	16.8	6.2	16.9
1.0	3.6	21.7	3.4	20.2	7.0	11.6	6.6	16.1	6.9	14.0	6.8	15.6	9.7	6.6	10.0	4.0	10.7	10.3	10.6	7.3
2.0	3.6	21.8	3.4	20.9	7.0	9.5	6.2	16.0	7.0	11.7	6.7	15.7	9.7	2.6	10.0	2.2	10.5	4.3	10.3	5.3
3.0	3.6	22.0	3.5	20.3	7.0	9.9	6.5	15.8	7.0	11.2	6.7	15.4	9.8	2.0	10.0	2.8	10.7	3.5	10.5	4.4
22.5	3.7	22.0	3.4	21.0	7.1	8.7	6.4	15.1	7.1	13.5	6.9	14.4	9.8	0.7	9.7	2.5	10.4	1.8	10.1	2.8

A pH of between 9.7 and 10 provided the most effective copper removal, with the concentration being reduced from 16.9 mg L^{-1} to 3.98 mg L^{-1} after 1 hr., 2.19 mg L^{-1} after 2 hr., and 2.02 mg L^{-1} after 3 hours, which is within 1% of reaching the World Health Organization limits (Fig 4-6A). Further experimentation is required to optimize the process in order to determine the most effective dose to reduce copper levels to within drinking water guidelines. By the end of the 22.5 hr. sampling period the copper concentration had been reduced to as low as 0.74 mg L^{-1}, well within the guidelines set by the World Health Organization. The removal can be attributed to the settling of the precipitated copper in the solution. The pH change fluctuated minimally over the sampling period; thus the removal of the copper ions did not have a major effect on the pH and therefore would not hinder the solution from removing more copper ions if the concentration was greater. This shows that more copper ions can be introduced to the system to increase the extent of disinfection without the risk of copper toxicity becoming a factor in downstream consumption (Fig 4-6 A & B). The overall settling time had a more minor impact on the copper concentration compared to the pH change (Fig 4-6 A & B). With further optimization, the amount of copper removed can be increased and the time required decreased, allowing for potable drinking water to be available within 1 or 2 hours of pH alteration, as these preliminary studies have come within 0.2 mg L^{-1} from that goal.

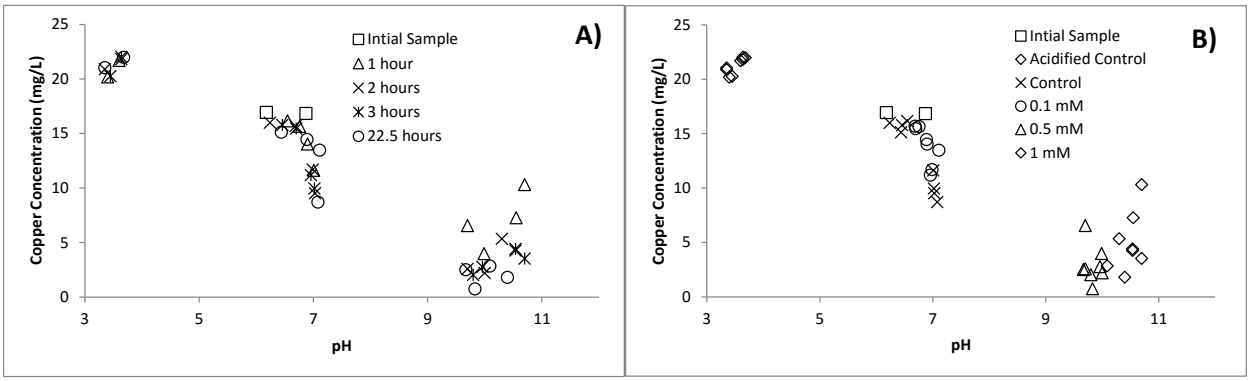

Figure 4-6: Copper concentration during the copper removal test over a range of pH values. A) The change in copper concentration over the sampling time. B) The change in copper concentration over a range of NaOH and HCl doses. (Temperature = 22.9 ± 2.1 °C)

4.4 Conclusions

Water safety and availability is a serious global challenge as millions of people in third world countries are suffering from waterborne diseases without proper drinking water treatment systems. We demonstrated bacterial inactivation using a small amount of electric energy so that the proposed method can be operated in isolated communities without grid electricity. We also demonstrated effective removal of ionic copper from the ionized water by adding a small amount of caustic chemical. In a serial experiment for disinfection and copper removal, the following conclusions were drawn from this proof-of-concept investigation.

- The electrochemical disinfection using copper achieved log 2.8 inactivation of *E. coli* in 2 hr. at 1.5 V application.
- The copper concentration governed the extent of microbial inactivation: the higher the copper concentration was, the greater the log inactivation was.
- The copper concentration attained (>10 mg L^{-1}) was higher by 2 orders of magnitude than that in conventional copper ionization (0.1-0.4 mg L^{-1}) (Cachafeiro et al., 2007, Landeen et al., 1989, Lin et al., 1996).
- At 2.0 V application, the log inactivation was lower than that at 1.5 V application because the oxygen evolution reaction competed against oxidative copper dissolution at the anode.

- Slightly acidic conditions (pH 6 or lower) were ideal for disinfection while pH of 7 resulted in precipitant formation at the anode, inhibiting oxidative copper dissolution at the anode and thus microbial inactivation.
- A small amount of caustic soda (4 mg NaOH in 0.2 L) successfully reduced ionic copper concentration to 2.2 mg L^{-1} in 2 hours.

These conclusions support that the demonstrated disinfection method has the potential to provide safe drinking water to people in under resourced communities.

4.5 Acknowledgements

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5. Conclusions

5.1 Silver disinfection and recovery

When 1.2 V was applied to the reactor for 30 minutes complete microbial disinfection was observed, with bacterial concentrations going from over 100,000 CFU mL^{-1} down to 0 viable cells. During this disinfection the silver concentration reached over 1 mg L^{-1}, a level that is substantially higher than most silver treatment methods, allowing for rapid and complete disinfection. This high concentration was achieved with less than 2 minutes of residence time within the reactor, displaying the rapid reaction rate of the silver oxidation. Throughout the disinfection test a linear relationship between the silver concentration in the solution and the induced electric current was displayed, allowing for accurate prediction of the silver concentration without the need for expensive analytical apparatus. The silver concentration in the solution was successfully brought from the high levels attained during disinfection to within WHO guidelines, with levels below 0.1 mg L^{-1} achieved. The total energy requirement for this system was small enough that photovoltaic solar panels or bicycle generators can successfully provide enough electricity to run the system. These conclusions support that this technology has the potential to provide a method for under resourced societies to obtain safe drinking water.

5.2 Copper disinfection and removal

When *E.* coli was treated with a high concentration (>10 mg L^{-1}) of copper ions a LRV of 2.8 was achieved after 2 hours of 1.5 V being applied to the system. A higher copper

concentration corresponded with a greater inactivation of bacteria, and the concentration of copper achieved was up to two orders of magnitude higher than typical copper ionization. When 2 V was applied to the cell less copper was released than when 1.5 V was used, and therefore less inactivation occurred. This was due to the oxygen evolution reaction competing with the copper oxidation reaction at the anode. pH was found to be a key characteristic to optimize the reactions that occurred, with slightly acidic conditions (pH of 6 or lower) resulting in a more favourable disinfection environment than a neutral pH. At a pH of 7 a precipitant formed on the anode, inhibiting the copper ion release via oxidation and thereby reducing the extent of microbial inactivation. A small amount of caustic soda (4 mg NaOH in 0.2 L) successfully reduced the residual copper concentration to 2.02 mg L^{-1} within 3 hr., and 0.74 mg L^{-1} within 22.5 hr. These conclusions support that the demonstrated disinfection method has the potential to provide safe drinking water to people in under resourced communities.

5.3 Future work

There are various directions future work can be taken with this research. The current work was all done at lab scale, so scaling up is a logical next step. By using larger electrodes and treating an increased volume of water the method can be further validated. Another possible avenue of future work is incorporating the removal/recovery step into the same cell as where the disinfection occurred. This was not done in the current research as the overall goal was to prove that the disinfection and removal/recovery concepts are possible, so side reactions and potential contamination sources were limited as much as possible. Future work could also look at the impact that typical contaminants or chemicals commonly found in tainted water have on the metal ions and their disinfection capabilities. Also, determining how the metal electrodes

withstand prolonged use is a viable next step for this research. Examining how the extent of disinfection is affected over time is a key point to see if this inactivation method is viable as a long term solution. A final option for future work is to try to optimize the process through the use of different materials/cell setup. Some options include: using different metals as anodes during silver recovery, optimizing the inter electrode distance, testing different common chemicals to affect pH for copper removal, and optimizing the applied voltage to minimize competing reactions while maximizing the ion production.

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Appendix A: Supplementary copper experimental data

Table S-1 Concentration of *E. coli* over time at open circuit conditions and no copper in cell (V=100 mL, T=22.9 ± 2.1 °C)

	Concentration (CFU/mL)						
Time (h)	pH=5		pH=6		pH=7		
0	126000	168000	96000	198000	145000	207000	162000
2	90000	165000	153000	210000	140000	237000	208000

Table S-2 Concentration of *E. coli* over time at open circuit conditions (pH=6, V=1 L, T=22.9 ± 2.1 °C)

Voltage	Time	Concentration (CFU/mL)		
O.C.C.	0	125000	131000	T.N.
O.C.C.	0.5	85000	57000	T.N.
O.C.C.	1	69000	47000	17700
O.C.C.	1.5	39000	9000	10500
O.C.C.	2	25000	8000	11800

***T.N.=Too numerous to count**

Table S-3 Concentration of *E. coli* over time at 1 V application (pH=6, V=1 L, T=22.9 ± 2.1 °C)

Voltage (V)	Time (h)	Concentration (CFU/mL)			
1	0	229000	96700	230000	T.N.
1	0.5	49300	75200	52000	52100
1	1	23400	21300	15600	10800
1	1.5	4900	9000	8300	6100
1	2	6200	4300	5700	6500

***T.N.=Too numerous to count**

Table S-4 Concentration of *E. coli* over time at 1.5 V application (pH=6, V=1 L, T=22.9 ± 2.1 °C)

Voltage (V)	Time (h)	Concentration (CFU/mL)			
1.5	0	183000	T.N.	170000	T.N.
1.5	0.5	36700	21200	57800	54400
1.5	1	4100	4300	18400	16800
1.5	1.5	800	300	6200	4200
1.5	2	400	300	300	100

***T.N.=Too numerous to count**

Table S-5 Concentration of *E. coli* over time at 2 V application (pH=6, V=1 L, T=22.9 ± 2.1 °C)

Voltage (V)	Time (h)	Concentration (CFU/mL)			
2	0	210000	207000	176000	T.N.
2	0.5	5200	21300	40500	53700
2	1	100	10300	26100	24400
2	1.5	0	7200	14000	15000
2	2	0	4100	8600	11200

***T.N.=Too numerous to count**

Table S-6 Concentration of E. coli over time at 1.5 V application (pH=5, V=1 L, T=22.9 ± 2.1 °C)

Time (h)	Concentration (CFU/mL)			
0	159000	T.N.	125000	T.N.
0.5	21000	36400	42000	42400
1	4300	4200	4900	15600
1.5	800	800	5900	7800
2	500	1500	600	500

***T.N.=Too numerous to count**

Table S-7 Concentration of E. coli over time at 1.5 V application (pH=7, V=1 L, T=22.9 ± 2.1 °C)

Time (h)	Concentration (CFU/mL)			
0	190000	T.N.	180000	T.N.
0.5	63300	69000	118000	T.N.
1	85400	63600	45900	66600
1.5	64200	74400	93600	73000
2	65000	49300	90400	95000

***T.N.=Too numerous to count**

Table S-8 Average anode potential over 2 hour disinfection test (V=1 L, T=22.9 ± 2.1 °C)

pH	Applied Voltage (V)	Average Potential of Anode (mV)
6	1	435.9
5	1.5	528.7
6	1.5	551.8
7	1.5	986.1
6	2	696.3

Table S-9 Copper concentration over removal test (V=200 mL, T=22.9 ± 2.1 °C)

	Copper Concentration (mg/L)									
Time (h)	Acidified control		Control		0.1 mM NaOH		0.5 mM NaOH		1 mM NaOH	
0	16.9	16.8	16.9	16.8	16.9	16.8	16.9	16.8	16.9	16.8
1	20.2	21.7	16.1	11.6	15.6	14.0	4.0	6.6	7.3	10.3
2	20.9	21.8	16.0	9.5	15.8	11.7	2.2	2.6	5.3	4.3
3	20.3	22.0	15.8	9.9	15.4	11.2	2.8	2.0	4.4	3.5
22.5	21.0	22.0	15.1	8.7	14.4	13.5	2.5	0.7	2.8	1.8

Table S-10 Resulting pH over removal test (V=200 mL, T=22.9 ± 2.1 °C)

	pH									
Time (h)	Acidified Control		Control		0.1 NaOH		0.5 NaOH		1 NaOH	
0	6.87	6.18	6.87	6.18	6.87	6.18	6.87	6.18	6.87	6.18
1	3.6	3.4	7.01	6.55	6.9	6.76	9.7	9.99	10.7	10.55
2	3.64	3.35	7.03	6.24	6.99	6.69	9.7	10	10.54	10.3
3	3.64	3.45	7.02	6.46	6.96	6.7	9.8	9.96	10.7	10.54
22.5	3.68	3.35	7.08	6.44	7.11	6.89	9.83	9.67	10.4	10.09

Table S-11 Copper concentration over disinfection test under open circuit conditions (pH=6, V=1 L, T=22.9 ± 2.1 °C)

Time (h)	Copper Concentration (mg/L)	
0	0	0
0.5	0.6	B.D.L
1	B.D.L	B.D.L
1.5	0.03	B.D.L
2	0.3	0.6

***B.D.L.=Below detection limit**

Table S-12 Copper concentration over disinfection test at 1 V application (pH=6, V=1 L, T=22.9 ± 2.1 °C)

Time (h)	Copper Concentration (mg/L)	
0	0	0
0.5	1.4	2.5
1	2.8	2.6
1.5	4.5	2.4
2	4.1	3.5

Table S-13 Copper concentration over disinfection test at 1.5 V application (pH=6, V=1 L, T=22.9 ± 2.1 °C)

Time (h)	Copper Concentration (mg/L)	
0	0	0
0.5	3.9	0.6
1	7.7	5.3
1.5	12.0	8.4
2	14.8	8.5

Table S-14 Copper concentration over disinfection test at 2 V application (pH=6, V=1 L, T=22.9 ± 2.1 °C)

Time (h)	Copper Concentration (mg/L)	
0	0	0
0.5	1.7	1.4
1	4.8	4.6
1.5	6.2	7.0
2	8.9	9.2

Table S-15 Copper concentration over disinfection test at 1.5 V application (pH=7, V=1 L, T=22.9 ± 2.1 °C)

Time (h)	Copper Concentration (mg/L)	
0	0	0
0.5	B.D.L	B.D.L
1	B.D.L	B.D.L
1.5	B.D.L	B.D.L
2	B.D.L	B.D.L

***B.D.L.=Below detection limit**

Table S-16 Copper concentration over disinfection test at 1.5 V application (pH=5, V=1 L, T=22.9 ± 2.1 °C)

Time (h)	Copper Concentration (mg/L)	
0	0	0
0.5	6.8	4.3
1	13.5	6.9
1.5	17.5	11.1
2	21.2	13.0