Identifying the optimal parameters for disinfecting microorganisms in an electrochemical water disinfection process

A dissertation submitted to The University of Manchester for the degree of Master of Science in the School of the Earth, Atmosphere and Environmental Science

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List of Contents

Abstract....4
Acknowledgement....5
Declaration....6
Copyright....6
1. Introduction....7
1.1 General Introduction....7
1.2 The Introduction of Electrochemical-adsorption Disinfection Process....9
1.3 Important Parameters Relating to Electrochemical-Adsorption Disinfection....11
1.3.1 Ion Composition....11
1.3.2 pH....13
1.3.3 Current Density....15
1.3.4 Other Parameters....16
2 Methodology....18
2.1 Experiment Installation....18
2.1.1 Adsorbent....18
2.1.2 Model microorganism....19
2.1.3 Sequencing Batch Reaction Mode and Electrochemical-Regeneration....20
2.2 Result Identification....21
2.2.1 Plate Counting....21
2.2.2 Live/Dead Bacterial Viability Kit(Baclight^{TM})....22
2.3 Current Intensity Experiment....23
2.4 Chlorine Experiment....24
2.5 pH Controlled Experiment....24
3 Results....25
3.1 Influent Bacterial Counts....25
3.2 Results from Plate Counting....26
3.2.1 Current Density Experiment....26
3.2.2 Chloride Experiment....28
3.2.3 pH Controlled Experiment....30
3.3 Results from Cell Staining....32

3.3.1 Cell Staining of the Influent Samples 32
3.3.2 Cell staining of the Effluent Samples 34
3.3.3 Cell Adsorption Observation 36
4. Discussions 38
4.1 The Effects of Different Current Density 38
4.2 Analysis of the Electrochemical Mechanisms 40
4.3 The Effects of pH 42
4.4 Further analysis 44
5. Conclusion 46
6. References 48
Appendix 53

Abstract

Electrochemical water disinfection is considered to be an environmentally friendly and cost-effective method for eliminating microorganisms and organic pollutants in wastewater. A new method called the Arvia process combines electrochemical treatment with physical adsorption using a graphite adsorbent Nyex. This report evaluates the treatment performance of the Arvia process over the 48-hour operation. Three key parameters, the current intensity, the presence of chlorine and pH in the bulk solution, are analysed to investigate the optimal conditions for operation. Test waters used in this project were artificially inoculated with *Shewanella oneidensis.* High current intensity (0.5A) and low current intensity (0.18A) were applied to examine the effect of applied current. The results sugges that the current contributes to *S.oneidensis* inactivation, but for a long-term operation, low current intensity is more appropriate because high current intensity causes Nyex degradation, which decreases adsorption capacity. Meanwhile, the presence of chlorine enhances an “electrochlorination” mechanism, resulting in more strong disinfectant hypochlorous acid being produced and causing cell death. pH is a critical issue since it not only influences the dominant chlorine species in the bulk solution but also affects the survival of *S.oneidensis.* In general, the optimal condition in this project was defined as a low current intensity (0.18A) with the presence of chloride and no pH control, which results in more than 99.9% of the viable bacteria present being removed over 48-hour treatment period. Further investigations should focus on the impact of a wider range of pH values as well as testing actual industrial wastewater. The mechanism of the latter treatment is challenging in that it may contain not only microorganisms, but also organic and inorganic pollutants.

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Declaration

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1. Introduction

1.1 General Introduction

Drinking water, consumed without appropriate treatment is a major source of pathogenic transmission. Waterborne diseases, including cholera, diarrhoea, paratyphoid fevers, and typhoid, are closely related to insufficient water disinfection. In India, only 35.1% of the population has the opportunity to use sanitation facilities in 2011 (UNICEF, 2013[1]). 19% of health care facilities in developing countries lack improved sanitary facilities and 35% of them lack cleaning detergents for daily disinfection. Approximately 842,000 people die annually because they fail to have access to safe drinking-water, sanitation, and hand hygiene (WHO, 2016[2]). The United Nations proposed 17 Sustainable Development Goals (SDGs), one of them is "ensure availability and sustainable management of water and sanitation for all". Therefore, improving and promoting sanitary technologies plays an important role in achieving the SDGs.

Conventional water disinfection methods can be classified as either physical or chemical. Various physical disinfection methods have been developed including thermal treatment, magnetic and electric fields treatment (Biryukov et al., 2005, Narsetti et al., 2006), dual-frequency ultrasound (Zou and Wang, 2017) and photo-catalytic treatment (Giannakis et al., 2016). Although these physical methods produce satisfactory disinfection efficiencies, they lack a significant "reservoir effect", which prevents recontamination of the treated water over time. When the treatment plant is far away from end users, the reservoir effect can prevent bacteria reproduction and reinfection of the water after primary treatment.

Chemical disinfection is a method that uses a strong oxidant as a disinfectant to kill the pathogens. The oxidant can exist in the water for a long time and form the reservoir effect, which enables long-term stability of treated water. Chlorination is the simplest chemical disinfection method and has worldwide acceptance. However, drawbacks are presented from three perspectives. Firstly, the remaining chlorine in treated water causes unpleasant

[1] Data were derived from UNICEF: https://www.unicef.org/infobycountry/india_statistics.html
[2] Data were derived from WHO: http://www.who.int/mediacentre/factsheets/fs391/en/

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odour and taste, especially when the chlorine is overdosed. Secondly, chlorination will not eliminate chlorine-tolerant organisms such as *Cryptosporidium parvum*, which survives in environments containing chlorine concentration up to 1000mg/L for 24 hours. The last but the most important reason is that chlorine can react with natural organic matter (NOM) in raw water, and then be converted into disinfection by-products (DBPs). DBPs are carcinogenic especially in the form of trihalomethanes(THMs) and haloacetic acids (HAAs). Exposure to high concentrations of THMs can lead to bladder cancer which has a high morbidity rate. Sufficient evidence shows that the exposure of THMs can cause pregnant woman and newborn babies physiological defects including low birth weight, preterm delivery and small for gestational age (Grellier et al., 2010) and, in the most serious cases, spontaneous abortion (Waller et al., 1998).

Therefore, there is a strong necessity for progressing research in the field of water disinfection alternatives. Electrochemical (EC) disinfection is considered as one of the leading contenders for physical-chemical disinfection because of its advantages on limiting the DBPs production and significant removal of unfavourable odour and taste (Miao et al., 2015). Compared to other treatment methods in the aspect of bacterial removal rate, EC disinfection has the best germicidal effect among chlorination, ozonation and Fenton reaction against the gut bacterium *Escherichia coli*. (Diao et al., 2004). In addition, the danger of storage and transportation of strong oxidants is greatly reduced.

The interest in EC disinfection has been raised since the 1990s. *Murphy et al* (1992) analysed direct electrochemical oxidations, with the help of a proton exchange membrane, successfully eliminating various organic compounds in wastewater. Although the treatment showed good efficiency, some disadvantages such as an increased energy consumption caused by high cell potentials due to low electrical conductivity (Hussain et al., 2015), and high investment for construction limit its application in some parts of the world.

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1.2 The Introduction of Electrochemical-adsorption Disinfection Process

Recently, a new method has been developed that combines an electrochemical process with a physical adsorption process, using the graphite interaction compound (GIC) as an adsorbent to reduce pathogenic content in water. The electro-regeneration of the used adsorbent subsequently takes place by applying current to the adsorption chamber so that the recycled adsorbent can be continuously used. This process is also termed the Arvia^{TM} process, invented by Arvia Technology Ltd.

The choice of adsorbent is essential for effective disinfection. Although the conventional adsorbent granular activated carbon can effectively adsorb bacteria because of its porous structure, the formation of a bacterial film on GAC surface is likely to increase the contact resistance as well as to restrict the electrical contact between particles. Therefore, this may decrease the treatment performance and would create difficulties in adsorbent regeneration (Matsunaga, Nakasono and Masuda, 1992; Hussain et al., 2016).

The adsorbent used in the Arvia^{TM} process is a GIC-bisulphate supported adsorbent, which is called the Nyex (produced by Arvia Technology Ltd). Nyex has high electrical conductivity compared with activated carbon (13 times greater), resulting in simple and quick electrochemical regeneration with low energy consumption. Nyex has been reported to obtain the highest efficiency of adsorbing organic pollutants among different graphite materials. Meanwhile, the regeneration process can recover 100% of adsorbent after the first five running cycles. (Asghar et al., 2013a; Asghar et al., 2013b).

The effectiveness of combined electrochemical-adsorption water disinfection in removing dissolved organic contaminants and pigments had been demonstrated by the research of Brown and colleagues (2004a). It used Nyex to remove atrazine in groundwater and investigated the electrochemical adsorbent regeneration in a sequencing batch mode. After one treatment cycle, the atrazine concentration was below 1μg/L. The regeneration of Nyex was proved to be successful after several cycles as the adsorption capacity remained constant. Similarly, further research successfully proved the removal efficiency of a selective pesticide metaldehyde. Following treatment, the effluent reached EU/UK

threshold without harmful by-products being formed (Nabeerasool et al., 2015). Furthermore, the same electrochemical-adsorption treatment has previously been proved to be effective in removing the colour from dyehouse wastewater without any loss of adsorption capacity during regeneration (Brown et al., 2004b).

Microorganisms surviving in water play a significant role in waterborne disease transmission. Generally, the term microorganism includes bacteria, archaea and protozoa. The Ariva™ process shows excellent sterilizing efficiency in removing pathogenic microorganisms. *Escherichia coli,* a Gram-negative bacterium, is an important model organism for laboratory studies for disinfection analysis as it is a key indicator of faecal contamination. The Ariva™ process can kill 99.98% of *E.coli* cells using Nyex in the first five running cycles (Hussain et al., 2014). Beyond that, other microorganisms can be chosen because they cause adverse effects on human health, including Gram-negative bacteria *Pseudomonas aeruginosa*, Gram-positive bacteria *Staphylococcus aureus*, yeast *Saccharomyces cerevisiae*, *Rhodosporidium turoloides* and the protozoan *Cryptosporidium parvum* which can all be effectively treated by Ariva™ process (Hussain et al., 2016).

The Ariva™ process is cost-effective and environmentally friendly. Harmful additives and by-products are theoretically minimised or avoided during the process (Technology Demonstration Project 31, 2013). Meanwhile, the applied current/voltage for regeneration is relatively low compared to the single electric treatment (Feng, et al., 2004) and so the treatment can be carried out with relatively low running costs. The Nyex adsorbent is cheap and easy to access (Patermarakis and Fountoukidis, 1990, Hussain et al., 2014), without reducing adsorption property during regeneration. For large-scale application, the construction is flexible, depending on the requirement of water treatment. For example, the electrochemical cells can be installed either in series or parallel, the operation mode can be in batch or continuous (Technology Demonstration Project 31, 2013)

1.3 Important Parameters Relating to Electrochemical-Adsorption Disinfection

The treatment efficiency of electrochemical-adsorption disinfection is determined by cell configuration, electrode materials, ion composition in water, the contaminants, and other experimental factors such as current density and flowrate (Kerwick, 2005). Numerous theories have been reported to explain electrochemical water disinfection mechanisms. For example, the electro-chlorination process is capable of producing free active chlorine by applying current and, the electric fields itself can inactivate and remove bacteria through direct cell destruction. Furthermore, some free radicals, such as s $^{\bullet}OH^{-}$ and $O2^{\bullet -}$, may be produced and increase the biocidal efficiency. (Diao et al., 2004). Therefore, it is vital to understand the key mechanisms, and their relative importance, involved in electrochemical-adsorption disinfection in order to achieve maximum treatment efficiency.

1.3.1 Ion Composition

Normally, after a primary treatment method, water contains solutes. In the UK, tap water consists of several chemical elements, most of which form naturally and are harmless, like calcium, magnesium, and iron. However, some of them are either artificially added for improving water quality or occur as contaminants, e.g. chlorine, copper, and zinc[3]. Chlorine species need to be highlighted because the transformation among chlorine species can result in pH change, DBPs generation and complex disinfection mechanisms. High treatment efficiency against *E. coli* was observed in the presence of chlorine in the Arvia™ process (Hussain, 2014), which suggest that chlorine species may pose positive contributions to bacteria removal.

The electrochemical-adsorption process involves an anode and a cathode, as well as a power supply providing continuous current. This generates oxidation reactions at the cathode and reduction reactions at the anode while the regeneration of the adsorbent take place at the same time. At the anode, the following oxidation reactions may occur:

$$2Cl^{-} - 2e^{-} \rightarrow Cl_2 (1)$$

[3] Content were derived from: http://freshlysqueezedwater.org.uk/waterarticle_watercontent.php

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$$4OH^- - 4e^- \rightarrow 2H_2O + O_2 (2)$$

$$Cl_2 + 4H_2O - 8e^- \rightarrow 2ClO_2 + 8H^+ (3)$$

At the cathode, the following reduction reactions may take place:

$$H^+ + 2e^- \rightarrow H_2 \quad (4)$$

$$Cl_2 + OH^- + e^- \rightarrow H_2O + OCl^- + Cl^- (5)$$

Chlorine gas formed will spontaneously react with water and produces hydrochloric acid and hypochlorous acid, whereby the chlorine ion is both oxidised and reduced. The residual chlorines species ClO^- and $HClO$ are also termed “free chlorine”,

$$Cl_2 + H_2O \rightarrow HCl + HClO (6)$$

Since HClO as a weak acid is very unstable, it further decomposes into ClO^- and H^+, depending upon pH and the temperature of the bulk solution as,

$$\mathrm{HClO} \leftrightarrow ClO^- + H^+ (7)$$

The bactericidal effect of chlorination primarily relies on hypochlorous acid produced at the anode. Hypochlorous acid is a small and electrically neutral molecule which can pass easily through the cell wall. Moreover, it is a strong oxidant so can inhibit cell division through disruption of DNA synthesis and extensive protein degradation (McKenna and Davies, 1988). Therefore, the treatment efficiency is strongly associated with the amount, and speciation, of chlorine in solution. Greater efficiency results from high free chlorine concentration which is capable of accelerating the above process due to higher production of hypochlorous acid.

As well as chlorine species, reactive oxygen species also enhance the bacterial inactivation process. This mechanism functions when the chlorine concentration is low or even zero (Kraft, 2008). Oxygen species can be generated by electrolysis of water at the anode. Any ozone and hydrogen peroxide formed can act as strong disinfectants to kill bacteria. Existing research has demonstrated these mixed oxidation species are more efficient in eliminating *E.coli* and chlorine-tolerant bacteria (*C. parvum*) than chlorination. (Venczel et

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al., 2004; Son et al., 2004, Jeong et al., 2007). The following reactions explain the formation of reactive oxygen species like ozone and hydrogen peroxide at electrodes (Martínez-Huitle and Brillas, 2008).

$$H_2O \rightarrow {}^{\cdot}OH + H^+ + e^- \quad (8)$$

$${}^{\cdot}OH \rightarrow {}^{\cdot}O + H^+ + e^- \quad (9)$$

$$2\ {}^{\cdot}O \rightarrow O_2 \quad (10)$$

$$2\ {}^{\cdot}OH \rightarrow H_2O_2 \quad (11)$$

$$O_2 + {}^{\cdot}O \rightarrow O_3 \quad (12)$$

1.3.2 pH

All wastewater treatment methods are expected to ensure the effluent has low environmental impact. Also, a recent MSc report (Hernandez, 2016) proved that lower pH contributes to better treatment efficiency by the Arvia™ process when removing *Shewanella oneidensis* due to intensified chlorination and oxidation. Similarly, the treatment of *Bacillus subtillis* presented low biocidal efficiency at pH 8, suggesting that the concentration of hydrogen has effect on electrochemical-adsorption process (Mezule et al., 2014)

The transformation of chlorine species is associated with the pH value in the bulk solution. The curve shown below (Fig 1.1) reveals the relationship of the distribution of free chlorine species and pH. The optimal pH range for hypochlorous is between pH 3.3 and 7.5 while the predominant free chlorine species at above pH 7.5 environment is ClO^-. Control of pH between 3.3 and 7.5 favours the equilibrium position of hypochlorous acid, resulting in a higher concentration of the most potent chloride disinfectant species.

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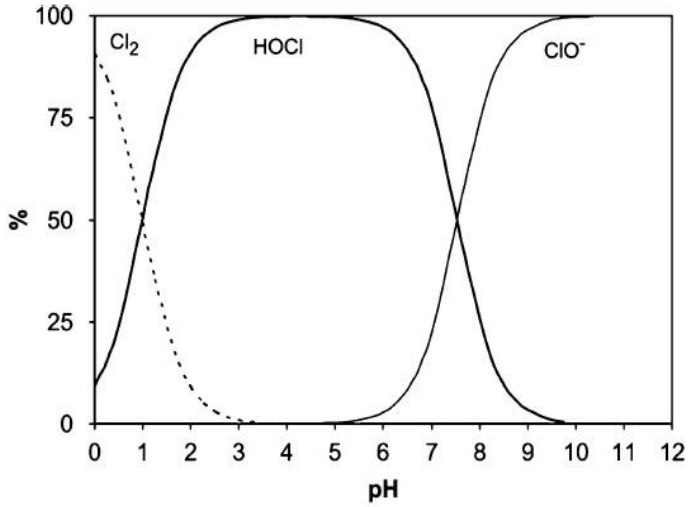

Fig 1.1 The distribution curve of Cl_2, HOCl and ClO^-. pH 3.3 to 7.5 favours the formation of HOCl while above pH 7.5 chloride is presented as ClO^-. Source: Deborde and von Gunten, 2008

Meanwhile, microorganisms live and thrive within specific pH range, beyond which cell death occurs. Most planktonic bacteria cannot survive electrochemical-adsorption disinfection due to this dual effect. However, the pH equilibrium around the cathode is affected by the decrease in hydrogen ions through reduction, producing hydrogen gas. Water is ionized and releases hydroxyl ions, which in turn increases the pH around the cathode. Overdosing chlorine ions often raises the pH value until the equilibrium is established, where chlorine cannot produce hypochlorous acid anymore. pH change also influences the oxidation of reaction oxygen species. As equations (8) to (12) show above, the hydrogen ion is formed during oxidation and influences the pH in the vicinity of electrodes. High hydrogen concentration slows the process (8) and (9), contributing to less ozone and hydrogen peroxide being formed thus influences the germicidal reaction.

In terms of the adsorption process, under a certain pH, the adsorbed species are likely to repel the adsorbent because they may carry the same electrical charge, leading to low adsorption efficiency. In contrast, when the adsorbate and the adsorbent possess opposite charges, or the adsorbate is neutral, the adsorbate can be packed densely on the adsorbent surface and maximize the adsorption process (Liu, 2015; Mohammod, 2011). This better explains why the adsorption of acid species has higher efficiency under alkaline

environment and vice versa (Cooeny, 1999). Therefore, it can be interpreted that the bacteria may have better adsorption efficiency under low pH environment because of the negative electrical charge carried by its cell walls.

1.3.3 Current Density

All chemical reactions in the Arvia^{TM} process, both chlorination and oxidation, rely on electrolysis. The regeneration of the Nyex adsorbent is also triggered by the applied current. Therefore, the current density becomes an important parameter in identifying the optimal treating conditions.

Current density determines the reaction rate. One research study (Xekoukoulotakis et al., 2014) reported that high current density results in more rapid and efficient electrochemical-oxidation performance against total coliforms. Under different current densities, 14.3 and 28.6 mA/cm2 respectively, the treatment performance of total coliforms was 96% reduction at 9 mins and 99.9% reduction at 6 mins. Electro-chlorination is also associated with current density. A series of experiments use four different current densities (0.25, 1.0, 2.5 and 8 mA/cm^2) to treat *E.coli* in the presence of chlorine. Parallel chlorine free solutions were also prepared to compare the treatment efficiency. Results showed that a large reduction of bacteria occurred in the current of the highest density due to increased free active chlorine (Schaefer et al., 2015).

It needs to be noted that the dominant mechanism of disinfection depends on the forms of ions in solution, corresponding to different optimal current densities. Meanwhile, very high current density means expensive energy consumption, which decreases the cost-effective benefit. Furthermore, the existing research has not investigated the current density in relation to the adsorbent regeneration which may be affected due to possible degradation of the adsorbent at high current densities. Therefore, it is not conclusive that better disinfection efficiency is attributed to higher current density. An optimum level, therefore, deserves deeper consideration.

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1.3.4 Other Parameters

Various studies have explored the importance of the electrode material which impacts on the species and yield of oxidants generated (Martínez-Huitle and Brillas, 2008). Improper electrode material may cause unwanted side reactions occurring on its surface, thus decreasing the efficiency of the disinfection. Furthermore, the electrocatalytic activity of electrodes accounts for the formation of desired oxidants and influences the dominant disinfection mechanism (Szpyrkowicz, Radaelli and Daniele, 2005). The production of active chlorine species of typical electrode materials boron-doped diamond (BDD), Ti/IrO_2, Ti/IrO_2, Ti/Pt-IrO_2 and Pt were investigated (Jeong, Kim, and Yoon, 2009). Among them, Ti/IrO_2 has the best performance while Pt is the worst. For this project, because the reaction column and electrodes are provided by Arvia, electrode material is regarded as a defined parameter. The analysis of different electrode materials is not included in the scope of this research.

Flow-rate determines the residence time of water in a reaction column. Slow flow-rates means longer residence times and adequate mass transfer on material surface. Low residence times account for low chlorine generation rates, further weakening the treatment efficiency in electrochemical disinfection against *A. salina* and *E.coli* (Lacasa et al., 2013). Hernandez (2016) showed that a long residence time contributes to a higher degree of reduction of *S.oneidensis* numbers, with 99% removal of bacteria observed at a flow-rate of 1200mL/ and 91.9% removal of bacteria at flow-rate 400mL/h. Hernandez also found that the pH value changes during testing of different flow-rate, resulted in strengthening the chlorination disinfecting mechanism. However, Nyex is observed to degrade as small flakes break off and this makes the effluent water turbid and black. Therefore, an optimal flow-rate also needs to be identified to avoid the above degradation of Nyex.

1.4 Aims and Objectives

Although existing references have revealed some achievements in identifying the optimal condition of the Ariva^{TM} process, (Kerwick et al., 2005; Gusmão, Moraes and Bidoia, 2010;

Hernandez, 2016; Hussain et al., 2016), the experiments were all conducted in short-term periods and limited sequencing cycles. It will therefore by necessary to test the long-term stability of these conditions for the large-scale promotion of the Arvia^{TM} process. Secondly, as mentioned above, the dominant mechanism in the Arvia^{TM} process can either be the electro-chlorination or electro-oxidation, depending on the major present ion species. Even the acidic environment produced could kill bacteria. Each potential mechanism may be affected by changes to current density, flowrate, pH so an optimal system is crucial for identifying the most efficient process. Therefore, recognising the best mechanisms and relevant parameters as well as identifying a "balance" among all parameters are essential for exploring and improving the Arvia^{TM} process.

The main objectives for this project are: 1) Evaluating the treatment performance of laboratorial based Arvia^{TM} process over 48-hour treatment; and 2) Identifying the optimal conditions for the treatment among current density, chlorine concentration and pH.

2 Methodology

A series of experiments were designed to identify the relative importance of current densities, chlorine concentration and pH during electrochemical disinfection over 48-hour operation. Initially, a baseline experiment was conducted without applied current to examine the removal of bacteria from sorption to the Nyex only with no additional electrochemical effect. Then, high current density (0.5A), extra chlorine, and pH controlled conditions were designed to identify the conditions needed to achieve the highest biocidal efficiency.

2.1 Experiment Installation

2.1.1 Adsorbent

Based on large empirical studies (Hernandez, 2016; Hussian et al., 2014; Mohammed, 2011), the Arvia^{TM} process uses Nyex as an adsorbent. The non-porous structure of this material results in a minimal surface area. The appearance of Nyex under microscope is shown in Fig 2.1. The mean particle diameter was not tested in this project, but a previous study measured the average partical size to be around 480μm (Hussian, et al., 2014). After each 48 hours experiment, the adsorbent was replaced with fresh Nyex in order to ensure all experiments have the same adsorption capacity at the beginning.

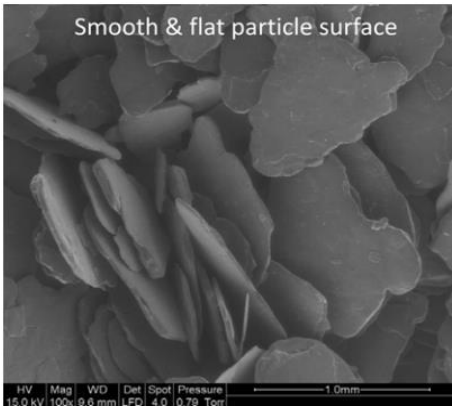

Fig 2.1. The microstructure of Nyex at 100 times magnifications. It shows a smooth and flat particle surface. Source: *Asghar et al., 2013*

2.1.2 Model microorganism

Most previous research studies have used *E.coli* as a model microorganism to analyse the performance of water treatment methods. The treatment against *E.coli* has already been demonstrated by Hussian. *Shewanella oneidensis* is also a gram-negative bacterium and has previously been shown to grow on electrode surfaces (Babauta, Nguyen and Beyenal, 2011) and so may be more difficult to treat through electrochemical disinfection. For this reason and its similarity to *E.coli* (both a facultative anaerobes of the Gamma Proteobacterial group), it was selected as our model organism. *S.oneidensis* can utilize a diverse range of organic compounds for its metabolism coupled to the reduction of a range of inorganic compounds, including toxic and metal elements. Therefore, this genus is has been studied widely in the field of metal bioremediation (Ornston, Gottesman and Harwood, 2007).

Fresh cultures of *S.oneidensis* were obtained from the University of Manchester Geomicrobiological laboratory, and then aerobically cultured by inoculating 30 mL autoclaved deionised water containing 0.9g tryptone soya broth (TSB), which is a common rich medium that provides adequate nutrients for microorganism growth. The solution was then incubated at 30°C in a 100mL flask and shaken at 110 rpm overnight. The resulting cell suspension was washed and resuspended in 20mL autoclaved deionised water with 0.369g/L Na_2SO_4 to avoid the influences from growth media and the same ion environment with the influent solution. The bacteria concentration was kept constant for all comparative trials which is defined as an optical density (OD) of 0.02 measured at 600nm absorbed wavelength (equal to around 5 x10^7 cells per millilitre). The volume of washed suspension can be calculated as,

$$C_{(cell\ in\ suspension)} \times V_{(Added\ volume)} = C_{(Designed\ cell\ concentration)} \times V_{influent}$$

where $C_{(Designed\ cell\ concentration)} = 0.02$;

$V_{influent} = 10L$;

$$C_{(cell\ in\ suspension)} = measurement \times dilution\ factor\ 10$$

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$$V_{(Added\ volume)} = \frac{0.02 \times 10L}{C_{(cells\ in\ suspension)} \times 10}$$

2.1.3 Sequencing Batch Reaction Mode and Electrochemical-Regeneration

This project used a similar sequence batch reactor but with a higher reaction column (26cm) than used in previous work (Shown in Figure 2.2). The influent contained 10L deionised water, 0.368g/L Na_2SO_4 to give 0.699mS/cm of conductivity and approximately 5 x10^7 cells per millilitre *S. oneidensis*. Adding Na_2SO_4 rather than other common solutes (e.g. NaC aimed to firstly increase the influent conductivity to allow lowering of the voltage at a given current density, consequently cutting down the operational costs (Anglada, Urtiaga and Ortiz, 2009), and secondly avoid the potential obstruction by the other important ions (e.g. chlorine), which would be analysed separately later. Deionised water was used instead of tap water since the tap water may contain too many non-defined impurities that may influence the biochemical reactions.

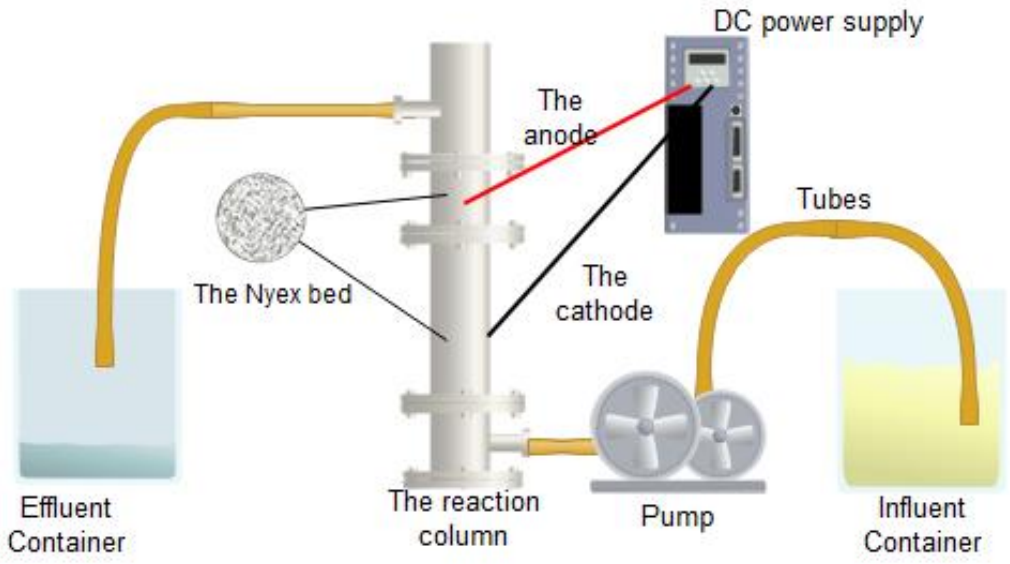

Fig 2.2. The schematic diagram presents a simplified Arvia process in a laboratorial scale.

The influent was continuously stirred using a magnetic stirrer and continuously pumped into the reaction column in an upward flow direction at a constant flowrate 150mL/h. The adsorption then happened in the reaction column which was fully filled with Nyex. The electro-regeneration of used absorbent was the last phase, achieved by applying a current to the reaction column in which each Nyex particle behaved as the anode, and the outer shell of the reaction column was acted as the cathode. The effective electrochemical disinfection zone was 15cm in height and made of stainless steel. After regeneration, the effluent gradually came out from the top of the reactor and was collected in an effluent container.

One treatment cycle was defined that continuous operated for 48 hours. Before each of the disinfection cycles, the column was flushed with 1L autoclaved deionised water containing 0.369g/L Na_2SO_4 to wash out any small flakes of the adsorbent. In order to maintain the bacteria concentration in the influent at a constant level, fresh saline solution (0.369g/L Na_2SO_4) with *S.oneidensis* was prepared in every 24 hours.

2.2 Result Identification

2.2.1 Plate Counting

The removal percentage of culturable bacteria was selected as the indicator of disinfection efficiency, thus counting the viable bacteria before the disinfection and viable bacteria after the treatment was necessary. The plate counting method was regarded as one of the best way to quantify the numbers of viable cells in samples (Sutton, 2011).

Fig 2.3 Viable cell colonies on the plate.

The effluent sample was taken at 1.5 hours, 24 hours and 48 hours. Fresh effluent was taken out from the effluent tube and then made in different dilutions

depending on different experimental scenarios, which are explained later. The influent was sampled and diluted 1:1000 and 1:10,000 with deionised water containing 0.369g/L Na_2SO_4 after selected cycles prior to plating onto 3 agar plates to give an average number of influent colony forming units number. Three replications at each dilution factor were essential for minimising operational and counting errors. The dilution and plate spreading was performed in a laminar flow cabinet in order to avoid contamination of the samples and the plates. The plates were put into the 30°C incubator overnight and colonies on the plates counted after 24 hours. After incubation, viable cells formed visible colonies on the plate, shown in Fig 2.3.

The plate counting method is very sensitive to a number of environmental conditions such as the external contamination mentioned above, temperature, nutrients, and the duration of cultivation. A reasonable and countable number on each plate should be within a range from 40 CFU to 200 CFU, beyond that the accuracy is largely reduced because colonies are too close to each other to count. Although in this project three replications were made, the data were sometimes inconsistent due to occasionally too few or too many CFU being present on each plate.

2.2.2 Live/Dead Bacterial Viability Kit(*Bac*light™)

An advanced method called Live/Dead Bacterial Viability Kit (*Bac*light™) has gained growing attention in the field of cell enumeration. The principle of *Bac*light™ is that the two nucleic acid-binding stains in this Kit, SYTO 9TM (green-fluorescent nucleic acid stain) and propidium iodide (red-fluorescent nucleic acid stain), can selectively penetrate health or compromised cell membranes resulting in the cells staining different colours. The SYTO 9™ penetrates all outer membrane and leaves green colour while the propidium iodide only passes through damaged membranes, changing the green colour to red. The differentiated cells therefore appear green or red by fluorescence reflection under a microscope (Boulos, 1999).

The application of this enumeration method has been proved in detecting “live”

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extremophilic microorganisms (Leuko et al., 2004) as well as identifying *Bacillus subtilis* and *Micro*coccus (Zhang et al., 2010). The validity of *Bac*light™ has been tested by comparing conventional direct counting methods, acridine orange direct count (Hobbie, Daley and Jasper, 1977) and CTC (5-cyano-2,3-ditolyl tetrazolium) in differentiating viable cells of *E.coli*. The results suggested that *Bac*light™ shows similar counting outcomes with other methods and slightly higher colony number when the environment restricts the other two direct counting methods (Boulos et al., 1999). The research concluded that the *Bac*light™ is a customer-friendly and time saving approach due to the relatively simple operation procedure and less time on incubation. Therefore, *Bac*light™ may become an alternative to back up the plate counting result.

For this project, a $1\mu L$ mixture of two dyes was mixed in the ratio 1:1, and added to 1mL effluent samples. Afterwards the samples with added dye were left in the dark for 15 minutes at room temperature to give sufficient incubation. Next, $10\mu L$ of the stained suspension was added to a glass slide with a cover slip placed over it and observed it under the microscope in the University of Manchester Geomicrobiology Laboratory. Both the influent and the effluent were stained under different experimental scenarios.

2.3 Current Intensity Experiment

Direct current was controlled to assess the effect on treatment performance. After a baseline experiment was conducted without applied current, either a high current (1.7 mA/cm^2) or low current (0.625 mA/cm^2) were applied via adjusting the power supply. Current density can be converted to current intensity as follows,

$$\text{Current intensity} = \text{internal area of the column} \times \text{current density}$$

$$\text{Internal area of the column} = 2\pi \times \text{radius} \times \text{length},$$

where radius and length were measured as 1.8cm and 26cm respectively,

$$\text{Internal area of the column} = 294.05 cm^2$$

$$\text{Low current intensity} = 294.05cm^2 \times \frac{0.625mA}{cm^2} = 0.18A$$

$$\text{High current intensity} = 294.05cm^2 \times \frac{1.7mA}{cm^2} = 0.5A$$

All the other experimental parameters were kept the same for making the results comparable. Because high current density accounted for a large bacteria reduction in bacterial numbers, the dilutions were selected as 1:10, 1:100 and 1:1000 for the first 1.5 hours. For the other effluent samples at 24 and 48 hours, 1:100, 1:1000 and 1:10,000 dilutions were used. Meanwhile, we monitored pH of the effluent as well as the voltage in the reaction cell.

2.4 Chlorine Experiment

The effect of chlorine on electrochemical disinfection was investigated by mixing the influent solution with 50mg/L chlorine ions (added as NaCl) with 0.18A current applied. The amount of NaCl can be interpreted as follows,

$$M_w(NaCl) = \frac{58.44g}{mol}; M_w(Na) = \frac{23g}{mol}; M_w(Cl) = \frac{35.45g}{mol}$$

$$\frac{M_w(Cl)}{M_w(NaCl)} = 60.6\frac{w}{w}\%$$

$$C_{NaCl} = \frac{50mg/L}{60.6\%} = 82.5\ mg/L$$

Hence, for 10 litres influent the amount of sodium chloride was 0.825g. For the plate counting, 1:10, 1:100 and 1:1000 were used for three sampling times.

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2.5 pH Controlled Experiment

Sodium hydrogen carbonate ($NaHCO_3$) can dissolve in water and release carbon dioxide to buffer the hydrogen ions in the influent. For the purpose of getting 0.03 mol/L concentration of $NaHCO_3$ in 10 litres influent, the amount can be calculated as,

$$\mathrm{w} = \mathrm{M} \times \mathrm{V} \times M_w$$

where the M means molar concentration, which is 0.03 mol/L; V equals to 10 litres and M_w is the molar weight of $NaHCO_3$ which is 84.01g/mol.

Then,

$$\mathrm{w} = \frac{0.03\mathrm{mol}}{\mathrm{L}} \times 10\mathrm{L} \times \frac{84.01\mathrm{g}}{\mathrm{mol}} = 25.203g$$

0.18A current intensity, 0.875g NaCl and 0.369g Na_2SO_4 were added in the influent as additives to ensure the experiment was comparable. The dilutions were defined as 1:10, 1:100 and 1:1000 since not so many bacteria were expected to survive after this treatment.

3 Results

3.1 Influent Bacterial Counts

The reduction in numbers of bacteria after treatment by the Arvia™ process was calculated as follows, using the number of colony number unites (CFU) before and after the disinfection treatment. High bacterial removal indicated high treatment efficiency.

$$\text{The percentage of bacterial removal(R)} = \left(\frac{N-n}{N}\right) \times 100\% \ ;$$

where N = the colony number before the treatment;

n=the colony number after the treatment.

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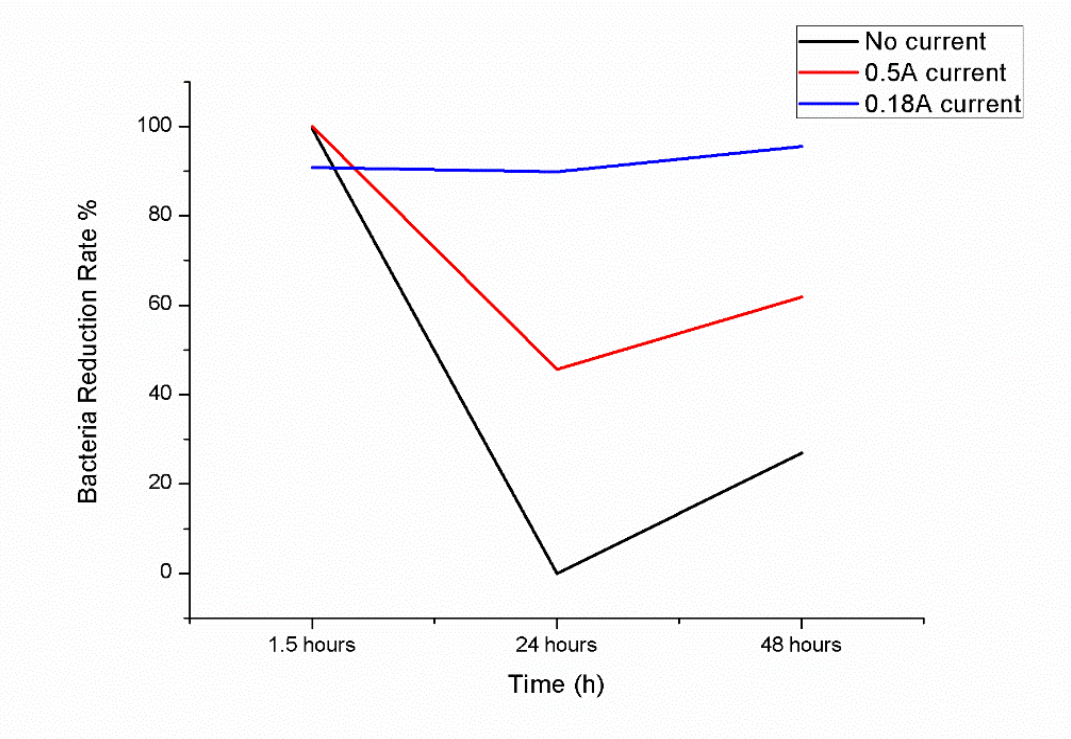
The number of cells in the influent was kept constant by always measuring the optical density of the inoculum at 600nm to achieve a final optical density of 0.02 in the influent. However, a number of large outliers occurred when counting the CFUs on the influent plates. A number of factors may be responsible for this including: the incubation conditions of the starting inoculum, the plate making procedure, the incubation conditions of the plates,

and the small concentration differences among influent samples because we changed the influent in every 24 hours. To minimize the error, a standardized N derived from calculating average number of all influent samples was used for each calculation.

3.2 Results from Plate Counting

3.2.1 Current Density Experiment

Three conditions: no current, high current(0.5A) and low current(0.18A), were analysed. In general, increasing the applied current increased disinfection efficiency as demonstrated by the bacteria counts from the two applied current experiments, which were higher than the no current experiment. The results are shown in Fig 3.1. For all three conditions, cell



	No current efficiency	0.5A current	0.18A current
1.5 hours	99.45%	99.98%	90.77%
24 hours	0.00%	45.89%	89.91%
48 hours	26.93%	61.82%	95.58%

Fig 3.2 The bacteria reduction rate under different current intensity. Blue line indicates low current group which shows generally the best and the most stable treatment than other two experiments. No current was the worst with only 26.93% of the bacteria being removed.

removal over the initial 24-hour declined and then rebounded after 48 hours. After the first 1.5 hours, the no current group and high current group showed more than 99% of bacteria were removed but only 90% of the bacteria were removed in the low current group. After 24 hours, the amount of bacteria being removed in both the high current group and no current group dropped significantly to 46% and 0%, respectively. However, only a negligible drop in bacteria removal was seen after 24 hours in the low current group. The high biocidal efficiency of the low current group was maintained at 48 hours.

Interestingly, during the high current experiment, after 24 hours the Nyex was observed to start breaking down, likely due to the high current intensity, resulting in a discoloured, brown effluent at 24 hours and then after 48 hours turning to black (shown in Fig 3.3). pH measurements showed no significant pH difference between the influent and the effluent in both experiments, while the effluent of the low current experiment had slightly higher pH values than in the high current experiment. pH change is shown in Fig 3.4. It can be concluded that the low current density is the most suitable scenario among the three conditions in terms of not only promising stable and high efficiencies in 48-hour treatment period, but also ensuring that most of the Nyex stays in the reaction column instead of being released into the effluent.

Fig 3.3 The black effluent after 48-hour treatment at 0.5A current intensity.

3.2.2 Chloride Experiment

The chloride experiment showed that increasing chloride concentrations in the influent enhanced the disinfection efficiency by comparing with the 0.18A current experiment. As shown in below Table 3.1, the percentage of bacteria removal in the chloride experiment was 99.98% after the first 1.5 hours and kept above 99% of bacterial removal until 48 hours, achieving 100% of removal at that time. For the 0.18A current without extra chloride group, the final bacterial removal was 95.58%. The results suggested that the chlorination positively affects the disinfection process and is able to kill all, or at least a significant majority, of the bacteria in the influent sample over the time period of the experiment.

	0.18A current	0.18A +Cl
1.5 hours	90.77%	99.98%
24 hours	89.91%	99.25%
48 hours	95.58%	100.00%

Table 3.1 The percentage of bacterial removal at no chloride and extra chloride scenarios. The applied current intensity equals to 0.18A.

However, a significant decrease of pH was observed after 48 hours in the chloride experiment (Fig 3.4). Initially, after the first 1.5 hours the monitored pH was close to neutral (6.95) and then increased to 7.14 after 24 hours. After 48 hours, the pH dropped dramatically to 3.64 and formed an acidic environment (shown in Fig 3.4). pH viability test indicated that *S.oneidensis* could survive in the environment at pH 4 to 6, but incubation out of that pH range resulted in 97% reduction in numbers of viable colonies (Hernandez, 2016). As a result of the viability test, the pH 3.64 environment is likely to have contributed to the high efficiency of *S.oneidensis* death. Thus, the increased treating efficiency can be attributed to both the effect of chlorination and the high degree of free hydrogen ion. Therefore, at 24 hours when little change in pH is seen, the increased efficiency of disinfection can be attributed to electrochlorination. However, at 48 hours when the maximum disinfection efficiency is seen, the contributing factors are likely to be both electrochlorination and a pH effect.

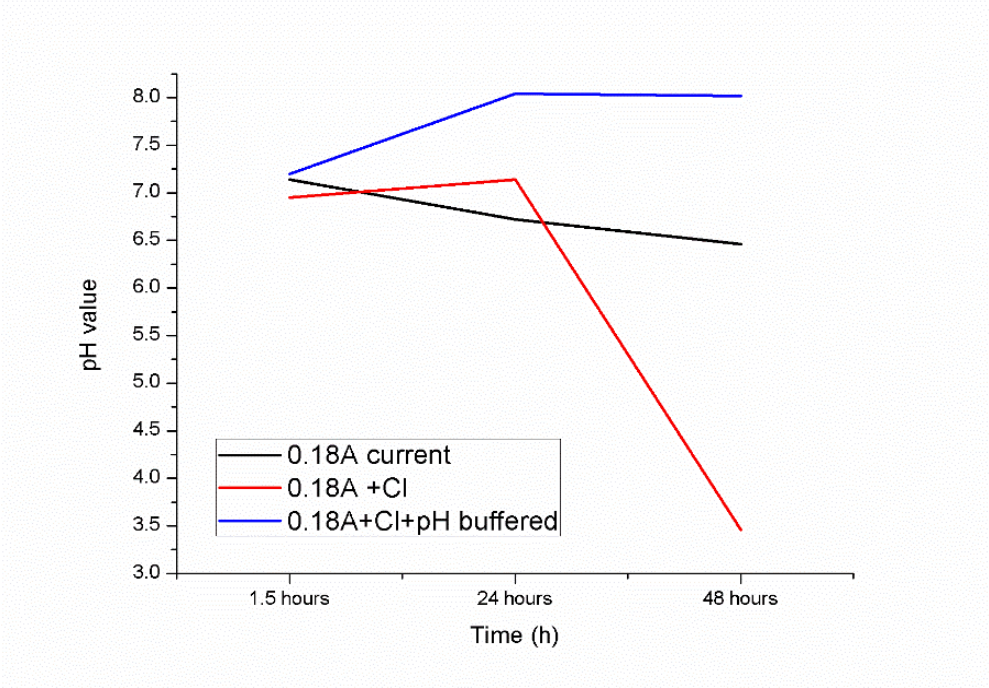


Fig 3.4 The pH change in three experiments. Black line is the baseline experiment. Red line indicates the extra chlorine concentration which dropped from 7.14 to 3.64 after 48-hour operation. Blue one is the pH buffered experiment. pH was buffered to 8 after 48 hours.

Further analysis focused on the ionic composition in the effluent sample. Ion chromatography is a typical analytic method that can identify anions and cations in aqueous phases. Here different ions dissolved in a suitable mobile phase pass through a chromatography column and exit with different retention times, dependent on relative

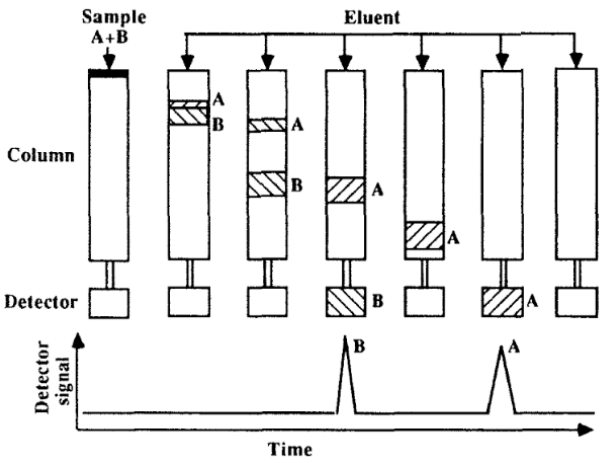


Fig 3.5 The schematic diagram of ion chromatography process.

charge and are quantified using an appropriate detector. Retention time is used to identify the ions present, and peak height used to quantify the concentration of ion (Haddad and Jackson.,1990). This analysis was conducted by the University of Manchester geochemical analytical group.

IC analysis of the effluent sample of extra chlorine experiment was conducted. The result is shown in the appendix. After 1.5 hours, the chloride concentration dropped from 54 mg/L to 5.84 mg/L, indicating that the chlorination took place. Chloride was converted into chlorine gas and was released from the liquid phase, or was oxidised into perchlorate, neither of which can be detected by the IC system used. The electrochlorination slowed down afterwards as the chloride concentration after 24 hours only decreased to 48.74mg/L and after 48 hours it remained steady at 55mg/L, which was the same as the influent concentration. This suggested that the main disinfection mechanism after 48 hours might be the high concentration of hydrogen ions instead of electrochlorination. One hypothesis could be after 48 hours the adsorption capacity of the Nyex reached maximum, so no more cell could be adsorbed on the surface and the desorption of dead cells did not work. Therefore, there was no room for further adsorption. Another hypothesis was the electrochlorination caused chlorine being consumed, while the new influent was prepared in every 24 hours so that the concentration of chlorine again increased.

3.2.3 pH Controlled Experiment

In order to analyse whether hydrogen ions can affect the bacterial counts, $NaHCO_3$ was added in the influent to neutralize the hydrogen ion. The influent was buffered to pH 8, which was slightly higher than expected. A successful neutralization happened as a consequence of the pH after 24 hours and 48 hours reaching all above 8 in the effluent. The results were shown in Fig 3.6.

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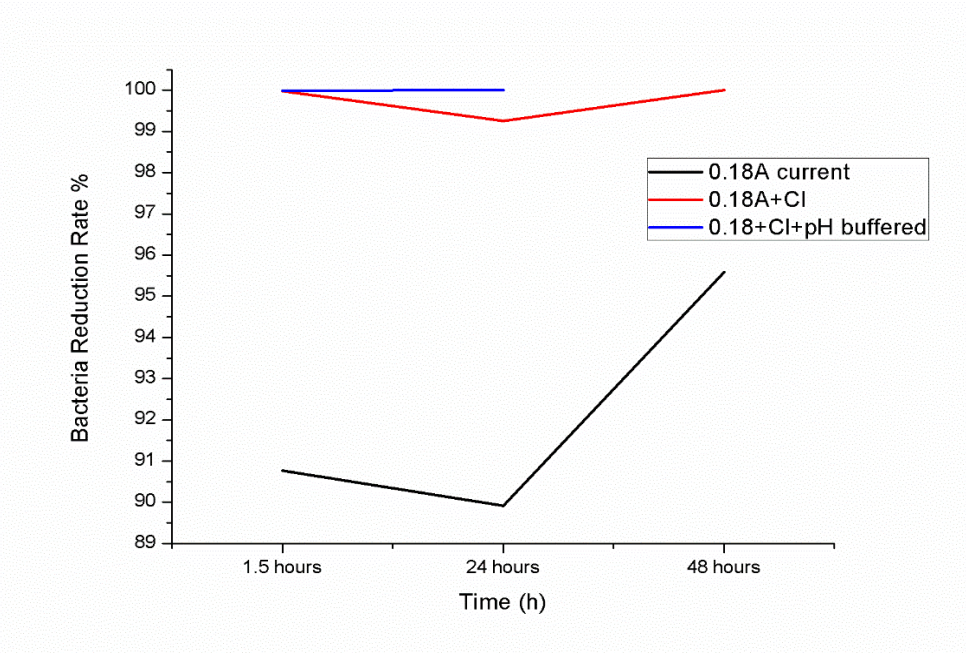
Despite getting outstanding cell removal efficiency in the first 1.5 hours and 24 hours, after 48 hours *S. oneidensis* did not grow on Agar plates in both the influent plates and the effluent plates in each dilution, suggesting that infection or operation errors might have happened instead of complete elimination of the bacteria. Cell staining results backed up this assumption, and will be presented in the next section. Only the percentage of bacterial removal after the first 1.5 hours and 24 hours were recorded and showed in the following table, which could not provide conclusive evidence to prove the effectiveness of pH buffered scenario. However, Hussain's group conducted pH control experiment against *E.coli*. This research suggested that neutral pH conditions are unsuitable for the long-term electrochemical disinfection based on five running cycles, where no disinfection was observed after regeneration or adsorption (Hussain et al., 2014). In general, the pH controlled scenario had greater treatment performance than the no additives scenario. The comparison with extra chloride scenario failed to suggest which one is better, indicating the further research should continuously focus on the influence of pH.



	0.18A current	0.18A +Cl	0.18A+Cl+Buffered
1.5 hours	90.77%	99.98%	99.99%
24 hours	89.91%	99.25%	100.00%
48 hours	95.58%	100.00%	

Fig 3.6 The bacteria reduction rate of extra chloride experiment and pH buffered experiment. 0.18A current experiment is used as baseline.

3.3 Results from Cell Staining

3.3.1 Cell Staining of the Influent Samples

Green live cells and red dead cells were clearly observed under the microscope. According to Fig 3.8, the influent contained numerous viable cells and dead cells. The occurrence of both viable and dead cells could be the result of the different growth phases happening at the same time. Growth curve theory (Fig 3.7) indicates that the number of viable cell have a relationship with time, and could be classified as four general phases: 1) the lag phase, where the cells are inoculated into growth media and are acclimatising prior to growth; 2) the log phase, where the cells reproduce exponentially; 3) the stationary phase, where the death rate and the growth rate are equal so that the total number of cells is kept constant; 4) the death phase, cells start to die because of inadequate nutrients and, the accumulation of toxic metabolites (or other deleterious changes in the medium)

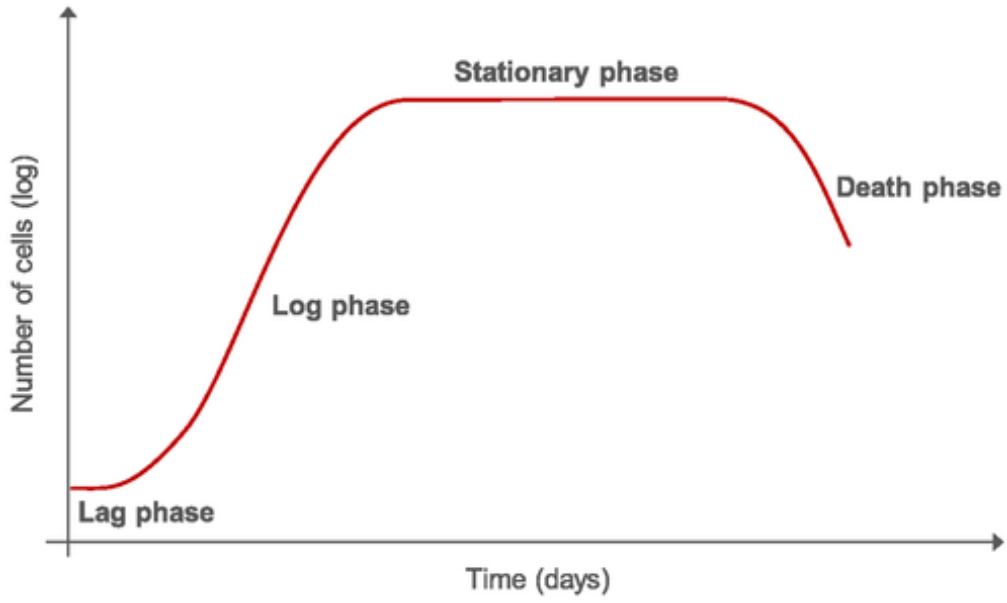


Fig 3.7 The Growth curve theory. Source:
http://www.leica-microsystems.com/science-lab/how-to-do-a-proper-cell-culture-quick-check/

Agrowth curve was not prepared for *S.oneidensis* in this project, but the growth of *S.oneidensis* MR-1 was previously assessed, having a specific growth rate and doubling time of 0.6885h^{-1} and 1.007h respectively at 30℃ (Jeong et al., 2006). Therefore, it was

possible that some cells were in the stationary phase while the other cells started to die. Meanwhile, this phenomenon made the staining method incapable of doing accurate enumeration because it could not distinguish the cell was either killed by the electrochemical-adsorption process, or it was already dead before passing through the reactor.

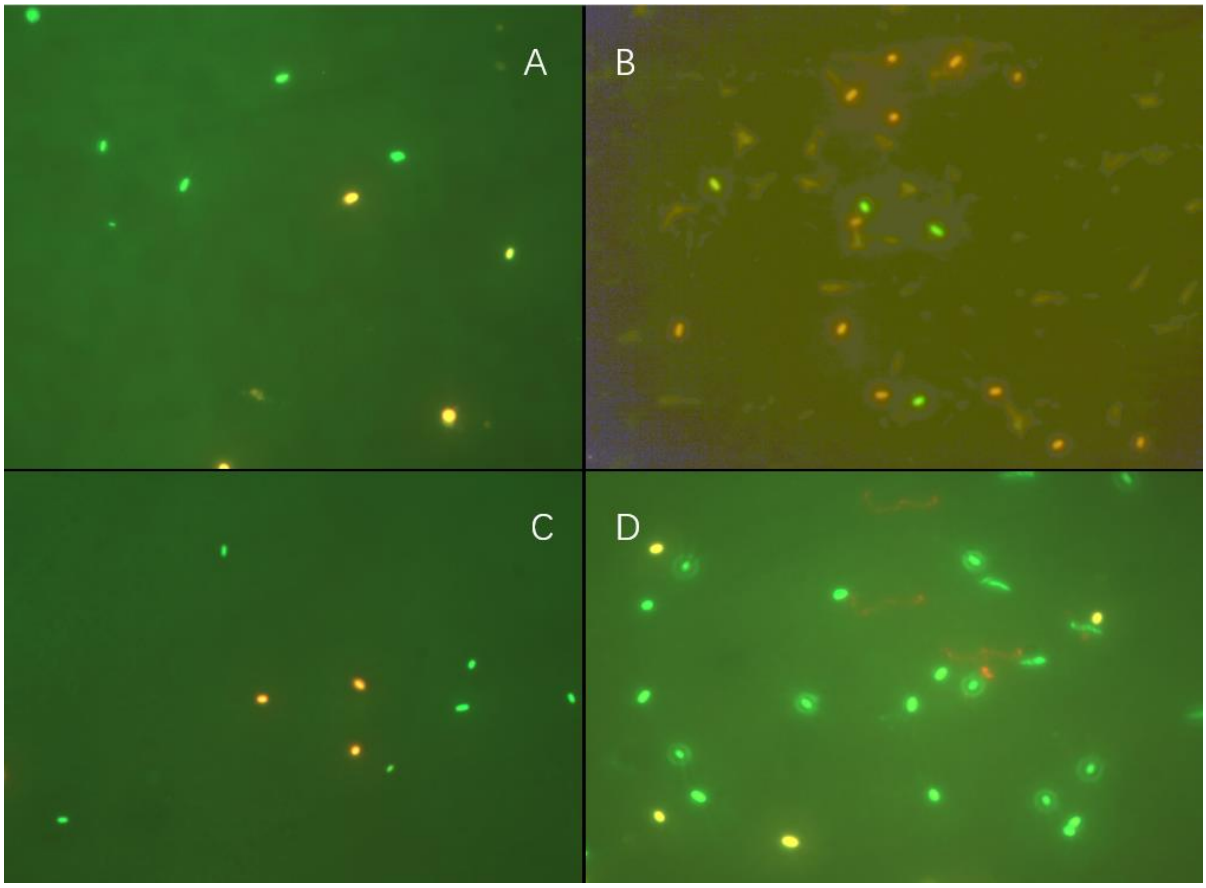


Fig 3.8 Cell staining of influent samples. Photo A, C and D were taken from 0.8 current with extra chlorine experiment. Photo B was taken from 0.18A current experiment. Different camera was applied so that the quality of the photos was slightly different. The distribution of green and red cells was very unequal.

3.3.2 Cell staining of the Effluent Samples

During the baseline experiment, after 24-hour operation large numbers of green cells appeared under the microscope, indicating that the electrochemical-adsorption disinfection did not work at all. The cells directly flowed out of the reactor. For the other experiments, the total number of observed cells was much less than the baseline. Although some red cells appeared, most of the cells were green. The number of the cells was impossible to compare due to unequal distribution. Large numbers were always found at the edge of the slide or the at the surface of the bubbles, which frequently appeared because of the operation. Meanwhile the use of counting chamber failed so that the number cannot be defined.

In general, the cell staining observation reinforces the previous plate counting results. Specifically, when plate counting cannot provide outcomes (no or too many colonies grows on the plate), the observation can evaluate the general growth conditions of the bacteria in samples. For example, during pH controlled experiment when plate counting method showed no colony was formed on the plate, some green viable cells were seen under the microscope, which presented in Fig 3.9, suggested that operation error occurred during the

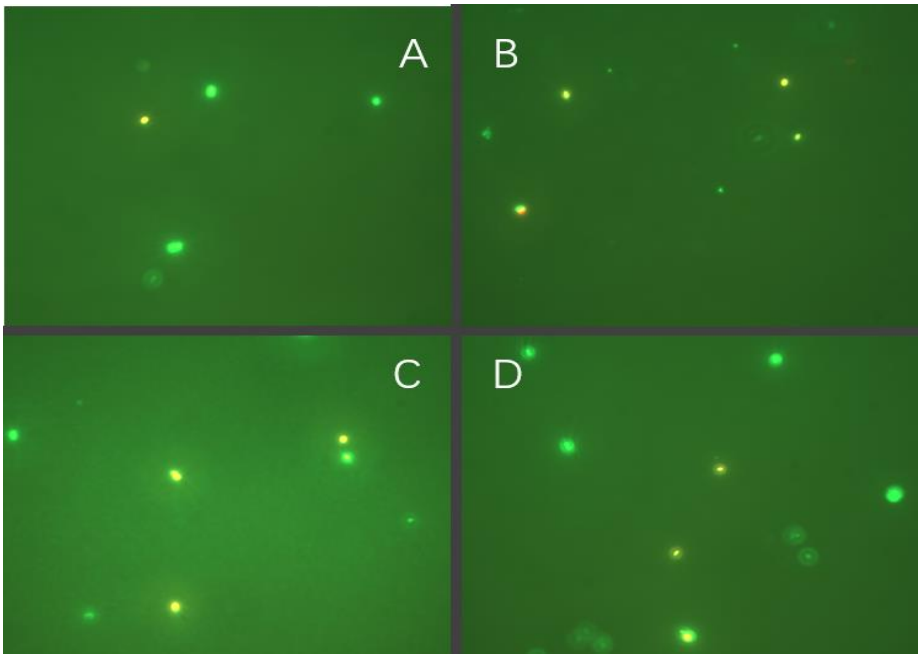


Fig 3.9 Cell staining results of 48-hour effluent of pH controlled experiment. The existence of viable and dead cells has been clearly expressed. A, B, C and D were captured at different positions on the slides.

incubation rather than the bacteria was completely inactivated. Compared with the chlorine experiment, the following pictures provided a general idea of the treatment performance by showing bacteria growth conditions. For the first 1.5 hours and 24 hours, no cells were observed under the microscope in both of two experiments. After 48 hours, no cells were detected in the added chlorine experiment effluents, but viable cells were detected in the pH controlled experiment, as shown in Fig 3.10. This result indicated that the performance of pH controlled scenario was worse than the non pH-controlled one.

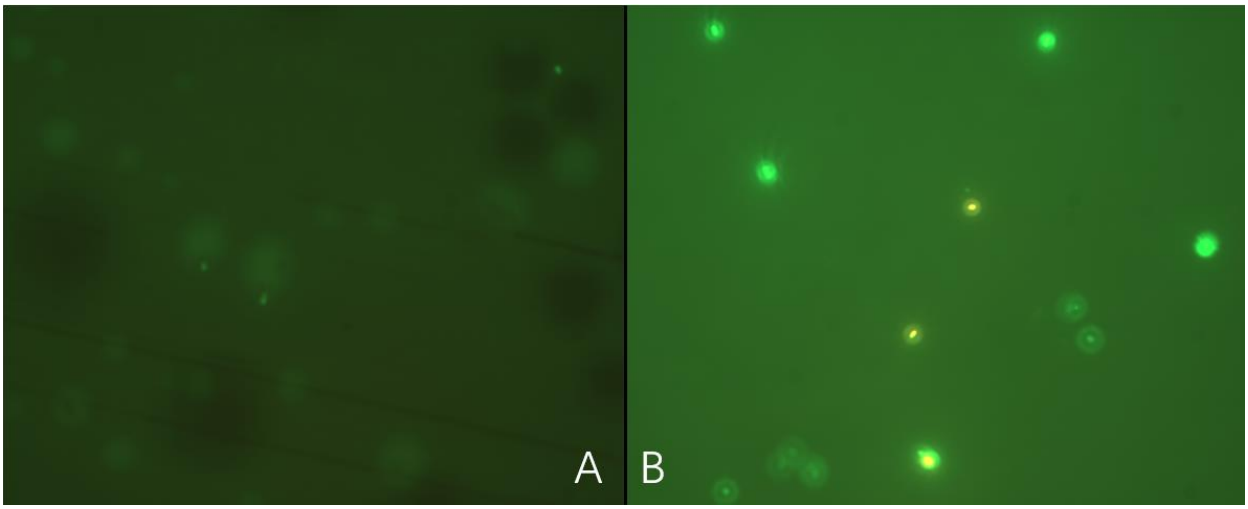


Fig 3.10 Cell staining results of A) Extra chlorine experiment after 48 hours, and B) pH controlled experiment after 48 hours.

Some difficulties were found during these experiments. Firstly, the background in the influent sample was filled with a mass of small fragments (shown in Fig 3.11), which created an obstacle in differentiation from live cells. These fragments were neither the broken graphite materials nor the stained cells. It could be possible that the fragments might be the added salts in the influents, but as the experiment went on, the amount of fragments decreased. This could suggest the material was washed out from the column or that it was a result of the staining preparation method which improved with time.

An additional problem arose from a number of cells which moved around and were not fixed on the slides, and caused difficulty in counting the cells. This was accentuated by the pressure applied to the cover glass and the slide during microscope using an oil immersion

objective. Furthermore, the cells seemed to attach to different layers under the microscope, which created problem in focusing objective lens on the cell.

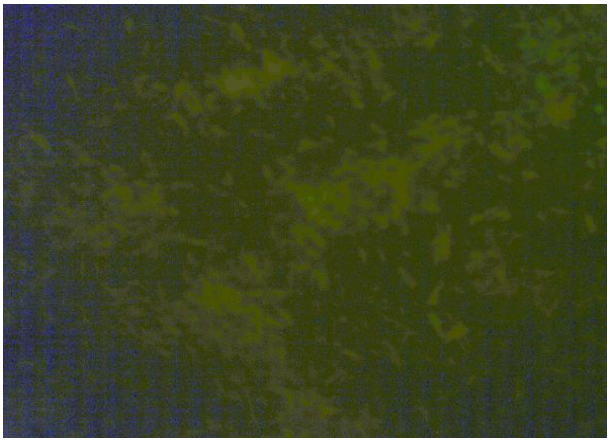


Fig 3.11 The background of the stained influent sample at 0.18 current density without any additives.

Furthermore, the bacteria can die on the slides. An observation once noticed that the number of dead (red) cells increased after leaving the slide for three more hours. As a result, the quality of the observation was largely reduced. Further improvement needs to be considered for more accurate quantification of viable bacteria.

3.3.3Cell Adsorption Observation

The microscopic observation (Fig 3.12) captured images which showed that the dead cells were adsorbed on the Nyex surface. Because the high current destroyed the Nyex, some graphite flakes came out of the reactor which did not happen when the current was lowered to 0.18A. Meanwhile, an unstained sample (shown in Fig 3.13) was also prepared, ensuring that the small flakes were the adsorbent rather than salt or something else. This finding successfully demonstrated that the cells could be adsorbed by this non-porous graphite material and then be killed on the adsorbent surface. It also suggested that little or no desorption was occurring which may explain why the biocidal efficiency decreased after 24 hours as discussed above. The dead cells might stay in the reaction column with the undamaged adsorbent instead of coming out with the effluent. Hence the total number of dead cells noted under the microscope was likely to be much less than the actual number.

The finding of significant numbers of dead cells located on the Nyex surface, and a limited number of dead cells seen in isolation, suggests that the primary mechanism of electrochemical disinfection occurs at the Nyex surface following adsorption of cells. In brief, it can be concluded that the cell staining method is not an ideal counting method for this project, but can identify whether cells are viable or dead, and thus provide mechanistic insights into the cell removal and disinfection process.

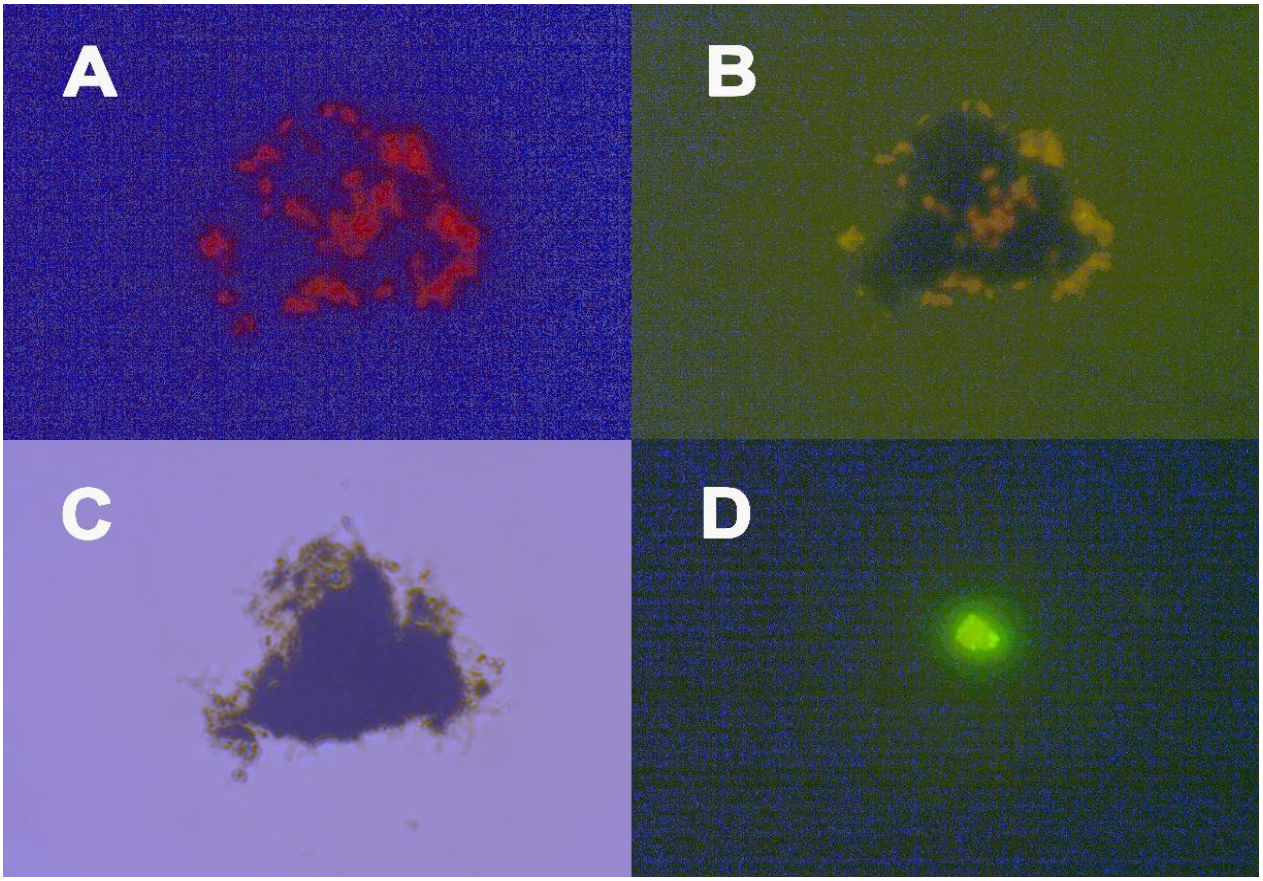


Fig 3.12 A) Dead cells observed under the microscope; B) Both dead and live observed under the microscope at the same position; C) The Nyex piece at the same position without applied any filter; D) Viable cells attached on the Nyex pieces at different location. All four photos were taken from the stained sample of 0.5A current applied without any additives.

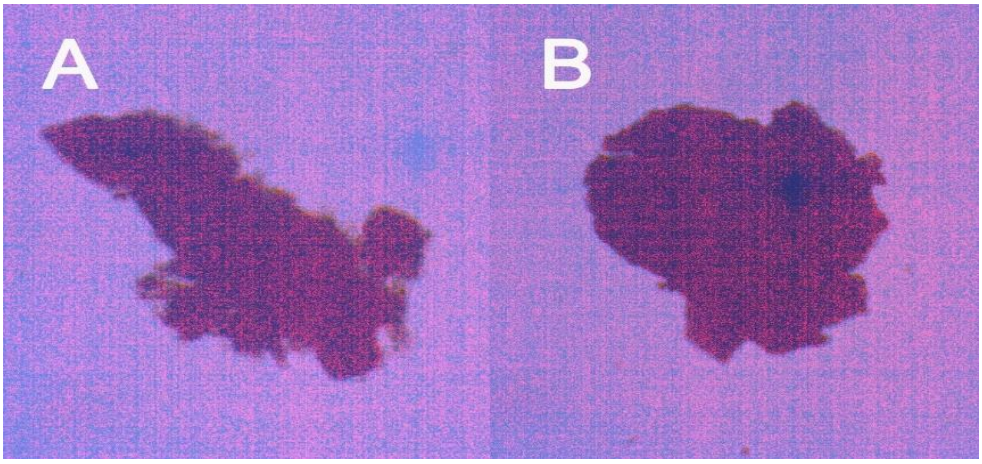


Fig 3.13 The Nyex pieces were shown in an unstained sample from 0.5A current without any additives.

4. Discussions

4.1 The Effects of Different Current Density

In this project, low current density resulted in more efficient bacterial cell removal than the high current density one. This result was not as same as the other electrochemical-adsorption experiments. Hussain's research (2014) suggested that high current density contributed to increased treatment efficiency by means of enhanced formation of free chlorine species. However, Hussain's experiment was only conducted over 140 minutes, which was much shorter than 48-hour operation. In our case, the system indeed showed removal of 99% of the bacteria added after the first 1.5 hours. However, long-term stability, and therefore system performance decreased significantly due to the Nyex degradation.

An appropriate current density is essential for optimal treatment especially when operating long-term, indicating that too high or too low current density may cause the occurrence of adsorbent degradation or inadequate oxidation (and disinfection) respectively. In this case,

the low current intensity is based on the preliminary calculations relating to the reactor structure and dimensions, although the optimal current applied will related to length and the diameter of the final effective reaction bed. Cost-effectiveness is another key factor, since high current density means large energy consumption and high operation costs.

In order to test the stability of the Nyex, a recycled flow experiment was conducted using 0.5A current intensity and a fast flow rate (1.5L/h) for 24 hours. The influent only consisted of deionised water and 0.369g/L Na_2SO_4. The effluent flowed back to the influent container rather than coming out of the system so that the system could be maintained at a high flow rate for 24 hours and also to allow any Nyex degradation to build up to levels that will be visible. The schematic diagram 4.1 described experimental components and the water flow direction.

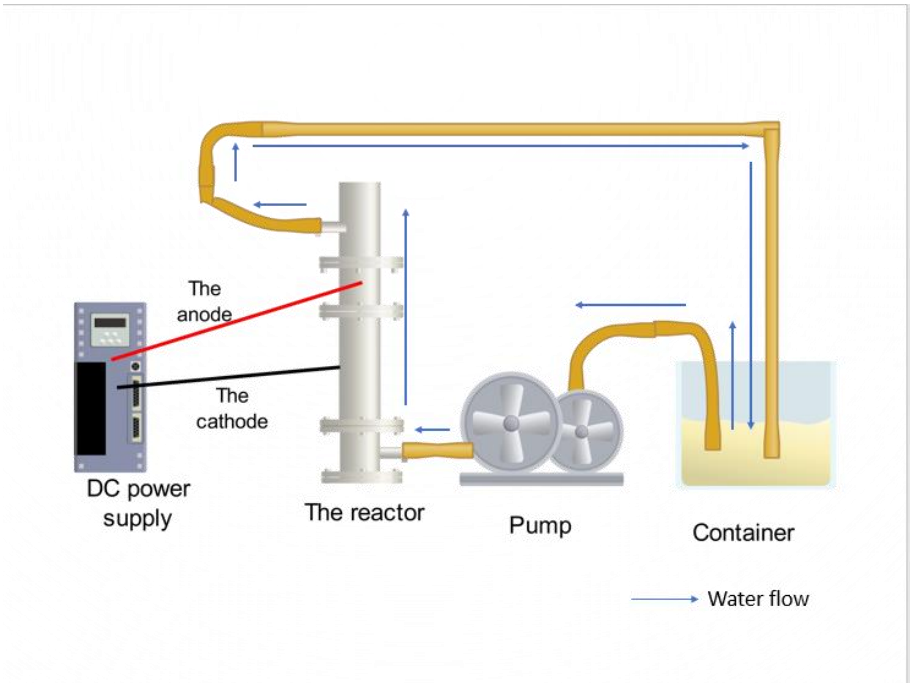


Fig 4.1 The schematic diagram of the recycled flow experiment.

This experiment aimed to analyse whether high flowrate could prevent the Nyex degradation at high current density. To quantitatively describe the Nyex in water, the spectrophotometer of treated water sample was analysed at a suitable wavelength using a spectrophotometer. High absorbance values means the presence of more suspended solids. Before analysing the water sample, the strongest adsorbed wavelength of the used Nyex was identified at 290 to 300nm. After 1.5 hours, the absorbance at 300nm was 0.111 and after 24 hours it was 0.019, suggesting that the suspended solid in the system before and after 24-hour operation was similar, most of the Nyex still stayed in the reactor column. Furthermore, the effluent was visually much cleaner than at the slow flowrate (150mL/h) in the 0.5A current experiment. Therefore, increasing the flow-rate can stabilise the Nyex in the system and prevent the graphite flakes coming out of the reaction column.

However, high flow-rate accounted for short residence time, that would decrease the treatment efficiency and was proved by Hernandez (2016) to be a key factor in controlling disinfection efficiency. Combining these two considerations, an alternative to overcome the adsorbent degradation problem perhaps can be modifying the design of the reactor. Applying lower current is another possible option.

4.2 Analysis of the Electrochemical Mechanisms

In general, two types of oxidation most likely happened in the reaction column. As shown in Fig. 4.2, direct oxidation would potentially destroy the bacteria on the surface of the Nyex, Indirect oxidation was achieved with the help of electro-generated mediators, in this case is free chlorine species, to carry out the oxidation.

In this project, the no-current applied group removed 99.45% of the bacteria after the first 1.5 hours, which means that bacteria can be physically adsorbed on the adsorbent surface spontaneously and inactivated without any applied current. No chemical oxidation happened. However, the capacity of physical adsorption was limited without the electro-regenerated adsorbent, resulting in achieving equilibrium very soon, shown by the fact that bacteria removal was only 26.9% after 48 hours. Therefore, this was not an

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appropriate approach for a long-term use or the treatment of highly polluted samples.

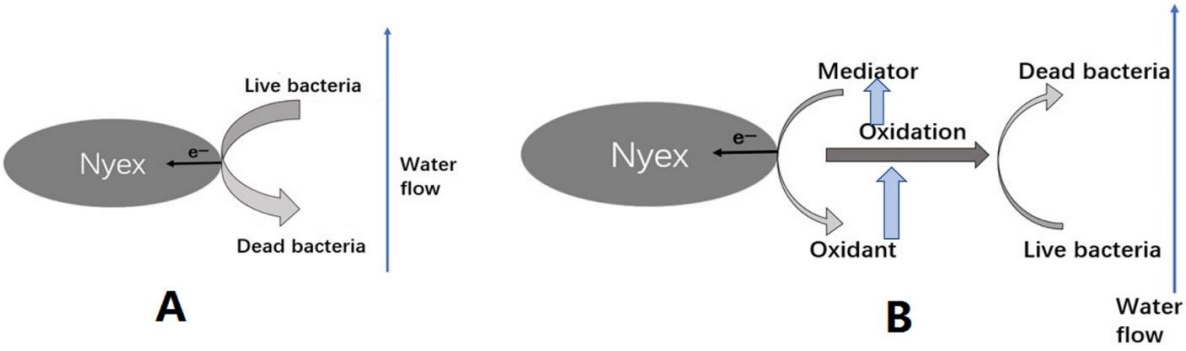

Fig 4.2 The demonstration of A) direction and B) indirection oxidation during the electrochemical-adsorption disinfection process.
Figure is modified from Anglada, Urtiaga and Ortiz, 2009.

One alternative could be the direct oxidation and regeneration of used adsorbent, triggered by applying current. First, the bacteria locolised on or around the adsorbent would be oxidized on the adsorbent surface by electron transfer, which demonstrated by Fig 4.2 (A). This process was examined by applying current without any additive experiments. The experiments ended up with 95% bacteria removal rate at the most appropriate current intensity 0.18A, which was not the optimal treatment efficiency among all scenarios. Incomplete treatment might have resulted from bacteria being densely attached to the adsorbent surface so that hindering regeneration of the Nyex surface. Nevertheless, it cannot guarantee that the direct oxidation was the only functional mechanism in this case because no ion-composition analysis was conducted. Without the presence of chloride, reactive oxygen species produced from electrolysis of water possibly reinforced the indirect oxidation and caused bacteria inactivation as well. This hypothesis could not be proved in this project. The conclusion merely could be the chloride free environment was less efficient when chloride was added, in keeping with the conclusion of other studies. (Hussain et al., 2014; Mezule et al., 2014).

Another alternative could be the indirect oxidation with intermediate chlorine species. The dominant disinfection form is HClO, which is simply produced by chlorine reacting with water under acidic environment. When the reaction proceeds, further oxidation would converted Cl^- to intermediate by-products ClO_2^- and ClO_2, finally giving chlorate ClO_3^- and the even more stable form perchlorate ClO_4^- as the end product.

Chlorate and perchlorate are the oxidized forms of chloride. Their adverse health effects both on human beings and animals have been revealed by Jung et al. (2010). Chlorate can damage the erythrocytes and further trigger the mutagenic activities in bacterial and mammalian cells while perchlorate influences the production of thyroid hormones. Furthermore, negative effect on the pituitary-thyroid axis has been found to associate with a short-term exposure of the mixture of chlorate and perchlorate (Khan et al., 2005). Aside from these health effects, these side reactions may influence the overall treatment performance because chlorate and perchlorate showed lower disinfection ability than HClO.

A detailed mechanism of transformations of chloride, chlorate and perchlorate and two important parameters, pH and reactive oxygen species, had been analysed by Jung et al (2010). The chlorate could be formed via different intermediate products and pathways while the perchlorate was only produced from oxidizing chlorate. During these reactions, pH and reactive oxygen species were specifically analysed. Lower pH resulted in less formation of ClO_3^- and ClO_4^-. The formation of ClO_4^- from ClO_3^- was reported to be independent of pH in the bulk solution. Reactive oxygen species played an important role on ClO_3^- and ClO_4^- formation through the direct oxidation and the chlorine electrolysis reaction. The concentration of $^{\cdot}OH$ positively contributed to ClO_3^- and ClO_4^- formation. With the purpose of generating more HClO but less chlorination by-products, an optimal condition may be to focus on low pH and $^{\cdot}OH$ production in the bulk solution.

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4.3 The Effects of pH

Seen from the current comparative experiments, a high current intensity causes lower pH

than low current intensity (shown in Fig 4.3) as a result of both chlorination and electrolysis of water. Once chloride is generated at the anode, it immediately reacts with water and releases hydrogen ions while the water undergoes the following reaction and generates hydrogen ions as well:

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^-$$

Theoretically, pH values between 3.3 and 7.5 may be the optimal for two reasons. First, HClO is the predominant disinfectant and second highly concentrated hydrogen ions can effectively kill the bacteria. However, UK water quality standard for treated water stipulates that the drinkable water should be between pH 6.5 to 9.5[4], thus the effluent could not be beyond that range. Controlling the pH at neutral is therefore important.

One study analysed the formation of chlorine species at neutral pH (Hussain et al., 2015). The result showed that, the generation of free chlorine species was hindered and the predominant reaction was electrolysis of water. Meanwhile, this process made the bulk solution acidic, and in turn favoured the evolution of chlorine. Another important point is that under neutral condition the *E.coli* is able to form a biofilm on the adsorbent's surface. This extracellular slime increases the resistance and limits the disinfection performance (Hussain et al., 2014). The observation of biofilm formation of *S. oneidensis* was conducted in this project. In summary, whether the optimal condition is either pH neutral or acidic remains controversial. The best operational scenario maybe a pH controlled one, but requires further investigation.

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[4] https://www.unitedutilities.com/globalassets/documents/pdf/phfactsheet_acc16.pdf

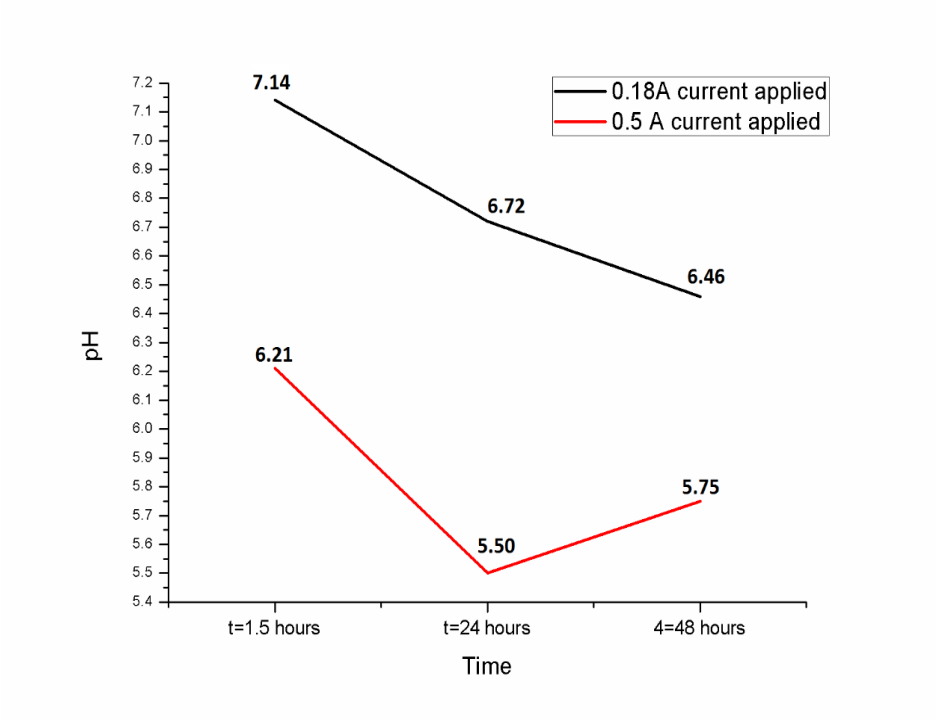

Fig 4.3 pH measurement in 0.18A current applied and 0.5A current applied experiments.

4.4 Further analysis

Firstly, more data points and repetitions are needed for further investigation. This project was only able to collect three data points and so for a better understanding of operating efficiency over a longer time period, additional data points would be beneficial. In addition, this project only used two different current intensities to test the electrochemical-adsorption performance. As has been proved in the previous stage of this research, current intensity influences Nyex degradation, thus, attempting to apply a different degree of current intensity can better show the most optimal condition in terms of the bearable capacity of the Nyex.

Analysing the treatment performance at different pH gradients is another critical point because pH controls the formation of free chlorine species and chlorination by-products. In this project, we adjusted the pH to slightly alkaline values and obtained acceptable treatment efficiencies after 24 hours. However, whether the efficiency can be maintained for 48-hour treatment period has not reached a conclusion. Furthermore, the ion

chromatographic analysis suggests that the transformation of chlorine species is very rapid so that minimizing the sample interval, such as sampling time after every 3 hours or even less, is likely to help identify any species produced.

Moreover, the execution of using electrochemical-adsorption disinfection to treat actual industrial wastewater must be the next step. Here the waters will contain not only microorganisms, but also organic and inorganic pollutants. These other pollutants may impact on disinfection efficiency. Further investations should also focus on costs of operation, as no energy consumption calculations or cost effectiveness analysis have been conducted in this project. This will be especially important for large-scale operations.

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5. Conclusion

Electrochemical-adsorption water disinfection can eliminate more than 99.9% of viable cells of the model bacterium *S. oneidensis* over the 48-hour continuous operation. The result of all five experiments is shown in Fig 5.1. The optimal current can be defined at 0.18A for this project, and this is related to the structure of the adsorbent bed. High current intensity can be applied in short-term operation, but it is unsuitable for long-term treatment due to the problems associated with the degradation of the adsorbent. In additions, high current intensity will translate to high energy consumption and expensive investment.

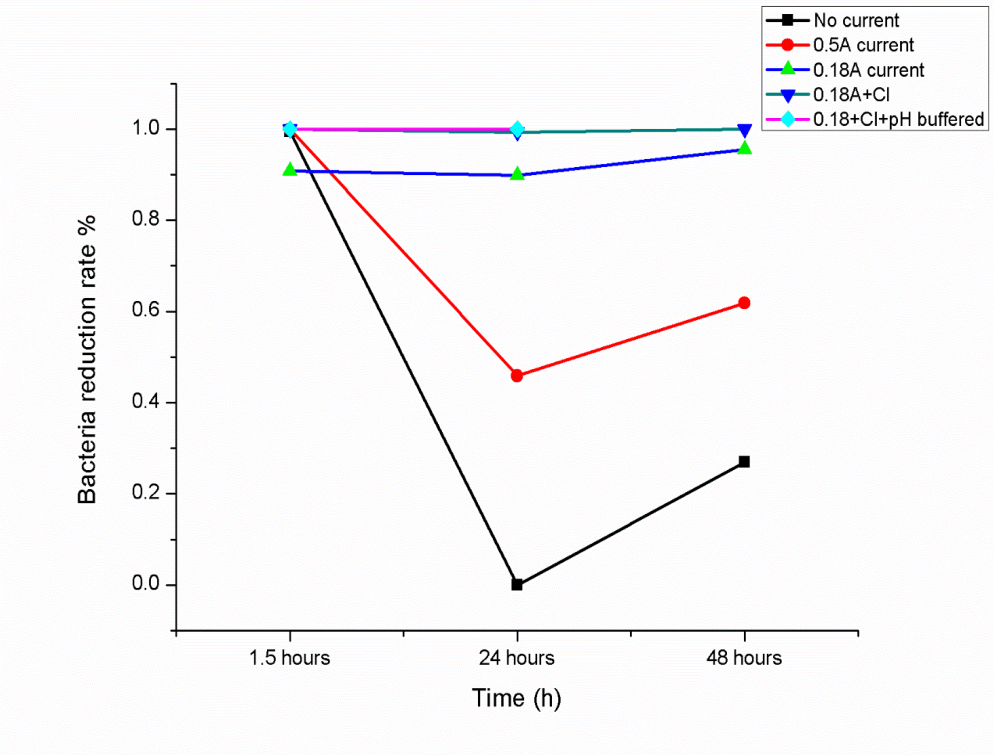

Fig. 5.1 The results of all five experiments in this project.

Free chlorine species play a key role in the disinfection through chlorination. Hypochlorous acid acts as a strong oxidant that would be expected to inactivate the cells of *S. oneidensis*. The concentration of hydrogen ion controls the dominant disinfection mechanism, and it also limits the formation of disinfectant as well as the chlorination by-products. In brief, direct oxidation may show lower treatment efficiency than indirect oxidation of free active

chlorine. Although plate counting failed to provide sufficient data to analyse whether pH controlled scenario is better than non-buffered one, the result was qualitatively supported by cell staining. The optimal condition is supposed to be the non-buffered experiment, for limiting the chlorination by-products and enhancing the chlorination process.

Since this project used very concentrated suspension of *S. oneidensis*, the energy consumptions are expected to be relatively high. In real applications, targeting lower cell densities, the cost of treatment is expected to be lower than would be expected from this experiment.

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Appendix

IC analysis report

Injection No.	Injection Name	Amount	Amount	Amount	Amount	Amount	Amount	Amount
Chloride	Chloride	Fluoride CD_1	mg/L Chloride CD_1	Nitrite CD_1	Sulphate CD_1	Bromide CD_1	Nitrate CD_1	Phosphate CD_1
1	RICK KIMBER 1	n.a.	50.0097	n.a.	n.a.	n.a.	n.a.	n.a.
2	2	n.a.	49.9578	n.a.	n.a.	n.a.	n.a.	n.a.
3	3	n.a.	5.8423	n.a.	n.a.	n.a.	n.a.	n.a.
4	4	n.a.	48.9826	n.a.	n.a.	n.a.	n.a.	n.a.
5	5	n.a.	44.0757	n.a.	n.a.	n.a.	n.a.	n.a.
6	6	n.a.	41.6955	n.a.	n.a.	n.a.	n.a.	n.a.
7	1 X10	n.a.	54.5494	n.a.	n.a.	n.a.	n.a.	n.a.
8	2 X10	n.a.	54.7928	n.a.	n.a.	n.a.	n.a.	n.a.
9	3 X10	n.a.	4.7288	n.a.	n.a.	n.a.	n.a.	n.a.
10	4 X10	n.a.	55.3094	n.a.	n.a.	n.a.	n.a.	n.a.
11	5 X10	n.a.	48.7422	n.a.	n.a.	n.a.	n.a.	n.a.
12	6 X10	n.a.	54.1228	n.a.	n.a.	n.a.	n.a.	n.a.
13	DIW	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
14	10mg/L FUM	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
15	3mg/L FUM	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
16	20mg/L FUM 40Cl	n.a.	39.9982	n.a.	n.a.	n.a.	n.a.	n.a.
17	20 Cl	n.a.	20.0092	n.a.	n.a.	n.a.	n.a.	n.a.
18	5 Cl	n.a.	4.9769	n.a.	n.a.	n.a.	n.a.	n.a.

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Rick Kimber: Ref 'Rick170517'

Dionex ICS5000 CAP I.C.

Note: Sample 3 was run neat; the rest were diluted 10x. All the values are corrected for dilution.

Note: No oxychlorides seen

Name	Amount mg/L Chloride	
40mg/L CAL3	39.9982	
20mg/L CAL2	20.0092	
5mg/L CAL1	4.9769	
DIW	n.a.	
2 X10	54.7928	Influent 26-4-17
3	5.8423	Effluent 1.5 hrs
4 X10	55.3094	Effluent 48 hrs
5 X10	48.7422	Effluent 24 hrs
6 X10	54.1228	Influent 24-4-17

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