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Investigating the effects of continuous electrochlorination on bacteria, bacteriophage and DNA.

By Ratish Ramyad Permala

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Acknowledgment of Country

We acknowledge that Curtin University works across hundreds of traditional lands and custodial groups in Australia, and with First Nations people around the globe. We wish to pay our deepest respects to their ancestors and members of their communities, past, present, and to their emerging leaders. Our passion and commitment to work with all Australians and peoples from across the world, including our First Nations peoples are at the core of the work we do, reflective of our institutions' values and commitment to our role as leaders in the Reconciliation space in Australia.

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I dedicate this thesis to my dad, Naresh Permala

ABSTRACT

Disinfection of drinking water is a vital step to generate pathogen-free water and decrease the incidence of infectious diseases associated with water-borne pathogens. Traditionally, chlorine is the most commonly used disinfectant to treat drinking water. However, the use of chlorine as a disinfectant has been associated with some serious hazards and health concerns. Electrochemical oxidation has gained a lot of attention as an alternative to the traditional use of chlorine as a disinfectant for the treatment of drinking water. In drinking water treatment, the primary goal of electrolysis is the *in situ* generation of disinfectants such as active chlorine (dissolved Cl_2, HOCl and OCl^-). Hydro-Dis® in collaboration with Water Corporation of Western Australia developed a Pilot continuous electrochlorination (CEC) unit and two Laboratory CEC units (Electrolytic cell 1 [EC1] and Electrolytic cell 2 [EC2]) using titanium electrodes coated with a mixed metal oxide (MMO) layer of Ti/Rh/Ru. EC1 and EC2 had a volume of 47 ml and 7.2 mL, respectively. The electrodes were 10 mm × 10 mm × 1 mm and 50 mm × 25 mm × 1 mm for EC1 and EC2, respectively. Each electrode for EC1 and EC2 had an active area of 0.0001 m^2 and 0.00125 m^2, respectively.

Chapter 2 focused on the performance of CEC technology as an alternative to conventional chlorination in drinking water treatment by investigating the impact of varying voltage, NaCl concentration, flowrate, and dissolved organic carbon (DOC) on chlorine production, disinfection and inorganic by-product formation for both the lab and the pilot unit. This study indicated that chlorine production was directly proportional to voltage applied and NaCl concentration but inversely proportional to flowrate on the lab unit. The same trend was observed with the pilot unit however, the mass production of chlorine (g/h) increased with increasing voltage and NaCl concentration but was unaffected with varying feed flowrates indicating the process is mass transfer limited and not diffusion limited.

Chlorate production on the lab unit increased with increasing voltage and NaCl concentration but decreased with increasing flowrate. However, the chlorate concentrations measured were well below the World Health Organisation (WHO) guidelines. Bromate and chlorite levels (< limit of detection) were also well below the limit of the Australian Drinking Water Guidelines. With the pilot unit, increasing DOC concentration increased adsorbable organic chloride (AOCl), trihalomethanes (THMs) and haloacetonitriles (HANs) concentrations. The predominant THM species was trichloromethane. Mostly chlorine containing disinfection by-products (DBPs) increased with increasing DOC content.

In **Chapter 3**, EC2 settings were optimised to examine the effects of CEC on common waterborne pathogens in a chloride-containing solution using plate counts and flow cytometry. The bacterial/phage suspensions tested consisted of 20 mg/L NaCl, 10 mM phosphate buffer (PB), pH 7.2 and the flowrate applied was set at 200 mL/min. Plate counts and plaque assays were performed

before and after CEC treatment to assess the disinfection efficiency of CEC. CEC was successfully able to inactivate 6 log of *E. coli* and *B. subtilis* with a CT (free chlorine concentration x contact time) of 0.07 mg.min/L, 6 log of *S. maltophilia* with a CT of 0.08 mg.min/L, 2.5 log of *L. pneumophila* with a CT of 0.20 mg.min/L, and 6 log of phage T4 and 2.5 log of MS2 phage with a CT of 0.18 mg.min/L, in a very short contact time (4.5 seconds).

Regardless of the different CT applied in EC2 setting, the total number of bacterial cells measured by SYBR Green I, was not affected by the free chlorine generated by CEC under the same experimental conditions. The findings suggested that there was no immediate measurable cell lysis and no DNA alteration after exposure to CEC treatment. This was likely due to the very short contact time of 4.5 seconds and the low concentrations of free chlorine (0.2 - 2.7 mg/L) generated by CEC on the bacterial cells under the conditions applied.

In the absence of chloride ions, EC2 was able to inactivate 0.5 log of *E. coli* within 0.08 min of contact time when operated in continuous mode. When operated in recirculating mode for 10 min, CEC inactivated 6 log of *E. coli.* No significant difference was observed between the levels of *E. coli* inactivation cause by CEC-treated water (in the presence of Cl^- ions) and standard chlorination (sodium hypochlorite) for the same contact time, suggesting that there were no additional microbe-inhibiting compounds produced by the CEC. The 6 log inactivation of *E. coli* in the absence of chloride ions was attributed to the direct oxidation of the *E. coli* cells on the surface of the electrodes.

Chapter 4 focused on the assessment of the microbiological quality of water after disinfection and the potential for *E. coli* to recover or regrow following CEC treatment of a chloride-containing solution using the lab unit. The ability of *E. coli* cells to recover in the presence or absence of nutrient addition following CEC treatment was analysed using flow cytometry and heterotrophic plate counts (HPC). The results indicated that no culturable cells were detected immediately after CEC treatment with CT ≥ 0.10 mg.min/L as previously shown in chapter 3. However, the flow cytometry results showed that the total number of SYBR-stained cells and their mean fluorescence intensity for each cell did not change significantly after treatment. This suggested that there was no measurable cell lysis and no measurable DNA alteration (by flow cytometry) after exposure to CEC treatment under the conditions applied in this study. In the absence of any nutrient addition (only phosphate buffer), no bacterial growth and no recovery of culturable cells were observed during the 96 hours of incubation after CEC treatment. When nutrients (diluted LB) were added after CEC treatment, cell recovery was observed after 24 hours. Recovery of cells only occurred for those treated with a CT 0.19 mg.min/L or less. No recovery was observed for the cells treated with CT of 0.30 mg.min/L. The rate of recovery was found to be slower for cells exposed to higher CT (0.19 mg.min/L), suggesting more membrane damage occurred at higher CT.

Since the number of recovered cells following CEC treatment and nutrient addition represented about 1/3 of the total number of cells stained by SYBR Green I, it seemed likely that the recovering cells were previously injured and unculturable by the standard plate counts (viable but non-culturable cells) or were undetected immediately after CEC treatment. A resuscitation test was performed to confirm if the cells that recover were previously injured cells (VBNC) or were undetected immediately after CEC treatment by plate counts. After 24 hours of recovery at room temperature (22 ^{o}C - 24 ^{o}C) in the presence of nutrients, culturable cells were observed for CEC treated samples to which nutrients were added. The observed culturable cells were indeed VBNC cells that slowly recovered in the presence of nutrients. Therefore, the results of this study emphasised the importance of drinking water utilities to routinely monitor the level of residual disinfection and the microbiological quality of water after the point of disinfection and implement flow cytometry as a diagnostic tool instead of the century-old HPC in order to safeguard the public from waterborne diseases.

Chapter 5 investigated the inactivation efficiency and the DBP (primarily THMs) formation of the pilot unit for ground and surface waters in Western Australia. Five different waters with different chemical characteristics were tested for disinfection by injecting the pilot unit with known concentrations of common waterborne pathogens, *E. coli*, *B. subtilis* and bacteriophage T4, and the number of viable cells were analysed before and after CEC treatment. The pilot unit consisted of two sets of cells, pilot unit cell 1 (PUC1) and pilot unit cell 2 (PUC2). PUC1 was designed for oxidation of reduced ions present in the waters and PUC2 was designed for disinfection. The pilot unit settings were optimized such that near zero free residual chlorine is produced after PUC1 and a target of 1 mg/L of free residual chlorine is achieved after PUC2 (immediately at the outlet of CEC). For each of the tested waters, varying the flowrate and the voltage applied allowed to detect low concentration of free residual chlorine (0 – 0.13 mg/L) after PUC1. The chlorine generated by the PUC1 were used to oxidise mainly reduced iron (Fe^{2+}) and manganese (Mn^{2+}) present in the ground waters to Fe^{3+} and Mn^{4+}, respectively. In the presence of reduced ions, low to no bacterial inactivation was observed after PUC1 due to the fast reaction of chlorine with Fe^{2+} and Mn^{2+}.

The pilot unit was able to meet the target of 1.0 mg/L of free residual chlorine after PUC2 for waters that contained low chloride, high DOC, high iron and high manganese. The chlorine produced by PUC2 were able to oxidise any remaining reduced ions and also inactivate at least 6 log of each microorganism (all population inactivated) that were injected into the pilot unit. Therefore, this pilot unit can effectively be used for pre-oxidation and disinfection processes.

THMs formation increased with increasing DOC in the source water. The highest total THM (86.64 µg/L) formation was recorded following treatment of Denmark River water, WA (highest DOC content 17.3 mg/L) after 7 days of contact time with the residual chlorine. Moreover, the concentration of all

THMs formed increased when the contact time increased from 15 min to 7 days. Since all the tested waters contained bromide ions, the relative abundance of brominated THMs also increased with an increase in contact time. Nevertheless, the total THMs formed after 15 min and 7 days of contact time with the residual chlorine for all the studied waters were well below the ADWG limit of 250 μg/L.

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Abbreviations

ADWG	Australian Drinking Water Guidelines
AOX	Adsorbable organic halides
ATP	Adenosine triphosphate
BCYE	Buffered charcoal yeast extract
BDD	Boron-doped diamond
CEC	Continuous electrochlorination
CFU	Colony forming units
COD	Chemical Oxygen Demand
CT	Product of the concentration of chlorine and the contact time of chlorine with the sample.
DBPs	Disinfection by-products
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DPD	N, N diethyl-1,4 phenylenediamine
DSA	Dimensionally stable anodes
DWU	Drinking water utility
EAOP	Electrochemical advanced oxidation processes
EC	Electrochemical
EC1	Electrolytic cell 1
EC2	Electrolytic cell 2
EDTA	Ethylenediaminetetraacetic acid
EF	Electric field
FC	Flow cytometry
FL-1	Fluorescence parameter 1
FL-2	Fluorescence parameter 2
FSC	Forward scatter
GC-MS	Gas chromatography-mass spectrometry
GWTP	Ground Water Treatment Plant
HAAs	Haloacetic acids
HANs	Haloacetonitriles
HKs	Haloketones
HOBr	Hypobromous acid
HOCl	Hypochlorous acid

HOI	Hypoiodous acid
HPC	Heterotrophic plate counts
LB	Luria Bertani
MMO	Mixed metal oxide
NOM	Natural organic matter
OEP	Oxygen evolution overpotential
PB	Phosphate buffer
Pt	Platinum
PUC1	Pilot unit cell 1
PUC2	Pilot unit cell 2
RCS	Reactive chlorine species
Rh	Rhodium
ROS	Reactive oxygen species
rpm	Revolution per minute
Ru	Ruthenium
SD	Standard deviation
SEM	Scanning electron microscope
SPME	Solid-phase microextraction
THMs	Trihalomethanes
Ti	Titanium
TOC	Total organic carbon
TOX	Total organic halides
TYGB	Tryptone yeast extract glucose broth
USEPA	US Environmental Protection Agency
UV	Ultra-violet radiation
VBNC	Viable but non-culturable
WA	Western Australia
WHO	World Health Organisation
WTP	Water Treatment Plant

Chapter 1. Literature Review

1.1 Disinfection

Disinfection is of unquestionable importance in the water treatment process to yield safe water free from pathogenic microorganisms. Disinfection of drinking water has made a great impact in the reduction of death and sickness rates through the 19th Century (Van Leeuwen, 2000). Waterborne diseases, including; amoebiasis, typhoid, diarrhoea, schistosomiasis, and cholera, are continuing to pose challenges in numerous nations, particularly in unindustrialized countries (Kerwick et al., 2005). Globally, 785 million people do not have access to a basic drinking-water facility and 2 billion people are affected by a drinking water source that is contaminated with faeces directly causing about 485,000 diarrhoeal cases annually. The World Health Organisation (WHO) predicts that by 2025, half of the global population will be living in water-stressed areas (WHO, 2019). Therefore, an efficient technique of removing pathogenic microbes from the treatment of drinking water involves coagulation/flocculation, sedimentation and filtration in addition to chemical processes. Primary disinfection is defined as the inactivation and the elimination of microbiological contaminants in raw water resources. Secondary disinfection is defined as the residual presence of disinfection products to avert regrowth of pathogenic microbes in the distribution system (Kerwick et al., 2005). Disinfection techniques can be classified into three broad groups; chemical, physical, and physicochemical.

1.1.1 CT concept

The CT value is the concentration of the disinfectant (C) in the water, multiplied by the contact time (t) the disinfectant has with the microorganism.

$$\mathbf{CT = C \times T}$$

where: C = the concentration of the disinfectant, usually measured in mg/L, and

T = time of contact of the microorganism with the disinfectant, measured in minutes.

CT values can provide a measurement that can be used to compare the efficacy of various disinfectant against a specific microbe (Grunert et al., 2018; NHRMC, 2011). The lower the CT value needed to inactivate the microorganisms, the stronger the disinfectant. For example, CT value of *Escherichia coli* for a 2-log/99% inactivation is less than 1 mg.min/L when chlorine is used, but 95-180 mg.min/L when chloramine is used (WHO, 2004). *E. coli* therefore requires a longer contact time with chloramine compared to chlorine to achieve the same 99% inactivation.

1.1.2 Physical disinfection

Physical disinfection does not involve chemicals and relies on physical means for disinfection. Physical disinfectants act directly on the pathogens without altering the elemental composition of the water (Biryukov et al., 2005). Some of the physical methods include thermal disinfection (heating/boiling),

membrane filtration, UV irradiation and ultra-sonication (Burch & Thomas, 1998). For instance, thermal disinfection (superheat-and-flush) is one of the most commonly used disinfection methods since it does not require any special equipment, making its operational conditions expeditious (Chen et al., 2005; Lin et al., 1998). This method is especially advantageous in the event of an outbreak (e.g. *Legionella*) when the water in the distribution network needs to be disinfected immediately (Chen et al., 2005). However, thermal disinfection is a time-consuming, labour intensive (requires many personnel to monitor flushing times and temperatures of the water tanks), is unable to disinfect biofilms and most importantly, is only effective for a short term (Blanc et al., 2005; Chen et al., 2005; Lin et al., 1998). On the other hand, UV irradiation is effective in killing microorganisms but it does not generate residual disinfection products and its efficiency is greatly affected by the turbidity of the water (Kraft, 2008).

1.1.3 Chemical disinfection

Chemical disinfection uses chemical substances to inactivate waterborne pathogens in potable water. The chemical substances together with their reaction products can act on the microorganisms in various ways, including disrupting the membrane, inhibiting active transport across the membrane, inhibiting metabolic activity, damaging nucleic acids leading to disruptions in replication, lysis and membrane leakage, coagulation of intracellular components, and denaturation of protein and inactivation of enzymes (Denyer & Stewart, 1998). Some of the chemical disinfectants used in drinking water are chlorine gas, sodium hypochlorite, (mono) chloramine, chlorine dioxide, ozone and hydrogen peroxide (Cho et al., 2006; Malato et al., 2009; WHO, 2004). Chemical processes of water disinfection are generally preferred as they provide both primary disinfection and residual disinfection. The most commonly used chemical agents are chlorine and chlorine derivatives (White, 1999).

Chlorine is the most widely used disinfectant in water treatment. Chlorine has a wide range of biocidal activity, it is easy to dose and monitor its concentration, is relatively cheap and provides both primary and secondary disinfection (Bridgewater et al., 2012). Despite chlorine's effectiveness as a disinfectant, it has several drawbacks. Chlorine is dangerous to transport and store as a gas, it exhibits an unfavourable taste and odour, and on its own is ineffective against microbes such as *Cryptosporidium parvum* (Kerwick et al., 2005). The formation of toxic and mutagenic disinfection by-products (DPBs) such as trihalomethanes, haloacetonitriles, haloacetic acids, trichloroacetaldehyde, haloacetaldehydes and halocetamide can affect consumers' health (Hrudey, 2009; Itoh et al., 2011). Strict guidelines to limit the presence of DBPs in drinking water have prompted research into alternatives to chlorine. These include chloramination, ozonation, titanium photocatalysis, UV irradiation and electrochemical disinfection. Alternatives such as UV irradiation and ozonation have

been accepted by water utilities for the disinfection of water, but they are limited by the fact they do not provide residual disinfection. Electrochemical disinfection has emerged as one of the most promising alternatives to chlorine as it generates both primary and residual disinfection products (Kerwick et al., 2005).

1.1.3.1 *Chlorine*

Chlorine has been used globally as a water disinfectant on a large scale since the early part of the 20th century and is still the predominantly used disinfectant since it is inexpensive and highly efficient. The high reactivity and efficiency of chlorine is due to its high oxidising capacity, E^o at 25 ^oC = 1.49 V (Glaze, 1990). When chlorine reacts with water, it forms hypochlorous acid (HOCl) and a chloride ion (Cl^-) (Equation 1)

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^- \text{ (Equation 1)}$$

Hypochlorous acid (HOCl) is a weak acid that dissociates into hypochlorite ions (OCl^-) and hydrogen ions (H^+) with pKa = 7.5at 25 ^0C. (USEPA, 1999)(Equation 2)

$$HOCl \leftrightarrows OCl^- + H^+ \text{ (Equation 2)}$$

Chlorine works best as antimicrobial agent when the pH is 4 – 7 (McDonnell, 2017). When the pH is between 4 -7, HOCl is the dominant species of chlorine present in water. At a pH of 7.5, both HOCl and OCl^- are present in equal amounts. However, in alkaline conditions (pH > 8), OCl^- ions dominate (LeChevallier & Au, 2004). HOCl is more reactive and is about 70 - 80 times stronger than OCl^- ions as a disinfectant (USEPA, 1999). HOCl has no electrical charge (neutral), hence it can penetrate cell walls of pathogenic microorganisms which are negatively charged (Silhavy, Kahne, & Walker, 2010) more easily. Negatively charged OCl^- ions experience a repulsive force by the cell walls of the pathogens, making it harder for the OCl^- ions to diffuse into the cells (White, 1999). The pH of the water being treated with chlorine is also important for these reasons. In Australia, the source water for water treatment has a pH 6.5 - 7.5 (Bridgewater et al., 2012). Within this range, both HOCl and OCl^- ions are present during disinfection with chlorine. As pH increases, the amount of HOCl decreases and this leads to a decrease in disinfection effectiveness (Richardson, 1998; White, 1999).

1.1.3.2 *Chloramine*

Chlorine reacts reversibly with ammonia in water to form three types of inorganic chloramine; monochloramine (NH_2Cl), dichloramine ($NHCl_2$), trichloramine (NCl_3), according to the following equations (3-5) respectively (Diehl et al., 2000).

$$NH_3 + HOCl \leftrightarrows NH_2Cl + H_2O \quad \text{(Equation 3)}$$

$$NH_2Cl + HOCl \leftrightarrows NHCl_2 + H_2O \text{ (Equation 4)}$$

$$NHCl_2 + HOCl \leftrightarrows NCl_3 + H_2O \quad \text{(Equation 5)}$$

Monochloramine is a preferred disinfectant of the three inorganic chloramine species since it is the most stable and produces the least taste and odour. Since the oxidising potential of monochloramine is low (E^o at 25°C = 0.75 V) (19), it is utilised as a secondary disinfectant. Monochloramines require a longer contact time to inactivate pathogenic microorganisms. For instance, a CT of 95 – 180 mg.min/L (5 ^{0}C) inactivates 2 log of *E. coli* (LeChevallier & Au, 2004). Dichloramine has a higher disinfecting capacity than monochloramine, however, it produces an unpleasant odour and is less stable. Trichloramine is the least stable and also generates an unpleasant odour (USEPA, 1999; UWRAA, 1990).

Generally, chloramination minimises chlorinated DBPs such as trihalomethanes and haloacetic acids, but does not eliminate them. Usually, the range of DBPs formation are similar as when chlorine is used, particularly when chlorine is added before the addition of ammonia (WHO, 2000). However, a greater quantity of cyanogen chloride and N-nitrosodimethylamine, other DBPs, are produced using monochloramine as a disinfectant compared to chlorine. For instance, the formation of N-nitrosodimethylamine increases the formation of nitrogenous-DBPs (USEPA, 1999). Whilst it is important to reduce the formation of DBP, the disinfection of the water is the highest priority because poor microbiological quality poses a greater and more immediate risk to the public health compared to short-term exposure to DBP (Hrudey, 2009)

1.1.4 Physicochemical disinfection

Physiochemical disinfection involves both chemical and physical approaches for disinfecting water. Electrolysis is a type of physicochemical disinfection that results in formation of mixed oxidants (disinfection agents) from electrolytic cells (Kerwick et al., 2005). Photocatalysis is another example of this method of disinfection whereby titanium dioxide, a nanoparticle sized photocatalyst, acts as an antimicrobial agent in water. TiO_2 alone or combined with copper and silver ions has shown prominent biocidal activity against a wide variety of pathogenic microorganisms including *Cryptosporidium* (Butterfield et al., 1997; Foster et al., 2011; Li et al., 2008; Maness et al., 1999). However, TiO_2 photocatalysis does not generate residual disinfection products (Bergmann et al., 2002).

1.1.5 Factors influencing disinfection

Some of the main factors affecting disinfection include the concentration of disinfectant, contact time, pH, temperature and the type of microorganisms (WHO, 2004). pH regulates the reaction kinetics, for

instance, at higher pH the antimicrobial activity of chlorine dioxide is increased, while free chlorine is greater at low pH. Chlorine dioxide is more effective and rapid at disinfecting chlorine-resistant pathogens such as *Cryptosporidium* oocysts at alkaline pH (pH 8) (USEPA, 1999). The influence of temperature on the disinfection kinetics can be modelled with the Arrhenius equation (Equation 6). Usually, disinfection efficiency increases with temperature (WHO, 2004). However, in some cases the disinfectant can be degraded at higher temperatures, thereby weakening its germicidal activity which can lead to serious health risks (William A. Rutala, 2008).

$$k = Ae^{-Ea/RT} \quad \textbf{(Equation 6)}$$

where k = rate constant, A = pre-exponential factor, E_a = activation energy, R = universal gas constant and T = absolute temperature.

Additional factors affecting the antimicrobial activity of some disinfectants are bacterial growth rate or growth phase, nutrient limitation and starvation, aggregation, encapsulation of bacteria and attachment of the bacteria to surfaces/particles (WHO, 2004). *Pseudomonas aeruginosa* is more resistant to chlorine dioxide, acetic acid, benzalkonium chloride (a quaternary ammonium compound) and glutaraldehyde when grown in distilled water compared to cells grown on tryptic soy agar [26]. Bacteria cultured at submaximal growth rates (low-nutrient environment) and at low temperatures are more resistant to a number of disinfectants (Berg, Matin, & Roberts, 1982; Harakeh et al., 1985). When aggregated, *Klebsiella pneumoniae* is 100-fold more resistant to hypochlorous acid and 2.3-fold more resistant to monochloramine (Stewart & Olson, 1992). Isolation of encapsulated coliforms from chlorinated water suggests an extracellular capsule can also make bacteria more resistant to free chlorine (Reilly & Kippin, 1983). Furthermore, microorganisms tend to be more resistant when attached or associated with different particulate surfaces such as glass, algae, carbon fines, particles associated with turbidity and macroinvertebrates (for e.g. *Crustacae*) (WHO, 2004). Some experiments have shown that most viable bacteria isolated on membrane filters (2.0 µm and 0.2 µm) from chlorinated water are either attached to suspended particulate matter or are aggregated (Ridgway & Olson, 1982). Besides being a ubiquitous reservoir of *Legionella* and other bacteria, free living amoeba give some opportunistic pathogen increased resistance against disinfectants by acting as "Trojan horses" for their host, that is, carrying and shielding amoeba-resistant bacteria such as *Legionella* species, in vesicles filled with the bacteria, from the effects of disinfectants (Greub & Raoult, 2004). Therefore, it is important to assess the disinfectant resistance patterns of bacteria in raw and treated waters in order to provide the public with safe drinking water.

1.2 Electrochemical disinfection

Over the past two decades, electrochemical technologies including electrochemical coagulation, electrochemical oxidation (referred to as electrochemical disinfection throughout this thesis) and electrochemical flotation have drawn a lot of attention as an alternative to the traditional, chlorine, disinfection of drinking water (Drees, Abbaszadegan, & Maier, 2003; Feng et al., 2004; Patermarakis & Fountoukidis, 1990). Electrochemical oxidation is a chemical reaction that occurs at the surface of the anode whereby an atom or a molecule loses one or more electrons during the passage of a direct current through the electrochemical unit. An electrochemical unit is made up of at least two electrodes, an anode (positively charged) and a cathode (negatively charged), an electrolyte, and an electrical circuit (Choi, Shim, & Yoon, 2013). The disinfection efficiency of the electrochemical cell relies on the type of electrode and electrolyte used (Jeong, Kim, & Yoon, 2009). Electrochemical (EC) disinfection is environmentally friendly since it can be powered by solar panels, making it suitable for use in developing countries and in remote communities (Cho et al., 2014; Huang et al.; Kraft, 2008). Compared to conventional chlorination, which is still the most popular disinfection method, EC disinfection does not require handling of chemicals such as chlorine gas or sodium hypochlorite, and the amount of disinfectants (such as hypochlorite), required can be adjusted according to the on-site demand, makes this mode of disinfection easier to operate (Gusmão, Moraes, & Bidoia, 2010; Kraft, 2008). EC disinfection can be relatively cheap considering it generates active chlorine species *in situ* from chloride-containing solutions and naturally occurring chloride ions in waters, eliminating the cost associated with the purchase, transport and storage of chlorine gas or sodium hypochlorite (De Battisti et al., 2018; Kraft, 2008). Furthermore, EC disinfection have been proven to be highly efficient at disinfecting a wide range of microorganisms such as bacteria, viruses and algae (Diao et al., 2004; Kraft, 2008; Shimada & Shimahara, 1982). EC disinfection is used in a wide range of industries and applications. For example, salt-water pool chlorination, power plant cooling systems, ship ballast water disinfection and desalination maintenance, food processing, wastewater treatment and reuse (Särkkä, Bhatnagar, & Sillanpää, 2015). Electrochemical oxidation methods in the presence of chloride ions are attractive as they have capacity to maintain residual disinfection. However, as with conventional chlorination, the risks associated with the production of toxic DBPs is a major drawback of this technology (Bergmann & Rollin, 2007).

1.2.1 Mechanism of action

Electrochemical oxidation mechanisms are: direct oxidation at the surface of the electrode- leading to the instantaneous inactivation of bacteria or by direct electron transfer/electric field (Jeong et al., 2007), and indirect oxidation by oxidants generated from either a chloride-containing solution or from the electrolysis of water. Indirect oxidation occurs when oxidants such as dissolved chlorine,

hypochlorous acid and hypochlorite are formed at the anode by chloride ions present in the electrolyte. Chloride is oxidised at the anode to form chlorine and chlorine in turn reacts with water to form hypochlorous acid and hypochlorite ions (Equations 7-9) (Anglada et al., 2011; Kraft, 2008). Dissolved chlorine, hypochlorous acid and hypochlorite ions are collectively referred to as active chlorine species and their formation relies on the pH of the solution (H. Bergmann & S. Koparal, 2005). The optimal pH for chlorine is between 4 and 7 (Penn, 2007). Furthermore, during the EC disinfection of a chloride containing solution using anodes with high oxygen evolution overpotential (OEP- electric potential at which water is hydrolysed into oxygen and hydrogen during the electrolysis) (such as boron doped diamond, lead dioxide and antimony-doped tin oxide), the chloride ions react with the produced hydroxyl radicals to form chlorine radical and hypochlorite ion (Equations 10 & 11) (Bergmann, 2010). The highly reactive and powerful oxidants generated from the electrolysis of water at the anode are hydrogen peroxide (H_2O_2) and reactive oxygen species (ROS) such as hydroxyl ($^{\cdot}OH$) radicals, ozone (O_3), and atomic oxygen (O_2^{-}) (Equations 12-15) (Liang et al., 2005; Martínez-Huitle & Brillas, 2008; Panizza & Cerisola, 2005). Therefore, the high disinfection efficacy of electrochlorination can be ascribed to the combined effects of direct oxidation at the surface of the electrode (Grahl & Märkl, 1996; Matsunaga et al., 2000), indirect oxidation by reactive chlorine species (RCS), free radicals (ROS) and H_2O_2 (Bergmann, 2010; Diao et al., 2004; Feng et al., 2004; Liang et al., 2005), and the electric field effect (Butterfield et al., 1997; Grahl & Märkl, 1996).

$2Cl^- \rightarrow Cl_2 + 2e^-$ (Equation 7)

$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$ (Equation 8)

$HOCl \leftrightarrows OCl^- + H^+$ (Equation 9)

$Cl^- + OH^{\cdot} \rightarrow Cl^{\cdot} + OH^-$ (Equation 10)

$Cl^- + 2OH^{\cdot} \rightarrow OCl^- + H_2O$ (Equation 11)

$H_2O \rightarrow {}^{\cdot}HO + H^+ + e^-$ (Equation 12)

${}^{\cdot}HO \rightarrow O^{\cdot} + H^+ + e^-$ (Equation 13)

$2{}^{\cdot}HO \rightarrow H_2O_2$ (Equation 14)

$O_2 + O^{\cdot} \rightarrow O_3$ (Equation 15)

1.2.2 Electrodes used in electrochemical disinfection

In electro-oxidation processes, choosing the right anode material is very important since the anode determines the disinfection efficiency as well as the electrode selectivity for ions present in the solution (Feng et al., 2016). Numerous electrodes have been used in the process of water treatment through electrochemical oxidation. Anode materials can be further divided into active electrodes and non-active electrodes.

The main active electrodes anodes used are titanium (Ti), ruthenium dioxide (RuO_2), iridium dioxide (IrO_2), commercially dimensionally stable electrode (DSA) formed by titanium metal with a thin layer of IrO_2 or RuO_2 (Miwa et al., 2006; Qu et al., 2012; Tavares et al., 2012; Wang, Kong, & Ma, 2007) and graphite (Ma, Wang, & Wang, 2007; Patel & Suresh, 2008; Wang, Gu, & Ma, 2007). On the contrary, non-active electrodes anodes are mainly produced by tin dioxide (SnO_2), lead dioxide (PbO_2) (Awad & Galwa, 2005; El-Ashtoukhy, Amin, & Abdelwahab, 2009; Flox et al., 2009; Hamza, Ammar, & Abdelhédi, 2011), commercial Ebonex ® electrode, constituted of magneli-phase (Ti_4O_7) and boron-doped diamond (BDD) {(Chen & Chen, 2006, 2011; Eric de Souza et al., 2020; Faouzi, Nasr, & Abdellatif, 2007; Kraft, Stadelmann, & Blaschke, 2003; Migliorini et al., 2011; Montilla et al., 2002; Panizza & Cerisola, 2005; Rodrigo et al., 2010).

There are numerous advantages and disadvantages associated with active and non-active electrodes anodes used in EC (**Table 1**). Lead and lead dioxide anodes are often utilized because of their low cost, stability, and elevated OEP, which slows O_2 generation in favour of Cl_2 generation (El-Ashtoukhy et al., 2009). The electrocatalytic activity of lead oxide electrode relies on the type and concentration of conductive electrolyte (Awad & Galwa, 2005) and its efficiency is enhanced when the electrode is doped with iron (Fe) (Jiang et al., 2014). Compared to conventional Ti/SnO_2-Sb/PbO_2, anodic oxidation by PbO_2 electrode shows a higher OEP and produces higher concentrations of hydroxyl radicals when degrading nitrobenzene (a carcinogenic pollutant) in a 0.1 M H_2SO_4 solution (Chen et al., 2014). Furthermore, the oxidation of textile effluents using Ti–Pt/β-PbO_2 electrode leads to higher chemical oxygen demand (COD) removal rates than DSA electrodes (Aquino et al., 2014). The higher COD removal rates are due to the generation of hydroxyl radicals, ozone and chlorine radicals generated from the reaction between chloride ions and hydroxyl radical (Pignatello, Oliveros, & MacKay, 2006). Iron (Fe) doping on PbO_2 electrode enhances OEP, which in turn favours the formation of Cl_2. Fe doped PbO_2 electrodes consisting of Ti/TiO_2 nanotube arrays electrodeposited by varying amounts of $Fe(NO_3)_3$ including 0.5M $Pb(NO_3)_2$ are efficient at generating oxidants electrochemically and have a great OEP (Jiang et al., 2014). While lead and lead dioxide electrodes are very good at generating oxidants in the presence of NaCl as electrolyte, in the presence of H_2SO_4 as the electrolyte, electrode poisoning may occur as a result of the formation of an adherent film on the surface of the anode

preventing further direct oxidation (Awad & Galwa, 2005). The use of these electrodes as anodes are also restricted due to the possible dissolution of Pb^{2+} ions which are toxic (Chen, 2004). Even with the potential presence of Pb^{2+} ions, these findings affirm that the Fe doped PbO_2 electrodes have the potential to improve electro-oxidation treatment and reduce operational costs.

Low-pressure conversion of carbon to crystals of diamond enable deposition a thin diamond layer film on appropriate substrates, including titanium, molybdenum, tungsten, niobium, and silicon (Chen & Chen, 2011). The production of active and stable BDD electrodes are facilitated using the hot filament chemical vapour deposition (HFCVD) method by means of titanium as substrate (Chen & Chen, 2006; Faouzi et al., 2007; Migliorini et al., 2011). Diamond-film conductors are a promising technology for electro-oxidation. They exhibit the highest OEP value (i.e. they generate the highest amount of hydroxyl radicals on the surface of the anode). Furthermore, diamond-film conductors have potential applications in non-aqueous media. Due to their inert surface, BDD electrodes are significantly corrosion resistant in the presence of strong acids. BDD electrodes have a far higher production rate of hydroxyl radicals, about 10 times greater than Ti/RuO_2 (Yoon, 2008). Ti/RuO_2 electrodes do not exhibit high hydroxyl radical production (Yoon, 2008). BDD electrodes have the ability to entirely degrade refractory organic pollutants without influencing the effectiveness of the process significantly (Rodrigo et al., 2010). In addition to the generation of hydroxyl radicals on the surface of the electrodes, diamond electrodes facilitate the so-called mediated-oxidation (whereby metal ions are oxidized to a higher and more reactive valence state on an anode) via other electrochemically formed compounds such as hypochlorite, perphosphate, or persulfate depending upon the electrolyte utilized (Särkkä, Bhatnagar, et al., 2015).

DSA (Ti electrodes with mixed metal oxide based coatings) are catalytic oxide electrodes that can efficiently produce active chlorine species due to their low OEP (Brillas, 2014). DSA materials include TiO_2-RuO_2 (Gusmão et al., 2010), SnO_2 (Watts et al., 2008), IrO_2-Sb_2O_5-SnO_2 (Fang, Shang, & Chen, 2006), IrO_2-RuO_2 (M. Bergmann & A. Koparal, 2005). The use of 3-dimensional electrodes ($Ti/Co/SnO_2$–Sb_2O_5) with a combination of activated carbon treatment are able to degrade organics in wastewater more efficiently due to the larger surface area (increased oxidant generation) compared to 2-dimensional electrodes (Wang, Kong, et al., 2007). Moreover, modifying the surface of MMO anodes by micro and nanostructures have shown to be more effective at degrading organic compounds and this is attributed to an increased surface area and thus higher rate of reactions (Wu, Huang, & Lim, 2014). While DSA electrodes such as TiO_2-RuO_2 (Gusmão et al., 2010) and IrO_2-RuO_2 (M. Bergmann & A. Koparal, 2005) are known to produce dominantly active chlorine species, BDD or titanium based electrodes that are used for Electrochemical Advanced Oxidation Processes (EAOP) are not so efficient for free chlorine production (Bergmann et al., 2008; Kraft, 2008). In addition to the oxidants generated

by electrochemical oxidation, EAOPs generate short-lived hydroxyl radicals which are powerful oxidants able to non-selectively destroy most organic and organometallic contaminants until their complete mineralisation into carbon-dioxide, water and inorganic ions (Sirés et al., 2014). However, the electrodes used in this study do not generate hydroxyl radicals and as such will focus primarily on electrochemical oxidation. Therefore, from a water treatment perspective, where active chlorine species are the main “desired” oxidants, DSA type electrodes with mixed metal oxide coatings are more applicable when it comes to the EC disinfection of natural waters that contain chloride salts.

Table 1 Advantages and disadvantages of the different active and non-active electrode anodes used in electrochemical disinfection (Eric de Souza et al., 2020; Särkkä, Bhatnagar, et al., 2015).

Anodes	Advantages	Disadvantages	Potential for O_2 evolution (V/standard hydrogen electrode)
Active electrode anodes			
Ti	Good stability	High cost	
RuO2	Good conductivity, good electrocatalytic activity	Low stability, May be corroded	1.4-1.7
IrO2	Good conductivity, Good electrocatalytic activity	Low stability	1.4-1.7
DSA (Dimensionally Stable Anode)	Good oxygen evolution potential, Good current efficiency, Low cost	Short life-span, Not electrochemically stable	1.4-1.8
Graphite	Low cost, Easy handling, Great applicability	Corrosion at high stresses	1.7
Non-active electrode anodes			
PbO_2	High oxygen evolution potential, Good current efficiency, Low cost, Easy handling	Toxic due to the possible formation of Pb^{2+} ions, corrosive	1.8-2.0
SnO_2	High electrical conductivity, High stability	Loses effectiveness after a short period of use	
Ebonex - Ti_4O_7	High oxygen evolution potential, Good current efficiency, Good conductivity, High chemical stability, Low cost, Easy handling,	Expensive	1.7-1.8
Boron-doped diamond (BDD)	High oxygen evolution potential, Good curent efficiency, Good conductivity, High chemical stability, Inert under extreme conditions	Very expensive, Can potentially form chlorate and perchlorate following oxidation of hypochlorite	2.2-2.6

1.2.3 Mechanisms of electrochemical microbial inactivation

Electrochemical eradication of yeast and bacterial cells have been reported in numerous studies (H. Bergmann & S. Koparal, 2005; Drees et al., 2003; Feng et al., 2004; Ghernaout et al., 2008; Ghernaout & Ghernaout, 2010; Ghernaout & Naceur, 2011; Ghernaout, Naceur, & Aouabed, 2011; Lopez-Galvez et al., 2012; Niepa, 2014). There are numerous mechanisms that describe the lethal effects of microorganisms following electrochemical exposure, such as oxidative stress and cell death due to the electrochemical oxidants generated, irreversible permeabilisation of the cell membranes due to the electric field applied, and electrochemical oxidation of vital cellular components when subjected to induced electric fields and electric current. However, the mechanism of EC killing of microorganisms is still not clear. Essentially, the physiological functions of bacteria depend on nucleic acids (DNA and RNA), cytoplasm and cell membrane. Hence, if any of these physiological aspects is compromised, it may lead to the bacterial cell death (Ghasemian et al., 2017). Disinfectants may deactivate bacteria through reactions with the cell membrane or reactions inside the cell (Huang et al., 2016). Predominantly, bacteria are inactivated following the diffusive transport of disinfectants into the cell whereby oxidants react with cell surface components leading to changes in cell membrane permeability and/or the disruption of enzymatic diffusion processes (Huang et al., 2016). Disinfectants can also inactivate bacteria by damaging the intracellular components of the cell, primarily resulting in the loss of DNA integrity (Huang et al., 2016). Furthermore, DNA damage can occur with or without distinct damage to cell surface components (Cho et al., 2010).

Electric fields (EFs) on their own can be lethal to cells due to permeabilisation of cell membranes (Weaver & Chizmadzhev, 1996). Studies carried out on artificial lipid bilayer membranes showed that when a membrane is subjected to an external EF, it accumulates charge, similar to a capacitor, and generates a transmembrane potential. When the membrane is completely charged, a short-lived-steady-state current is established, inducing the permeability of the membrane to hydrophilic molecules. This process is characterised by the formation of temporary pores in the membrane when subjected to the external EF. Two important factors affect the reversibility of this electro-permeabilisation: the amplitude of the induced transmembrane potential and the length of time exposed to the external EF. Generally, in cells, irreversible permeabilisation and cell death occur when the transmembrane potential is greater than 1 V and when the cells are exposed to longer pulse times. The transmembrane potential generated by an external EF relies on the size of the cell membrane. Larger cells experience a greater transmembrane potential from an applied EF. Consequently, the amplitude of EF required to deactivate yeast cells is usually less than that needed to deactivate bacteria (Gášková et al., 1996). Inactivation happens as a result of either the generation of permanent pores leading to the disruption of the cell membrane, or the loss of vital cell constituents (e.g. proteins)

and disruption of chemical gradients by diffusive transport across the temporary pores (Weaver & Chizmadzhev, 1996). In the presence of electrochemically generated oxidants, these pores can enable the oxidants to enter freely inside the cell, facilitating inactivation of the cells (Drees et al., 2003).

EFs are also able to inactivate cells without disrupting their membranes. The EF generated by applying a constant voltage of 0.7 V against a saturated calomel electrode in a carbon-cloth electrochemical reactor inactivates only *E. coli* K-12 cells that are attached to the electrode. The inactivation is due to the direct electrochemical oxidation of the intracellular coenzyme A, which dimerises by forming disulfide bonds, leading to a decrease in the rate of respiration and eventually cell death (Matsunaga et al., 1992).

Depending on the CT value and the type of bacterial cell, some disinfectants can have a more significant impact on cell membrane permeability than on intracellular components of the cells. *Enterococcus* and *E. coli* behave differently when exposed to low doses (< 2 mg/L as Cl_2) of electrochemically generated oxidants (Huang et al., 2016). Free chlorine doses of < 0.5 mg/L as Cl_2, mostly affect the cell membrane components of both bacteria (*Enterococcus* and *E. coli*). However, the differences lie in the disinfection kinetics, which are attributed to the variations in their cell surface structure (Gram-negative and Gram-positive) (Phe et al., 2005; Tree, Adams, & Lees, 2003). Chlorine doses ranging between 1.5 mg/L and 3 mg/L have a greater impact on nucleic acid integrity (Phe et al., 2005; Ramseier et al., 2011).

The generation of powerful intermediate chemical species like free radicals (chlorine, hydroxyl and atomic oxygen radicals) and other ROS which have higher oxidising potential than chlorine, can improve bacterial inactivation by EC disinfection (Diao et al., 2004). Scanning electron microscope (SEM) examination shows leakage of intracellular components from *E. coli* cells following EC disinfection of a model wastewater, containing 500 mg/L of NaCl, using DSA type of electrodes (titanium meshes coated with RuO_2, TiO_2 and ZrO_2) (Diao et al., 2004). The leakage observed following hydroxyl radical (Fenton reaction) treatment and EC treatment is similar but higher than cells treated with ozonation and chlorination using DSA type electrode (Diao et al., 2004). EC disinfection using BDD electrodes, showed changes in the cell walls and intracellular components of *E. coli* as a result of the actions of hydroxyl radicals generated (Jeong, Kim, & Yoon, 2006). Lipid peroxidation in the cell membranes of disinfected bacteria is attributed to the generation of ROS following EC treatment using a honeycombed platinum-coated titanium electrode to disinfect seawater (Tanaka et al., 2013).

EC disinfection using BDD anode in three different electrolytes: phosphate, sulfate and chloride showed different subcellular mechanisms of *E. coli* deactivation (Long, Ni, & Wang, 2015). EC disinfection of the phosphate solution shows that *E. coli* inactivation arise due to the mineralisation

of organic intracellular components of the cells. In the sulfate solution, some vital membrane proteins such as K^+ ion transport systems are destroyed leading to an excessive leakage of K^+ ions from the cytoplasm. The loss of K^+ ions disrupt the membrane potential of the cell, thereby hindering the subcellular localization of proteins and ATP synthesis associated with cell division, leading to the inactivation of the *E. coli* cells. In the chloride solution, damage to the intracellular enzymatic systems is responsible for cell inactivation.

While numerous studies have focused on the EC inactivation of bacteria and yeast, little research has been conducted on the EC inactivation of viruses. The knowledge of vital virus functions within the host is essential to understand and predict the inactivation mechanisms, which are poorly understood. The inactivation of virus is complicated by the fact that closely related viruses exhibit different disinfection kinetics when treated with the same disinfectant (Wigginton et al., 2012). For instance, Poliovirus 1 Mahoney is twice as sensitive to chlorine as the very similar Poliovirus 1 Bruhilde (Floyd, Sharp, & Johnson, 1979; Sharp & Leong, 1980). These varying disinfection kinetics suggest that even minor alterations in structural or genomic components can have a significant impact on viral resistance to inactivation (Wigginton et al., 2012).

The virucidal activity of electrochemical disinfection of drinking water (Drees et al., 2003; Julian, Trumble, & Schwab, 2014; Venczel et al., 2004) and wastewaters (Huang et al., 2016) is attributed to the direct electric field/current and electrochemically generated active chlorine species in the presence of a chloride-containing electrolyte. Bacteriophage MS2, a +RNA virus with an icosahedral, tailless capsid about 25-27 nm in diameter, is the most commonly used indicator for enteric viruses due to its similarities in shape, size and physicochemical properties and *in vitro* culturability to enteric pathogenic viruses (Abbaszadegan et al., 2007; Bae & Schwab, 2008). The electrochemical disinfection of a 150 mM KCl, 30 mM Tris at pH 8 using platinum electrodes showed the inactivation rate of *E. coli* to be 2.1 - 4.3 times higher than MS2 phage after the exposure of the cells to currents ranging from 25 to 350 mA in 5 s pulses (similar time period) (Drees et al., 2003). The free chlorine generated by an electrochemical cell using titanium electrodes (coated with Group VIII metal oxides) and a chloride containing solution at 25 ^{0}C and pH of 6, inactivated 4 log of MS2 phage (Venczel et al., 2004). The EC oxidation of a brine solution using the Smart Electrochlorinator 200 (SE-200) (Cascade Design, Inc., Seattle, WA, USA) inactivates MS2 and Mouse Norovirus (MNV-1) based on their infectivity and genomic RNA inactivation (Julian et al., 2014). The free chlorine generated (2500 mg/L as Cl_2) inactivates 7 log and 2 log of the infectivity of MS2 and MNV-1, respectively whereas genomic RNA inactivation (using a Taqman-based qRT-PCR assay to quantify viral inactivation) was about 5 log and 1.5 log respectively. The inactivation mechanism is attributed to the free chlorine generated which acts by denaturing capsid proteins and/or viral genomes (Julian et al., 2014). The EC disinfection of

laboratory water in the presence and absence of chloride and ammonium ions at a pH of 7.4-7.5, inactivates 5 log of MS2 phage using BiOx/TiO_2 semiconductor anode and stainless steel cathode (Huang et al., 2016). The free chlorine generated is the decisive factor controlling virus inactivation, whereby the genome-mediated replication and protein-mediated injection of viral functions are disrupted (Huang et al., 2016). Considering these observations and the fact that human enteric viruses such as rotaviruses and enteroviruses can be found in wastewater and contaminated drinking water sources and are more resistant to EC disinfection (Drees et al., 2003), current EC disinfection technologies intended to inactivate bacteria for drinking water treatment may not be adequate to inactivate all viruses.

1.3 Aquatic Natural Organic Matter

Aquatic natural organic matter (NOM) is an operational term used to define a complex mixture of water-borne organic materials that originate from living or dead organisms (microorganisms, vegetation and animals), including their waste and degradation products (Chow et al., 1999). NOM is present in lakes, rivers and ground waters due to the breakdown of terrestrial plants through soil leaching and the decomposition of water-borne organisms (Thurman, 2012). Furthermore, the quantity, character and properties of NOM in waters vary considerably depending on the geology, climate and topography (Franchi & O'Melia, 2003).

NOM is made up of a variety of organic compounds, ranging from aliphatic compounds to highly-coloured aromatic components. Some of this organic matter is negatively charged and vary in molecular sizes and chemical compositions (Świetlik et al., 2004; Thurman, 2012), NOM in waters can be broadly classified as hydrophilic or hydrophobic. Hydrophilic NOM is made up of a higher amount of aliphatic carbon and nitrogenous compounds (such as amino acids, proteins, carbohydrates and sugars). On the other hand, hydrophobic NOM contains a higher proportion of aromatic carbon, including phenolic structures and conjugated double bonds. Hydrophobic acids represent the major components of NOM and account for more than 50 % of the dissolved organic carbon (DOC) in water (Świetlik et al., 2004; Thurman, 2012)

DOC contains humic and non-humic fractions. The humic portion (i.e., hydrophobic fraction) makes up about half of the DOC present in natural waters and are described as organic compounds that consist of polyelectrolytic organic acid with varying molecular sizes, including in the macromolecular range (molecular weight ranging from 5000 to 10000 Da)(Owen, Amy, & Chowdhury, 1993). The non-humic part is classed as known bio-molecular groups of compounds of lower molecular weight fulvic acids with higher proportions of N-containing organics and carbohydrates (McDonald et al., 2004). Based on their chemical characteristics and significant impacts for water treatment processes, aquatic

humic acids and fulvic acids are the two major constituents of aquatic carbon in water. As such, in this research, the term NOM refers to both fulvic and humic acids. Even though a lot of studies have been carried out on aquatic humic and fulvic acids, their chemical structures are still not fully identified. It is well known that the main functional groups are aromatic and aliphatic carboxylic groups, phenols, ketones and alcohols (Leenheer, 2004; Schulten & Leinweber, 1996).

The presence of NOM in natural waters is a major concern for drinking water quality and drinking water treatment processes (Jacangelo et al., 1995; Murray & Parsons, 2004). The colour and off-tastes and odours of waters containing high levels of NOM directly impact water aesthetics. In the presence of high concentrations of NOM, water treatment processes use high doses of disinfectant (to reach appropriate CT) and coagulant which, in turn, result in increased formation of noxious by-products and sludge (Jacangelo et al., 1995; Murray & Parsons, 2004; Semmens & Field, 1980). Furthermore, the presence of NOM in treated drinking waters promotes microbiological growth in distribution systems (Jegatheesan et al., 2004). Additionally, NOM can result in an increase in concentrations of organic pollutant (e.g. phenolic compounds) and heavy metals (e.g. copper (II), zinc and lead) in raw water sources as a result of metal-NOM complexation and adsorption (Cabaniss, 2009; Liu, Zhang, & Talley, 2007; Schmitt et al., 2003). Therefore, it is crucial to understand and predict the reactivity of NOM or its composition throughout different stages of water treatment.

Techniques to efficiently remove NOM vary according to the composition and quantity of NOM in the source water. Due to the high variability of NOM, no single method can treat NOM (Särkkä, Vepsäläinen, & Sillanpää, 2015). Commonly and economically practical processes available to remove NOM are coagulation and flocculation followed by flotation/sedimentation and filtration. Additional options for NOM removal involve activated carbon filtration, magnetic ion exchange resin (MIEX®) and membrane filtration methods (Särkkä, Vepsäläinen, et al., 2015).

Advanced oxidation processes, in particular electrochemical technologies for water purification and disinfection, such as electro-coagulation, electro-flotation and electro-oxidation, are of increasing interest in recent years (Chen, 2004; Mollah et al., 2004). Traditional water purification processes such as biological treatment, chemical coagulation or UV oxidation cannot effectively remove some toxic and refractory organic pollutants. Electrochemical processes are emerging as efficient means of treating NOM as they are inexpensive, innovative, simple to operate (in situ generation of oxidants either directly at the surface of the electrode or indirectly from chemicals in the treated water) and efficient at degrading many harmful pollutants (e.g. hydrophobic and hydrophilic colloids) from wastewaters before discharge into water networks or circulation back into processes (Chen, 2004; Holt, Barton, & Mitchell, 2005; Mollah et al., 2004; Mollah et al., 2001; Vepsäläinen et al., 2009).

1.3.1 Electro-oxidation of NOM

The concept behind electrochemical processes is to utilise the redox reactions occurring at both the anode (oxidation of organics/pollutants) and cathode (reduction of heavy metals) to remove pollutants, and has been mainly used for the remediation of heavy metals (e.g. Nickel) from industrial wastes (Coman, Robotin, & Ilea, 2013; Nancharaiah, Mohan, & Lens, 2015). However, in the last decade, the use of electro-chemical processes have been able to remove organic pollutants either by partial degradation or complete mineralisation by electro-oxidation at the anode (Garcia-Segura, Ocon, & Chong, 2018). As such, the electrocatalytic characteristics of the anode material play a vital role in organic removal efficiency of the electrochemical processes. However, electro-oxidation removal of organics also depend on the composition of the electrolyte and the experimental conditions (e.g. current density) (Panizza & Cerisola, 2009). Furthermore, the electro-oxidation of organic compounds can occur by two different mechanisms, direct oxidation and indirect oxidation, as previously described in section 1.2.1 (Brillas & Martínez-Huitle, 2015; Garcia-Segura & Brillas, 2011; Panizza & Cerisola, 2009).

1.3.1.1 *Direct oxidation of organics*

Direct oxidation involves direct charge transfer reactions between the anode surface and the organic compounds (**Figure 1**). The mechanism only relies on the mediation of electrons, capable of oxidising the organic compounds at specific potentials more negative than OEP (Garcia-Segura et al., 2018; Panizza & Cerisola, 2009). Generally, the direct oxidation requires prior adsorption of organics onto the surface of the anode, making the process rate-limiting and does not lead to the complete degradation of organics (Panizza & Cerisola, 2009). The voltages required for oxidation of organics are usually high (Rodgers, Jedral, & Bunce, 1999; Rodrigo et al., 2001). This means water can be oxidised and oxygen is generated as the main side reaction. In this case the reaction is not favourable for the oxidation of organics and significantly affects the efficiency of degradation of organics. Theoretically, direct oxidation is only feasible at potentials lower than OEP. However, at these potentials, the reaction rate has low kinetics depending on the electrocatalytic activity of the anode material. The decrease in the catalytic activity of the anode is known as the poisoning effect. The poisoning effect happens when a polymer layer forms on the surface of the anode and further inhibits the electro-oxidation process. This deactivation due to the poisoning effect relies on the adsorption characteristics of the anode, nature and concentration of the organic compound being oxidised. Moreover, the deactivation can be accentuated in the presence of aromatic substrates like pyridine, chlorophenols, phenol and napthol (Rodgers et al., 1999; Rodrigo et al., 2001). An example of direct oxidation is the electrochemical combustion of phenol (Panizza & Cerisola, 2009). Phenol is completely oxidised to carbon dioxide at the platinum anode by the electrochemical transfer of oxygen from water to phenol

at pH 6 (Equation **16**). The hydrogen ions formed at the anode is then reduced at the cathode to form hydrogen gas (Equation **17**)(Panizza & Cerisola, 2009).

$C_6H_{12}O_5OH + 11H_2O \rightarrow 6CO_2 + 28H^+ + 28e^-$ (Equation 16)

$28H^+ + 28e^- \rightarrow 14H_2$ (Equation 17)

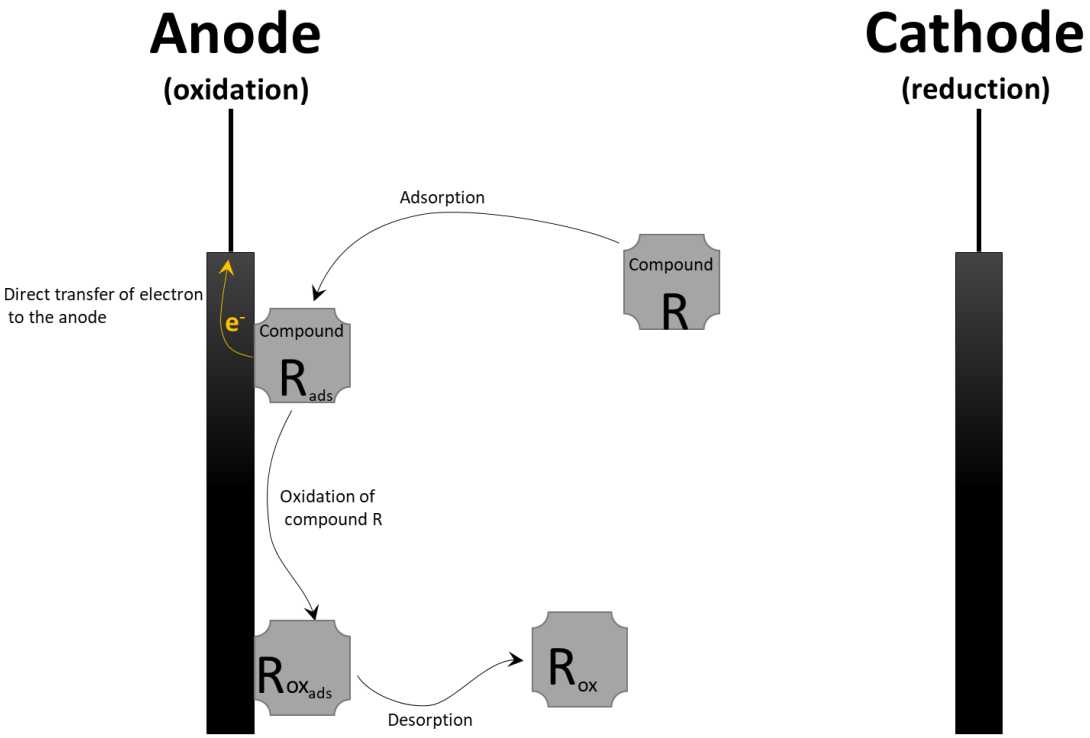


Figure 1 Direct electro-oxidation of organics at the anode surface. (R: the organic compound, Rads: adsorbed organic compound, Rox_{ads}: adsorbed oxidised organic compound, Rox: oxidised organic compound). Adapted from Garcia-Segura, Ocon [141].

1.3.1.2 *Indirect oxidation of organics*

Indirect electro-oxidation of organics occurs when mediators in the form of highly oxidative species such as active chlorine species (dissolved Cl_2, HOCl and OCl^-), ROS (hydroxyl radicals, hydrogen peroxide and ozone) are generated in situ at the surface of the anode (see section 1.2.1 for the reaction mechanisms).

1.3.1.2.1 Indirect oxidation by reactive oxygen species

ROS formation during indirect oxidation depends on the electro-generation of adsorbed hydroxyl radicals (E^o = 2.8 V/SHE) onto the surface of the anode as an intermediate of the OEP (Cavalcanti et al., 2013; Martínez-Huitle & Ferro, 2006; Panizza & Cerisola, 2009) (Equation**18**). As previously mentioned in section 1.2.2, electrodes with high OEP are better at generating hydroxyl radicals. These electrodes can be further sub-divided into active and non-active electrodes based on the enthalpy of adsorption of the hydroxyl radicals on the surface of the anode (Kapałka, Fóti, & Comninellis, 2008).

$$\mathbf{M + H_2O \rightarrow M(^{\bullet}OH) + H^+ + e^- \ (Equation\ 18)},$$

where M is the anode and M(˙OH) is the adsorbed hydroxyl radical on the anode surface.

Active anodes (**Figure 2a**) electrochemically convert organics into more biodegradable molecules, for instance short-chain carboxylic acids. However, they are not able to completely mineralise organics into carbon dioxide (Cavalcanti et al., 2013; Comninellis & Pulgarin, 1991; Scialdone, Galia, & Filardo, 2008). This is because the metal or metal oxides of the anodes have oxidation states that are higher than the standard potential for OEP (E° = 1.23 V/SHE), resulting in the favoured formation of higher oxide oxidants at the anode surface by the chemisorption of hydroxyl radicals. The organic compound is then oxidised by the higher oxide oxidants (Garcia-Segura et al., 2018). Some of the active anode materials are DSA of ruthenium (IV) oxide (RuO_2) (Chanworrawoot & Hunsom, 2012; Santos, Dezotti, & Dutra, 2013), platinum (Cavalcanti et al., 2013; Hammami et al., 2009; Sala & Gutiérrez-Bouzán, 2014) and iridium (IV) oxide (IrO_2)(Fóti et al., 1999; Rajkumar & Palanivelu, 2004).

In the case of non-active anodes **(Figure 2b**), the electro-generated hydroxyl radicals (Equation **18**) remain physisorbed on the surface of the anode (Kapałka, Fóti, & Comninellis, 2010). Due to their higher oxidant power, major lability and reactivity, the physisorbed hydroxyl radicals can completely degrade organic compounds into carbon-dioxide (Garcia-Segura et al., 2018). Some of the non-active anode materials are boron-doped diamond (Cavalcanti et al., 2013; Gargouri et al., 2014; Rodrigo et al., 2001), tin (IV) oxide (SnO_2) (Comninellis & Pulgarin, 1993; Martínez-Huitle et al., 2008) and lead (IV) oxide (PbO_2) (Aquino et al., 2014; Gargouri et al., 2014; Martínez-Huitle et al., 2008).

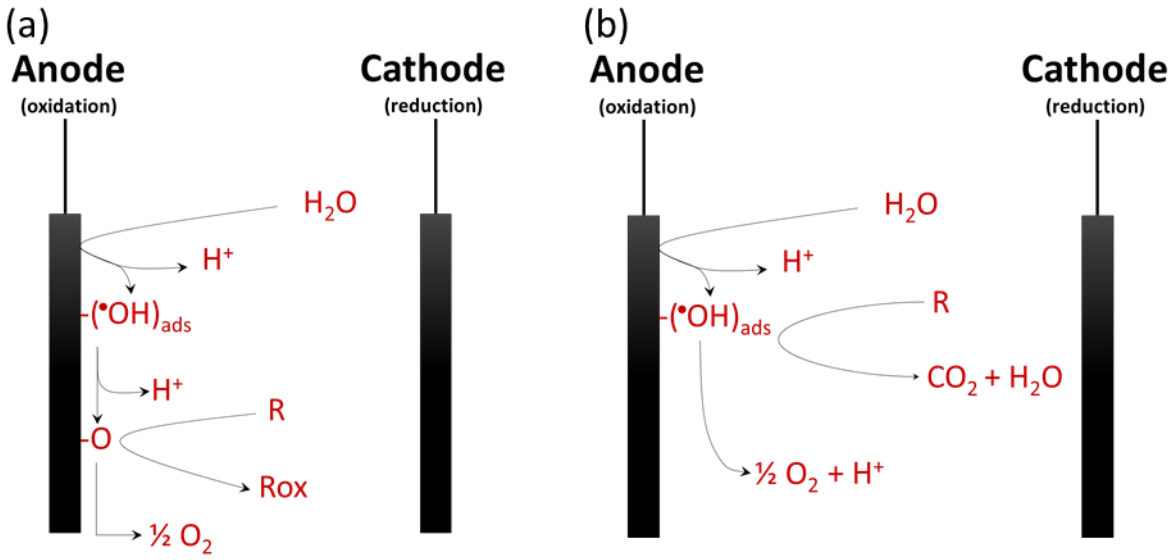


Figure 2 Indirect electro-oxidation of organics in the presence of (a) active anodes and (b) non-active anodes. [(·OH)ads: adsorbed hydroxyl radicals, R: organic compound, Rox: oxidised organic compound]. Adapted from Garcia-Segura, Ocon [141].

1.3.1.2.2 Indirect oxidation by active chlorine species

Organic compounds can also be oxidised indirectly by the *in situ* generation of active chlorine species (dissolved Cl_2, HOCl and OCl^-) from the electrochemical oxidation of a chloride containing solution as shown in **Figure 3** (refer to section **1.2.1** for reaction mechanism of active chlorine species formation).

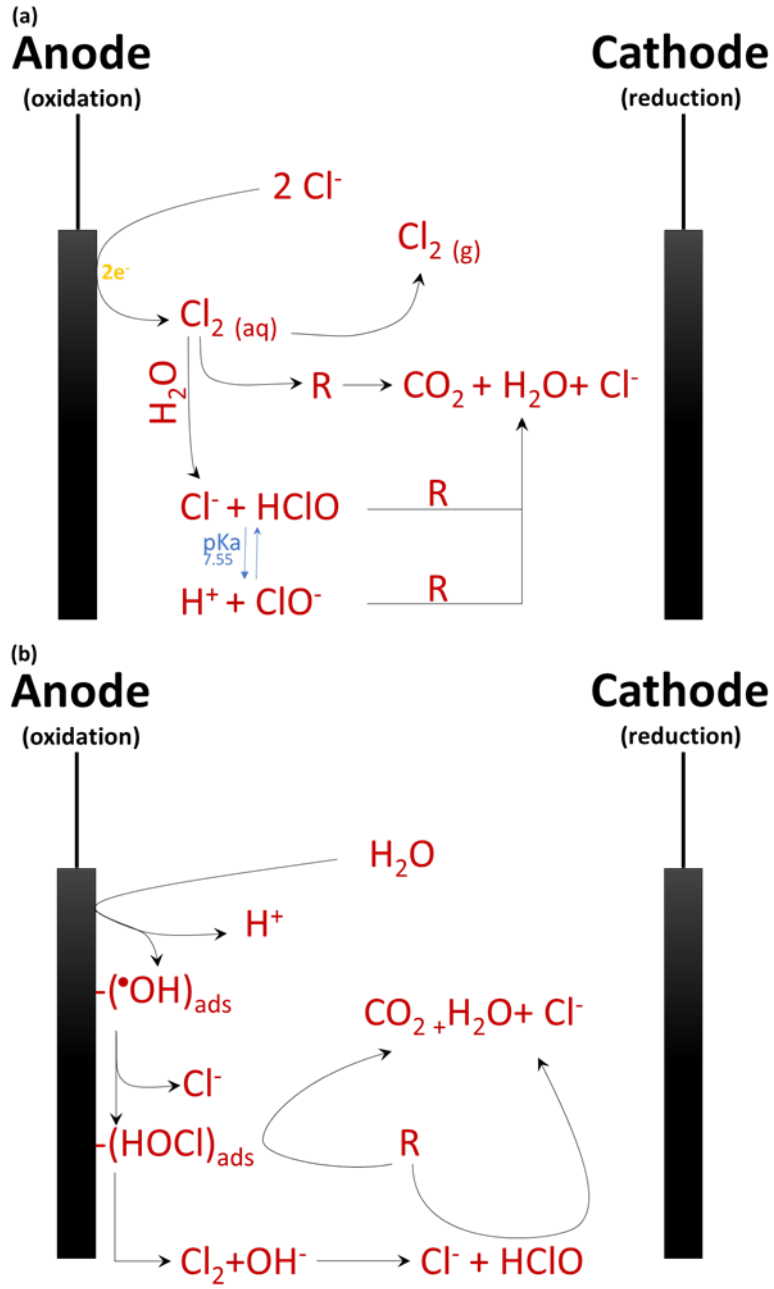


Figure 3 Indirect oxidation of organics by active chlorine species via (a) direct chloride oxidation and (b) chloride oxidation mediated by reactive oxygen species. (R: organics, $(^{\cdot}OH)_{ads}$: adsorbed hydroxyl radical, $(HOCl)_{ads}$: adsorbed hypochlorous acid). Adapted from *Garcia-Segura et al. (2018)***.**

The pH of the oxidants generated by the indirect oxidation of chloride ions in generating active chlorine species plays an important role. The standard reduction potential of aqueous Cl_2 (E^o = 1.36 V/SHE) and hypochlorous acid (HOCl) (E^o = 1.49 V/SHE) are much higher than hypochlorite ion (OCl^-) (E^o = 0.89 V/SHE). Therefore, under acidic pH conditions, the rate of organics oxidation is higher when mediated by active chlorine species (De Moura et al., 2014; Deborde & von Gunten, 2008).

Depending on the type of electrode used, ROS can act as mediators to generate active chlorine species from a chloride containing solution as previously described in section 1.2.1 (Bonfatti et al., 2000; Neodo et al., 2012; Rosestolato et al., 2014). This is possible due to the oxygen transfer reactions between the adsorbed oxychlorinated species produced from equation 19 as intermediates for chlorine production (**Equation 20**), as shown in **Figure 3b**. However, the oxidation of organics through this process is slower compared to the oxidation by homogenous active chlorine species.

$$M(OH) + Cl^- \rightarrow M(HOCl) \quad \textbf{(Equation 19)}$$

$$M(HOCl) \rightarrow {}^1/_2Cl_2 + OH^- \quad \textbf{(Equation 20)}$$

The anode materials (active and non-active) used for the indirect oxidation of organics by active chlorine species are similar to those used for the indirect oxidation by ROS. However, as discussed in section 1.2.2, active anodes (IrO_2, TiO_2, RuO_2 and Pt) have better electrocatalytic characteristics to generate active chlorine species than non-active anodes and non-active anodes (e.g. BDD) generate unwanted non-oxidising chlorine species (perchlorate) due to the further oxidation of dissolved Cl_2 and $HOCl/OCl^-$ (Ferro et al., 2000; Garcia-Segura et al., 2015).

The indirect oxidation/degradation of organics facilitated by active chlorine species has been of great industrial interest since chloride ions are omnipresent in industrial effluents and mainstream water bodies. While electro-generated active chlorine species can degrade organics, they can also generate undesirable organo-chlorinated/brominated by-products (such as halomethanes and haloacetic acids) and toxic ionic species (such as chlorate and perchlorate) (Bergmann, Rollin, & Iourtchouk, 2009; Ghernaout et al., 2011; Plewa et al., 2010; Radjenovic & Sedlak, 2015). Therefore, it is important to define a threshold for chlorine concentration when it comes to organic removal with a lesser formation of toxic by-products and this can be one of the biggest challenges of electro-oxidation technologies before scaling up to pilot and industrial scale implementation (Radjenovic & Sedlak, 2015).

1.4 Disinfection by-products formation

Generation of DBPs is a major problem associated with chlorination (both EC and sodium hypochlorite injection) for the treatment of drinking water. Chemical disinfectants (such as chlorine, chlorine dioxide, ozone and chloramines) are known to generate different types of DBPs. Commonly found DBP precursors found in naturally occurring water sources are NOM, bromide and iodide. Bromide is usually detected in the range of 20 to 2000 µg/L while iodide is found at much lower concentrations, generally varying between 0.4 and 100 µg/L worldwide, in natural waters (Agus, Voutchkov, & Sedlak, 2009). In Western Australia, the bromide levels in source waters range from 400 to 8400 µg/L. The

presence of such high bromide concentrations might be due to the prevalence of saline soils (Gruchlik et al., 2014). Bromide and iodide found in source waters can be rapidly oxidised by oxidants such as chlorine to generate hypobromous acid (HOBr) and hypoiodous acid (HOI), respectively, as shown in equations 21 and 22 (Kumar & Margerum, 1987; Nagy, Kumar, & Margerum, 1988).

$$Br^- + HOCl \rightarrow HOBr + Cl^- \quad k = (1.55 – 6.84) \times 10^3 \text{ M/s (Equation 21)}$$

$$I^- + HOCl \rightarrow HOI + Cl^- \quad k = 4.3 \times 10^8 \text{ M/s (Equation 22)}$$

HOBr and HOI are active oxidants that can then react with NOM to form brominated and iodinated DBPs, in a similar way to chlorine (hypochlorous acid, HOCl) generating chlorinated DBPs. The amount of DBP formation relies on the type and dose of disinfectant, the nature and concentration of precursors, the contact time of the disinfectant with the precursor and several other water quality characteristics (e.g. pH and temperature) (Hong et al., 2013). Halogenated DBPS that can be identified individually include trihalomethanes (THMs) and haloacetic acids (HAAs), the two most abundant types of DBPs, as well as haloketones (HKs), haloacetonitriles (HANs), haloacetamides, halonitromethanes, chloral hydrate and halopyrroles (Chowdhury, Champagne, & McLellan, 2009; Croué, Violleau, & Labouyrie, 2000; Harrington et al., 1996; Richardson et al., 2007; Wu et al., 2000). The DPB formation potential can be measured by a group parameter, Total Organic Halogen (TOX) that is widely used as an estimate of the total amount of halogenated organics in water (Dressman & Stevens, 1983). Since the discovery of DBPs (Bellar, Lichtenberg, & Kroner, 1974; Rook & JJ, 1974), more than 600 DBPs have been identified (Richardson & Postigo, 2015). However, this accounts for only a fraction (not more than 50 %) of the TOX formed in disinfected drinking water, while the other 50 % of the halogenated organic material contributing to TOX still need to be identified (Li et al., 2011; Singer, 1994).

The components of NOM of interest in water treatment are humic and fulvic acids, as previously mentioned in section 1.3. The reactivity of these substances with chlorine has been studied (Reckhow, 1990). The major disinfection by-products that have been identified as a result of reacting NOM with chlorine are THMs, HAAs, HKs and HANs. Furthermore, when natural waters are treated with free chlorine, THMs and HAAs combined account for about 50 % of the TOX (Reckhow & Singer, 1984). Even though their contribution to the bulk water is low, humic acids consume a greater concentration of chlorine, and at the same time produces more TOX, THMs, haloacetic acids and haloacetonitriles than the equivalent quantity of fulvic acids on a carbon molecule basis (Reckhow, 1990).

DBPs can be cytotoxic, mutagenic, genotoxic, neurotoxic, carcinogenic and teratogenic towards mammalian cells (Richardson et al., 2007). Individuals who consume chlorinated drinking water have

a slightly increased risk of having adverse health effects (Richardson et al., 2007) including cancer of the rectum (Bove, Rogerson, & Vena, 2007b), colon (King, Marrett, & Woolcott, 2000; Rahman et al., 2010) and bladder (Bove, Rogerson, & Vena, 2007a; Costet et al., 2011; Villanueva et al., 2004), primarily due to the exposure to DBPs in the drinking water (Wagner & Plewa, 2017). These qualities are clearly undesirable in human drinking water. The AMES test for mutagenicity *Salmonella typhimurium* classified the DBPs hydroxy-2-furanone, bromoacetic acid, bromoform, dibromoacetic acid, tribromoacetic acid and chloroform as the most cytotoxic (in descending order) and all but chloroform are mutagenic (McMillan, 2000). Furthermore, cytotoxicity and genotoxicity analyses of DBPs on a single Chinese Hamster Ovary (CHO) cell line (AS52) indicate that brominated-DBPS are more toxic than their chlorinated analogues and nitrogenous-DBPs and iodinated DBPs are even more toxic than their brominated analogues as well as the DBPs that are regulated by the US Environmental Protection Agency (USEPA) and listed by World Health Organisations (WHO) (Wagner & Plewa, 2017).

During the disinfection of bromide-containing water with chlorine, hypobromous acid (HOBr) and hypobromite (OBr^-) are formed, as shown by equation 23.

$$\mathbf{Br^- + ClO^- \rightarrow BrO^- + Cl^-} \qquad \mathbf{k = 9 \times 10\text{-}4\ M/s} \quad \textbf{(Equation 23)}$$

Aqueous bromine, constituting of HOBr and OBr^-, is known to be a better substituting agent and more reactive (an order magnitude faster with an initial reaction rates estimated as 500 – 5000 M/s) than chlorine (HOCl and OCl^-) (Bond et al., 2014; Heeb et al., 2014) when reacting with NOM to generate brominated DBPs via electrophilic substitution and addition (Criquet et al., 2015). As such, brominated DBPs will be the dominant species compared to chlorinated DBPs when disinfecting bromide-containing waters with chlorine. Furthermore, bromine can oxidise NOM and release Br^- via electron transfer. Once released, the bromide may create a recycling effect leading to high formation of brominated DBPs where a great proportion of the free bromide is eventually integrated into organic brominated compounds. In the presence of excess chlorine, the reduced bromine (measured as bromide) is again oxidised to HOBr or OBr^-. HOBr or OBr^- then reacts further with remaining NOM in waters to generate more brominated DBPs (Heeb et al., 2014). The role of bromide ions in the oxidation processes in water treatment is shown in **Figure 4**.

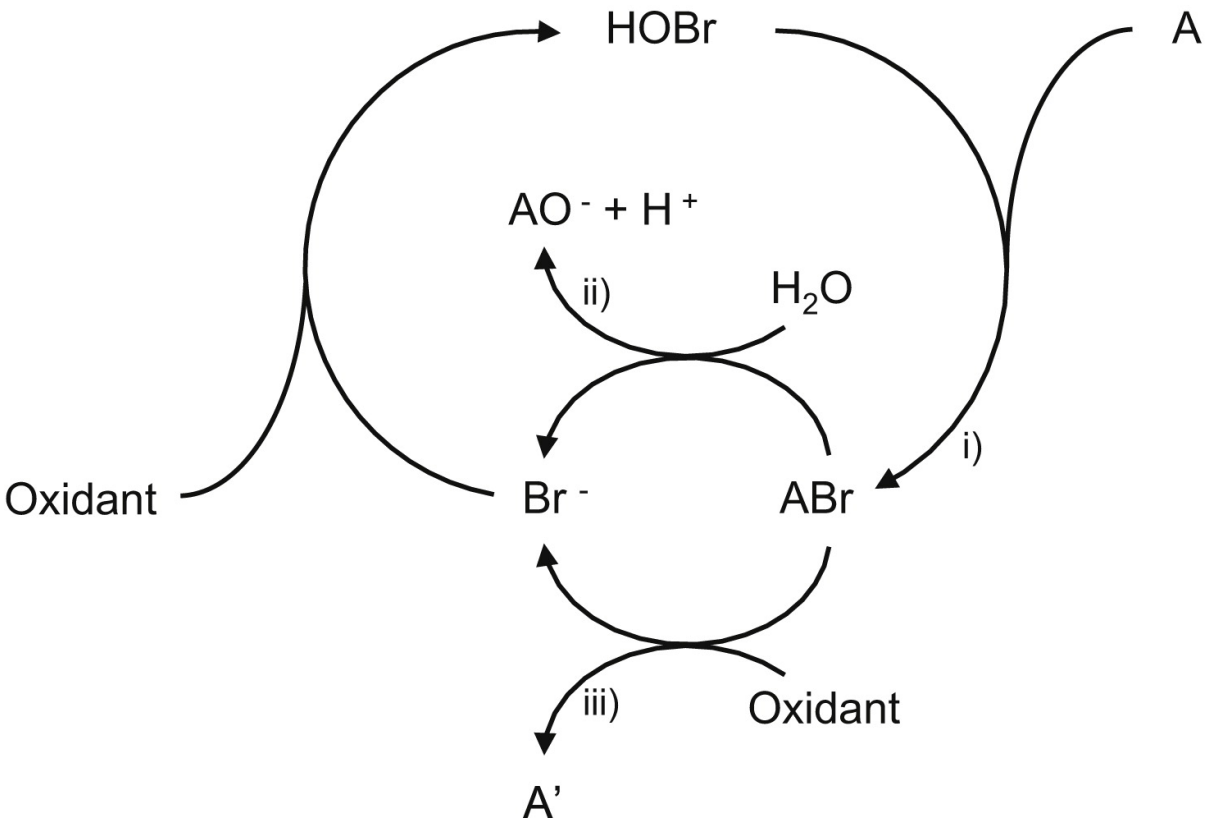


Figure 4 Role of bromide (Br^-) in oxidative water treatment. Br^- is oxidised to bromine (HOBr for simplicity), which reacts with organic or inorganic reactants (A), to generate (i) brominated compounds (ABr), (ii) AO^- via hydrolysis of ABr, or (iii) A, after further oxidation of ABr. Adapted from Heeb et al. (2014)**.**

During the electrochlorination process, halogenated ions generated react with inorganic matter in the water to form inorganic by-products. Such inorganic by-products include chlorite, chlorate and bromate. Chlorite and bromate are on the World Health Organisations control list, with guideline maximum concentration limits of 0.7 mg/L and 0.01 mg/L, respectively. The Australian National drinking water guidelines give a limit value of 0.8 mg/L for chlorite and 0.02 mg/L for bromate (NHMRC, 2011). The same guidelines (NHMRC, 2011) do not contain a concentration limit for chlorate and instead recommend adhering to the guideline set by the World health organisation (Richardson et al., 2007) (**Table 2**). Chlorate is currently on the monitoring list of unregulated contaminants from the US Environmental Protection Agency (USEPA). It was not included in the initial guidelines for monitoring DPBs because at the time there was insufficient data on the health effects of the compound (USEPA, 1999).

The presence of chlorate and perchlorate in water can lead to serious health issues for humans as a result of their harmful effects (Srinivasan & Sorial, 2009; Urbansky & Schock, 1999). Chlorate is a known neurotoxin that causes haemolytic anaemia by destroying red blood cells (Siddiqui, 1996). Perchlorate can cause thyroid issues by disrupting the production of hormones that are required for normal growth and development (Alfredo, 2015). As a result of these unwanted side effects of chlorate and perchlorate, the World Health Organisation has set a guideline of 700 µg/L as the threshold for chlorate; however, several countries have standards higher than this (Canada 1000 µg/L, New Zealand 800 µg/L). USEPA is looking to establish a guideline of 210 µg/L for chlorate depending on the challenges water utilities face. (Alfredo, 2015)

The formation of chlorate and perchlorate depend on the type of oxidants generated during electrochemical oxidation which in turn rely on multiple key parameters (electrode material, temperature and pH of the solution, composition of the electrolyte and the applied current) as described in section **1.1.5**. During electrolysis, chlorate and perchlorate can be formed by the following reactions (Yoon et al., 2015):

Chlorate (ClO_3^-)

$6HOCl + 3H_2O \rightarrow 2ClO_3^- + 4Cl^- + 12H^+ + 3/2O_2 + 6e^-$ (Equation 24)

$6OCl^- + 3H_2O \rightarrow 2ClO_3^- + 4Cl^- + 6H^+ + 3/2O_2 + 6e^-$ (Equation 25)

$Cl^- + 4OH^- \leftrightarrows ClO_2^- + 2H_2O + 4e^-$ (Equation 26)

$ClO_2^- + 2OH^- \leftrightarrows ClO_3^- + H_2O + 2e^-$ (Equation 27)

$O_3 + ClO_2^- \rightarrow ClO_3^- + O_2$ (Equation 28)

$OH^\cdot + ClO_2^- \rightarrow ClO_2^\cdot + OH^-$ (Equation 29)

$OH^\cdot + ClO_2^\cdot \rightarrow ClO_3^- + H^+$ (Equation 30)

Perchlorate (ClO_4^-)

$ClO_3^- + H_2O \leftrightarrows ClO_4^- + 2H^+ + 2e^-$ (Equation 31)

$H_2O \rightarrow (OH^\cdot)_{ad} + H^+ + e^-$ (Equation 32)

$ClO_3^- + (OH^\cdot)_{ad} \rightarrow ClO_4^- + H^+ + e^-$ (Equation 33)

$ClO_3^- + OH^\cdot \rightarrow ClO_4^- + H^+ + e^-$ (Equation 34)

Table 2 World Health Organisation disinfection by-product contaminant guidelines

Disinfection by-product	Guideline value (mg/L)
Bromate	0.01
Bromodichloromethane	0.06
Bromoform	0.10
Chlorite	0.70
Chlorodibromomethane	0.10
Chloroform	0.20
Cyanogen chloride	0.07
Dibromoacetonitrile	0.07
Dichloroacetic acid	0.05
Dichloroacetonitrile	0.02
Formaldehyde	0.90
Trichloroacetaldehyde	0.01
Trichloroacetic acid	0.20
2,4,6-Trichlorophenol	0.20

1.5 Assessment of water quality

Water harbors indigenous waterborne bacteria and foreign bacteria such as opportunistic pathogens originating either from an animal source or from faecal contamination from humans (Cabral, 2010) (Pavlov et al., 2004). Hence, it is important to implement national and international regulatory standards to provide the public with safe drinking water. These include the Water Directive (USEPA, 2009) (USA), the European Council Directive 98/83/EC on the quality of water intended for human consumption (Commission, 1998), the WHO guidelines (WHO, 2017), and in Australia, the Australian Drinking Water Guidelines (NHMRC, 2011).

In order to detect these waterborne pathogens at limited cost, faecal indicator bacteria (FIB) are used as surrogate for pathogenic bacteria in environmental samples such as soil and water and therefore should act in the same way as the pathogens. The term FIB describes the range of bacteria that inhabit the gastrointestinal tract of homeothermic animals and includes *Escherichia coli* and the faecal coliforms, *Enterococcus* spp., all of which are permanently excreted in faecal material (Rochelle-Newall et al., 2015). Ideally, indicator bacteria should be present in the intestinal tract of the same animal as the pathogens; should be present only in contaminated samples and not in uncontaminated ones; should have similar survival patterns as pathogens outside the host; should not be able to grow and proliferate in the environment; should be easily detectable; be of low risk to the person conducting the analyses and, ideally, should be relatively cheap to use (Ferguson & Signoretto, 2011; Ishii & Sadowsky, 2008). Assessment of water quality commonly involves monitoring the microbiological activity of faecal indicators such as *E. coli*, coliforms, *Enterococcus spp.*, and opportunistic pathogen such as *Mycobacterium* species (non-tuberculous mycobacteria being one of the major genus), *Legionella pneumophila* and *Pseudomonas aeruginosa* by analysing the heterotrophic plate count (Gensberger et al., 2015; Perrin et al., 2019).

1.5.1 Heterotrophic Plate Count (HPC)

A common method used to measure microbial contamination of water is to count heterotrophic culturable bacteria on a non-selective solid medium under specific incubation time and temperature (Allen, Edberg, & Reasoner, 2004). Heterotrophic plate count (HPC) is also often referred to as "colony-forming units", "standard plate count", "total viable count" and "total bacterial count". The use of HPC as a water quality factor was first proposed by Robert Koch in 1883 (Bartram et al., 2004). During the last century, the application of HPC for the routine monitoring of the microbiological quality of drinking water has undergone numerous changes to enable detection of the highest proportion of bacteria in a particular sample (Reasoner, 2004). For instance, different agar-based media such as R2A agar, yeast extract agar and plate count agar are used. The incubation temperatures vary from 20 ^{o}C to 40 ^{o}C. Moreover, the incubation times can vary from hours to weeks, depending on the standard drinking water legislation and guidelines of different countries (Allen et al., 2004; Bridgewater et al., 2012; WHO, 2017). The Australian Drinking Water Guidelines recommends the use of either yeast extract agar at 20 – 22 ^{o}C for 72 – 120 hours or R2A agar at 35 – 37 ^{o}C for 24 – 48 hours (NHMRC, 2011). The most commonly used method of HPC determinations include the pour-plate technique, spread plate method and membrane filtration technique (Sartory, Gu, & Chen, 2008). HPC results can differ depending on the type of media, incubation time and temperature applied. Despite this, HPC is still considered the standard method for viability testing of drinking water by authorities. (Van Nevel et al., 2017).

One of the main advantages of HPC is that a positive result (colony formation) is an undeniable characteristic of bacterial viability (Hammes, Berney, & Egli, 2011). HPC is also relatively inexpensive, uncomplicated technique implemented for over a century. HPC counts also provide a reference from which to gauge the efficacy of alternative water treatment regimens (Allen et al., 2004; Chowdhury, 2012; Douterelo et al., 2014; Reasoner, 1990; Sartory, 2004). Applications include evaluating the efficacy of chlorine as a disinfectant (LeChevallier et al., 1984), investigating microbiological changes due to the biological instability and regrowth of drinking water (Francisque et al., 2009; Prest et al., 2016; Uhl & Schaule, 2004), analysing microbiological activity in biofiltration systems (Camper et al., 1986), enumerating the number of bacteria grown in batch during stagnation of unfiltered (Uhl & Schaule, 2004), nano-filtered drinking water (Liikanen, Miettinen, & Laukkanen, 2003) and in overnight household plumbing (Lautenschlager et al., 2010; Pepper et al., 2004).

HPC is however labour intensive and (Van Nevel et al., 2017) can take 2 to 10 days to produce results. This is not convenient when operators are faced with drinking-water quality issues and need to take rapid action. Furthermore, routine HPC data of drinking water are not a true representation of the diversity and abundance of bacteria in the water (Van Nevel et al., 2017). Amann (1911); Winterberg (1898) were among the first microbiologists to report that the number of colonies on solid agar plates represent only a small fraction of the actual number of bacteria in a given water sample using direct microscopy. HPC yields only about 0.01 % (Bartram et al., 2004), 0.0001 – 6.5 % (Hammes et al., 2008) and 0.05 – 8.3 % (Burtscher et al., 2009) of the actual total cell numbers depending on the source of the water sample, the type of HPC and total cell count methods applied. Numerous studies have investigated the “unseen majority” of bacterial cells undetected by HPC methods but observed by culture-independent techniques such as next generation sequencing, flow cytometry and microscopy (Bogosian & Bourneuf, 2001; Epstein, 2013; Green & Keller, 2006; Kell et al., 1998; Oliver, 2010). Apart from the dead or lethally damaged bacteria, the general consensus is that the “unseen majority” consist of two main groups; bacterial strains which enter into the viable but non-culturable (VBNC) state (HPC results are false negatives) and bacteria which are not culturable by HPC techniques (Van Nevel et al., 2017).

Culture-dependent techniques for bacterial isolation clearly underestimate the microbiome complexity in drinking water (Bautista-de los Santos et al., 2016; Proctor & Hammes, 2015). The use of 16S rRNA gene amplification shows a complete difference in the bacterial community of HPC isolates and the total bacterial population (Burtscher et al., 2009; Farnleitner et al., 2004). The 16S rRNA gene profiles following HPC isolation are completely different from the isolates obtained directly from the water samples (Burtscher et al., 2009). Furthermore, the HPC isolated community consists

primarily of copiotrophic bacteria and the drinking water community consists primarily of oligotrophic marine bacteria (Burtscher et al., 2009).

1.5.2 Flow cytometry

Flow cytometry (FC) is emerging as a valuable alternative to the traditional use of HPC for microbial water quality assessment (Hammes et al., 2008). FC is a cultivation-free method that detects bacteria irrespective of their culturability. While FC has been used to characterise and quantify microorganisms present in natural aquatic environments for many decades (Legendre & Yentsch, 1989; Troussellier, Courties, & Vaquer, 1993), its use as a technique to analyse drinking water has recently been recognised (Hammes et al., 2008; Hoefel et al., 2003; Daniel Hoefel, PT Monis, et al., 2005; Daniel Hoefel, Paul T Monis, et al., 2005). FC was first applied in drinking water treatment to detect *Cryptosporidium* oocysts (Vesey, Slade, & Fricker, 1991). However, bacteria were first detected using FC while investigating the adhesion of *E. coli* onto corrosion products (e.g. Iron oxyhydroxide) in drinking water distribution networks (Appenzeller et al., 2002). In 2003, FC was used to detect physiologically active bacteria in drinking water and reported significant difference in total number of bacteria and number of culturable bacteria recorded using FC and HPC (Hoefel et al., 2003). Thereafter, numerous drinking water studies contrasting FC and HPC data claimed that FC is more appropriate to use as a diagnostic tool, and cast a doubt on the future application of HPC for routine microbial assessment of water quality (Gillespie et al., 2014; Hammes et al., 2008; Ho et al., 2012; Daniel Hoefel, PT Monis, et al., 2005; C. Liu et al., 2013).

There is robust literature available concerning the application of FC in drinking water analysis. Notable studies include microbial characterisation of disinfection processes (Phe et al., 2005; Ramseier et al., 2011; Wert, Dong, & Rosario-Ortiz, 2013), water treatment processes (Hammes et al., 2008; Helmi et al., 2014; Ho et al., 2012; Van Nevel et al., 2012; Vital et al., 2012) and viability assessment (Berney et al., 2008; Pianetti et al., 2005). FC is used to investigate the potential regrowth and biological stability of microorganisms in drinking water distribution networks and plumbing of buildings (Gillespie et al., 2014; Daniel Hoefel, PT Monis, et al., 2005; Daniel Hoefel, Paul T Monis, et al., 2005; Lautenschlager et al., 2010; Lautenschlager et al., 2013; Lipphaus et al., 2014; C. Liu et al., 2013; Nescerecka et al., 2014; Prest et al., 2013; Van Nevel et al., 2016; Vital et al., 2012; Wen et al., 2014). FC combined with fluorescence staining of total nucleic acids enables the enumeration of bacteria in water samples (Hammes & Egli, 2005; Van Nevel, De Roy, & Boon, 2013). High sensitivity dual channel flow cytometers (FC with dual fluorescence) can rapidly detect and enumerate pathogens of interest as well as the total number of bacteria in a mixture (Yang et al., 2010). During the last decade, the use of FC fingerprinting (which uses a range of statistical tools to interpret raw FC data) has facilitated the

characterisation and monitoring of changes in bacterial communities in drinking water samples (De Roy et al., 2012; Douterelo et al., 2014; Kahlisch et al., 2010; Prest et al., 2014).

FC is quantitative, fast (10 min staining, < 1 min analysis), reproducible (< 7 % irregularity between laboratories) and accurate (< 3 % relative standard deviation between measurements) method for enumerating the total bacterial cell concentrations in the presence of a conventional nucleic acid stain (e.g. SYBR Green I/II) (Hammes et al., 2008; Prest et al., 2013; Wang et al., 2010). FC also allows the enumeration of viable bacteria when a combination of viability stains (e.g. SYBR/SYTO combined with propidium iodide (PI)) is used (Berney et al., 2008; Helmi et al., 2014).

Despite the great wealth of information offered by FC that is not available using HPC, FC is still not widely implemented in national and international drinking water guidelines as a tool to assess microbial water quality (Safford & Bischel, 2019). So far, Switzerland is the only country who has published and implemented the use of FC to determine the total bacterial cell counts and to evaluate the ratio of bacteria with high and low nucleic acid content in fresh water (Book, 2012). However, some water utilities and regulatory bodies have started applying FC (Safford & Bischel, 2019). Scottish Water (Scotland's water utility company) are developing FC protocols for full-scale drinking water treatment plants and drinking water distribution systems (Water, 2014). A report from the State Water Resources Control Board of California has recognised FC as amenable to automation and a fast technique to assess the microbial quality of water (Olivieri et al., 2016). However, some major obstacles need to be overcome before adopting FC as the alternative tool for the routine microbial assessment of water quality over HPC.

One of the main challenges is the interpretation of FC data in terms of water quality since it is the first and foremost step before setting advisory limits or standards. The lack of standardisation can be due to several factors. First, there can be substantial variability between the flow cytometers from different manufacturers but also between models from the same companies. The main causes of variability can be the wavelength, number and power of excitation lasers; the type and number of detectors and the operational settings for handling samples (Safford & Bischel, 2019). Secondly, operators apply varying instrument settings and manual gating (to distinguish the bacterial signal from the background) for FC quantification purposes. Hence, the lack of standardisation makes the results subjective and operator dependent (Aghaeepour et al., 2013; De Roy et al., 2012; Prest et al., 2013).

In order to overcome the problem with instrument- and user-bias, a reproducible staining procedure using a fixed gating approach (i.e. eliminating the manual gating step by the operator) is introduced to generate consistent and stable water quality results, irrespective of the type of instrument utilised (Prest et al., 2013). Some researchers (Aghaeepour et al., 2013; De Roy et al., 2012; Koch et al., 2014)

tried to eliminate gating completely by subjecting the FC data to a gating-independent statistical analysis, while others (Castillo-Hair et al., 2016) have developed software that converts FC data from arbitrary units to calibrated units. It is suggested that final scientific papers should include the publication of experimental data as Flow Cytometry Standard (FCS) files (source of important metadata) to enable comparison of FC data from different experiments and different instruments (Safford & Bischel, 2019).

Another drawback of FC that restricts its use for the routine microbial assessment of water quality is the fact that it cannot distinguish between single cells and aggregated cells (Shapiro, 2005). FC underestimates the number of bacteria when colonized suspended particles or clumps of biofilm are present in the samples (Van der Kooij & Van der Wielen, 2014). In order to overcome this challenge, mild sonication is used to disrupt clusters and clumps of bacterial cells that are present in biofilm and wastewater samples and uses microscopy to validate the technique (Foladori et al., 2010; Ma et al., 2013). Sonication can however damage bacterial cells (Buesing & Gessner, 2002) and affect viability assessments. Moreover, the introduction of a sonication step considerably lengthens the processing time, diminishing one of the main advantages of FC (Van Nevel et al., 2017). (G. Liu et al., 2013).

Considering the complexity and diversity of microbial communities in water samples, FC can detect and quantify the total number of cells, however, it is unable to distinguish between faecal indicative bacteria from opportunistic pathogens and viable cells from dead cells (Van Nevel et al., 2017). The assessment of cellular viability is amongst one of the most frequent uses of FC, however, there is neither a globally accepted definition of the term viability nor a globally accepted method to assess viability (Safford & Bischel, 2019). The most common method to assess cellular viability by FC involves the combination of two nucleic acid stains; a cell permeable stain such as SYBR and SYTO, and a cell-impermeable stain such as PI (Hammes et al., 2011). FC analysis of cells stained with SYBR Green represent the total cell counts (TCC) since SYBR Green is cell permeable and enters all the cells while cells stained with PI represent the proportion of intact cell counts (ICC) since the dye stains cells only with damaged membrane. The combination of SYBR Green I and PI is widely used in FC for the characterisation of membrane integrity, primarily to distinguish between cells with either intact or compromised membrane (Vital et al., 2012). However, FC fails to identify cells that have neither a compromised membrane nor an intact membrane but exist in an intermediate state (viable but non culturable state) (Berney et al., 2007; Kaur, Karthikeyan, & Smith, 2013). Furthermore, cells with membrane damage does not always correlate directly with reduced cell viability. While cells with severe cytoplasmic membrane damage can be regarded as dying or dead, cells with intact membranes are not always viable (Hammes et al., 2011). For example, UV-C inactivate cells by damaging nucleic acids without compromising the cell membrane integrity thereby leaving the damage unnoticed by PI

staining. (Kong et al., 2016; Nie et al., 2016; Yoon et al., 2017). In this example, HPC analysis accurately classifies these cells as dead while FC does not. A wide range of stains are readily available and have been assessed, however, the challenge centers on what type of stain or combination of stains to use. Stains choice should take into consideration the mechanism of action of the disinfectant and the mechanism of action of the dye before a standardised viability assessment procedure is established (Nescerecka, Hammes, & Juhna, 2016).

The implementation of FC for routine water assessment could be simpler if a correlation existed between TCC or ICC and HPC, but regrettably, no such correlation exists. A number of studies comparing FC data with HPC data has found them to be only loosely correlated (Burtscher et al., 2009; Hoefel et al., 2003; Nescerecka et al., 2014; Siebel et al., 2008). While FC and HPC techniques can be used together as complementary approaches, the practicality and financial cost associated with applying both techniques for the same purpose increases costs (Van Nevel et al., 2017). (Dong et al., 2020).

1.5.3 Bacteria in the viable but non-culturable state

The viable but non-culturable (VBNC) state is a survival strategy for bacteria to deal with stressful environmental conditions (Oliver, 2010). VBNC are unable to replicate and form colonies on routine bacteriological media, but indeed remain viable and maintain low metabolic activity (Oliver, 2010). Over 100 species of microorganisms (bacteria and fungi) are known to enter into the VBNC state following exposure to some kind of stress (Dong et al., 2020). VBNC bacteria can resuscitate into a culturable state under favorable conditions (James D. Oliver, 2000). While in the VBNC state, a number of important metabolic activities decrease such as the synthesis of macromolecules, the rate of respiration and the transport of nutrients (James D Oliver, 2000; Porter, Edwards, & Pickup, 1995). Furthermore, in the VBNC state, bacteria produce shock and starvation proteins (McGovern & Oliver, 1995; Morton & Oliver, 1994). While a rapid decrease in ATP levels are observed for dead cells, cells in VBNC state maintain a high level of ATP (Beumer, De Vries, & Rombouts, 1992; Federighi et al., 1998). VBNC bacteria usually exhibit significant morphological changes such as dwarfing (Liu et al., 2017; James D Oliver, 2000; Wei & Zhao, 2018; Zhao et al., 2013). For instance, *Vibrio cholerae* changes from an arc shape to a spherical shape (Chaiyanan et al., 2007), and *E. coli*, *Helicobacter pylori* and *S. typhimurium* change from a rod shape to a spherical shape (Gupte, De Rezende, & Joseph, 2003; Orta de Velásquez et al., 2017; Signoretto, del Mar Lleo, & Canepari, 2002). Morphological changes are strongly correlated to alterations in cell wall content, for instance glycan strand shortening, increase in muropeptides which are covalently bonded to lipoproteins (Signoretto et al., 2002) and increase in the cross linking of peptidoglycans (Signoretto et al., 2000). While fatty acids present in cytoplasmic membranes of the VBNC cells are significantly altered (Day & Oliver, 2004; Linder & Oliver, 1989), the

cells are still able to maintain their membrane potential (Porter et al., 1995). Hence, VBNC bacteria are able to maintain the fluidity of the cell membrane when they are exposed to harsh environmental factors (Dong et al., 2020). As a result of these changes, cells in the VBNC state become more resistant to antibiotics (rifampicin, ciprofloxacin and ampicillin) (Lin et al., 2017; Ramamurthy et al., 2014), physical stresses (ultrasound, heat and glass beads homogenization) (Signoretto et al., 2000; Zhao et al., 2013) and chemical stresses (chlorination, heavy metal toxicity, salinity and acidity) (Nowakowska & Oliver, 2013; Su, Jane, & Wong, 2013) compared to culturable cells.

While some studies have demonstrated that some pathogens in the VBNC state can maintain their virulence, others have reported pathogens to enter into an avirulent VBNC state. VBNC pathogens such as *H. pylori*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *E. coli* O157:H7 and *Aeromonas hydrophila* are unable to cause infections until they resuscitate and become culturable again (Baffone et al., 2003; Boehnke et al., 2017; Du et al., 2007; Zhao et al., 2016). On the other hand, VBNC waterborne pathogens such as *Legionella pneumophila* and some *Vibrio* continue to produce toxins in the VBNC state (Alleron et al., 2013; Vora et al., 2005). Moreover, VBNC *Campylobacter jejuni,* induced following incubation at low temperature (4 ^{o}C), invade the epithelial cells of human intestine (Chaisowwong et al., 2011). VBNC *Listeria monocytogenes*, induced by chlorine stress, maintain their infectivity and reduce the lifespan of *Caenorhabditis elegans* (Highmore et al., 2018). As such, it is crucial to assess the viability and virulence of VBNC pathogen when monitoring the microbial water quality in order to provide the public with safe water.

1.6 Significance and Objectives of this research

Continuous electrochlorination has proved to be a highly reliable and efficient technique of generating chlorine on site and providing a chlorine residual in water treatments. Recent field scale trials conducted by the Water Corporation of Western Australia (WA) at Horrocks Ground Water Treatment Plant (GWTP) indicate CEC may be suitable for use in drinking water applications. In particular, CEC technology is suitable on ground water with high total dissolved solids. This study will provide insight into the understanding of various operational parameters on the electrogeneration of chlorine and its impact on bacteria and bacteriophage. This study will also contribute to the knowledge on the assessment of the microbiological quality of drinking water using heterotrophic plate count and flow cytometry before and after CEC treatment. From an operational point of view, CEC treatment (in-situ generator of chlorine) is a safe - reduce/eliminate manual handling of hazardous chemicals such as chlorine gas, has a lower environmental impact – green technology as it reduces reliance on existing source water supply (brackish, reuse and wastewater), lowers logistical impacts (fuel/road- no need

to transport and store Cl_2 gas to and on site) and can be used as a source of energy recovery (reuse or recombination of the Hydrogen generated by CEC).

Objective 1: Impact of various operational parameters at laboratory and pilot scale CEC units on the electrogeneration of chlorine, disinfection and inorganic by-products

Hydro-Dis® and Water Corporation of Western Australia have developed a laboratory and pilot scale CEC unit consisting of Titanium based electrodes coated with a mixed metal oxide (MMO) layer of Ti/Rh/Ru. In this study, we investigated chlorine production, disinfection and inorganic by-product formation of the laboratory and pilot CEC units. This study focused on the effects of water flowrate, NaCl concentration, voltage and DOC on chlorine and inorganic by-product formation for both the lab and pilot unit. Furthermore, the effects of naturally occurring salts, sulfate and bicarbonate, on chlorine production, and the presence of commonly found bromide concentrations in surface waters were investigated only on the laboratory scale CEC.

Objective 2: Impact of electrochemically generated oxidants by CEC on bacteria and bacteriophage.

In this study, we investigated the disinfection efficiency of CEC on *E. coli*, *B. subtilis*, *S. maltophilia* (a multi-drug resistant strain), *L. pneumophila* (serogroup-1 Philadelphia strain), phage T4 and MS2 in a chloride containing electrolyte and in continuous mode (very short contact time of 4.5 seconds). Flow cytometric analysis combining with SYBR Green I and SYTOX Orange in parallel with plate counts were used to investigate the effects of the oxidants on *E. coli*, *B. subtilis* and *S. maltophilia* in continuous mode. We also investigated the disinfection efficiency of CEC on *E. coli* in a chloride-free electrolyte when the CEC was operated in continuous and recirculating modes. Lastly, we compared the disinfection efficiency of CEC in continuous mode using a chloride-containing solution with conventional chlorination (sodium hypochlorite solution) based on the same contact times on an *E. coli* suspension.

Objective 3: Investigate the potential recovery/regrowth of bacteria after CEC treatment and the importance of routine assessment of the microbiological quality of drinking water after disinfection.

This study aimed at better understanding the potential for bacteria to recover or regrow following electrochemical disinfection of a chloride-containing solution. In order to mimic the presence of organic matter along distribution networks, two sets of experiments, with and without nutrient addition post-disinfection, were investigated. This study also aimed at contributing to the differences between HPC and flow cytometry when it comes to bacterial viability and cell counting. Moreover, a resuscitation test was performed to determine if culturable cells observed post disinfection treatment

and nutrient addition were a result of true resuscitation of injured cells or the growth of culturable bacteria not detected by the standard plate counts immediately after disinfection.

Objective 4: Impact of different chemical characteristics of natural waters on the disinfection efficiency of the Pilot CEC unit.

The aim was to assess the efficacy of the pilot unit including two successive electrolysis cells (i.e., pre and post oxidation) on the disinfection of waters with different chemical characteristics focusing on: chloride content, dissolved organic carbon (DOC) concentration, iron and manganese contents. The effect of the oxidants generated by the CEC on the disinfection of water were tested on 5 drinking water treatment plants (one in Perth and four in the Great Southern Regions of WA). Natural waters (ground water and surface water) samples were spiked with known concentrations of common waterborne pathogens, *E. coli*, *B. subtilis* and bacteriophage T4, and the number of viable cells was analysed both pre and post CEC treatment. In addition, the DBPs formation associated with all the microbial experiments were investigated in order to evaluate if the DBPs formed were within the limits of the Australian Drinking Water Guidelines.

Chapter 2 . Impact of various operational parameters at Laboratory and Pilot Scale CEC units on the electrogeneration of chlorine, disinfection and inorganic-by-products.

2.1 Introduction

This chapter focused on the performance of continuous electrochlorination (CEC) technology as an alternative to conventional chlorination in drinking water treatment. In drinking water treatment, the primary goal of electrolysis is the *in situ* generation of disinfectants such as active chlorine (dissolved chlorine, HOCl and OCl^-) (Yoon et al., 2015). The electrochemical oxidation of water containing chloride ions produces active chlorine, one of the key species in reducing the incidence of waterborne diseases (Diao et al., 2004). However, the presence of residual chlorine in water could produce toxic organic and inorganic by-products such as trihalomethanes, haloacetonitriles, chlorate and bromate as described in Chapter 1 section **1.4**.

Among the various parameters that affect the generation of oxidants, having an appropriate anode material is the key determinant for the efficacy of disinfection. The anode material dictates the species and amount of oxidants generated (Martínez-Huitle & Brillas, 2008). Anode materials often used include titanium coated with a MMO layer (also known as Dimensionally Stable Anodes (DSA)) (Gusmão et al., 2010), boron-doped diamond (BDD) (Lacasa et al., 2013; Long et al., 2015) and platinum (Jeong et al., 2007). While DSA electrodes (active electrode anodes) such as TiO_2-RuO_2 (Gusmão et al., 2010) and IrO_2-RuO_2 (M. Bergmann & A. Koparal, 2005) are known to produce dominantly active chlorine species, BDD electrodes (non-active electrode anodes) that are used for Electrochemical Advanced Oxidation Processes (EAOPs) are not so efficient for free chlorine production (Bergmann et al., 2008; Kraft, 2008). Furthermore, the electrolysis of chloride-containing solutions using BDD electrodes generate ROS, RCS but also chlorate and perchlorate (not formed at DSA and Pt anodes) from further oxidation of hypochlorite (Kraft, 2008; Palmas et al., 2007) and their levels are only acceptable at very low concentrations in drinking water (Kraft, 2008). A recent study conducted by Curtin Water Quality Research Centre group has used a continuous electrochlorination cell (EC3) with Ti based electrodes with a MMO layer of Ti/Rh/Ru in the presence tertiary alcohol showed no hydroxyl radicals were generated (Personal communication, unpublished data). Therefore, for the aim of generating chlorine, as discussed previously in the literature, DSA type electrodes are more suitable for the EC treatment of drinking water with the objective to generate free chlorine.

Oxidation at the anode can also lead to the formation of dichlorine ($Cl_2^{\cdot-}$), sulfate ($SO_4^{\cdot-}$) and carbonate ($CO_3^{\cdot-}$) radicals in the presence of their corresponding anions. The electro-oxidation of naturally occurring salts, generates $Cl_2^{\cdot-}$, $SO_4^{\cdot-}$ and $CO_3^{\cdot-}$ radicals at the anode and these radicals may also have a significant impact on the degradation processes of Natural Organic Matter (NOM) (Barazesh, Prasse, & Sedlak, 2016; Farhat et al., 2015). While most of the studies that have investigated the production and possible effects of $Cl_2^{\cdot-}$, $SO_4^{\cdot-}$ and $CO_3^{\cdot-}$ radicals from the oxidation of chloride-, sulfate and

bicarbonate-containing water focused on BDD electrodes, there is a knowledge gap on the impact of these commonly found anions on chlorine production using titanium electrodes coated with a MMO layer of Ti/Rh/Ru.

The main objective of this study was to investigate chlorine production, disinfection and inorganic by-product formation of the laboratory and pilot units. This study focused on the effects of flowrate, NaCl concentration, voltage and DOC on chlorine production, disinfection and inorganic by-product formation for both the lab and the pilot unit. Furthermore, the effects of naturally occurring salts, sulfate and bicarbonate, on chlorine production, and the presence of commonly found bromide concentrations in surface waters were investigated on EC1. EC1 was essentially used to study the performance of the electrode material of CEC while EC2 was used for all microbiological studies. Only limited experiments were performed with EC2 as this cell was provided by Hydro-dis® in the 3rd year of this thesis work and there was insufficient time to repeat all tests carried out on EC1 (first generation of cell provided by Hydro-Dis®).

2.2 Materials and methods

Hydro-Dis® in collaboration with Water Corporation of Western Australia developed a Pilot CEC unit and two Laboratory CEC units using titanium electrodes coated with a mixed metal oxide (MMO) layer of Ti/Rh/Ru electrodes. The pilot unit (**Figure 5**) was designed as a two-stage treatment unit, pre-treatment stage (pre-oxidation) and post-treatment (disinfection) stage and consisted of four treatment lines (2 cells per treatment line) per stage (**Figure 6**). Each cell had a volume of 750 mL, consisted of an annulus electrode (610 mm in length with an outer diameter of 60 mm) and a surface area of 0.0096 m^2. Water flows through the pre-treatment cells first then into the post-treatment cells. The pilot unit has been designed such that it can utilise/switch on 8, 6, 4 or 2 cells at one time (for instance 2 cells from pre-treatment and 2 cells from post-treatment). The pilot unit has four sampling points labelled as follows (**Figure 7**):

1. Post-treatment (after the Pilot unit cell 2 [PUC2]- disinfection cells)
2. Pre-treatment (after the Pilot unit cell 1 [PUC1]- pre-oxidation cells)
3. Raw water after Bacteria/Phage injection
4. Raw water

Figure 5 Pilot unit depicting the electrolytic cells (a, c), power supply for the pre- and post-treatment cells (b), the water inlet and outlet and the sampling points (d).

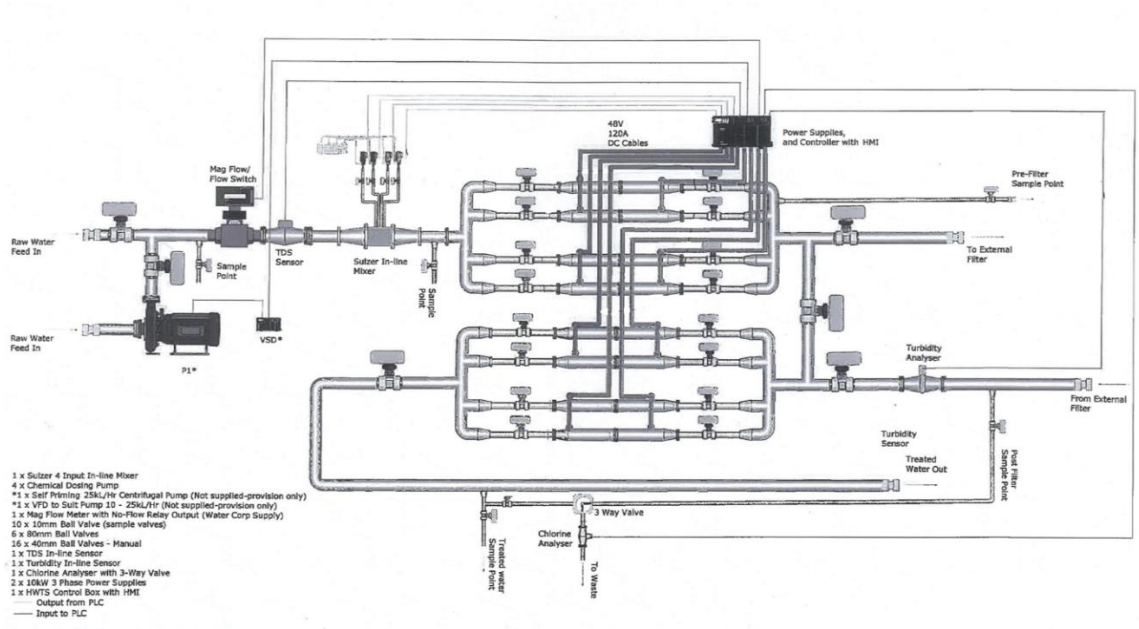


Figure 6 Schematic diagram of the pilot unit illustrating the two sets of four treatment lines for pre-treatment and post-treatment.

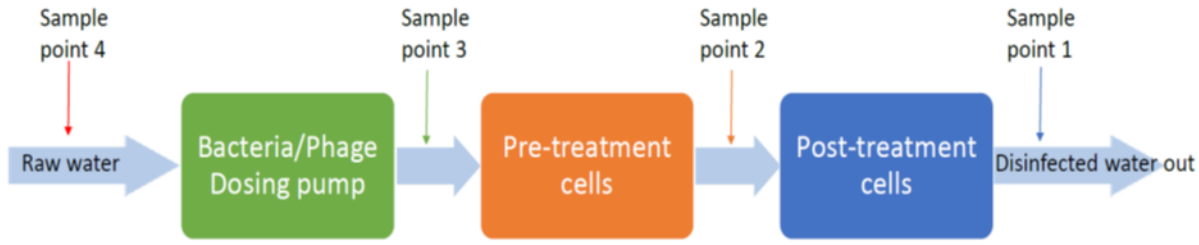


Figure 7 **Pilot unit flow diagram indicating the four sampling points.**

The two laboratory scale CEC units were, electrolytic cell 1 (EC1) and electrolytic cell 2 (EC2) (**Figure 8**). Both EC1 and EC2 consisted of non-divided cells with two titanium metal plates as substrates coated with ruthenium and iridium oxides in the ratio of 7:3 as electrodes (anode and cathode), connected to a 100 W DC power supply (IT6720, ITECH Electronics Co., Ltd, CHINA) with voltage capacity ranging from 0 V to 60 V and a peristaltic pump (Masterflex®L/S®, Cole Palmer) (**Figure 9**). The differences between the two cells were the volume of the cells and the size of the electrodes. EC1 (cylindrical reactor) and EC2 (cuboid reactor) had a volume of 47 ml and 7.2 mL, respectively. The electrodes were 10 mm × 10 mm × 1 mm and 50 mm × 25 mm × 1 mm for EC1 and EC2, respectively. Each electrode (each anode and cathode) for EC1 and EC2 had an active area of 0.0001 m^2 and 0.00125 m^2, respectively. The electrodes were positioned in the middle of the reactors. The gap separating the two electrode plates were 4 mm and 2 mm for EC1 and EC2, respectively. The cells were set up vertically with water flowing in the bottom and out of the top to make sure the reactor is always filled when operated.

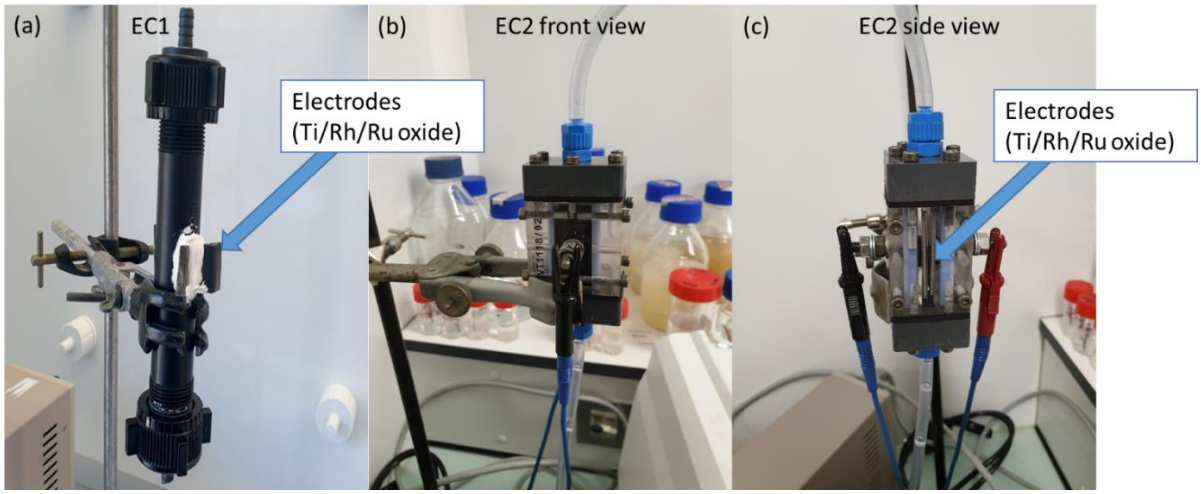


Figure 8 Laboratory units used in this study. Electrolytic cell 1 (a), front view of electrolytic cell 2 (b) and side view of electrolytic cell 2 (c). The electrodes used in EC1 and EC2 were titanium plates coated with a MMO layer of Ti/Rh/Ru.

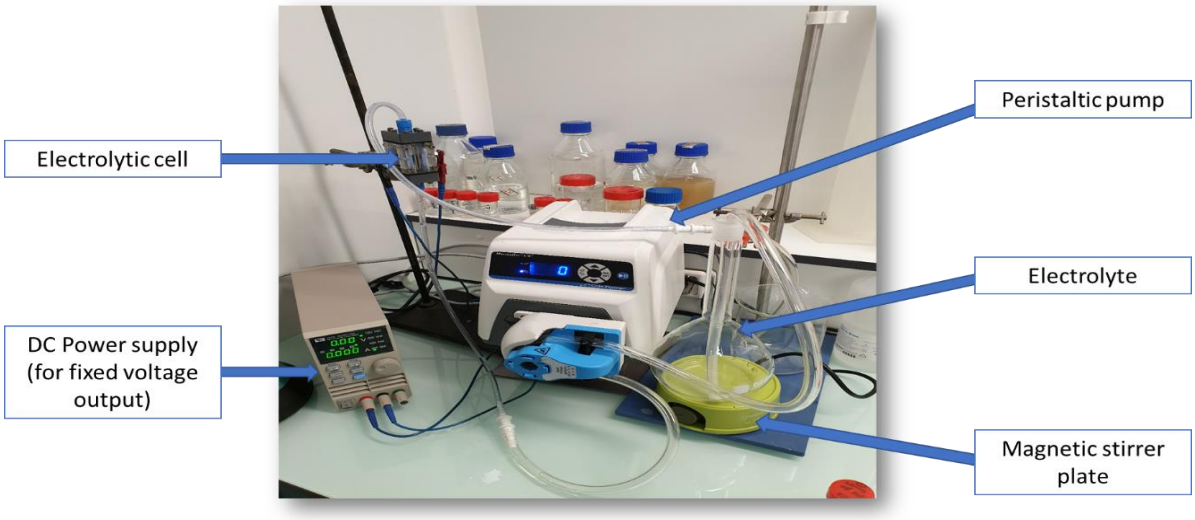


Figure 9 Laboratory setup of CEC cell.

2.2.1 Analytical Methods

2.1.1.1 Chlorine Analysis

For the pilot unit, chlorine concentration analysis (on-site) was done using a pocket colorimeter (HACH 2800, Loveland, CO, USA). Free chlorine and Total chlorine were analysed using the "DPD Free Chlorine Reagent" and "DPD Total Chlorine Reagent" powder pillows, respectively. The Hach method 10245 for

medium range chlorine detection up to 4.00 mg/L as Cl_2 was used. Samples with readings higher than 4.00 mg/L as Cl_2 were diluted accordingly.

For the lab unit, the chlorine generated by the CEC cell was measured using the N, N diethyl-1,4 phenylenediamine (DPD) colorimetric method (Bridgewater et al., 2012). Briefly, 3 mL of sample was added to a glass cuvette containing 150 µL of DPD buffer and 150 µL of DPD solution. The absorbance at 525 nm of the resulting magenta coloured solution is then measured using a spectrophotometer (UV-1280, Shimadzu). The DPD solution was prepared by combining 0.275 g of DPD sulfate anhydrate (Sigma-Aldrich, 07672), 2 mL concentrated sulphuric acid diluted 1 in 3 (Sigma-Aldrich, 339741), 6.25 mL of 0.8 % sodium ethylenediaminetetraacetic acid (EDTA)(Sigma-Aldrich, E9884) in a final volume of 250 mL using Milli-Q® water. The DPD buffer was prepared by combining 11.5 g of monopotassium phosphate, 7.52 g of disodium hydrogen phosphate, 25 mL of 0.8 % sodium EDTA in a final volume of 250 mL using Milli-Q® water. Total chlorine was measured by adding a few crystals of potassium iodide to a cuvette containing 150 µL of DPD solution, 150 µL of DPD buffer and 3mL of the electrolyte. The absorbance was then measured at 525 nm using the spectrophotometer (Harp & Hach, 2003).

2.1.1.2 Iron Analysis

Iron was measured using a Hach® pocket colorimeter according to Hach method 8008. This method employs the 1.10 phenanthroline method and uses Hach FerroVer® reagent pillows. Filtered and total Iron was differentiated by measuring the total iron content in an unfiltered sampled and in an identical sample filtered by 0.45 µm porosity filter (Millex-syringe filter unit, Merck, Millipore).

2.1.1.3 Adsorbable Organic Halides (AOX)

AOX was analysed according to a method optimized by Kristiana et al. (Kristiana et al., 2015). Briefly, samples were acidified with nitric acid (HNO_3) to pH 2. 50mL of the acidified sample was passed through a column of activated carbon to absorb any organically bound halides. Inorganic halides were removed by washing the samples with 5mL of 5 mg/L HNO_3 to avoid contamination. The activated carbon samples were placed in sample boats in the Mitsubishi AQF-100 combustion system where the samples were systematically combusted. The hydrogen halide gasses produced were dissolved into MilliQ water for analysis with ion chromatography (ICS-3000, Dionex, Sunnyvale, CA, USA) using an IonPac® AS19 ion chromatography with an IonPac® AG19 guard column (Dionex). **Table 3** shows the limit of detection of the THMs and Hans species measured in this study.

Table 3 Limit of detection of THMS and HANs.

DBPs		LOD (ng/L)
THMs	TCM	10.8
	BDCM	31.6
	DBCM	11.2
	TBM	8.1
HANs	TCAN	0.6
	DCAN	0.9
	BCAN	3.6
	DBAN	2.8

2.1.1.4 Dissolved Organic Carbon (DOC) Analysis

DOC concentration was measured using a Total Organic Carbon Analyser (Shimadzu TOC-L) using combustion catalytic oxidation method after filtration through a 0.45 µm membrane according to the Standard Method 5310C (Eaton et al., 2005).

2.1.1.5 Trihalomethanes (THMs) and Haloacetonitriles (HANs) Analysis

THMs and HANs were analysed by solid-phase microextraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS) based on a method adapted from Allard et al. (2012) and Bagastyo et al. (2012). Briefly, sodium sulfate (3.60 g) was added to samples (10 mL) previously spiked with internal standard (1,2-dibromopropane at 5 µg/L). Samples were extracted and analysed (within 12 hrs) automatically by SPME. Instrumental conditions that were modified are: inlet temperature: 220°C, oven programming; initially held at 220°C for 3.5 minutes, then heated to 260°C at 1°C/s and held for 3.0 minutes, finally heated to 270°C at 5.0 °C/s and held for 6 minutes, resulting in a total chromatographic run time of 29 minutes.

2.1.1.6 Anions Analysis

Chloride, bromide and chlorate analysis was performed by ion chromatography (ICS-3000, Dionex, Sunnyvale, CA, USA) using an AG-S9 column (Dionex). The analysis of bromate and chlorite were performed by post column reaction and UV detection on the same equipment. An acidic KI solution was introduced to the eluent stream producing triiodide ion (I_3^-) which was detected at a wavelength of 288 nm (Salhi & von Gunten, 1999). Bromate, chlorate and chlorite were analysed after residual chlorine quenching with the addition of sodium thiosulfate ($Na_2S_2O_3$) at 1.2 times the molar ratio of free chlorine present.

2.1.1.7 Hydrogen Analysis

The gas generated was determined at the outlet of PUC2 using the water displacement method (the volume of gas separating from the aqueous phase was measured using a volumetric flask, see **Figure 10**). The hydrogen content of the gas samples was analysed by Gas Chromatography-Thermal Conductivity Detection-Flame Ionisation Detection (GC-TCD-FID) at the ChemCentre Laboratory (Perth, Australia).

Figure 10 Volumetric flask used to measure the gas separating from the chlorinated water produced by the pilot unit.

2.2.2 Experimental Procedures

In this study, experiments with EC2 and the pilot unit were performed in continuous mode to investigate the effects of varying flowrate, NaCl concentration, DOC concentration and voltage on chlorine production. Additional experiments with EC1 were carried out in continuous and recirculation mode to further characterise the performance of CEC based on the same electrode material used in EC2 and the pilot unit. Experiments with EC1 and EC2 were carried out separately and in triplicate. Based on the volume of EC1 (47 mL) and EC2 (7.2 mL), in continuous mode, both reactors needed 3 residence times (equivalent to a total outflow volume of 140 mL for EC1 and 21 mL for EC2) in order to achieve steady chlorine production and making sure the current remains stable within this time.

2.2.2.1 Laboratory scale CEC

In all the experiments, a magnetic stirrer set at 400 revolutions per minute (rpm) was used to ensure uniform distribution of electrolyte and chlorine generated. In order to investigate the effects of different variables; voltage, flow rate and NaCl concentration, on chlorine production and inorganic

by-products formation, a base experiment was established. The laboratory scale setup is shown in **Figure 11**.

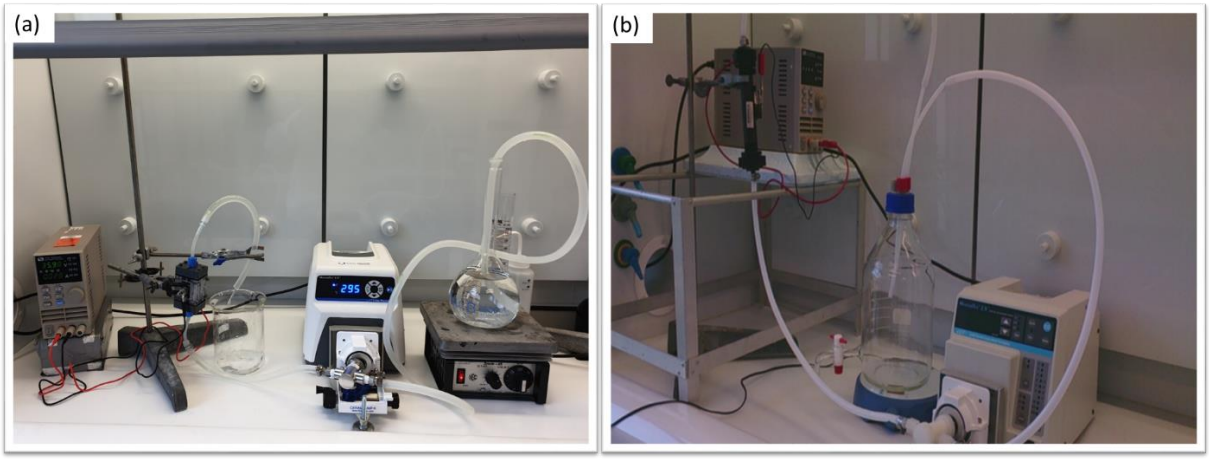


Figure 11 : Laboratory scale CEC setup in (a) continuous mode with EC2 and (b) recirculating mode with EC1.

2.2.2.1.1 Experiments conducted in recirculating mode

2.2.2.1.1.1 In the absence of Natural Organic Matter (NOM)

The base case consisted of 150 mg/L NaCl dissolved in MilliQ water (pH 6) and a final volume of 500 mL of working solution inside the reactor. A potential difference of 12V was applied across EC1. Each experiment was run for 30 minutes with samples taken at different time intervals. From this base case, parameters were varied or added to investigate their effects on chlorine production and inorganic by-products formation. The flow rate and voltage across the cell varied respectively from 17.5 mL to 200 mL and from 12 V and 24 V.

The effect of chloride was investigated with concentrations ranging from 75 to 300 mg/L of NaCl in solution. Bromide ions was spiked at concentrations relevant to real surface waters (500 µg/L and 1mg/L bromide) to investigate the production of bromates.

The effect of other commonly occurring salts in source waters i.e., sodium sulphate (Na_2SO_4) and sodium bicarbonate ($NaHCO_3$) on chlorine formation was studied.

Additional experiments were performed in the presence of 264 mg/L of sodium bromide (NaBr) as electrolyte, corresponding to the same molarity as NaCl in the base case.

The formation of hazardous inorganic by products, namely chlorate, chlorite and bromate was analysed over time following residual chlorine quenching.

2.2.2.1.1.2 AOX experimental procedure for CEC and conventional chlorination treated samples in the presence of NOM

A series of 200 mL solution were made using Suwannee River NOM extract at a concentration of 1.68 mg/L DOC. The solutions used for CEC treatment consisted of 150 mg/L NaCl prepared in MilliQ water (pH 6) while solutions for conventional chlorination contained 10 mM PB at pH 8. Four experiments were carried out for each treatment. Samples treated with conventional chlorination were spiked with 15 mg/L of Cl_2 (from a hypochlorite solution). Samples were taken after 15, 30, 60 and 120 minutes of contact time. Immediately after sampling, the chlorine concentration was measured. The sample was then transferred to an amber glass bottle and the free chlorine was quenched with $Na_2S_2O_3$, 1.2 times the molar quantity of the free chlorine produced. AOCl extraction and analysis was then performed. Chlorine doses were expressed in C.T (mg.min/L).

C.T = Concentration of chlorine (mg/L as Cl_2) × Time of contact (min)

2.2.2.1.2 Experiments conducted in continuous mode (In-line electrolysis)

2.2.2.1.2.1 Impact of CEC on NOM UV absorbing moieties

The effect of CEC treatment (EC1) on NOM character (UV absorbance) was evaluated on a solution of 10 mg/L NOM (Suwannee River humic substances) prepared in 10 mM PB at pH 8. The solution was treated with CEC at flowrates of 50, 100 and 200 mL/min and voltages of 12, 24 and 48 V. The UV absorbance at 254 nm for the control (before CEC treatment) and the CEC treated samples were analysed using a HACH® spectrophotometer (HACH 2800, Loveland, CO, USA).

2.2.2.1.2.2 Effect of NOM on chlorine generated by CEC and conventional chlorination

The chlorine demand of NOM following CEC (EC1) and conventional chlorination treatment was compared using a series of solutions of Blavet River humic substances i.e., 2, 4, 8 and 10 mg/L of DOC.

For the CEC tests, 150 mg/L NaCl solution as in the base case were prepared with 10 mM PB adjusted at pH 8. Electrolysis was carried out at a flowrate of 200 mL/min and 24 V. Under these conditions and in absence of NOM the chlorine production from the electrolysis cell was 4.4 mg/L of Cl_2. Each NOM solution was then electrolysed separately and the chlorine residual was measured at different time intervals (i.e., from 0 to 120 minutes).

Conventional chlorination (using a hypochlorite solution) was conducted on similar NOM solutions using a chlorine dose of 4.4 mg/L of Cl_2. The initial reading of Cl_2 residual was conducted 24 seconds after the addition of sodium hypochlorite to ensure the sample has the same contact time as that of the CEC treated sample (includes 14 s in the cell and 10 s from the CEC cell to the sampling point-

actual contact time with the electrode is 6 s). The chlorine residuals were measured at regular time intervals (up to 120 min).

2.2.2.2 Effect of voltage, flow rate and NaCl concentration on chlorine production using EC2

The effect of varying feed flowrates, voltages and NaCl concentrations on chlorine production was investigated using 10 mM phosphate buffer solutions at pH 7.2 in a final volume of 1000 mL. Voltages applied were: 6, 12, 18 and 24 V. Flowrates tested were: 100, 200 and 400 mL/min. NaCl concentrations tested were: 0, 10, 20, 30, 50, 100 and 150 mg/L.

2.2.2.3 Pilot scale CEC

2.2.2.3.1 Effect of feed flowrate, voltage, NaCl concentration and DOC concentration on Cl_2 production, disinfection and inorganic by-products formation

The effect of feed flowrate, voltage, NaCl concentration and DOC concentration on chlorine production and inorganic by-products formation were investigated using feedwater (140 mg/L Cl^-, 180 µg/L Br^-, 1.37 mg/L DOC, and pH 6.95) from a reticulated standpipe at Gwelup GWTP. The current generated by the CEC cells for each treatment were measured and recorded. Samples were collected from the PUC1 and PUC2 sampling points for chlorine analysis and inorganic by-products formation.

The effect of feed flowrate was investigated by varying the flowrate from 7 m^3/h and 19 m^3/h in increments of 4 m^3/h. A constant voltage of 20 V was applied to both PUC1 and PUC2.

The effect of voltage was investigated by applying 12, 18, 24, 30 and 36 V, for both PUC1 and PUC2. A constant feed flowrate of 20 m^3/h was used.

The effect of NaCl concentration was investigated by injecting a brine solution (164 g/L NaCl) into the feed water through a dosing pump at flowrates of 0, 6, 12, 18, 24 and 30 L/h. A constant feed flowrate and voltage of 20 m^3/h and 18 V, respectively were applied for these tests.

The effect of increasing DOC concentration in the feed water on CEC treatment was investigated by injecting 11 g/L as carbon of an organic matter concentrate i.e., MIEX brine (treated by the variable shear enhanced filtration process (VSEP) to remove a large part of the salts) into the feed water before the electrochlorination cells to vary the influent DOC from 2.35 – 4.25 mg/L. Chlorine was analysed immediately after treatment, after 15 minutes, 30 minutes and every day for 7 days to follow the chlorine demand. THMs, HANs and AOX were analysed after 7 days of reaction time to establish the formation potential of chlorination by-products.

2.2.2.3.2 Effect of chloride and flowrate on hydrogen production

The production of hydrogen is one of the major cathodic processes that occurs during the electrolysis of water (Scott, 2020): $2H_2O + 2e^- \rightarrow 2OH^- + H_2$. Due to its explosive nature and in order to better understand the degassing requirements for a CEC system, the evolution of hydrogen gas was studied. The hydrogen content of the gas produced during the operation of the pilot unit was measured using bore W257 (35 mg/L Cl^-, 1.6 mg/L DOC, pH 7.95, conductivity 297 µS/cm and Temperature 42 ^{0}C) from Wanneroo GWTP with 35 and 300 mg/L of chloride addition. The experiments were performed at two different flowrates, i.e., 5 and 10 m^3/h and at 80 Amps for both PUC1 and PUC2. The gas generated was determined at the outlet of PUC2 using the water displacement method (**Figure 10**). The hydrogen content of the gas samples was analysed by Gas Chromatography-Thermal Conductivity Detection-Flame Ionisation Detection (GC-TCD-FID) at the ChemCentre Laboratory (Perth, Australia).

2.3 Results

2.3.1 Laboratory scale experiments conducted in recirculating mode using EC1

2.3.1.1 In the absence of Natural Organic Matter (NOM)

2.3.1.1.1 Effect of flowrate on Cl_2 production

The effect of flowrate on chlorine production over time was investigated by applying varying flowrates to solutions that had the same NaCl concentration, pH and voltage across the cell. The effect of flowrate is inversely proportional to chlorine production (**Figure 12a**). Furthermore, the production of chlorine (mg.min/L) is directly proportional to the residence time in the cell (**Figure 12b**). With increasing flowrate, the percentage of chloride that was converted to chlorine (Mole/Mole) decreased. After 30 min of electrolysis time, 7.7 %, 6.9 %, 5.4 % and 2.7 % of the chloride was converted to chlorine when 17.5 mL/min, 35 mL/min, 70 mL/min and 200 mL/min was applied, respectively. Therefore, increasing the flowrate decreased the amount of chlorine produced over time.

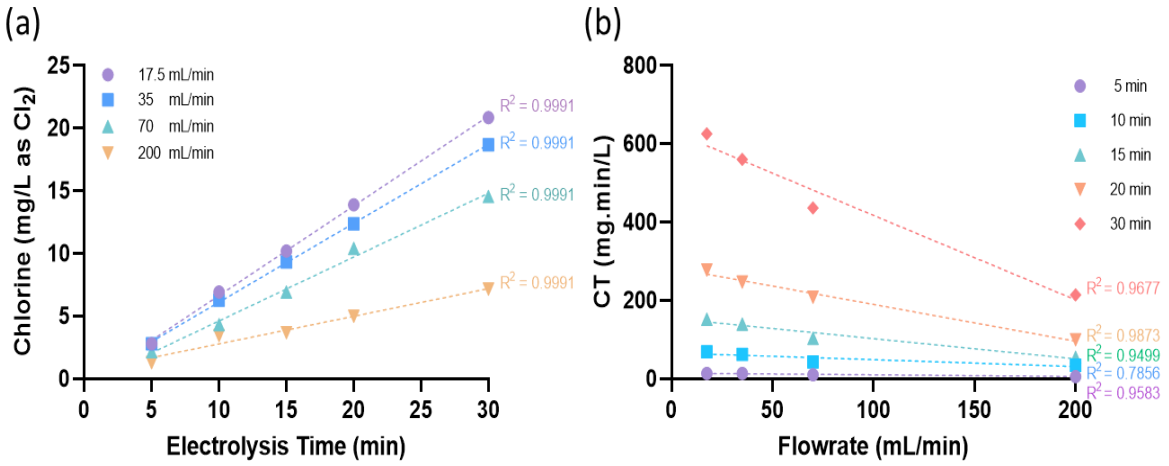


Figure 12 Effect of varying flowrates on (a) chlorine production and (b) CT while keeping NaCl concentration, pH and voltage applied constant at 150 mg/L, 6 and 12 V, respectively, using EC1 in recirculation mode.

2.3.1.1.2 Effect of voltage and NaCl concentration on Cl_2 production

The effect of varying voltage on chlorine production over time was tested by applying 12 V and 24 V across the cell for the same NaCl concentration (150 mg/L), flow rate (200 mL/min) and pH 6. Increasing the voltage applied increased chlorine production and the current measured (**Figure 13**). The chlorine generated at 24 V (150 mg/L NaCl) was 2.8 times higher than that obtained at 12V (**Figure 13**). The current measured through the cell at 24 V was 2.5 times higher than at 12V (**Figure 13b**).

The effect of varying NaCl concentration on chlorine production over time was studied by using different solutions made up of 150 and 300 mg/L NaCl and applying a constant voltage of 12 V and flow rate of 200 mL/min across the cell at pH 6. Increasing NaCl concentration increased chlorine production and current measured (**Figure 13**). The chlorine generated at 300 mg/L was 2.5 times higher than at 150 mg/L (**Figure 13a**). The current measured in the 300 mg/L NaCl solution was twice than in the 150 mg/L NaCl solution at 12 V (**Figure 13b**). Therefore, doubling either the voltage or the chloride concentration doubles chlorine production.

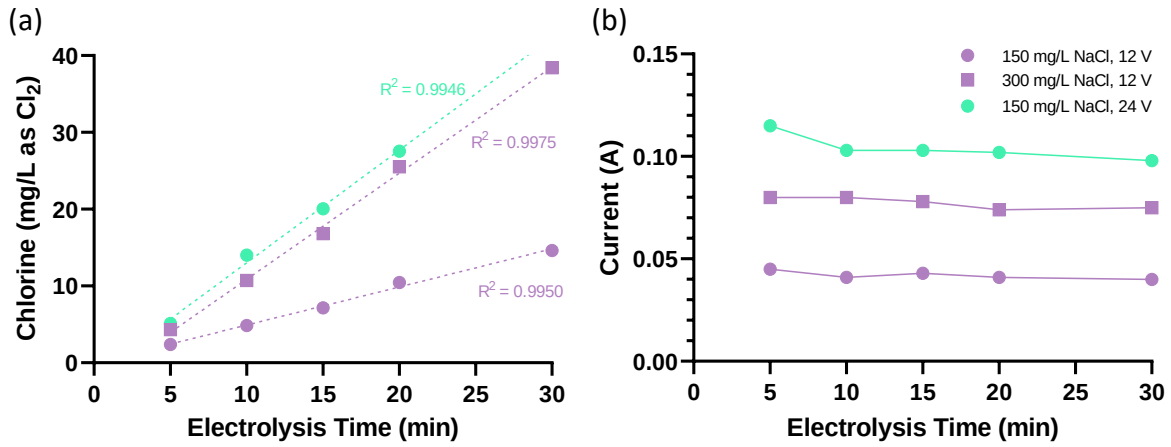


Figure 13 Effect of varying voltage and NaCl concentration on (a) chlorine production and (b) current, using EC1 in recirculation mode. Flow rate of 200 mL/min and pH 6 were kept constant.

2.3.1.1.3 Effect of sulfate and bicarbonate salts on Cl_2 production

The addition of similar molar concentrations of sodium sulfate and sodium bicarbonate salts to 150 mg/L NaCl solutions at 12 V and flowrate of 200 mL/min on chlorine production was tested. In the presence of both sodium sulfate and sodium bicarbonate, higher currents (0.083 A and 0.085 A) were recorded compared to the solution containing only 150 mg/L NaCl (0.04 A). Compared to the sample with only 150 mg/L NaCl, the chlorine production increased by 29 % - 61 % in the presence of sodium bicarbonate but decreased by 28 % - 32 % in the presence of sodium sulfate (**Figure 14**). The interaction of sulfate ions with metal oxides on the surface of the electrode seems to inhibit the chlorine production.

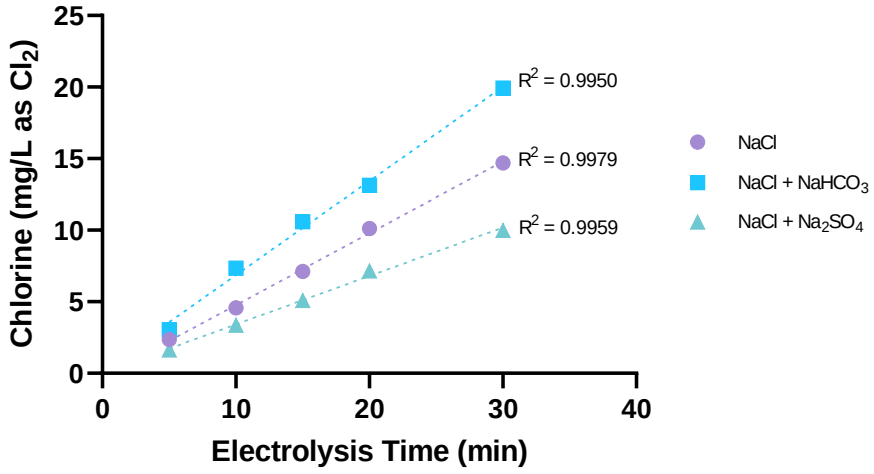


Figure 14 Chlorine formation in the presence of 150 mg/L NaCl only, 150 mg/L NaCl + 215 mg/L sodium bicarbonate and 150 mg/L NaCl + 182 mg/L sodium sulfate. A voltage of 12 V, a flowrate of 200 mL/min and pH 6 was applied in each experiment, using EC1 in recirculation mode.

2.3.1.1.4 Effect of NaCl concentration on chlorate formation

The effect of chloride concentration on the production of chlorate was tested by the electrolysis of solutions with varying concentration of NaCl at 12 V and flowrate of 200 mL/min. Samples were taken at 5, 15, 30 and 60 minutes of electrolysis. Increasing the chloride concentration in the solutions increased the concentration of chlorate generated linearly (**Figure 15**). In the base case (150 mg/L NaCl), after 30 min of electrolysis, the conversion of chloride to chlorate was 0.08 %. For the same base case, the chlorate to chlorine production after 30 min of electrolysis was 0.47 %.

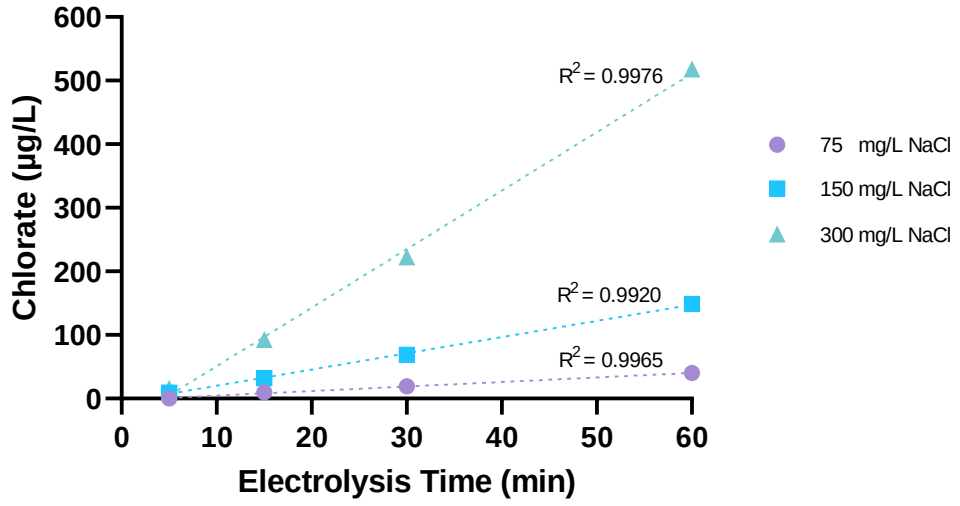


Figure 15 Chlorate formation during the electrolysis of solutions with increasing NaCl concentrations (75, 150, 300 mg/L) at 12 V, 200 mL/min and pH 6, using EC1 in recirculation mode.

2.3.1.1.5 Effect of bromide ions on bromine and bromate formation

Bromine and bromate production was investigated using the base case (150 mg/L NaCl, 12 V, 200 mL/min, pH 6, current 0.049 A) but instead of NaCl, 264 mg/L of sodium bromide (current 0.091 A), equimolar bromide concentration to 150 mg/L NaCl was used. After 30 min of electrolysis under these conditions, 2845 µg/L of bromate was formed. This corresponds to a conversion rate of 0.86 % of bromide to bromate (ten times more than chloride to chlorate conversion which was 0.08 %). **Figure 16** shows that the bromine production was expressed as mg/L equivalent to Cl_2 as such could not distinguish between HOCl and HOBr formation, was much lower than the chlorine production using the same molar concentration (i.e., 2.56 mmol/L). The decrease in chloride and bromide concentration was not measured. However, a slightly higher bromine production (expressed as mg/L equivalent to Cl_2) was observed when a solution containing both NaCl and NaBr was used compared to the electrolysis of only 150 mg/L NaCl (**Figure 16**). This was likely due to the higher current recorded in the presence of NaCl and NaBr (0.091 compared to 0.049 A for NaCl only). Furthermore, after 30 min

of electrolysis, the number of moles of chlorine + chlorate (0.21 mM + 0.0008 mM) produced was more than that of bromine + bromate (0.06 mM + 0.0222 mM).

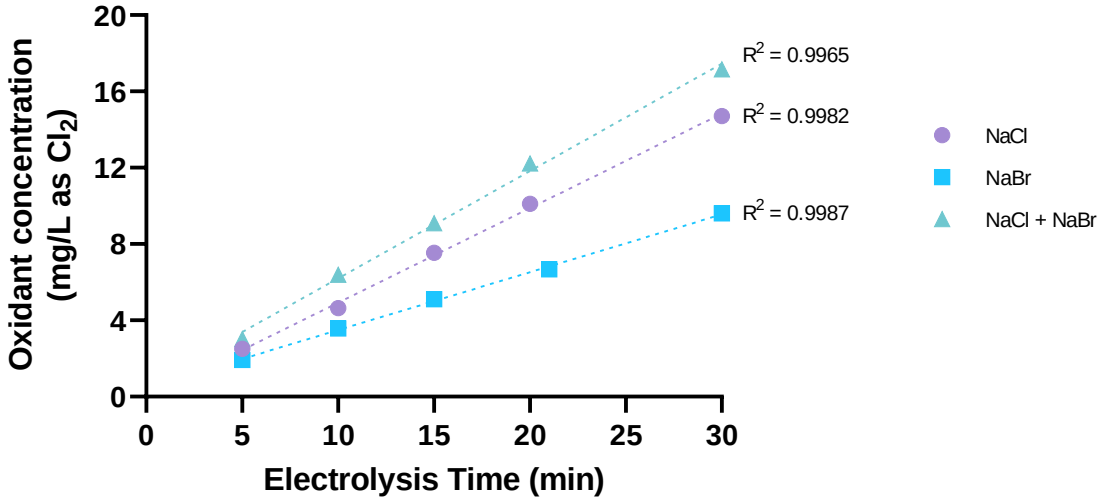


Figure 16 Oxidant formation in the presence of 150 mg/L NaCl, 264 mg/L NaBr and 150 mg/L NaCl + 264 mg/L NaBr solutions over 30 min of electrolysis at 12 V, 200 mL/min and pH 6, using EC1 in recirculation mode.

2.3.1.2 Effect of NOM isolate on AOCl formation following CEC and conventional chlorination treatment

The production of Absorbable Organic Chloride (AOCl) during CEC treatment of DOM containing solution was compared to the AOCl production obtained during conventional chlorination (carried out in a beaker) based on CT exposure. Similar AOCl concentrations were obtained for CT values below 200 mg.min/L with CEC and conventional chlorination (**Figure 17**). At higher CT values (i.e., >600 mg.min/L), the AOCl generated was found to be lower in the CEC-treated solution. This might be ascribed to the loss of volatile DBPs during recirculation.

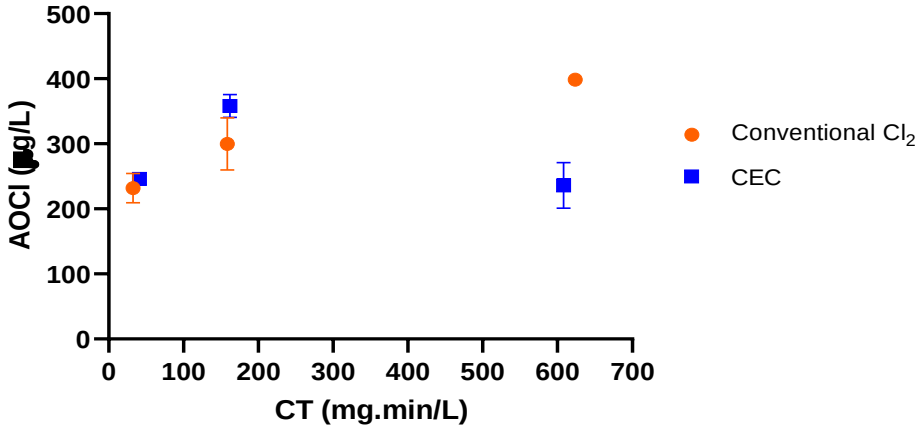


Figure 17 AOCl production following CEC treatment (150 mg/L NaCl, 12 V, 200 mL/min, EC1 in recirculation mode) and conventional chlorination (sodium hypochlorite) in the presence of 2.68 mg/L of dissolved organic carbon.

2.3.2 Laboratory scale experiments conducted in continuous mode (In-line electrolysis)

2.3.2.1 Impact of CEC on NOM UV absorbing moieties

The effect of electrolysis on NOM character was investigated using synthetic water by applying varying flowrates and voltages and measuring the UV absorbance at 254 nm. **Table 4** shows that CEC treatment of NOM (10 mg/L NOM - Suwannee River humic substances, 10 mM PB solution buffer at pH 8, no chloride content) did not change the UV absorbance of the solution, suggesting that the aromatic character of the organic matter is not impacted by this treatment under the experimental conditions applied.

Table 4 Impact of electrolysis on NOM UV absorbing moieties.

Voltage (V)	Flowrate (mL/min)	Absorbance at 254 nm
Before Electrolysis		0.188
12	50	0.188
12	100	0.182
12	200	0.180
24	100	0.184
48	50	0.181
48	100	0.189

2.3.2.2 Effect of NOM on chlorine generated by CEC and conventional chlorination

The chlorine demand of NOM following CEC and conventional chlorination treatment was compared using solutions spiked with Blavet River humic substances with DOC of 2 – 10 mg/L based on the same applied contact time. The initial chlorine dose (i.e., 4.4 mg/l as Cl_2) was determined as the chlorine

production recorded at the outlet of the CEC cell under similar experimental conditions (200 mL/min, 150 mg/L NaCl, 10 mM phosphate buffer, 24 V) but in absence of NOM. For the conventional chlorination experiment (also carried out in 10 mM PB), the initial reading of Cl_2 residual was conducted 24 seconds after the addition of sodium hypochlorite to ensure the sample has the same contact time as that of the CEC treated sample (includes the time in the cell and the time from the CEC cell to the sampling point). Each NOM solution was then treated (electrolysis or sodium hypochlorite injection) separately and the chlorine residual was measured at different time intervals (i.e., from 0 to 120 minutes). **Figure 18** shows that the immediate chlorine demand (where Time 0 = 24 seconds) was significantly higher ($p < 0.0001$) in the CEC cell (EC1) than during conventional chlorination. The increase in chlorine demand with time followed the same trend suggesting a similar reaction mechanism might be involved between conventional chlorination and post-CEC treatment.

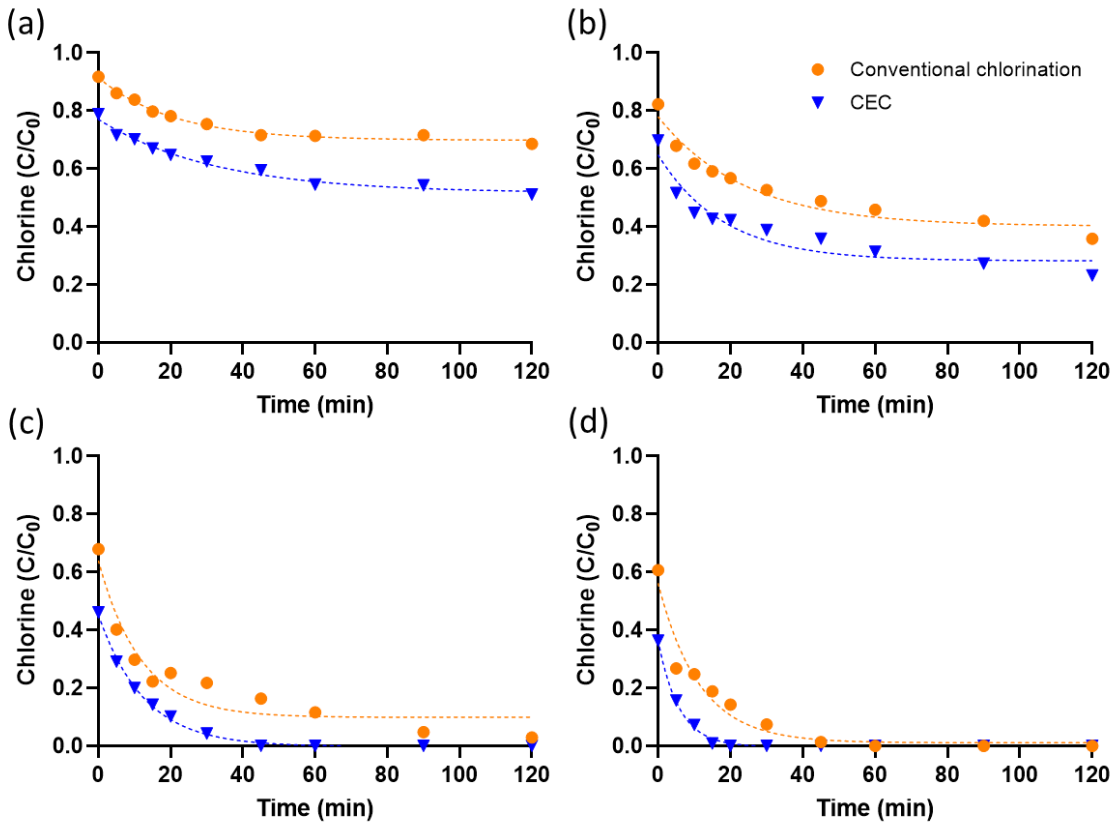


Figure 18 Chlorine demand of (a) 2 mg/L, (b) 4 mg/L, (c) 8 mg/L and (d) 10 mg/L DOC of NOM solutions (150 mg/L NaCl, 10 mM PB, pH 8) following CEC (200 mL/min, 24 V, EC1, continuous mode) and conventional chlorination. The initial chlorine dose was 4.4 mg/L. The CEC effluent was collected in a tube containing sodium thiosulfate solution. Conventional chlorination was also performed in 10 mM PB, the initial reading of Cl2 residual was conducted 24 seconds after the addition of sodium hypochlorite to ensure the sample has the same contact time as that of the CEC treated sample. The

measured chlorine concentration (C) were normalised against the initial chlorine concentration (C_0) of 4.4 mg/L as Cl_2. All the concentration ratios (C/C_0) were fitted with a pseudo-first-order kinetic decay.

The difference in immediate chlorine demand between the two chlorination condition increases with increasing NOM concentration (**Figure 19**). As expected, the higher the NOM concentration the higher the immediate chlorine demand. It was also observed that increasing the NOM concentration increased the difference between the two treatment conditions. Due to the very short contact time (24 seconds), the difference in immediate chlorine demand between the two treatments could be assigned to mixing difference (in situ generation of chlorine oxidants in the CEC reactor compared to dosing of sodium hypochlorite in a beaker containing a magnetic stirrer) since electrolysis experiments did not show any significant impact on UV_{254} absorbing moieties known as chlorine consumer sites.

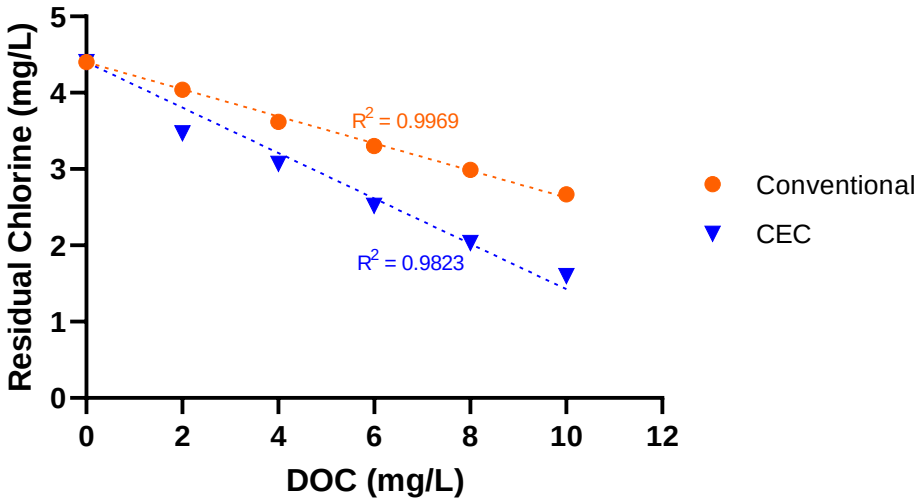


Figure 19 Residual chlorine concentrations for different DOC concentrations measured after 24 seconds of contact time with chlorine in 150 mg/L NaCl, 10 mM PB and pH 8 solutions, using EC1 in continuous mode.

2.3.3 Effect of voltage, flow rate and NaCl concentration on chlorine production in the absence of NOM using EC2

The impact of varying voltages, flowrates and NaCl concentrations on Cl_2 production was studied using 10 mM PB solutions at pH 7.2 with EC2. As observed previously with EC1 (**Figure 12 & Figure 13**), increasing voltage and NaCl concentration, increased the chlorine production of the cell, while an increasing flowrate decreased chlorine production linearly (**Figure 20a, b**). With increasing voltage from 6 V to 24 V (fixed flowrate of 100 mL/min and NaCl of 20 mg/L), the chloride to chlorine

production (mole/mole) increased from 7 % to 40 %. With increasing NaCl concentrations of 10 mg/L to 150 mg/L (fixed voltage of 6V and flowrate of 100 mL/min), the chloride to chlorine production remained constant at 7 %. With increasing flow rate (fixed voltage of 6 V), a decrease in chloride to chlorine production was observed. At 100 mL/min, 200 mL/min and 400 mL/min, 7 %, 3 % and 1 % of chloride was converted to chlorine, respectively.

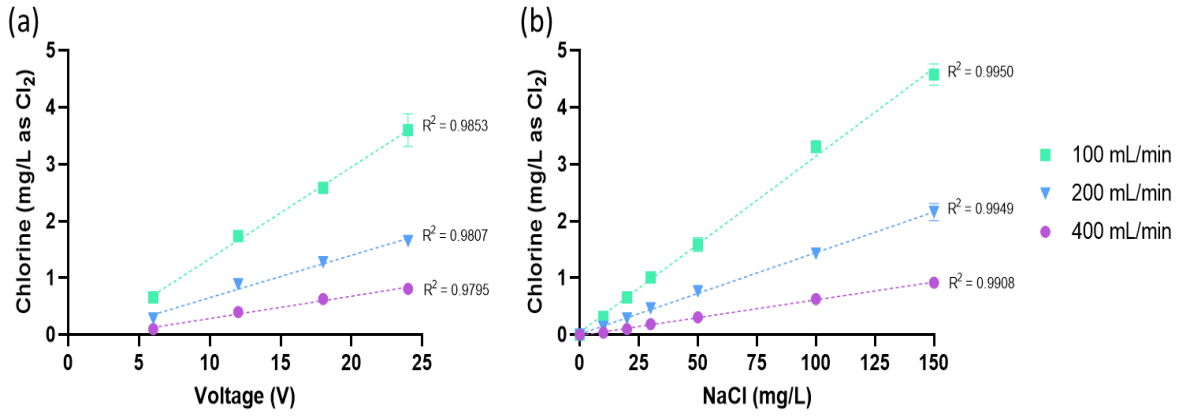


Figure 20 Effect of varying voltage (6 - 24 V), NaCl concentration (0 -150 mg/L) and flowrate (100 - 400 mL/min) on chlorine production in a 10 mM Phosphate buffer, pH 7.2 solution using EC2 in continuous mode. The impact of voltage on (a) chlorine production was carried out in 20 mg/L NaCl solutions. The impact of varying sodium chloride concentration on (b) chlorine production was carried out at a constant voltage of 6 V. The contact times (include the time in EC2 to the sampling tube) at flowrates of 100 mL/min, 200 mL/min and 400 mL/min were 6.6 s, 4.5 s and 3.4 s, respectively.

2.3.4 Pilot scale CEC Experiments

2.3.4.1 Effect of feed flowrate on Cl_2 production and inorganic by-products formation

The impact of varying the flowrate of the feedwater from the reticulated standpipe (Gwelup GWTP) on CEC performance was investigated by applying a constant voltage of 20 V. **Figure 21a** shows the relationship between the measured chlorine concentration, current (after both PUC1 and PUC2 treatment) and the flowrate of the feed water. The production of chlorine increased with a decrease of flowrate through the cell. The free chlorine to chloride ratio (mole/mole) ranged between 0.48 % to 1.50 % for PUC1 and 0.99 % to 2.87 % for PUC2 when flowrate varied from 7 m^3/h – 19 m^3/h. The inputs of PUC1 and PUC2 systems to the total chlorine production were very close to equal i.e., the total chlorine concentration increased by the same amount after PUC1 and PUC2 in the absence of significant chlorine consumers.

Irrespective of the feed flowrate, **Figure 21b** shows that PUC1 and PUC2 produced the same quantity of chlorine per hour (mass production of Cl_2 in g/h). **Figure 21c** shows the effect of different feedwater flowrates on the production of chlorate in electrolysed water after both PUC1 and PUC2. Chlorate was detected in the Gwelup pre-treated water (pre-chlorination step) at a concentration of 100 µg/L (presence could be due to the presence of chlorate in the commercial sodium hypochlorite). A slight production of chlorate was observed after treatment with PUC1 and PUC2. The final concentration decreased with increasing flowrate as observed for chlorine content (lower contact time). The free chlorine to chlorate formation (mole/mole) ranged between 0.65 % – 1.34 % for PUC1 and 0.35 % - 1.26 % for PUC2. Bromate and chlorite levels were below the detection limit (1.3 µg/L) of the IC analysis method.

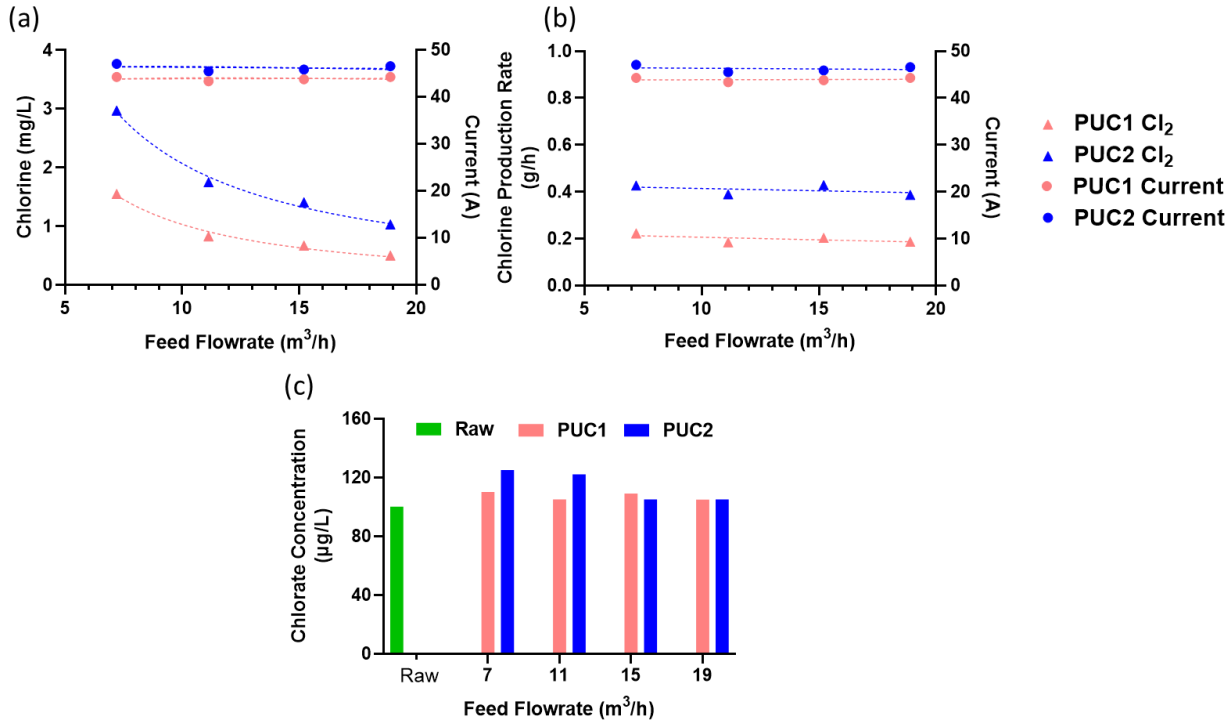


Figure 21 Impact of varying feed flowrate (7 m^3/h to 19 m^3/h) on (a) chlorine production, (b) mass chlorine production and (c) chlorate formation. A constant voltage of 20 V was applied in all experiments using Gwelup GWTP pre-treated water from a reticulated standpipe (140 mg/L Cl^-, 180 µg/L Br^-, 1.37 mg/L DOC, pH 6.7). *PUC1 = Pre-treatment cells and PUC2 = Post-treatment cells.*

2.3.4.2 Effect of voltage on Cl_2 production and inorganic by-products formation

The impact of varying voltages on the pilot unit was studied by applying a constant flowrate of 20 m^3/h. **Figure 22a** shows the effect of voltage across the cell on the production rate of chlorine and concentration of chlorine in the treated water. Both the rate of chlorine production and the concentration of chlorine in the treated water increased linearly with an increase in the voltage

applied. The inputs from PUC1 and PUC2 to the final total chlorine content were equal. The measured chlorine concentration from PUC2 was always double that of PUC1. This is because chloride containing water flows through PUC1 first then through PUC2. Chloride was oxidised to chlorine by PUC1 and by PUC2. PUC2 produced the same amount of chlorine as PUC1 but when measuring the chlorine from the sample point after PUC2, it contains chlorine generated by PUC1 and also by PUC2. With increasing voltage, the free chlorine to chloride ratio ranged from 0.1 % - 1.2 % for PUC1 and from 0.2 % - 2.5 % for PUC2.

Figure 22b shows the effect of changing the voltage on the current applied to the cells. The current (for both PUC1 and PUC2) increased linearly with an increase in the voltage applied.

Figure 22c shows the effect of applied voltages to the system on the concentration of chlorate in the electrolysed water. For voltages lower than 24 V, the chlorate content remained almost constant and equal to the concentration recorded in the pre-treated water that was sourced from the reticulated standpipe (results in accordance to **Figure 21c**). At higher voltages (30 and 36 V) a greater formation of chlorate was detected. The highest chlorate formation (150 µg/L) was recorded at 36 V after PUC2. With increasing voltage, the formation increased from 0.9 % to 2.2 % after PUC1 and from 0.5 % to 2.7 % after PUC2. Chlorite and bromate levels were below the limit of detection (1.3 µg/L) in all cases.

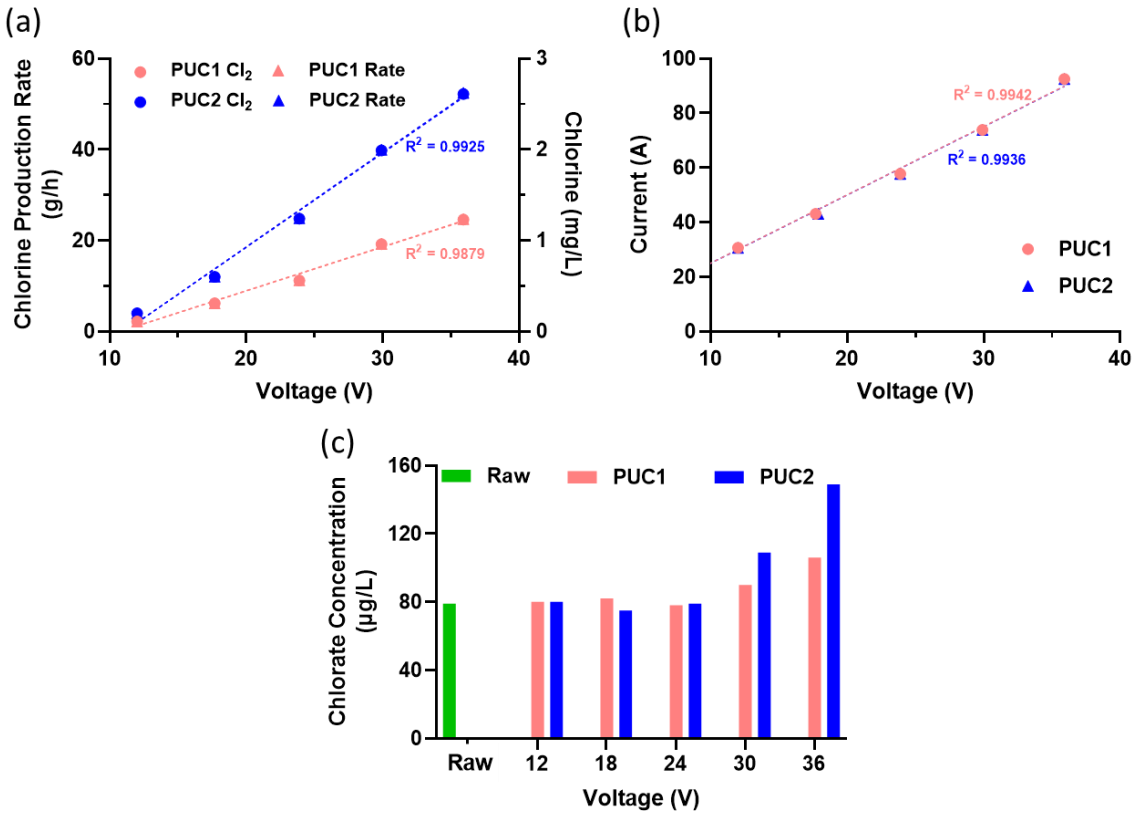


Figure 22 Impact of varying voltages (12 V - 36 V) on (a) chlorine and mass chlorine production, (b) current generated and (c) chlorate formation. A constant feed flowrate of 20 m^3/h was applied in all experiments using Gwelup GWTP pre-treated water from a reticulated standpipe (140 mg/L Cl^-, 180 µg/L Br^-, 1.37 mg/L DOC, pH 6.7). *PUC1 = Pre-treatment cells and PUC2 = Post-treatment cells.*

2.3.4.3 Effect of NaCl concentration on Cl_2 production and inorganic by-products formation

The influence of varying chloride concentrations entering the CEC cells on chlorine production was studied by injecting a 164 g/L brine (NaCl) solution into the feed water at varying flowrates using a dosing pump. **Figure 23a** shows the effect of different chloride concentrations in the feed water on the production rate of chlorine and the chlorine concentration of the CEC treated water in the pilot unit when the voltage across the cells remained constant. As expected, increasing the chloride concentration in the feed water led to the increase of concentration of chlorine in the treated water (linear increase, similar behaviour for both PUC1 and PUC2) and therefore an increase in the rate of chlorine production (doubled at PUC2 as compared to PUC1). For all the applied chloride concentrations, the free chlorine to chloride ratio after PUC1 was about 0.5 % and after PUC2 about 1.0 %.

With more electrolyte (higher conductivity) and under the same voltage a greater current is transferred; a progressive increase in electrical current was measured (**Figure 23b**).

Figure 23c shows a slight increase in the concentration of chlorate in the electrolysed water (as compared to the background concentration) with increasing chloride concentration in the feed water; however, a small fraction of the added chloride is converted to chlorate. The background chlorate concentration is due to the use of pre-treated water from the reticulated standpipe in this experiment. However, it is also possible that chlorate is further oxidised to perchlorate (Yoon et al., 2015). The chlorate concentration is, in general, higher after PUC2 than after PUC1. For the highest studied chloride concentrations (278 and 303 mg/L) the increase in chlorate content after PUC2 was double the increase observed after PUC1 (40 µg/L and 20 µg/L, respectively). Furthermore, for chloride concentrations greater than 176 mg/L, the free chlorine to chlorate formation was 1 % after both PUC1 and PUC2. Overall, the chlorate production remained very low (≤ 148 µg/L) and the final concentrations are well below the WHO recommended threshold concentration for drinking water (700 µg/L). The concentrations of chlorite and bromate were below the limit of detection (1.3 µg/L) in all samples.

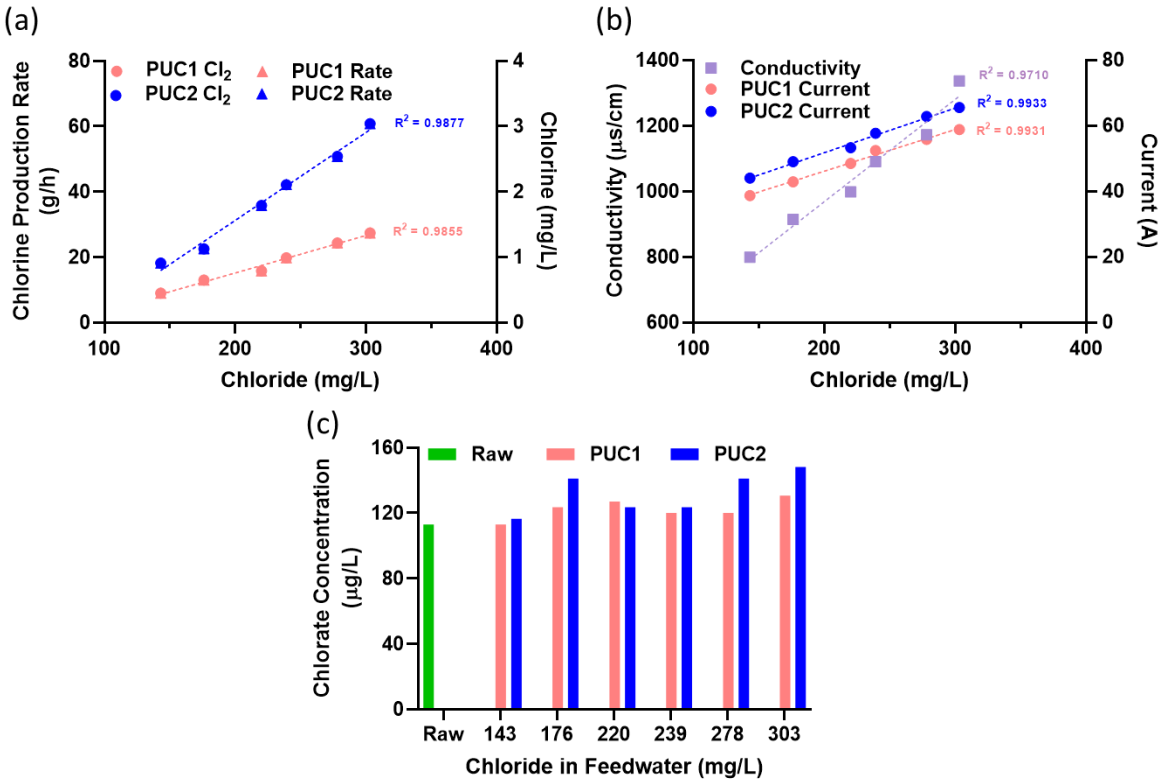


Figure 23 Impact of varying chloride concentrations on (a) chlorine and mass chlorine production, (b) conductivity and current generated and (c) chlorate formation. A brine solution of 164 g/L NaCl was injected in the feedwater via a dosing pump at different flowrates between 0 and 30 L/h in increments of 6 L/h. A constant feed flowrate of 20 m^3/h and a constant voltage of 18 V was applied in all experiments using Gwelup GWTP pre-treated water from a reticulated standpipe (140 mg/L

Cl^-, 180 µg/L Br^-, 1.37 mg/L DOC, pH 6.7). ***PUC1 = Pre-treatment cells and PUC2 = Post-treatment cells.***

2.3.4.4 Effect of DOC on Cl_2 production, disinfection and inorganic by-products formation

The effect of increasing DOC concentration in the feedwater on CEC treatment was investigated by injecting 11 g/L as C of a MIEX brine (VSEP treated to remove a large part of the salt) into the pilot unit to vary the DOC concentrations from 2.35 - 4.25 mg/L. **Figure 24a** shows the chlorine residual for the first 7 days post-CEC treatment of Gwelup GWTP pre-treated water. The initial chlorine concentration of the different waters was not affected by the increase in DOC content, however and as expected, the higher the DOC content, the higher the chlorine demand was over time.

Figure 24b, **c** and **d** show the formation of adsorbable organic chloride (AOCl) and bromide (AOBr), THMs and HANs after 7-day chlorine contact time, respectively. The higher the DOC concentration, the higher the AOCl formation. The amount of AOBr produced was lower than AOCl and remains relatively constant for the three different feed waters tested. For THMS, the three brominated species (Bromodichloromethane, dibromochloromethane and tribromomethane) were observed in addition to chloroform (trichloromethane), with chloroform remaining the predominant species for all three feed waters tested. Increasing DOC content mainly led to an increase in trichloromethane and bromodichloromethane; the production of dibromochloromethane and tribromomethane remained relatively constant. On average, THMs incorporated 50% of the AOCl and 70% of the AOBr generated (data not shown); result that is commonly observed from the chlorination of bromide containing surface waters (Yoon et al., 2015). For haloacetonitriles (HANs), dichloroacetonitrile (DCAN), bromochloroacetonitrile (BCAN), bromoacetonitrile (BAN) and dibromoacetonitrile (DBAN) were detected. The production of HANs was significantly lower that the production of THMs. As noticed for THMs, the chlorine containing species are more abundant than the bromine containing species. Again, the higher the DOC the higher the HANs formation.

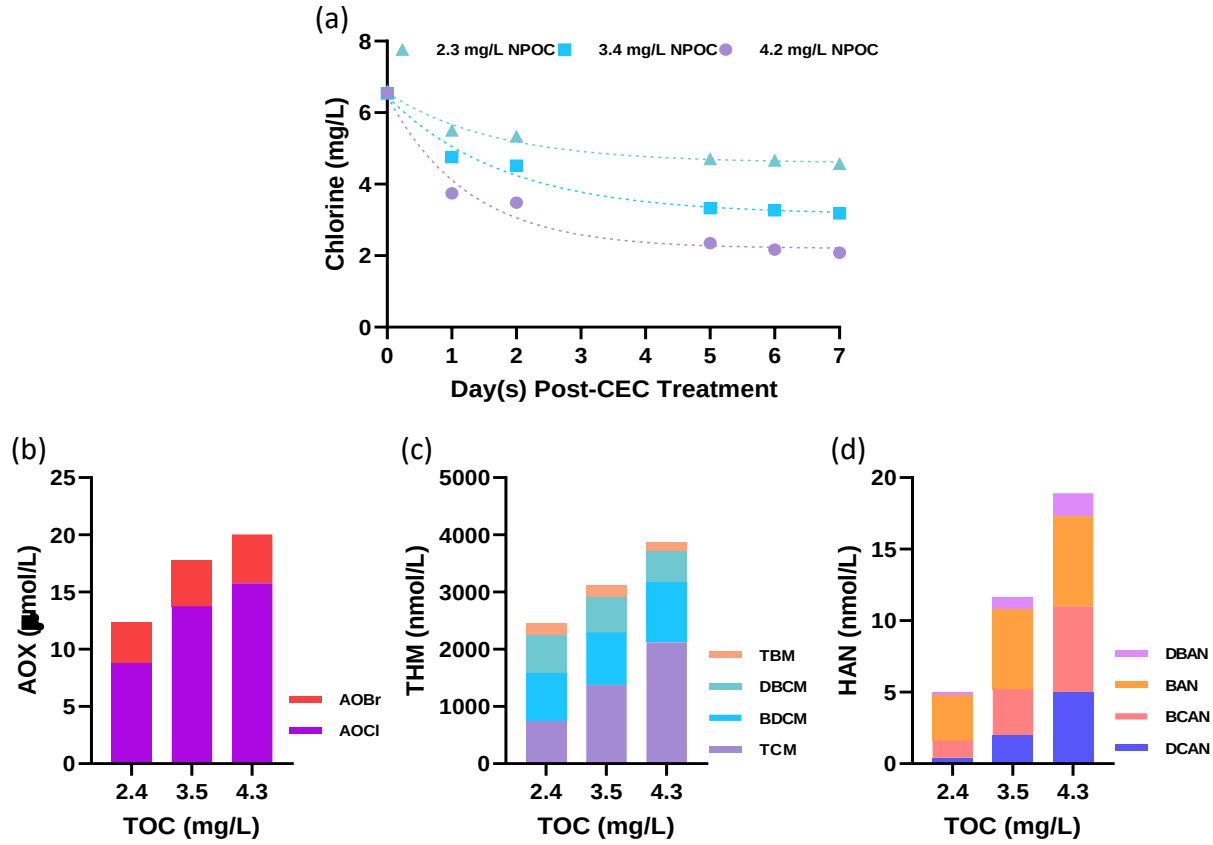


Figure 24 Impact of 2.35 - 4.25 mg/L dissolved organic carbon on (a) chlorine demand over 7 days, (b) Adsorbable Organic Halide formation, (c) Trihalomethanes formation and (d) Haloacetonitriles formation after 7 days of chlorine contact time following the electrolysis of Gwelup GWTP treated water (flowrate 9.5 m^3/h, Pre-treatment: 35.7 volts/77 Amps, Post-treatment: 35.9 volts/90 Amps, 140 mg/L Cl^-, 180 µg/L Br^-, pH 6.7).

2.3.4.5 Effect of chloride and feed flowrate on hydrogen gas production

The hydrogen produced during the operation of the pilot unit (flowrates: 5 and 10 m^3/h; 80 Amps for PUC1 and PUC2) with bore W257 with chloride addition, i.e., 35 and 300 mg/L Cl^-) was determined at the outlet of PUC2 using the water displacement method. The flowrate (L/s) of water from the PUC2 sampling point was determined by measuring the amount of time taken to fill a 1 L volumetric cylinder. The gas produced was collected in gas sampling bags (in duplicates) connected to the top of the volumetric flask (**Figure 10**) and brought to ChemCentre (Perth, Australia) for the analysis of its composition.

The gas produced during electrolysis was mainly composed of H_2, N_2 and O_2 (Table 5**Table 5**). Results obtained with the duplicates are comparable showing that our experimental protocol was repeatable. Interestingly, at higher flowrate the measurable production of gas as volume of gas per volume of

water is much lower than at low flowrate, observation that is independent of the concentration of chloride. Furthermore, the gas composition was relatively constant for the different experimental conditions applied with 40-45% H_2, 35-40% of N_2 and 15-25% O_2. A different composition with lower H_2 content and higher N_2 content was found for the low chloride (35 mg/L) and high flowrate (10 m^3/h) condition.

Table 5 Gas composition produced during chlorine production by the pilot unit

Chloride (mg/L)	Feed flowrate (m^3/h)	mL gas produced /L water	Gas composition (%)				
			CO2	CO	H2	N2	O2
35	5.2	1180	0.7	<0.2	43	33	23
35	5.2	1128	0.7	<0.2	40	35	24
35	10	91	0.3	<0.2	15	61	23
35	10	95.2	0.3	<0.2	24	54	22
300	5.32	960	4	<0.2	41	35	23
300	5.32	996	0.4	<0.2	32	43	25
300	10	102.9	0.4	<0.2	44	40	15
300	10	100.1	0.3	<0.2	48	37	14

It is important to note that by the end of this project, the pilot unit treated a range of different ground and surface waters, totalling approximately 25 ML of water and no scaling was observed at the cathode. Similarly, with the lab scale units (EC1 and EC2), no scaling was observed.

2.4 Discussion

Electrochlorination has emerged as an alternative to conventional chlorination primarily because it is an *in-situ* generator of active chlorine species. However, to our knowledge, the effects of varying flowrate, voltage, NaCl concentration, DOC, sulfate and bicarbonate salts, and bromide on chlorine production, disinfection by-products and inorganic by-products formation have not been investigated on electrochemical cells involving Titanium electrodes coated with a MMO layer of Ti/Rh/Ru.

Irrespective of the mode of operation (continuous/in-line electrolysis or recirculating), for a given voltage and NaCl concentration, chlorine production in mg/L was inversely proportional to flowrate from the lab units (EC1 and EC2) in the absence of NOM (**Figure 12 & Figure 20**). This is because a greater retention time in the cells allow more chloride ions to be converted to chlorine. Overall, a lower flowrate increases chlorine production (in mg/L).

Similarly, with the pilot unit, the chlorine production of PUC1 and PUC2 increased with decreasing flowrates (**Figure 21a**). However, the amount of chlorine (mg/L) generated by PUC1 and PUC2 were almost the same with increasing flowrates in the absence of significant chlorine consumers from the Gwelup GWTP pre-treated water (DOC: 1.37 mg/L). Furthermore, regardless of the feed flowrate applied, the mass production of chlorine (g/h) were the same for both PUC1 and PUC2 (**Figure 21b**). This indicates that the process is mass transfer limited and not diffusion limited under our flowrate condition i.e., chlorine formation was constant. Increasing the feed flowrate induced greater dilution of the chlorine produced and therefore a smaller concentration of chlorine was measured at the outlet. This suggests that any difference in chlorine production cannot be attributed to a change in the energy conditions in the cell.

Chlorine production and current measured were directly proportional to the voltages applied on the lab unit regardless of the mode of operation and the presence of NOM, for a given flowrate and NaCl concentration (**Figure 13 & Figure 20**). It is well known that the voltage applied is one of the main driving force of an electrolytic cell, the higher the voltage, the greater the current passing through the cell (Ohm's law). A greater current leads to the production of more chlorine as observed in this study.

Likewise, with the pilot unit, the concentration of chlorine (mg/L), the rate of chlorine production (g/h) and current measured in Gwelup GWTP treated water also increased linearly with an increase in the voltage applied (**Figure 22a & b**). The measured chlorine concentration after PUC2 was always double that after PUC1. This indicates that in the absence of significant chlorine consumers, both PUC1 and PUC2 are able to produce the same amount of chlorine when a specific feed flowrate is used.

Increasing NaCl concentration increased chlorine production as well as the current measured, irrespective of the mode of operation applied on the lab and pilot unit, for a given voltage and flowrate (**Figure 13**, **Figure 20** & **Figure 23a,b**). This was expected as with more ions in solution, the electrolyte exerts a greater capacity for carrying the current. With a greater current the oxidation of chloride to chlorine is expected to be greater as shown and explained above, by increasing the voltage and thereby current. Likewise, the electrolysis of similar molar concentrations of sodium sulfate and sodium bicarbonate in the presence of 150 mg/L NaCl generated higher currents (0.083 A and 0.085 A) than a solution containing only 150 mg/L NaCl (0.04 A). However, chlorine production increased in the presence of sodium bicarbonate but decreased in the presence of sodium sulfate compared to the 150 mg/L NaCl solution when the pH was controlled at pH 6 (**Figure 14**).

The electrolysis of sodium sulfate in the presence of chloride ions has shown to decrease the average chlorine production by 29 %. The adsorption of sulfate anions, having a stronger affinity for electrode surface functional sites, is known to slow down the rate of formation of HOCl (Trasatti, 1987).

Therefore, the decrease in chlorine production observed is more likely due to the competition at the anode for the oxidation of sulfate ions (Amikam, Nativ, & Gendel, 2018) or the complexing of sulfate ions on the surface of the anode might be limiting the number of electrode surface sites accessible for the direct oxidation of chloride ions to chlorine (Karlsson & Cornell, 2016).

The electrolysis of sodium bicarbonate in the presence of chloride ions has shown to increase the average chlorine production by 41 %. Until date, no study has been done to show the effects of bicarbonate salts on chlorine production using titanium electrodes coated with a Ti/Rh/Ru oxide layer. In the absence of electrochemically generated hydroxyl radicals by the electrodes used in this study, the increase in chlorine production is likely due to the increase in conductivity of the solution. Further studies to investigate the impact of varying pH and cyclic voltammetry analysis in phosphate buffer solutions have to be performed to confirm the effect of bicarbonate and sulfate ions on the electrochemical generation of chlorine.

Increasing the feed flowrate of the pilot unit, slightly decreased chlorate production after PUC1 and PUC2 treatment using Gwelup GWTP pre-treated water (**Figure 21c**). As observed for chlorine production (**Figure 21a**), increasing the feed flowrate induced greater dilution of the chlorate produced and therefore a smaller concentration of chlorate was measured at the outlet. In the presence of chloride as an electrolyte during electrolysis, chlorate can be produced as a by-product, in addition of the electrochemically generated oxidant, active chlorine (Yoon et al., 2015). The highest concentration of chlorate formation was recorded after PUC2, 125 µg/L and 122 µg/L at low flowrates of 7 m^3/h and 11 m^3/h, respectively. This indicates that operating the pilot unit at a low flowrates (< 15.2 m^3/h) will increase the formation of chlorate.

Applying voltages lower than 24 V on the pilot unit kept the chlorate concentration almost the same as the concentration measured in the source water (Gwelup GWTP pre-treated water from the reticulated standpipe) while at higher voltages (30 and 36 V) a greater concentration of chlorate was observed (**Figure 22c**). In order to evaluate the operating conditions of the pilot (mainly) and lab units, the concentration ratio of free chlorine to chlorate formation during electrolysis was calculated. The concentration ratio of free chlorine to chlorate formation exhibited the same trend as observed with the formation of free chlorine with increasing voltages. The highest concentration ratios recorded were 2.2 % and 2.7 % after PUC1 and PUC2, respectively at the highest voltage (36 V) applied. This means in order to limit chlorate production, ≤ 24 V can be applied. Therefore, depending on targeted chlorine residual of the water treatment plant, it is crucial to consider the voltage applied.

Increasing the NaCl concentration on the lab unit increased the formation of chlorate linearly (**Figure 15**) while on the pilot unit; only a slight increase was observed (**Figure 23c**). The linear increase in

chlorate formation for the lab scale can be attributed to the absence of NOM (only MilliQ and NaCl in the solution) and the increased chlorine production and therefore, favoured further oxidation of $HOCl/OCl^-$ to form chlorate. However, with increasing NaCl concentrations, for both the lab and pilot (PUC1 and PUC2) unit, the concentration ratios of free chlorine to chlorate formation were almost constant, 0.4 % and 1.0 %, respectively.

In the absence of hydroxyl radicals, the formation of chlorate during electrolysis in this study can be via two possible pathways. The direct oxidation of chloride ions into chlorate and/or the direct oxidation of the electrochemically generated free chlorine ($HOCl/OCl^-$) into chlorate (Yoon et al., 2015). The chlorate formed might have been further oxidised by direct oxidation to generate perchlorate (ClO_4^-) (Yoon et al., 2015), however, the concentration of perchlorate was not measured in this study. Further studies have to be done in the presence of sodium thiosulfate to quench any free chlorine generated to confirm if chloride ions are directly oxidised to chlorate and if chlorate is further oxidised to form perchlorate to allow the completion of chlorine atom mass balance.

Overall, even at the lowest flowrate, the highest voltage and the highest NaCl concentration applied, the chlorate concentrations were well below the WHO guidelines of 700 µg/L. The fact that bromate and chlorite were below the detection limit of the IC analysis (1.3 µg/L), means the levels of these inorganic by-products were also well below the limit of the ADWG (bromate: 0.02 mg/L and chlorite: 0.80 mg/L) (NHMRC, 2011).

Bromine production was much lower than chlorine production (expressed as mole of Cl_2/L) when using the same molar concentration (i.e., 2.56 mmol/L) while a slightly higher production of oxidant was observed when a solution of 150 mg/L NaCl and 264 mg/L NaBr was used compared to the electrolysis of only 150 mg/L NaCl (**Figure 16**). This shows that in the presence of bromide ions, HOCl generated is rapidly converted to HOBr by competitive replacement of the halogen ion (Jong, 2014). However, the fact that after 30 min of electrolysis (NaCl solution only compared to NaBr solution only), the number of moles of chlorine + chlorate (0.21 mM + 0.0008 mM) produced was more than that of bromine + bromate (0.06 mM + 0.0222 mM) shows that the oxidation of chloride and bromide have different yields and the production of less bromine is not compensated by the conversion to bromate. This means at the voltage applied in this experiment (12 V), the difference in yield might be due to a diffusion phenomenon (rate of diffusion of chlorine gas or bromine gas formed on the surface of the anode in the electrolyte to form aqueous HOCl and HOBr and the further conversion of HOCl to form HOBr by competitive replacement). Therefore, it is very important to consider the concentration of bromide ions present in the water being treated with CEC when upscaling the experiments to pilot unit testing in order to limit the production of bromate but also other brominated DBPs which are

more toxic and carcinogenic than their chlorinated analogues (Liu & Zhang, 2014; Plewa et al., 2002; Richardson et al., 2007).

The immediate chlorine demand (24 seconds: includes 14 s in the cell and 10 s from the CEC cell to the sampling point- actual contact time with the electrodes is 6 s) of varying concentrations of NOM solutions (DOC = 2 to 10 mg/L) was significantly higher ($p < 0.0001$) in the lab unit than during conventional chlorination based on the same applied chlorine dose (4.4 mg/L as Cl_2) (**Figure 18**). The immediate chlorine demand for chemical and electrochemical oxidation were compared in an equal manner with initial chlorine concentration of 4.4 mg/L for both treatments as explained in detailed in section **2.2.2.1.2.2**. Increasing DOC concentration increased the chlorine demand but also the difference in immediate chlorine demand between the two treatments (**Figure 19**). DOC reacts with chemical oxidants (such as $HOCl/OCl^-$) and accelerates their consumption (von Gunten, 2003; Wert, Rosario-Ortiz, & Snyder, 2009). The increase in chlorine demand with time followed the same trend suggesting a similar reaction mechanism might be involved after CEC and conventional chlorination treatment. The difference in immediate chlorine demand between CEC and conventional chlorination might be due to the difference in mixing conditions i.e. mixing was faster with CEC than with the introduction of the sodium hypochlorite solution. Another possible explanation could be as a consequence of direct electrooxidation, which might have slightly enhanced the reactivity of the dissolved organic matter (DOM) with chlorine (not observed by UV measurement). For instance, low doses of ozone (for pre-treatment) can enhance the DBP formation (e.g. chloral hydrate, Haloketones and trichloronitromethanes) by changing the NOM characteristics forming transformations products that react with chlorine species to form DBPs after subsequent chlorination (De Vera et al., 2015; Yang et al., 2012).

The difference might also be due to competition between chloride ions and phenolic compounds oxidation at the anode. Phenol is taken as an example since it is an important constituent of DOM and studies investigating electrooxidation of NOM focused mainly on phenolic moieties (Abou-Taleb, Hellal, & Kamal, 2021; Li et al., 2019). Phenolic compounds are known to undergo electrochemical oxidation on electrochemical advanced oxidation processes (EAOPs) electrodes (Chaplin, 2014). In the absence of hydroxyl radicals, two reaction pathways are feasible. Phenols mainly oxidise via a direct electron transfer (DET) mechanism to form phenoxy radicals at anodic potential > 0.5 V versus SHE. The phenoxy radicals readily undergo polymerisation via C-C coupling or ether linkages to form a polymer layer around the anode. The second pathway is through a second DET mechanism that converts the phenoxy radicals into phenoxium ion. The phenoxium ion is quickly converted to 1,4 benzoquinone through nucleophilic attack of water (Chaplin, 2014). However, the fact that the chlorine demand for both treatments with time follows the same trend suggests that similar reaction

mechanism, reaction of DOC with HOCl/OCl^-, are involved in the solution after electrolysis. In order to determine the exact reaction mechanism taking place, the analyses should be extended to DBPs formation (more specific than chlorine demand) and analysis of organics on the surface of the electrodes.

The formation of absorbable organic chloride (AOCl) in the presence of 2.68 mg/L of DOC, were similar for CT values below 200 mg.min/L but lower for CT values greater than 600 mg.min/L for CEC (recirculating mode, 150 mg/L NaCl, 12 V) compared to conventional chlorination (**Figure 17**). The decrease in AOCl formation observed with CEC for CT values > 600 mg.min/L can be due to either degassing of the volatile DBPs during recirculation or due to the change in DBP precursors. AEOPs are known to oxidise DOM which consist of several reactive moieties such as phenols and aliphatic acids via DET mechanism (Chaplin, 2014). Even though each of these moieties react with oxidants differently, their oxidation via DET mechanism can minimise AOCl precursor formation (de Vera, Gernjak, & Radjenovic, 2017). Then, the oxidation of phenols should have changed the UV absorbance (254 nm) but the absorbance of CEC treated NOM solutions did not change in this study (**Table 4**). Electrochlorination of organic matter in solution using MMO-stainless steel electrodes (active electrodes) show an increase in UV_{254} absorbing species such as aromatic rings at pH 6.5 (current density of 10 mA/cm^2), whereby the organic load of the synthetic water was reduced. However, the remaining organic matter had a higher ratio of aromatic character than in the initial state (Rathod et al., 2021). Previous studies also concluded that aromaticity does not correlate with the increase of humic substances but was rather an indicator of the fraction of aromatic character and the general relation to the formation of DBPs (Edzwald, Becker, & Wattier, 1985). This suggests that CEC treatment might have oxidised phenolic structures but not to a significant way that is measurable by UV absorbance.

Similarly, the pilot unit followed the same trend, increasing the DOC content increased the chloride demand over time (**Figure 24a**). However, the initial chlorine concentration of the different waters was not affected by the increase in DOC content and this might be due to the very short contact time (0.28 seconds with the electrodes) in the electrolysis cells. In the presence of bromide ions (180 µg/L), AOBr was produced but in lower concentration than AOCl, and remained relatively constant with increasing DOC concentrations. The higher the DOC concentration, the higher the THMs and HANs formation (**Figure 24c & d**). With increasing DOC concentration, the chlorine containing species (trichloromethane - predominant species, bromodichloromethane, dichloroacetonitrile and bromochloroacetonitrile) increased. On average, THMs incorporated 50% of the AOCl and 70% of the AOBr generated.

The DBPs formation results from the pilot unit are similar with previous studies. Chlorinated and brominated DBPs formation increase with increasing DOC concentration due to the availability of more organic sites (Neale & Leusch, 2019). The extent and speciation of DBPs formation depend on the competition between chlorine and bromine reaction with NOM. Generally, the reaction of aqueous bromine with NOM is much faster than with chlorine (Acero, Piriou, & Von Gunten, 2005; Westerhoff, Chao, & Mash, 2004). In the presence of a low bromine to chlorine ratio, HOCl can outcompete HOBr to react with NOM, thereby producing less brominated DBPs (Bond et al., 2014; Tan et al., 2016). Bromine substitution in THMs follows the same trend (Hua, Reckhow, & Kim, 2006). Considering brominated DBPs have more adverse health effects compared to chlorinated-DBPs (Richardson et al., 2007), the chlorine residual in Australia (2-3 mg/L) tends to be higher than other countries at comparable bromide concentration in order to minimise the production of brominated DBPs.

The amount of hydrogen gas (15-48 %) generated from the electrolysis of Wanneroo GWTP ground water was well below the upper explosive limit for hydrogen in air, irrespective of the chloride content and feed flowrate applied (**Table 5**).The production of hydrogen is one of the major cathodic processes that occurs during the electrolysis of water (Scott, 2020). Due to its explosive nature and in order to better understand the degassing requirements for a CEC system, the evolution of hydrogen gas was studied. The explosive range for hydrogen in air, given by the Lower Explosive limit (LEL) is 4% and the Upper Explosive Limit (UEL) is 75%. Outside of this range the gas mixture is either to rich or too lean to burn. The explosive range for hydrogen in oxygen, given by the Lower Explosive Limit (LEL) is 4% and the Upper Explosive Limit (UEL) is 94% (Yaws). In a hypothetical system, if the treated water is fed straight into a closed environment, the gaseous mixture does not present as a hazard further upstream in the treatment process. Therefore, no specific degassing requirement is needed following the electrolysis of ground water under the conditions applied in this study.

2.5 Conclusion

This study indicates that increasing voltage and NaCl concentration increased chlorine production while an increase in flowrate decreased chlorine production on the lab scale CEC. The same trend was observed with the pilot unit however, the mass production of chlorine (g/h) increased with increasing voltage and NaCl concentration but was unaffected with varying feed flowrates indicating the process is mass transfer limited and not diffusion limited. Increasing the conductivity (increasing current for similar voltage) generally increase chlorine production. However, different anions have different impacts. For the most commonly found, opposite trend was observed for bicarbonate and sulfate. The decrease in chlorine production in the presence of sulfate anions could be due to the interaction of sulfate on the electrode surface. Bromine was found to be less reactive than chlorine with DSA cell

and this could be due to difference in diffusion properties. Direct electrolysis might be having an impact on DOM, enhancing its reactivity with chlorine. More studies have to be done to confirm this hypothesis. Chlorate production increased with increasing voltage and NaCl concentration but decreased with increasing flowrate. However, the chlorate concentrations measured were well below the WHO guidelines. Bromate and chlorite were below the detection limit of the IC analysis, therefore were also well below the limit of the ADWG. Increasing DOC concentration increased the chlorine demand for both the pilot unit and the lab unit. With the pilot unit, increasing DOC concentration increased AOCl, THMs and HANs concentrations. The predominant THM species was trichloromethane. Mostly chlorine containing DBPs increased with increasing DOC content. Overall, the results of the lab scale unit reflect the results obtained with the pilot unit.

Chapter 3. Impact of electrochemically generated oxidants by CEC on bacteria and bacteriophage.

3.1 Introduction

Electrochemical disinfection mechanisms are: direct oxidation at the surface of the electrode- leading to the instantaneous inactivation of bacteria or by direct electron transfer/electric field (Jeong et al., 2007), and indirect oxidation by oxidants generated from either a chloride-containing solution or from the hydrolysis of water (described in more details in section **1.2.1**). The high disinfection efficacy of electrochemical disinfection can be ascribed to the synergistic effects of direct oxidation on the surface of electrode (Grahl & Märkl, 1996; Matsunaga et al., 2000), indirect oxidation by active chlorine species, free radicals (ROS and $Cl^{\cdot}$) and H_2O_2 (Bergmann, 2010; Diao et al., 2004; Feng et al., 2004; Liang et al., 2005), and the electric field effect (Butterfield et al., 1997; Grahl & Märkl, 1996). However, as previously mentioned in Chapter 2, the electrodes of the laboratory unit used in this study do not produce radicals during electrolysis (Curtin Water Quality Research Centre, Personal communication, 2018).

The electrochemical inactivation of bacteria and yeast cells are well known (Davis et al., 1994; Gášková et al., 1996; Grahl & Märkl, 1996; Matsunaga et al., 1992; Patermarakis & Fountoukidis, 1990; Tokuda & Nakanishi, 1995; Velizarov, 1999). There are numerous mechanisms that describe the lethal effects of microorganisms following electrochemical exposure, such as oxidative stress and cell death due to the electrochemical oxidants generated, irreversible permeabilisation of the cell membranes due to the electric field applied (principle for the process of electroporation), and electrochemical oxidation of vital cellular components when subjected to induced electric fields and electric current (described in more detail in section **1.2.3**).

While numerous studies have focused on the EC inactivation of bacteria and yeast, little research has been conducted on the EC inactivation of viruses. EC disinfection can result in damage to multiple vital functions of viruses. A virus is infective when it is able to carry out its vital functions: binding to its host, injecting its genome into the host and replicating its genome within the host. Severe disruptions to one or more of the three vital functions by disinfectants lead to inactivation of the virus (Wigginton et al., 2012) (described in more details in section **1.2.3**).

Flow cytometry (FC) is emerging as a valuable alternative to the traditional use of heterotrophic plate count (HPC) for microbial water quality assessment (Van Nevel et al., 2017) mainly because HPC is unable to detect bacteria in the viable but non-culturable (VBNC) state. Under different stresses, bacteria enter into the VBNC state where they can no longer form colonies on solid media (James D. Oliver, 2000; Xu et al., 1982). FC is a cultivation-free method that detects bacteria irrespective of their culturability. The combination of FC and fluorescent stains such as SYBR Green I and propidium iodide (PI) have been used to assess the impact of disinfectants on cellular membrane integrity and to

investigate the disinfection mechanisms of bacteria in water treatment processes (Safford & Bischel, 2019; Van Nevel et al., 2017). The combination of these two dyes followed by FC analysis enables the differentiation between intact and membrane damaged cells (Van Nevel et al., 2017) (described in more detail in section **1.5**).

In this study, we investigated the inactivation efficiency of CEC on *E. coli*, *B. subtilis*, *S. maltophilia* (a multi-drug resistant strain), *L. pneumophila* (serogroup-1 Philadelphia strain), phage T4 and MS2 in a chloride containing electrolyte and in continuous mode (very short contact time of 4.5 seconds). Flow cytometric analysis combining with SYBR Green I (cell-permeant) and SYTOX Orange (cell-impermeant) in parallel with plate counts were used to investigate the effects of CEC-generated chlorine on *E. coli*, *B. subtilis* and *S. maltophilia* in continuous mode. We also investigated the inactivation efficiency of CEC on *E. coli* in a chloride-free electrolyte when the CEC is operated in continuous and recirculating modes. Lastly, we compared the inactivation efficiency of CEC in continuous mode using a chloride-containing solution with conventional chlorination (sodium hypochlorite solution) based on the same contact times on an *E. coli* suspension.

3.2 Materials and methods

3.2.1 Bacterial culture and growth conditions

E. coli B and *B. subtilis* (ATCC 6633) strains were obtained from the School of Pharmacy and Biomedical Sciences of Curtin University. *S. maltophilia* AB550, a multi drug resistant strain was isolated in Western Australia (Glady-Croue et al., 2018). A loop of pure bacterial culture maintained at -80 °C in Luria Bertani (LB) broth (Tryptone 10.0 g/L, Sodium chloride 5.0 g/L and Yeast extract 5.0 g/L) and 10 % dimethyl sulfoxide (DMSO) (Chem-Supply, DA013-500M) was spread onto an LB agar (LB broth formulation + agar 16 g/L) plate and incubated overnight at 37 °C. For each experiment, an isolated colony of was picked from a fresh LB plate to inoculate 20 mL of diluted, sterilised LB (LB diluted 1:3 using filter (0.22 µm filters, Millex-syringe filter unit, Merck, Millipore) sterilised Milli-Q® water). The inoculum was then incubated at 37 °C for 20 hours in a shaking incubator at 170 rpm to reach the stationary growth phase. After 20 hours of incubation, the cells were harvested by centrifugation (Allegra® X-12R, Beckman Coulter) at 3270 × g for 10 min. The supernatant was carefully discarded and the pellet was resuspended in 10 mL of 10 mM sterilised phosphate buffer (PB) (1.431 g of sodium phosphate dibasic heptahydrate (Sigma, S9390) and 0.644 g of sodium phosphate monobasic monohydrate) at pH 6.9 (background electrolyte). The resuspended cells were centrifuged, the supernatant discarded and pellet resuspended in 10 mM PB for another two times to remove as much growth media (LB) to avoid the chlorine demand from LB. The final cell suspension was then adjusted to 0.5 McFarland using the background electrolyte to achieve about 1-2 × 10^8 CFU/mL.

L. pneumophila serogroup 1 (Philadelphia-1) strain was obtained from Professor Laurence Mathieu (LCPME, Nancy, FRANCE). A loop of pure *L. pneumophila* culture maintained at -20 °C in buffered charcoal yeast extract (BCYE) and 10 % DMSO was spread onto a CYE agar plate (Thermo Fisher Scientific) and incubated for 3 days at 37 °C. Before each experiment, an isolated colony was picked to inoculate a 250 mL volumetric flask containing 100 mL of yeast extract (autoclaved) and Legionella BCYE supplement (Thermo Fisher Scientific). The inoculum was then incubated at 37 °C for 24 hours in a shaking incubator at 170 rpm. After 24 hours of incubation, the cells were washed and resuspended as described above.

3.2.2 Amplification, purification and enumeration of Phage.

Bacteriophage T4 was obtained from the School of Pharmacy and Biomedical Sciences of Curtin University. Bacteriophage T4 was amplified, purified and quantified using the soft agar overlay (double-agar overlay) method of plaque assay adapted from Mullan (2001) using *E. coli* B as the host. Briefly, phage T4 was quantified by adding 500 µL of the appropriate phage dilution, 1 mL of LB and 500 µL of *E. coli* B in the exponential phase in a 15 mL Falcon tube. The tube was then vortexed and incubated at 37 °C for 15 min in a shaking incubator at 170 rpm. 4 mL of soft overlay agar (3 g/L agar) was then added to the content of the tube. The tube was then vortexed and poured onto LB agar plates (16 g/L agar). The plates were then incubated at 37 °C for 18 hours. Plates with 10 – 100 plaques were used to calculate the phage titre (PFU/mL). Phage T4 stock was prepared by scrapping gently the soft overlay agar using a glass spreader into a 50 mL Falcon tube containing 10 mL of PB (10 mM, pH 6.9) after lyses to confluence. The content of the tube was vortexed and then centrifuged (Allegra® X-12R, Beckman Coulter) at 3270 × g for 20 min. The supernatant was then filtered (0.22 µm filters, Millex-syringe filter unit, Merck, Millipore) to remove bacterial cells. The purified stock was then stored at 4 °C before use.

MS2 phage (ATCC 15597-B1) was obtained from Professor Christophe Gantzer (LCPME, Nancy, FRANCE). Amplification of MS2 phage was adapted from the International Organisation for Standardisation (ISO 10705-1,1995) (without the chloroform treatment step) using *E. coli* Hfr K12 (ATCC 23631) as the host. MS2 phage were then concentrated and purified as described previously (Brié et al., 2016). Briefly, the amplified phage were then centrifuged twice at 8000 × g for 20 min at 4 °C. The supernatant was then filtered (0.22 µm filters, Millex-syringe filter unit, Merck, Millipore) and ultracentrifuged at 42 500 × g for 18 h at 4 °C (Thermo Scientific A-621 fixed rotor, USA). The pellet (concentrated phage) was then resuspended in 20% iodixanol solution (Optiprep; Axis-Shield, Dundee, Scotland) and placed on a 40 % iodixanol layer in a Cone-Top PA tube (14 × 47 mm, Thermo Fisher Scientific). The content of the tube was centrifuged at 160 000 × g for 7 h at 15 °C (Thermo Scientific

TH-641 swing rotor). The phage was then carefully removed using a syringe. A Float-A-Lyzer G2 dialysis device (MW100 kD; Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) was used to dialyse the phage twice (overnight and for 7 h) against 10 L of 1 mM PBS (1mM Na_2HPO_4 and 14mM NaCl, pH 7.4) and stored at 4 ^oC before use. MS2 phage was enumerated using the double-agar overlay method of plaque assay according to the International Organisation for Standardisation (ISO 10705-1,1995) using *E. coli* Hfr K12 as the host. Briefly, 500 µL of the appropriate MS2 phage dilution, 500 µL of *E. coli* Hfr K12 (grown to exponential phase in tryptone yeast extract glucose broth (TYGB: tryptone 10g/L, yeast extract 1 g/L, NaCl 8 g/L, $CaCl_2.2H_2O$ 30 g/L, glucose 100 g/L) were added to a 15 mL Falcon tube. The tube was then vortexed and incubated at 37 ^oC for 15 min in a shaking incubator at 170 rpm. 4 mL of soft overlay TYG agar (3 g/L agar) and 40 µL of calcium-glucose solution ($CaCl_2.2H_2O$ 30 g/L, glucose 100 g/L) were then added to the content of the tube. The tube was then vortexed and poured onto TYG agar plates (16 g/L agar). The plates were then incubated at 37 ^oC for 18 hours. Plates with 10 – 100 plaques were used to calculate the phage titre. The final concentration of MS2 phage was around 6.0×10^{13} PFU/mL.

3.2.3 Chlorine analysis

The free chlorine generated by the CEC cell was measured using the N, N diethyl-1,4 phenylenediamine (DPD) colorimetric method (Bridgewater et al., 2012) as previously described in Chapter 2 section 2.1.1.1. The free chlorine (mg/L) measured was expressed as CT values (mg.min/L).

CT value = concentration (C) of free chlorine × contact time (T) of free chlorine with the bacterial/phage suspensions

3.2.4 Experimental apparatus and procedures

The treatment process involved the laboratory scale CEC unit - EC2 as described in section **2.2**. A schematic diagram of the CEC treatment process with the microbiological and chemical analyses carried out is shown in **Figure 25**. In this study, the CEC cell was operated in two modes, continuous and recirculating as shown in **Figure 25** and **Figure 26**. When operated in the continuous mode, the solution passes through the CEC cell only once and has a fixed contact time of 4.5 seconds (2.2 s in the cell and 2.3 s from the cell to the sampling tube). In the recirculating mode, the solution keeps running through the cell, thereby, increasing the contact time of the solution with the CEC cell.

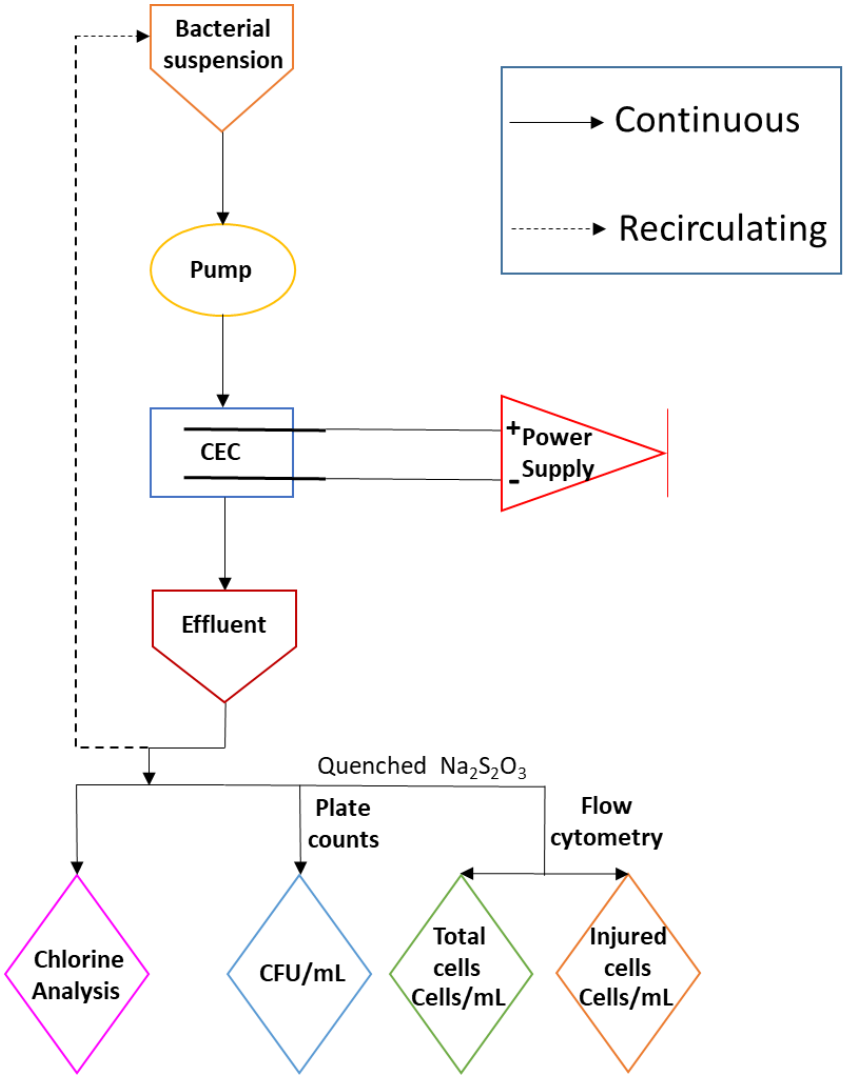


Figure 25 Schematic diagram illustrating the process of disinfection using CEC and the analyses performed at the different voltages applied.

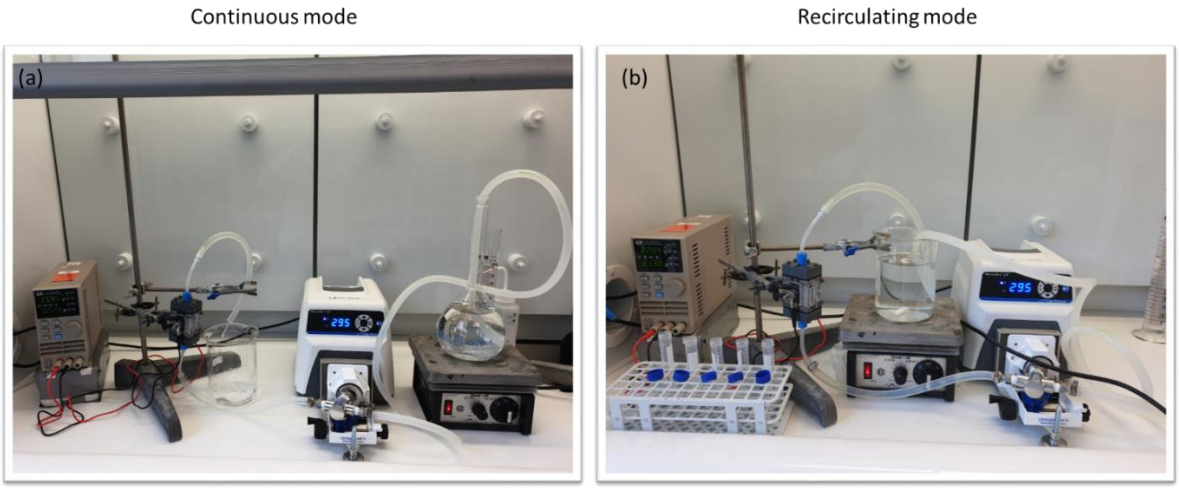


Figure 26 CEC (EC2) operating in (a) continuous mode and (b) recirculating mode.

3.2.4.1 *Inactivation efficiency of bacteria and bacteriophage by the free chlorine generated by CEC*

The CEC cell in continuous mode was fed with bacterial suspensions of *E. coli*, *B. subtilis, S. maltophilia* and *L. pneumophila* at a final concentration of 10^5-10^6 CFU/mL and suspensions of T4 phage and MS2 phage at a final concentration between 10^6 – 10^9 PFU/mL. Preliminary tests showed that there were no major differences in the chlorine demand of phage concentrations of 10^6 and 10^9 PFU/mL. Each suspension (1 L) contained a final concentration of 20 mg/L of sodium chloride (NaCl), 10 mM PB, pH of 7.2 which was pumped into the CEC cell at a constant flow rate of 200 mL/min. Voltages were adjusted between 4 V and 36 V for each experiment. The effluents were then analysed for residual chlorine (expressed as CT = mg/L of Cl_2 × min) and quenched with an excess of 0.1 M $Na_2S_2O_3$. Once quenched, the effluent was analysed by plate counts and plaque assay to determine the number of CFU/mL and PFU/mL, respectively. The CT value depends on the concentration (C) of disinfectant (chlorine) and the contact time (T). For all the experiments carried out in continuous mode, the flow rate was kept constant at 200 mL/min, therefore, the contact time of the solution with the oxidants generated by the cell was constant (4.5 s). Hence, CT varied according to the chlorine concentration (C) generated by the CEC when exposed to varying applied voltages as previously shown in Chapter 2.

3.2.4.2 *Effects of free chlorine generated by CEC on bacteria*

The effects of free chlorine generated by the CEC was measured on *E. coli*, *B. subtilis* and *S. maltophilia* at a final concentration of 10^5-10^6 CFU/mL. The bacterial suspensions were subjected to the same CEC experimental conditions as described in the previous section. Once quenched, the effluent was analysed by plate counts to determine the number of CFU/mL and by flow cytometry to determine the total number of cells/mL (SYBR-stained cells) and the number of membrane damaged cells/mL (SYTOX-stained cells).

3.2.4.3 *Inactivation efficiency of CEC on E. coli in the absence of chloride ions*

The CEC cell was operated in continuous and recirculation modes. In continuous mode, *E. coli* suspension of 6.3×10^5 – 2.1×10^6 CFU/mL in 20 mM PB was pumped into the CEC cell at a constant flow rate of 200 mL/min with increasing voltages ranging from 0 V to 48 V. The influent (the initial *E. coli* suspension in 20 mM PB) and effluents were analysed by plate counts to determine the number of CFU/mL before and after CEC treatment.

In recirculating mode, *E. coli* suspension of 1.2 – 2.1×10^6 CFU/mL in 20 mM PB was pumped into the CEC cell at a constant flow rate of 200 mL/min at 36 V. Effluent samples were collected after 5, 10, 15 and 20 min of recirculation in the cell. The influent and effluent samples were analysed by plate counts to determine the number of CFU/mL before and after CEC treatment.

3.2.4.4 *Inactivation efficiency of CEC v/s Standard chlorination*

The comparison was done only for *E. coli* only. In order to compare CEC with standard chlorination, a fresh stock solution (1.0 g/L as Cl_2) of sodium hypochlorite (NaOCl) (Sigma Aldrich) was prepared. The NaOCl stock solution was diluted using filter sterilised MilliQ water to match the free residual chlorine concentrations generated by the CEC cell at the voltages applied (6 V to 36 V) in the presence of 20 mg/L NaCl and 10 mM PB (pH 7.2, without bacteria). A stopwatch was started immediately when the respective NaOCl dilutions were spiked under vigorous stirring (400 rpm) with 1.5 – 2.1 × 10^6 CFU/mL of *E. coli*. After 0.08 min (4.5 seconds) of contact time (same contact time as *E. coli* undergoing CEC treatment at a constant flow rate of 200 mL/min), free residual chlorine was analysed. The reaction was quenched with an excess of 0.1 M $Na_2S_2O_3$ and the bacterial samples were analysed by plate counts to determine the number of CFU/mL before and after sodium hypochlorite treatment.

3.2.5 Enumeration of culturable bacteria using plate counts

The number of culturable bacteria in a sample was determined using the drop plate method (Herigstad, Hamilton, & Heersink, 2001) by counting the number of colony-forming units (CFU)/mL formed on LB agar plates for *E. coli*, *B. subtilis* and *S. maltophilia* and on BCYE agar plates for *L. pneumophila*. In the context of this study, the term inactivation refers to the culturable fraction of bacteria/phage.

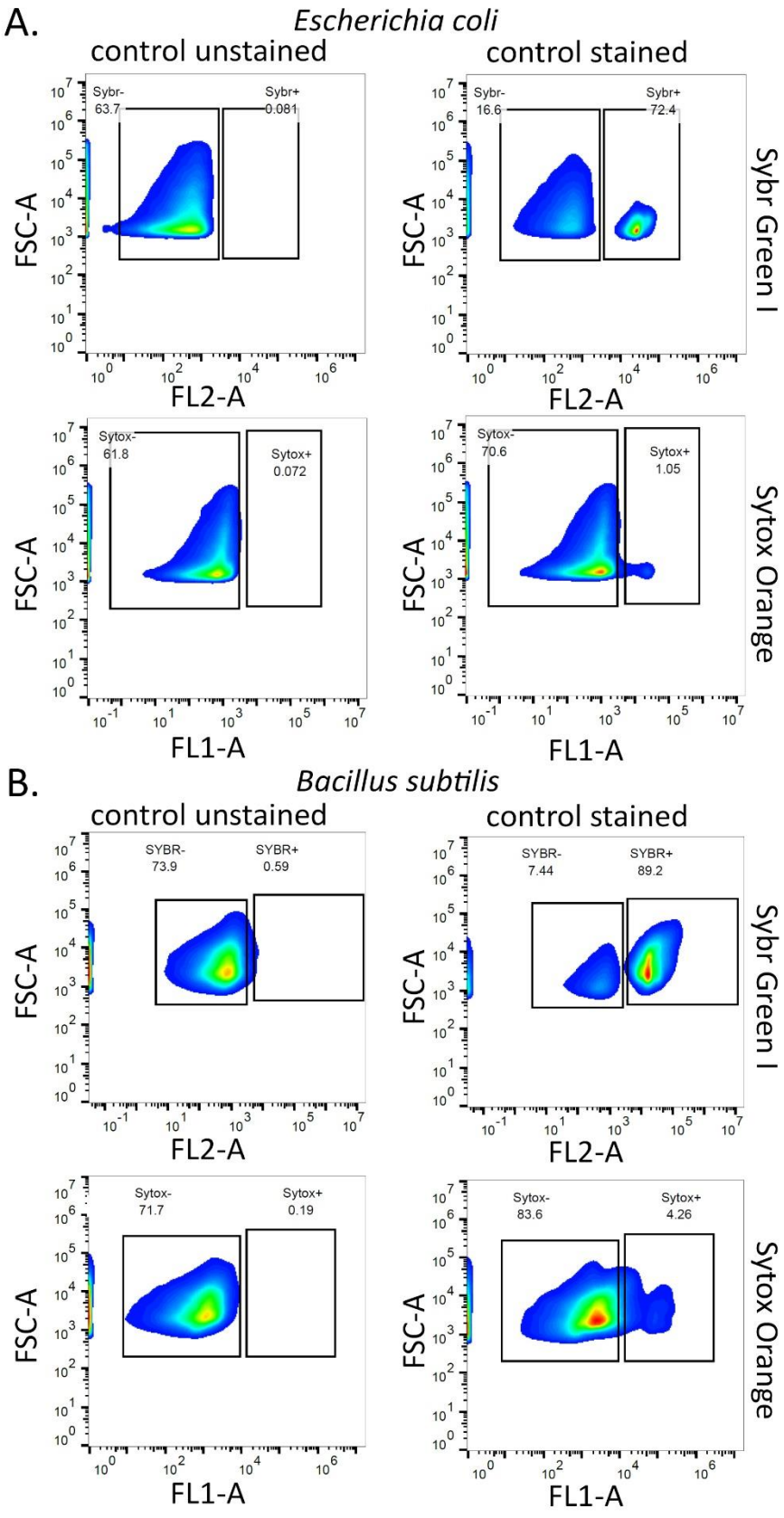
3.2.6 Enumeration of the total and membrane damaged number of bacteria using flow cytometry

The number of total and membrane damaged cells in the feed water and effluent were measured using SYBR® Green I and SYTOX® Orange (1 X, Invitrogen, Life Technologies Ltd, France), respectively. The SYBR® Green I (10 000 X) and SYTOX® Orange stocks were diluted to make secondary stock solutions of 10 X using filter sterilised water, from which 100 µL of each was used to stain samples with a final volume of 1 mL. After the addition of SYBR® Green I and SYTOX® Orange, the samples were mixed gently by vortexing and then incubated for 20 min at room temperature in the dark. The samples were then analysed by the BD Accuri™ C6 flow cytometer (BD Biosciences) equipped with laser excitations at 488 nm and 640 nm. Each run was set for 2 minutes and the flow rate was set to medium (35 µL/min). Samples with over 10^6 events/mL were diluted using filter-sterilised water (0.22 µm filters, Millex-syringe filter unit, Merck, Millipore). A 2 min wash of the flow cytometer was carried out between different samples using filter-sterilised water. The FL1 and FL2 (fluorescence parameters) channels were used to record orange and green fluorescence at 585 nm and 533 nm, respectively (**Table 6**). *E. coli*, *B. subtilis* and *S. maltophilia* samples were analysed using a primary threshold of 3000, 2000 and 1000 on the forward-angle light scatter channel (FSC), respectively. An additional

secondary threshold was added to SYBR-stained cells on the FL2 channel at 2000 for all 3 bacteria to minimise the background noise (**Figure 27**). The BD Accuri™ C6 software was used to analyse the results. Calculations for the total and membrane damaged number of cells per mL stained by SYBR® Green I and SYTOX® Orange were based on the FL2 and FL1 data, respectively. Flow cytometry analysis were performed using the median values for both SYBR and SYTOX fluorescent cells due to the bimodal distribution of the bacteria tested. In the context of this study, the total number of culturable cells were measured by plate counts. The total number of cells refers to SYBR-stained cells measured by flow cytometry. SYTOX orange was used to stain the nucleic acids of cells with compromised membranes.

Table 6 Fluorophores and detectors used in flow cytometry analysis.

Excitation λ	Detector	Filter	Fluorophore	Cell Permeability	Manufacturer (Item #)
547	FL-1	533/30	SYTOX® Orange	Impermeant	Invitrogen™ S11368
497	FL-2	585/40	SYBR® Green I	Permeant	Invitrogen™ S7567


A.
Escherichia coli
control unstained
control stained
Sybr-
63.7
Sybr+
0.081
Sybr-
16.6
Sybr+
72.4
Sytox-
61.8
Sytox+
0.072
Sytox-
70.6
Sytox+
1.05
FSC-A
FL2-A
FL1-A
Sybr Green I
Sytox Orange
B.
Bacillus subtilis
control unstained
control stained
SYBR-
73.9
SYBR+
0.59
SYBR-
7.44
SYBR+
89.2
Sytox-
71.7
Sytox+
0.19
Sytox-
83.6
Sytox+
4.26

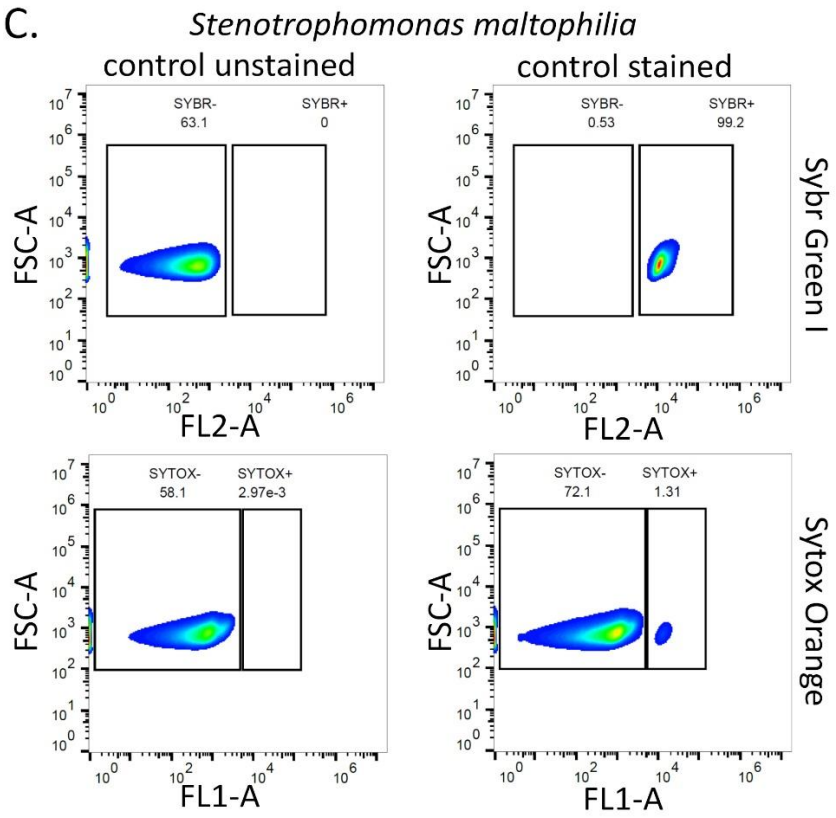


Figure 27 Flow cytometry thresholds applied to analyse *E. coli, S. maltophilia* and *B. subtilis* samples. *E. coli* (A), *B. subtilis* (B) and *S. maltophilia* (C) samples were analysed using a primary threshold of 3000, 2000 and 1000 on the forward-angle light scatter channel (FSC), respectively. An additional secondary threshold was added to SYBR-stained cells on the FL2 channel at 2000 for all 3 bacteria.

3.2.7 Statistical Analysis

All experiments were conducted in triplicates and the data were analysed using GraphPad Prism 8.3.0 software (La Jolla California, USA). The data are represented as mean ± standard deviation (SD)

3.3 Results

3.3.1 Inactivation efficiency of bacteria and bacteriophage by the free chlorine generated by CEC

As previously shown in Chapter 2, an increase in voltage increases the current and chlorine production. The disinfection efficiency of the free chlorine generated by CEC in continuous mode (contact time of 0.08 min) on bacterial and bacteriophage suspensions were investigated in the presence of 20 mg/L NaCl, 10 mM PB (pH 7.2) and at a constant temperature, pH and flow rate of 200 mL/min. Previous controls conducted in Chapter 2 using the same solutions have shown that the free residual chlorine

(1.0 mg/L as Cl_2 at 12 V) remained stable in the absence of bacteria for the same experimental times. Plate counts and plaque assays were performed before and after CEC treatment to assess the disinfection efficiency of CEC. 10^5 - 10^6 CFU/mL of *E. coli*, *B. subtilis*, *S. maltophilia* and *L. pneumophila,* 10^6 PFU/mL of phage T4 and 10^9 PFU/mL of MS2 phage were exposed to different CT as the applied voltage increased from 0 V to 36 V.

In terms of the pH of the solution, no significant difference was observed before and after CEC treatment. The pH of the electrolyte changed from 7.2 ± 0.05 to 7.3 ± 0.05. The temperature of the electrolyte was the same before and after CEC treatment (24 °C). When exposed to a CT between 0.01 - 0.03 mg.min/L, we observed 1.5, 2.5, 0.75 and 0.50 log inactivation of *E. coli*, *B. subtilis*, *S. maltophilia* and *L. pneumophila,* respectively (**Figure 28**). When exposed to a CT of 0.07 - 0.08 mg.min/L, we observed a 6 log (complete) inactivation of *E. coli*, *B. subtilis* and *S. maltophilia* and 1 log inactivation of *L. pneumophila.* At the highest CT (0.2 mg.min/L), 2.5 log of *L. pneumophila* was inactivated (**Figure 28**). Furthermore, the rate of inactivation was *B. subtilis* > *E. coli* > *S. maltophilia* > *L. pneumophila. B. subtilis* was the most sensitive bacterium and *L. pneumophila* the most resistant bacterium to the free chlorine generated by CEC in this study.

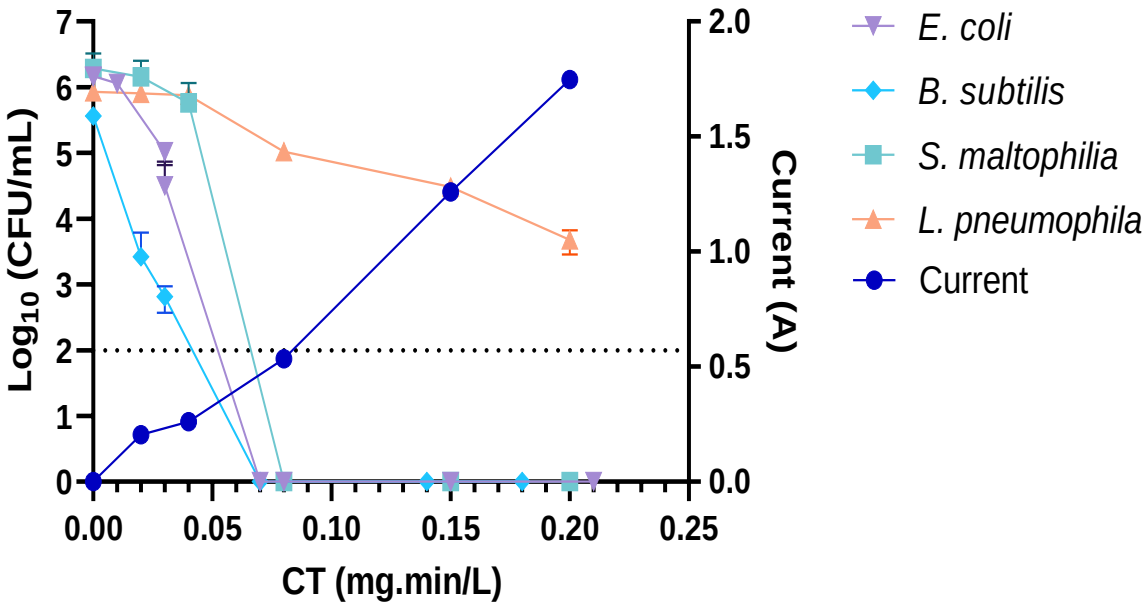


Figure 28 Inactivation of *E. coli, B. subtilis, S. maltophilia* and *L. pneumophila* after CEC treatment in continuous mode at 200 mL/min, 20 mg/L NaCl, 10 mM PB, pH 7.2 when exposed to increasing CT. Initial concentrations of bacteria were 10^5 - 10^6 CFU/mL. Each point is an average value of 3 independent experiments and the errors bars correspond to standard deviations.

For the phage inactivation, CT of 0.02` - 0.03 mg.min/L inactivated 2.5 and 0.4 log of phage T4 and MS2, respectively (**Figure 29**). At the highest CT (0.18 mg.min/L), a 6 log inactivation was observed for phage T4, however, 2.5 log of MS2 phage was inactivated (**Figure 29**), making MS2 phage resistant to complete inactivation by the free chlorine generated under the conditions applied in this study.

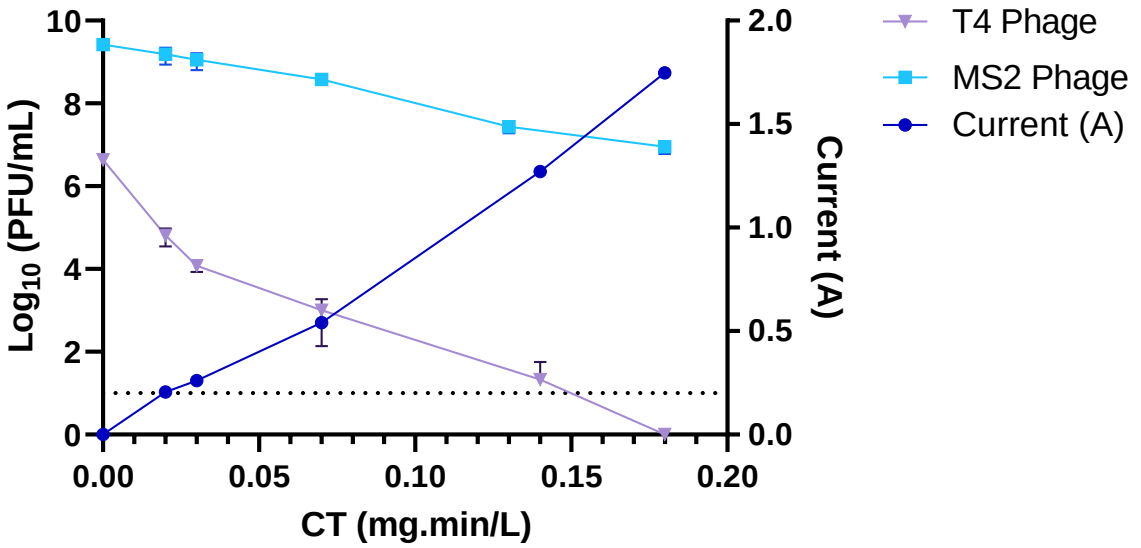


Figure 29 Inactivation of phage T4 and MS2 after CEC treatment in continuous mode at 200 mL/min, 20 mg/L NaCl, 10 mM PB, pH 7.2 when exposed to increasing CT. Initial concentrations of phage T4 and MS2 were 10^6 PFU/mL and 10^9 PFU/mL, respectively. Each point is an average value of 3 independent experiments and the errors bars correspond to standard deviations.

3.3.2 Effects of free chlorine generated by CEC on bacteria measured by flow cytometry

The cellular damage of the free chlorine generated by the CEC in continuous mode on bacterial suspensions were investigated in the presence of 20 mg/L NaCl, 10 mM PB, at a constant temperature (24 °C), pH (7.2) and flow rate of 200 mL/min using flow cytometry. 10^5 - 10^6 CFU/mL of *E. coli*, *B. subtilis* and *S. maltophilia* were exposed to varying CTs. Flow cytometry of SYBR- and SYTOX-stained cells was carried out to establish the total and membrane damaged number of cells before and after CEC treatment. Plating of samples on LB media was carried out in parallel to assess the number of culturable cells.

Whereas for no longer culturable bacteria treated with CT of 0.01 mg.min/L (6 log inactivation - **Figure 30**), flow cytometry showed that the number of SYBR-stained cells and the median SYBR fluorescence intensity did not change with increasing CT irrespective of the bacteria tested (**Figure 30**). This confirms that the number of cells present in the solution did not change and suggests that both no cell lysis and no measurable nucleic acids alterations occurred during the CEC under the applied conditions. Flow cytometry also showed that the number of SYTOX fluorescent cells and their median fluorescence intensity increased with increasing CT for all three bacteria (**Figure 30**) and in Appendix **Figure 34**, **Figure 35** and **Figure 36**). This could suggest that at the highest CT (0.21, 0.18 and 0.20 mg.min/L) applied, 55 %, 70 % and 21 % of the total number of cells of *E. coli*, *B. subtilis* and *S. maltophilia*, respectively, became permeable to SYTOX as a result of the cellular membrane damage caused by free chlorine generated by CEC under the conditions applied.

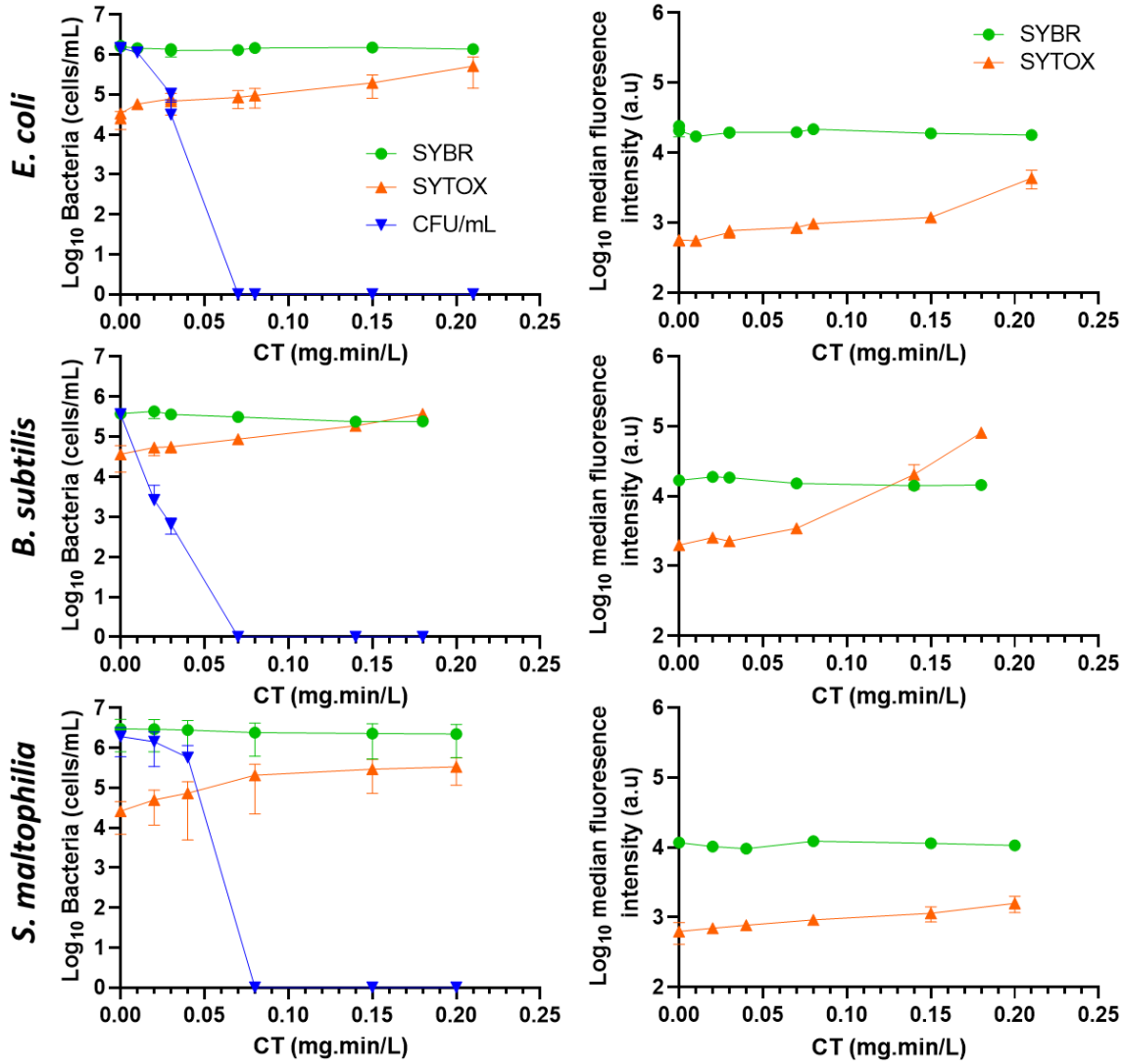


Figure 30 Effects of the free chlorine generated by CEC in continuous mode on the number of CFU/mL, number of bacteria stained with SYBR Green I and SYTOX Orange (graphs on the left) and their median fluorescence intensity (graphs on the right) before and after CEC treatment. Each point is an average value of 3 independent experiments and the errors bars correspond to standard deviations.

3.3.3 Inactivation of *E. coli* when exposed to CEC treatment without chloride ions

The length of contact time of the oxidants generated by the CEC in the absence of chloride ions was investigated by running the CEC in continuous and recirculating modes. 10^5 - 10^6 CFU/mL of *E. coli* were resuspended in 20 mM PB and treated with CEC in both continuous and recirculating mode at a constant flow rate of 200 mL/min, pH of 7.2 and temperature of 24 °C. In continuous mode, voltages ranging from 0 V to 48 V (corresponding to 0 A – 1.98 A) were applied. In recirculating mode, a constant

voltage of 36 V was applied over 30 min of recirculation. Plating of samples on LB media was carried out to determine the number of *E. coli* cells present before and after CEC treatment.

In the absence of chloride ions, no chlorine was measured. When the CEC was operated in continuous mode, voltages ≥ 10 V and current ≥ 1.9 A, CEC inactivated 0.5 log of *E. coli* within 0.08 min of contact time (**Figure 31a**). However, when operated in recirculating mode at 36 V, CEC was able to inactivate 1.5 and 6 log of *E. coli* after 5 and 10 min of recirculation, respectively (**Figure 31b**). In addition, the voltage potential dropped from 36 V to 21-23 V and a constant current of 1.98 A were observed when operated in recirculating mode. The decrease in culturability could be due to a longer contact time of the *E. coli* cells with the surface of the electrodes.

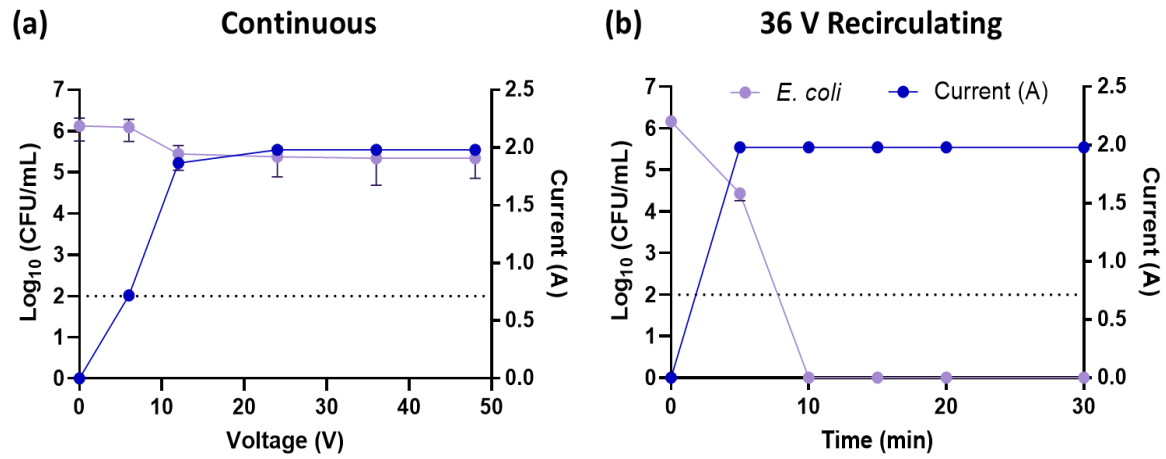


Figure 31 Inactivation of *E. coli* in the absence of chloride ions in (a) continuous mode with varying applied voltages and in (b) recirculating mode at 36 V over 30 min of contact time. Initial concentration of *E. coli* was 10^5 - 10^6 CFU/mL. Each point is an average value of 3 independent experiments and the errors bars correspond to standard deviations.

3.3.4 Inactivation efficiency of CEC v/s Standard chlorination

CEC was compared with standard chlorination (using sodium hypochlorite) to investigate its disinfection efficiency at inactivating *E. coli*. Initial concentrations of 1.5 – 2.1 × 10^6 CFU/mL *E. coli* resuspended in 20 mg/L NaCl and 10 mM PB were treated with CEC in continuous mode at 200 mL/min with varying voltages (6 V – 36 V). Different concentrations of hypochlorite (0.1 – 2.7 mg/L as Cl_2) were prepared according to the free residual chlorine concentrations generated by the CEC cell at the different voltages applied in the absence of bacteria. The bacteria were exposed to the chlorine generated by CEC and the hypochlorite solutions for the same contact time (0.08 min). Plate counts were carried out before and after each treatment to determine the number of CFU/mL.

There was no significant differences (*p*-value = 0.159) in the inactivation of *E. coli* by CEC and standard chlorination (**Figure 32**). The results suggest that bacterial inactivation by CEC in the presence of chloride ions is attributed to the active chlorine species generated.

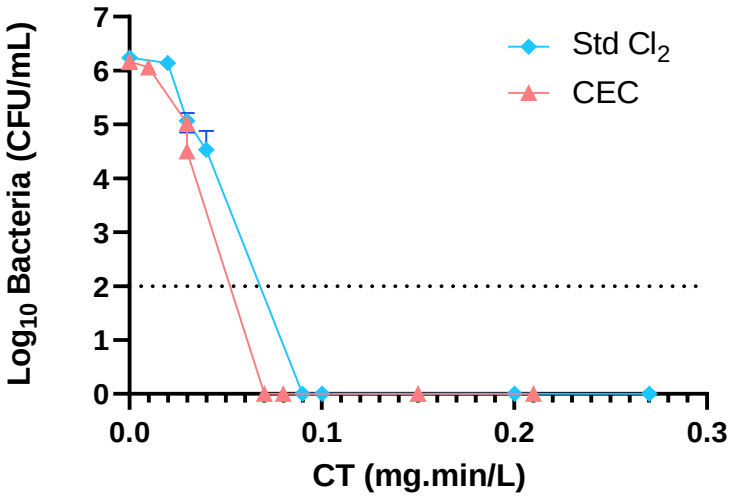


Figure 32 Inactivation of *E. coli* by CEC in continuous mode and standard chlorination (hypochlorite solution) at varying CT. Initial concentration of E. coli was 1.5 – 2.1 × 10^6 CFU/mL. Each point is an average value of 3 independent experiments and the errors bars correspond to standard deviations.

3.4 Discussion

Over the past several decades, electrochemical disinfection has been gaining more attention for its potential to replace traditional chlorination disinfection processes in the water treatment industry (Yoon et al., 2015). In this study we evaluated the disinfection efficiency of CEC, made up of titanium electrodes coated with a MMO layer of Ti/Rh/Ru electrodes, on common waterborne bacteria and bacteriophages based on culturability.

CEC treatment of the bacterial/phage suspensions was conducted under quasi-steady conditions. When operated in continuous flow in the presence of 20 mg/L NaCl, 10 mM PB (pH 7.2) and 10^6 CFU/mL or 10^6 – 10^9 PFU/mL of a single microorganism the pH of the solution remained constant. This can be explained by the very short contact time (0.08 min) and the amount of H^+ formed at the anode was counterbalanced by the amount of OH^- formed at the cathode (Cho et al., 2004; Sirés et al., 2014). The temperature of the electrolyte (24 ^{0}C) also stayed the same before and after CEC treatment. The main effects of the oxidants generated by the CEC were targeted at the cellular and subcellular level rather than altering the macroscopic characteristics of the bacterial/ phage suspensions.

In the absence of chloride ions, CEC inactivated 0.5 and 6 log of *E. coli* in continuous and recirculating mode for 10 min, respectively. Energy-rich and short-lived intermediate products such as free radicals ($^{\bullet}OH$, $^{\bullet}O_2^-$, $^{\bullet}HClO_2^-$ and $^{\bullet}ClO_2^-$) are known to contribute to the germicidal capacity of EC disinfection (Li et al., 2004). However, as previously mentioned in section 3.1, the electrodes of EC2 do not produce radicals during electrolysis. Therefore, the 0.5 log inactivation of *E. coli* observed while running the CEC cell in continuous mode (0.08 min) (**Figure 31a**) suggests that the contact time of the bacterial solution in the cell was too short for electric field effect/electron transfer on the surface of the electrodes but also confirms that there was no production of highly germicidal and short-lived free radicals by EC2. Likewise, the EC disinfection of a solution containing 0.2 M potassium dihydrogen phosphate (KH_2PO_4) at a pH of 7.1 and temperature of 25 ^{o}C using Ti/RuO_2 electrodes for a contact time of 3 min resulted in < 0.5 log inactivation of *E. coli* (Jeong et al., 2009). However, no significant bacterial inactivation (i.e. minimal/no production of ROS) is observed at Ti/Pt-IrO_2 and Ti/IrO_2 electrodes (Jeong et al., 2009).

Furthermore, there was no significant difference between the inactivation of *E. coli* by the free chlorine generated by CEC (in the presence of 20 mg/L NaCl) and standard chlorination (sodium hypochlorite) for the same contact time (**Figure 32**). This confirms that free chlorine is the main disinfecting compound that is generated by CEC. Therefore, the 6 log inactivation of *E. coli* observed in the absence of chloride ions after 10 min of recirculation is due to the direct oxidation of the *E. coli* cells on the surface of the electrodes which is characterised by the instantaneous inactivation of bacteria (Jeong et al., 2007). Indeed, 20 % of *E. coli* are killed instantaneously in the absence of chloride ions using Pt electrodes due to the direct oxidation on the surface of the electrodes whereby negatively charged *E. coli* cells are adsorbed on the surface of the anode (+ve), followed by a direct electron transfer reaction. (Jeong et al., 2007).

The free chlorine generated by CEC under our experimental conditions (20 mg/L NaCl, 10 mM PB, pH 7.2 and temperature of 24 ^{0}C, flow rate of 200 mL/min and in continuous mode) did not affect the total number of bacterial cells (both intact and damaged, measured by flow cytometry - *E. coli*, *S. maltophilia* and *B. subtilis*), irrespective of the treatment (CT: 0 – 0.21 mg.min/L) applied (**Figure 30**). This suggests that there is no measurable cell lysis and no DNA alteration after exposure to CEC treatment. If CEC treatment would have led to cell lysis, we would expect to see a decrease in the total number of cells and a decrease in their fluorescence intensity but this was not the case (Nie et al., 2016; Phe et al., 2007). Since the three bacteria tested showed similar trend when exposed to increasing CT of free chlorine generated by CEC, the flow cytometry results can be divided into two groups (**Figure 33**): low CT (0 - 0.08 mg.min/L, free chlorine 0 – 1.0 mg/L) and high CT (0.08 - 0.22

mg.min/L, free chlorine 1.0 – 2.7 mg/L). When exposed to a low CT, we suggest that the free chlorine generated are enough to cause cellular membrane damage and enter the cytoplasm of the cell. Whereas at the high CT, the free chlorine generated caused more extensive cellular membrane damage and altered the nucleic acid but not enough to cause conformational changes to the binding sites of SYBR and SYTOX. If the binding sites of the fluorophores were damaged, we would have seen a decrease in the fluorescence intensity of SYBR and SYTOX stained bacteria but that was not the case in our study. Hypochlorous acid (HOCl) is known to react with nucleic acids (DNA and RNA) in a specific way that can lead to chemical and structural changes such as the dissociation of double stranded DNA into single strands following oxidative damages due to HOCl (Bernofsky, 1991; Dennis, Olivieri, & Krusé, 1979; Hoyano et al., 1973; Prütz, 1998; Whiteman, Jenner, & Halliwell, 1997). Chlorination damages cellular surfaces when exposed to < 0.5 mg/L of free chlorine and nucleic acids of *E. coli* cells when exposed to 1.5 – 3.0 mg/L of free chlorine, injected as a hypochlorite solution, for a contact time of 90 min (Phe et al., 2005). However, in this study, no DNA damage was observed. This could be due to the very short contact time of 0.08 min and the lower concentrations of chlorine, 0.2 - 2.7 mg/L of free chlorine generated by CEC on the bacterial cells under the conditions applied.

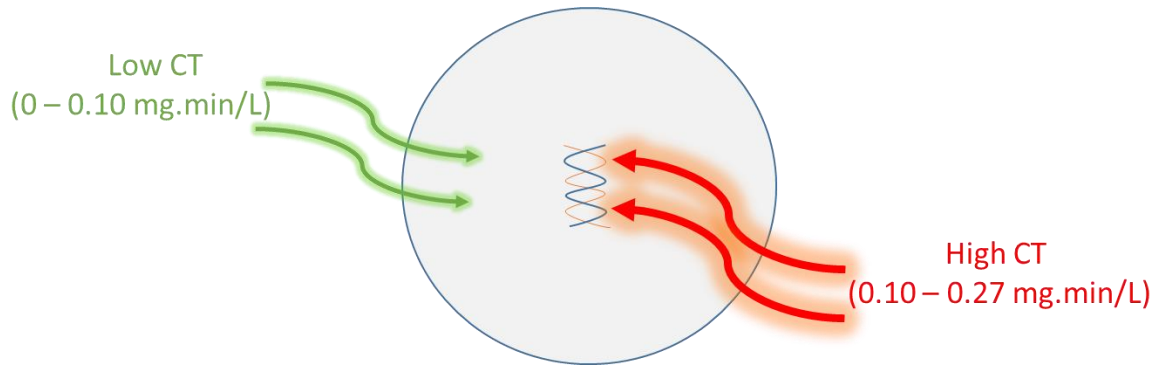


Figure 33 Potential effects of chlorine with increasing CT generated by CEC in continuous mode on bacteria following flow cytometry analysis.

However, the plate counts results showed that the free chlorine generated by the CEC decreased the culturability of the *E. coli* cells when exposed to the low CT (0 - 0.08 mg.min/L, free chlorine 0 – 1.0 mg/L) (**Figure 30**). This means that either the cells are dead, or the cells have entered into the VBNC state where they would no longer be detected by plate counts. The flow cytometry and plate counts results are in accordance with previous studies (Wang et al., 2010). Electrochemically generated free chlorine from an Ecodis® device equipped with coated titanium electrodes showed that *E. coli* cells start to lose their culturability on LB agar when exposed to as low as 0.05 mg/L of free chlorine and lose their culturability completely when exposed to a CT of 2.0 mg.min/L (free chlorine of 0.2 mg/L for 10 min of contact time) (Wang et al., 2010). Furthermore, at a CT of 2.0 mg.min/L, 70 % of the total number of *E. coli* cells after treatment are in the VBNC state (Wang et al., 2010).

L. pneumophila showed the greatest resistance to free chlorine generated by CEC (2.50 mg/L) followed by *S. maltophilia*, *E. coli* and *B. subtilis* (**Figure 28**). Interestingly, *B. subtilis* (in the vegetative form in this study), a rod-shaped Gram-positive organism, showed the fastest inactivation rate when exposed to the oxidants generated by CEC. Disinfectants are effective when they enter the cell wall of bacteria and reach a high enough concentration to exert antimicrobial activity at the target sites (Virto et al., 2005). One would think that the thick murein (peptidoglycan) layer of Gram positive *B. subtilis* could increase their resistance to the disinfectants but there's no clear correlation between Gram-positive bacteria and having higher resistance to disinfectants (Grunert et al., 2018). Compared to *E. coli*, *P. aeruginosa*, *S. aureus* and *E. hirae*, *B. atrophaeus* (previously reported as *B. subtilis* var. *niger*) is the most sensitive bacteria when exposed to the oxidants generated by the electrolysis of a low conductivity sulfate medium using BDD anode (Bruguera-Casamada et al., 2016). This might be due to the sensitivity of *B. atraphaeus* to changes in pH during electrochemical oxidation (Bruguera-Casamada et al., 2016). However, in this study, the changes in pH before and after CEC treatment were not significant.

The effectiveness of electrochemical disinfection is known to vary according to the bacterial strain and the CT value (chlorine concentration and contact time of the bacteria with chlorine (Cervero-Aragó et al., 2015; Kuchta et al., 1983). The results from this study showed good agreement with previous studies that reported *L. pneumophila*, in particular (Philadelphia strain) is the most resistant strain to the chlorine generated from a hypochlorite solution (Cervero-Aragó et al., 2015; Kuchta et al., 1983). Given their high level of resistance to free chlorine, compared to other bacteria such as *E. coli*, *S. aureus*, *K. pneumonia* and *E. aerogenes*, a higher CT of 4.0 mg.min/L is required for *L. pneumophila* (Cervero-Aragó et al., 2015).

A CT of 0.08 mg.min/L (1.0 mg/L free chlorine and contact time = 0.08 min) was required to inactivate 6 log of *S. maltophilia*. *S. maltophilia* AB550 is a multidrug- and solar radiation-resistant strain isolated from the effluents of an urban wastewater treatment plant in Western Australia (Glady-Croue et al., 2018). The resistance of *S. maltophilia* cells to free chlorine could be attributed to the formation of viable ultramicrobial cells (UMC) (Silbaq, 2009). *S. maltophilia* forms UMC (0.1 – 0.2 µm) in response to stress (Silbaq, 2009). In this study, the free chlorine generated by CEC could trigger the stress response. Furthermore, these viable UMC are unable to grow on standard heterotrophic plate counts (HPC) (Silbaq, 2009). This phenomenon can be described as the viable but non-culturable (VBNC) state that some bacteria enter when exposed to environmental stresses (McDougald et al., 1998). Flow cytometry cell sorting with PCR-DGGE showed the presence of VBNC cells of *S. maltophilia* in water after disinfection (1.06 mg/L free chlorine) treatment and the cells are metabolically active in the distribution system (D. Hoefel et al., 2005). The presence of VBNC bacteria after disinfection and along

the distribution network highlight the importance of better techniques other than HPC to monitor the microbiological quality of water (D. Hoefel et al., 2005).

E. coli (Chen et al., 2018; Lin et al., 2017) and *L. pneumophila* (Alleron et al., 2013; Dusserre et al., 2008) are also known to enter into the VBNC state following exposure to environmental stresses such as exposure to disinfectants (chlorine). As such, the effects of free chlorine on these bacterial cells shown in **Figure 28** could be even lower, since viability was assessed by plate counts, which is still recognised as the gold standard in many countries even though it is unable to detect VBNC cells (Cervero-Aragó et al., 2015).

MS2 phage has shown to be more resistant to the chlorine generated by CEC as compared to phage T4 (**Figure 29**). A CT of 0.18 mg.min/L inactivated 6 and 2.5 log of phage T4 and MS2, respectively. The susceptibility of viruses depend on their structure (naked or enveloped) and chemical composition (for e.g. hydrophobicity and charge of capsid proteins) (Wigginton & Kohn, 2012). The inactivation results of MS2 phage were comparable to previous results where a CT of 0.142 mg.min/L of free chlorine, injected as a hypochlorite solution, inactivated 3 log of MS2 phage at 20 ^{0}C and pH of 7.2 (Lim, Kim, & Ko, 2010). Similarly, a CT of 0.156 mg.min/L of free chlorine, injected as a sodium hypochlorite solution was able to inactivate 2 log of MS2 phage at 15 ^{0}C and pH of 7.5 (Grunert et al., 2018). However, the results of this study were different to previous findings which showed that a CT of 7.0 mg.min/L is able to inactivate 4 log of MS2 phage following the generation of 2.0 mg/L of free chlorine (for 3.5 min) by an electrochemical cell using titanium electrodes coated with Group VIII metal oxides in a solution at 25 ^{0}C and pH of 6 (Venczel et al., 2004). The differences could be due to the very short contact time (0.08 min) and the higher pH (7.2) used in this study. Different mechanisms of virus inactivation are involved when chemical oxidants are used (Wigginton et al., 2012). The infectivity of viruses depends on their binding capacity to a host cell, genome insertion inside the host and replication of their genome inside the host. Free chlorine affects both the protein- and genome-mediated functions of MS2 phage (used as a model for pathogenic enteric viruses) which involves mainly genome injection and replication, but does not affect its binding ability to its host cells (Wigginton et al., 2012). On the other hand, compared to MS2 phage, phage T4 is a tailed phage (made up of a broad range of receptor-binding proteins) and consists of linear dsDNA (about 165 Kb in length) (Nobrega et al., 2018). The reason why phage T4 was more sensitive to the oxidants generated by CEC than MS2 phage could be primarily due to the free chlorine damage to the receptor-binding proteins in the phage tail, such that the phage lost its binding capacity to its host cell. If the phage T4 cannot bind to its host then it automatically loses its infectivity.

3.5 Conclusion

This study examined the effects of the oxidants generated by CEC (EC2) on different microorganism in a chloride-containing (using plate counts and flow cytometry in parallel) and chloride-free solution (plate counts only). In a chloride-containing solution, CEC in continuous mode was successfully able to inactivate 6 log of *E. coli* and *B. subtilis* with a CT of 0.07 mg.min/L, 6 log of *S. maltophilia* with a CT of 0.08 mg.min/L, 2.5 log of *L. pneumophila* with a CT of 0.20 mg.min/L, and 6 log of phage T4 and 2.5 log of MS2 phage with a CT of 0.18 mg.min/L, in a very short contact time (0.08 min).

Irrespective of the CT applied, the total number of bacterial cells (both intact and damaged - *E. coli*, *S. maltophilia* and *B. subtilis*) measured by SYBR Green I, was not affected by the free chlorine generated by CEC under the same experimental conditions. The findings suggest that there was no immediate measurable cell lysis and no measurable DNA alteration after exposure to CEC treatment using flow cytometry under the conditions applied in this study.

The bacteria (*E. coli*, *S. maltophilia* and *B. subtilis*) tested showed similar trend when exposed to increasing CT of free chlorine generated by CEC in continuous mode. When exposed to a low CT (0-0.08 mg.min/L), the total number of cells measured by SYBR Green I and the number of membrane damaged cells measured by SYTOX Orange suggest that the free chlorine generated was enough to cause cellular membrane damage and enter the cytoplasm of the cell. Whereas at the high CT (0.08 – 0.22 mg.min/L), the free chlorine generated caused more extensive cellular membrane damage and altered the nucleic acid but not enough to cause conformational changes to the binding sites of SYBR and SYTOX.

In a chloride-free electrolyte, CEC was able to inactivate 6 log of *E. coli* when operated in recirculating mode for 10 min and this was primarily attributed to the direct oxidation of the cells on the surface of the electrode.

Lastly, there were no major microbiological differences between CEC generated oxidants (in continuous mode) and standard chlorination at inactivating *E. coli*, confirming the CEC-generated free chlorine was the key oxidant responsible for inactivating the different microorganisms.

3.6 Appendix

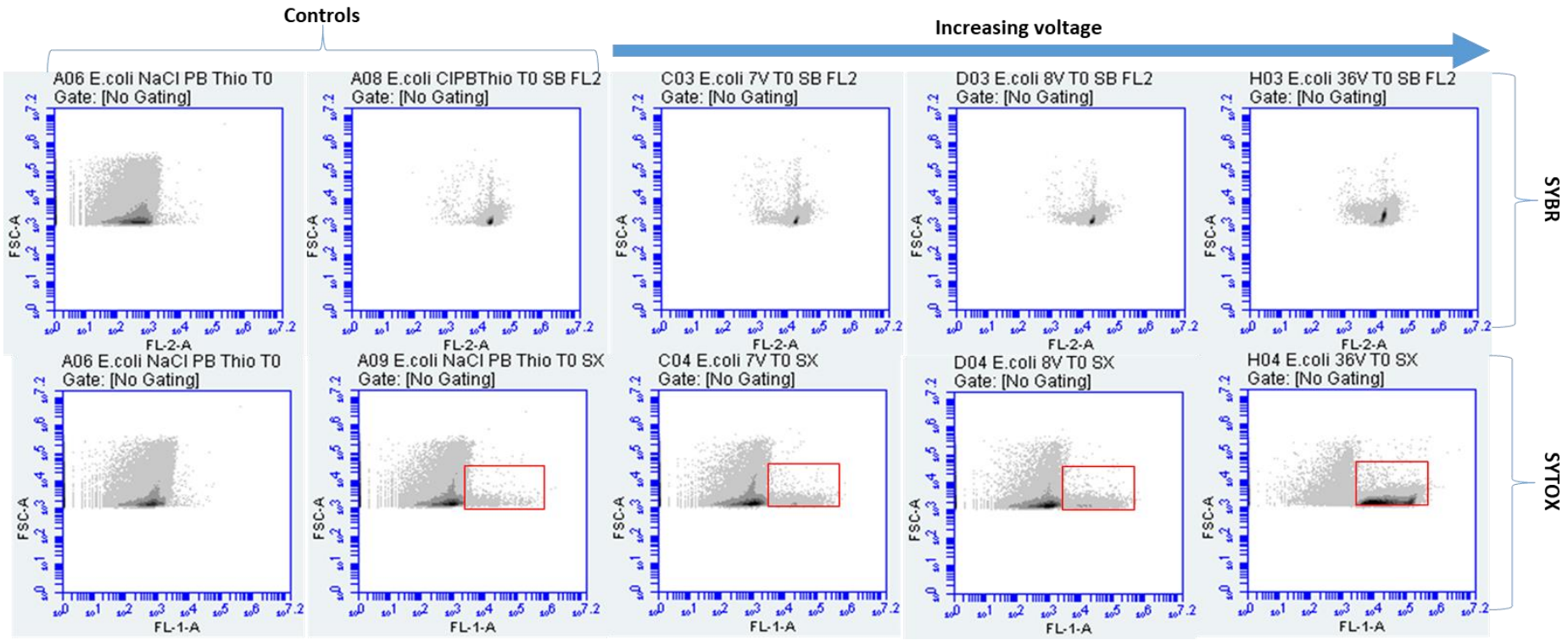


Figure 34 Flow cytograms of controls and CEC (in continuous mode) treated *E. coli* cells. The first row represents the forward scatter channel (FSC) versus the fluorescent parameter 2 (FL-2: for SYBR Green I) channel. The second row represents the FSC versus the fluorescent parameter 1 (FL-1: for SYTOX Orange). From left to right, each flow cytogram represents: unstained cells (control), SYBR or SYTOX stained cells before CEC treatment (control), CEC treated samples at 7 V, 8 V and 36 V, respectively. The average free chlorine concentrations (corresponding CT values) for 7, 8 and 36 V were 0.40 mg/L (CT = 0.4 mg.min/L), 0.90 mg/L (CT = 0.09 mg.min/L) and 2.64 mg/L (CT = 0.26 mg.min/L), respectively. For all samples, a threshold of 3000 was applied on the FSC and a secondary threshold was applied on the FL-2 channel (SYBR) at 2000. The red boxes represent the fraction of cells stained with SYTOX to measure their number and fluorescence intensity.

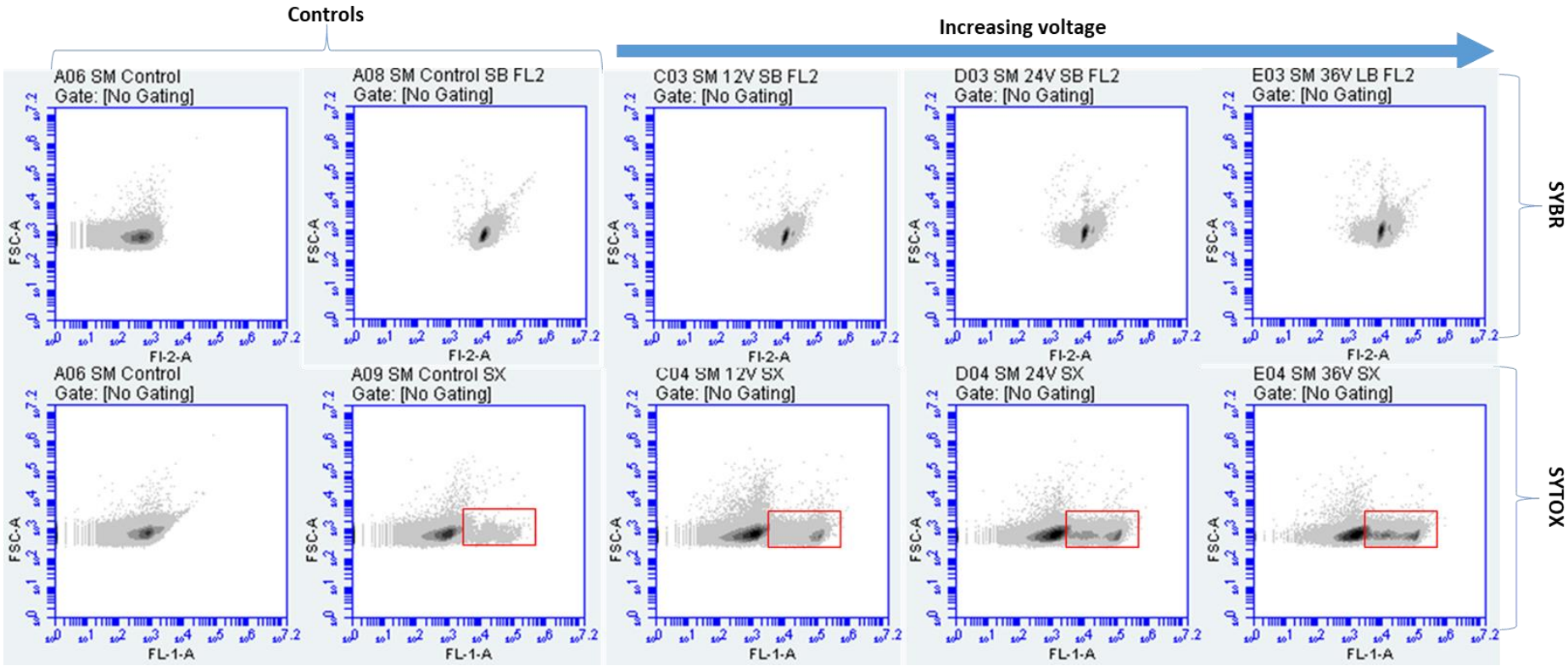


Figure 35 Flow cytograms of controls and CEC (in continuous mode) treated *S. maltophilia* cells. The first row represents the forward scatter channel (FSC) versus the fluorescent parameter 2 (FL-2: for SYBR Green I) channel. The second row represents the FSC versus the fluorescent parameter 1 (FL-1: for SYTOX Orange). From left to right, each flow cytogram represents: unstained cells (control), SYBR or SYTOX stained cells before CEC treatment (control), CEC treated samples at 12 V, 24 V and 36 V, respectively. The average free chlorine concentrations (corresponding CT values) for 12, 24 and 36 V were 0.99 mg/L (CT = 0.10 mg.min/L), 1.90 mg/L (CT = 0.19 mg.min/L) and 2.45 mg/L (CT = 0.25 mg.min/L), respectively. For all samples, a threshold of 1000 was applied on the FSC and a secondary threshold was applied on the FL-2 channel (SYBR) at 2000. The red boxes represent the fraction of cells stained with SYTOX to measure their number and fluorescence intensity.

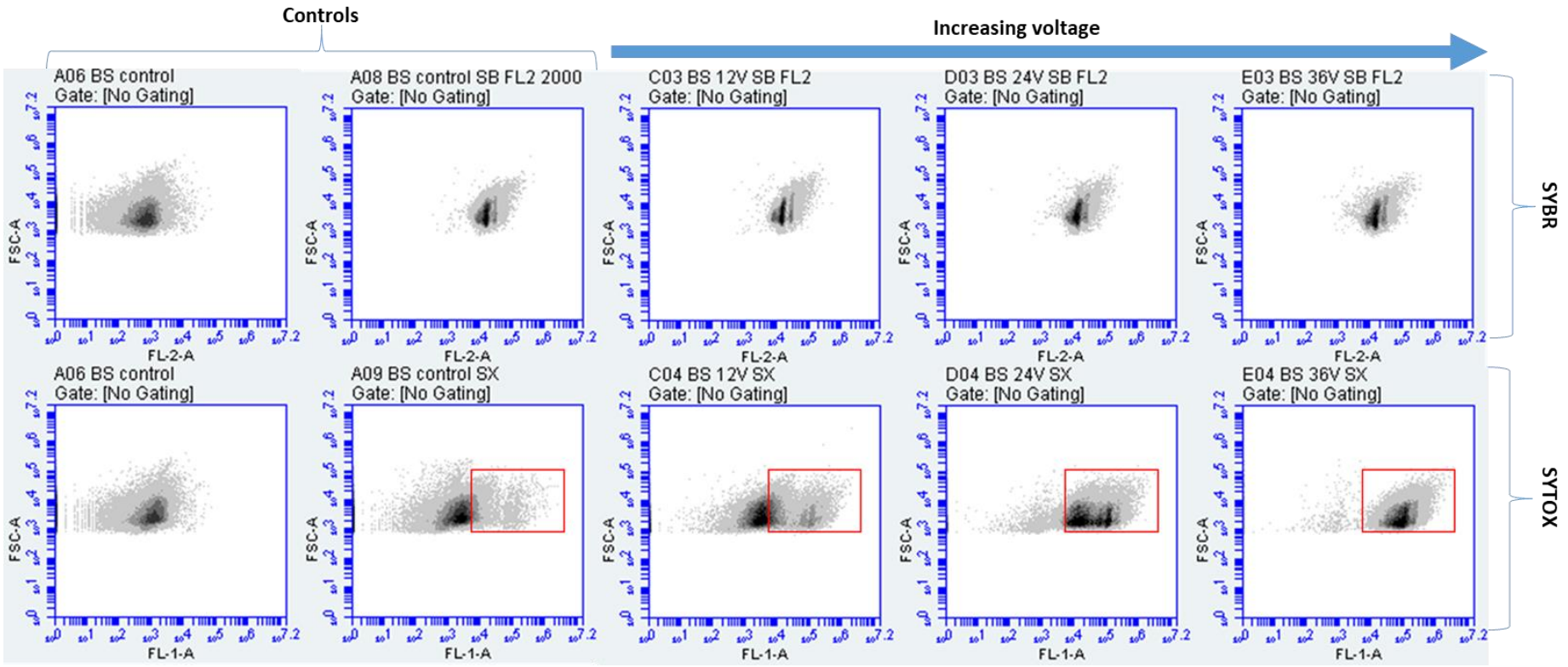


Figure 36 Flow cytograms of controls and CEC (in continuous mode) treated *B. subtilis* cells. The first row represents the forward scatter channel (FSC) versus the fluorescent parameter 2 (FL-2: for SYBR Green I) channel. The second row represents the FSC versus the fluorescent parameter 1 (FL-1: for SYTOX Orange). From left to right, each flow cytogram represents: unstained cells (control), SYBR or SYTOX stained cells before CEC treatment (control), CEC treated samples at 12 V, 24 V and 36 V, respectively. The average free chlorine concentrations (corresponding CT values) for 12, 24 and 36 V were 0.87 mg/L (CT = 0.09 mg.min/L), 1.71 mg/L (CT = 0.17 mg.min/L) and 2.21 mg/L (CT = 0.22 mg.min/L), respectively. For all samples, a threshold of 2000 was applied on the FSC and a secondary threshold was applied on the FL-2 channel (SYBR) at 2000. The red boxes represent the fraction of cells stained with SYTOX to measure their number and fluorescence intensity

Chapter 4. Investigate the potential recovery/regrowth of bacteria after CEC treatment and the importance of routine monitoring of the microbiological quality of drinking water after disinfection.

4.1 Introduction

It is vital for drinking water utilities (DWUs) to monitor the hygienic and aesthetic quality of the water from the water treatment plant to the consumer's tap. The need to monitor the microbiological cleanliness of treated water after the disinfection stage is crucial to safeguard public health from waterborne diseases. This ensures there is no potential contamination or regrowth of waterborne pathogens due to the biological instability of the water in the distribution network, particularly under varying environmental and operational circumstances (Lautenschlager et al., 2013; Pinto et al., 2014; Pinto, Xi, & Raskin, 2012; Prest et al., 2016; Reasoner, 1990). From a DWU viewpoint, the ideal microbiological methods for the usual monitoring of water quality will have to be appropriate, easy to operate, quick and cost-effective (Van Nevel et al., 2017).

Currently, the heterotrophic plate counts (HPC)/ conventional plating methods are analytic techniques that DWUs primarily use as a standard operational tool to assess the general microbiological quality of potable water (Bartram et al., 2004). However, its drawback – the "great plate count anomaly" phenomenon, whereby HPC number are high but operational decision-makers deal with zero count samples, has been apparent for a long time (Staley & Konopka, 1985) (as described in section **1.5.1**). The fact that HPC can detect only about 1 % of the total bacterial population in drinking water as observed by direct microscopy made researchers move towards cultivation-independent methods (Gillespie et al., 2014; Kooj, 2003; Wagner et al., 1993). In the last couple of years, the combination of flow cytometry with fluorescent stains has emerged as one of the most promising technique in monitoring the microbiological quality of water and also act as an alternative to the century-old HPC (Hammes & Egli, 2010)(as described in section **1.5.2**).

Despite all the advances in disinfection technologies to find alternatives to standard chlorination, studies focusing on monitoring the microbiological cleanliness of electrochemically treated water after the point of disinfection are very scarce. This study is aimed at better understanding the potential for *E. coli* to recover or regrow following electrochemical disinfection of a chloride-containing solution. In order to mimic the presence of organic matter along distribution networks, two sets of experiments, with and without nutrient addition post-disinfection, were investigated. This study also aimed at contributing to the differences between HPC and flow cytometry when it comes to bacterial viability and cell counting. In this study, the total number of cells refer to cells measured by flow cytometry and the total number of culturable cells refer to cells measured by plate counts. Moreover, a resuscitation test was performed to determine if culturable cells observed post disinfection treatment and nutrient addition are a result of true resuscitation of injured cells or the growth of culturable bacteria not detected by the standard plate counts immediately after disinfection.

4.2 Materials and method

4.2.1 Bacterial culture and growth conditions

E. coli B was obtained from the School of Pharmacy and Biomedical Sciences of Curtin University. E. coli was grown as described in section 3.2.1. The final cell suspension was adjusted to 0.5 McFarland using the background electrolyte to achieve 1-2 × 10^8 CFU/mL.

4.2.2 Experimental apparatus and procedures

A schematic diagram of the CEC treatment process is shown in **Figure 37a**. The treatment process involved the CEC cell 2 (EC2) as described in section 2.2. EC2 was fed with a bacterial suspension at a final concentration of about 10^6 CFU/mL in 20 mM of sodium chloride (NaCl), at a constant flow rate of 200 mL/min and a constant voltage ranging between 5 V to 36 V for each experiment. Effluent was analysed for residual chlorine (expressed as CT values) and the chlorine was quenched with an excess of 0.1 M sodium thiosulfate ($Na_2S_2O_3$). Once quenched, effluent was analysed by plate counts and flow cytometry to determine the number of CFU/mL and the total number of cells/mL, respectively (**Figure 37b**). In order to investigate whether the CEC treated cells can recover and regrow in the presence of nutrients, diluted LB (diluted 1:3, TOC 2.2 mg/L) was added to the quenched samples and allowed to stand at room temperature for 5 h, 24 h and 48/96 h. Diluted LB was added to mimic the presence of organic matter in distribution networks (for e.g. pipelines). The samples were then analysed by plate counts and flow cytometry (**Figure 37b**).

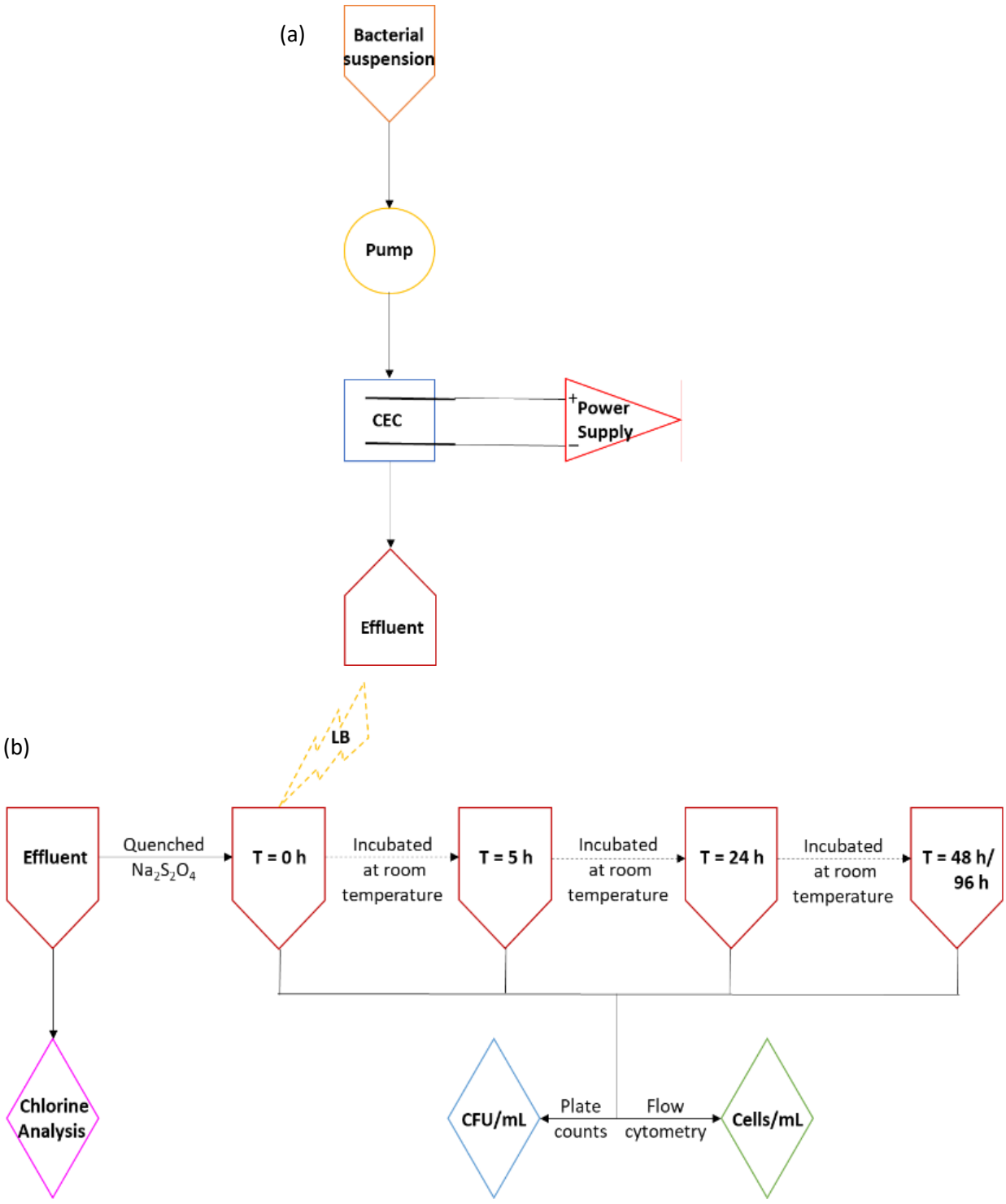


Figure 37 Schematic diagram illustrating (a) the process of disinfection of *E. coli* using CEC and (b) the analysis carried out at the different time-points.

4.2.3 Chlorine analysis

The free chlorine generated by EC2 was measured on the effluent (after CEC treatment) using the N, N diethyl-1,4 phenylenediamine (DPD) colorimetric method (Bridgewater et al., 2012) as described in section 2.1.1.1. The free chlorine generated was expressed as CT values (mg.min/L), according to the equation below.

CT value (mg.min/L) = concentration (C) of free chlorine (mg/L) × contact time (T) of free chlorine with the *E. coli* suspension (min)

4.2.4 Enumeration of culturable *E. coli* using plate counts

The number of culturable bacteria in a sample was determined using the drop plate method (Herigstad et al., 2001) using 10 µL of each sample and counting the number of colony-forming units (CFU)/mL formed on LB agar plates (LOD = 100 CFU/mL)

4.2.5 Enumeration of the total number of *E. coli* using flow cytometry

The number of culturable *E. coli* in a sample was determined using the drop plate method (Herigstad et al., 2001) by counting the number of colony-forming units (CFU)/mL formed on LB agar plates. For flow-cytometry-based detection of cell numbers, SYBR® Green I (1 X, Invitrogen, Life Technologies Ltd, France) was used for estimation of total cell numbers (including non-viable cells) in the effluent as described in section 3.2.6. All samples were analysed using a primary threshold of 3000 on the forward-angle light scatter channel (FSC). An additional secondary threshold was added to SYBR-stained cells on the FL2 channel at 2000 to minimise the background noise. No compensation and no gating were used. The BD Accuri™ C6 software was used to analyse the results (**Figure 38**). The mean fluorescence intensity for the total number of cells per mL stained by SYBR® Green I were based on the fluorescence parameter 2 (FL2) data.

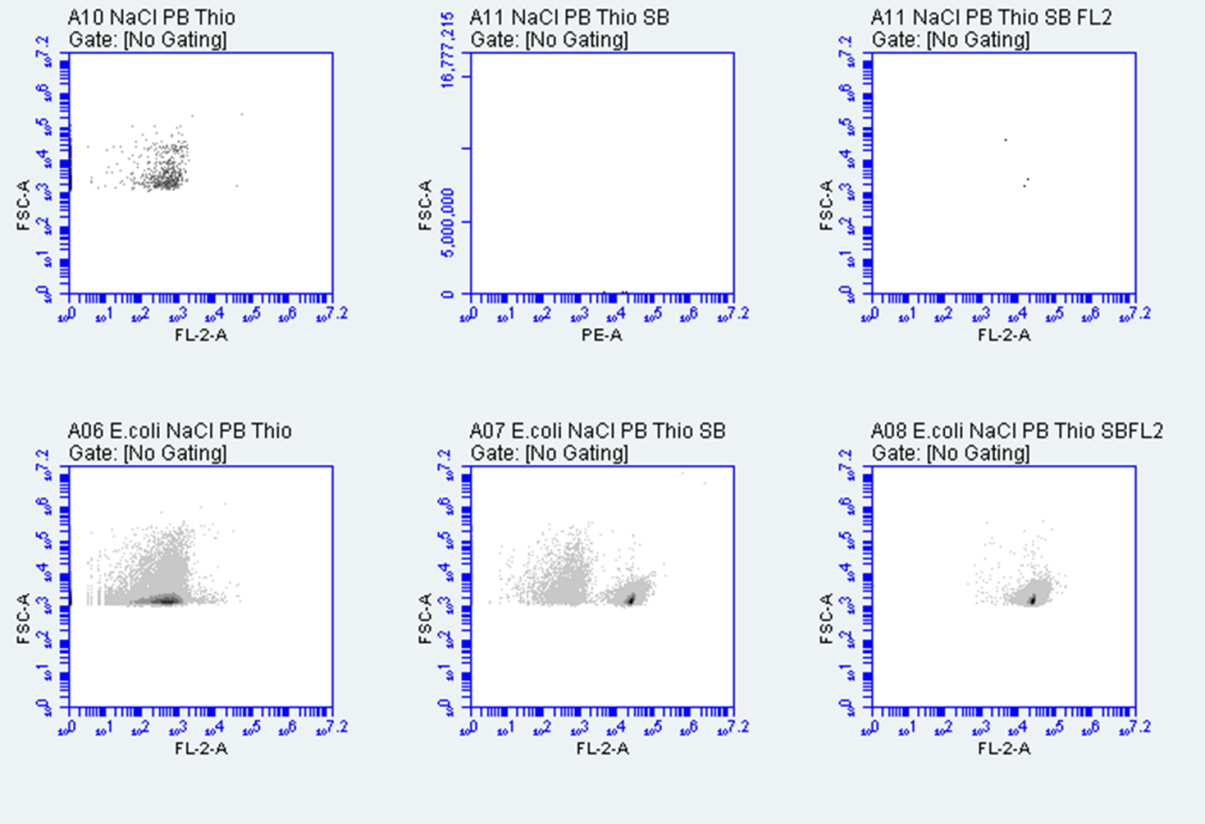


Figure 38 Flow cytograms of *controls and E. coli* before CEC treatment in continuous mode. All samples were analysed using a primary threshold set at 3000 on the forward-angle light scatter (FSC). Rows 1 and 2 represent the forward scatter channel (FSC) versus fluorescent parameter 2 (FL-2 for SYBR) for: the buffer solution (20 mg/L sodium chloride, 10 mM phosphate buffer pH 7.2 and sodium thiosulfate) without bacteria (control) and buffer solution with *E. coli*, respectively. From left to right, each flow cytogram represents unstained, SYBR stained and SYBR-stained with secondary threshold of 2000 on the FL-2 channel, respectively.

4.2.6 Resuscitation test

In order to determine if culturable cells observed after CEC treatment and nutrient addition are a result of true resuscitation of damaged cells or the growth of culturable bacteria not detected by the standard plate counts immediately after CEC treatment, a resuscitation test was conducted. The experimental setup was adapted from the publication of Whitesides and Oliver (1997).

In this study, three 10 mL aliquots of a sample that has undergone CEC treatment was collected and diluted to 10^{-1} and 10^{-2} using the background electrolyte. Sterilised LB (diluted 1:3, TOC 2.2 mg/L) was added to each bottle that was then allowed to stand at room temperature (22 °C - 24 °C) for 24 hours. After the incubation time, the number of culturable bacteria (CFU/mL) in each sample was determined using the drop plate method.

4.2.7 Statistical Analysis

The data were analysed using GraphPad Prism 8.3.0 software (La Jolla California, USA). Statistical significance (95 % confidence interval) was determined using the Student's t-test, one-way or two-way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD). The data are represented as mean ± standard deviation (SD) and *P*-values < 0.05 were considered significant.

4.3 Results

4.3.1 A population of CEC-treated cells are able to recover following incubation in fresh culture medium

Previously (in **section 3.3.1**) we demonstrated that immediately following CEC treatment of *E. coli* the number of culturable cells markedly decreased. However, we wondered if all these cells were permanently inactivated or were non-culturable but potentially viable following a recovery period. Flow cytometry of SYBR-stained cells was carried out to establish the total number of cells before and after CEC treatment. SYBR stains both living and dead bacteria in 0.1 M phosphate buffer before and after CEC treatment. Plating of samples on LB media was carried out in parallel to assess the number of viable cells. Flow cytometry confirmed that total number of SYBR-stained cells (**Figure 39a**) and the mean fluorescence intensity (**Appendix 1**) for each cell did not change significantly after treatment (p-values: Control = 0.99, CT 0.02 = 0.49, CT 0.19 = 0.44). The estimated cell concentration was 1.5×10^6 cells/mL in both controls and immediately after CEC treatment (**Figure 39a**). Plating of these samples following CEC treatment confirmed that the vast majority of cells were non-culturable following treatment. After CEC treatment with CT = 0.02 mg.min/L, 65 % of cells were non-culturable. No cells were culturable following a treatment with CT = 0.19 mg.min/L (**Figure 39b**).

To test if the CEC-treated cells were able to recover following an incubation period, CFU/mL was calculated from the same samples following a 24 h and 96 h period without any additional nutrient addition. The remaining viable biomass did not increase over this period, suggesting that either the cells were non-viable or that conditions were not optimal for their recovery following treatment. The total number of SYBR-stained cells and CFU/mL was near identical to values obtained immediately following CEC treatment (**Figure 39a and b**). The results suggest no measurable bacterial growth and no measurable recovery of culturable cells up to 96h following treatment.

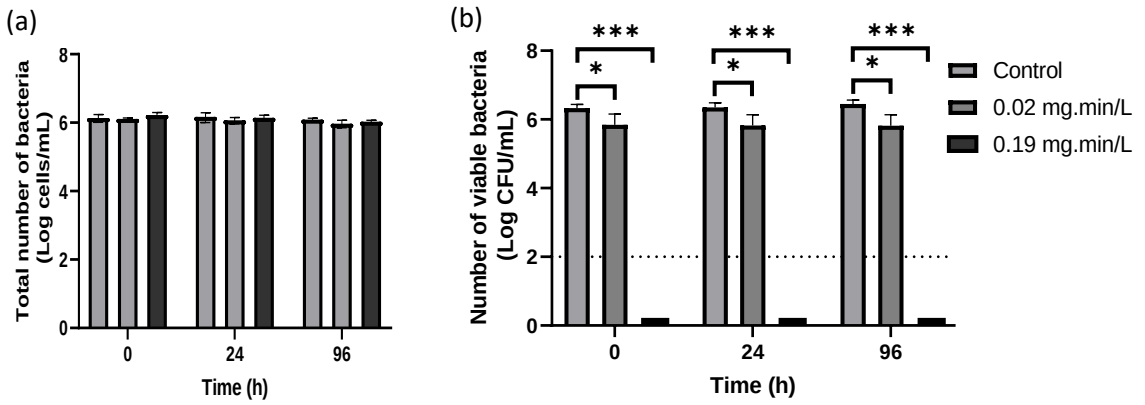


Figure 39 Recovery of *E. coli* after CEC treatment in continuous mode. (a) The total number of cells stained by SYBR and (b) The number of viable cells (CFU/mL) at T= 0 h, 24 h and 96 h, respectively. T=0 h refers to samples that were quenched immediately after CEC treatment. T = 24 h and 96 h refer to samples that were quenched immediately after CEC and then incubated at room temperature for 24 and 96 h, respectively. Control = sample passed through the CEC without CEC treatment and was incubated at room temperature for 24 and 96 hours. Error bars represent SD, * represents the level of significance and n= 3-17.

Previous studies have shown that seemingly non-viable cells can recover if incubated in the presence of fresh media (De Roy et al., 2012; Morton, Zhang, & Edwards, 2005). To examine this possibility, the recovery experiments were repeated but a final concentration of 1/3rd LB medium was added immediately following CEC treatment. Interestingly, cells treated with 0.10 mg.min/L or 0.19 mg.min/L CT were able to recover to 1 x 10^6 CFU/ml following a 24-h incubation period in 1/3rd LB medium. Cells treated with 0.3 mg.min/L did not recover (**Figure 40b**).

These experiments were performed at 3 different CT; 0.10 mg.min/L, 0.19 mg.min/L and 0.30 mg.min/L which are known to have no culturable cells (< limit of detection, i.e. 100 CFU/mL) immediately after CEC at (T= 0 h).

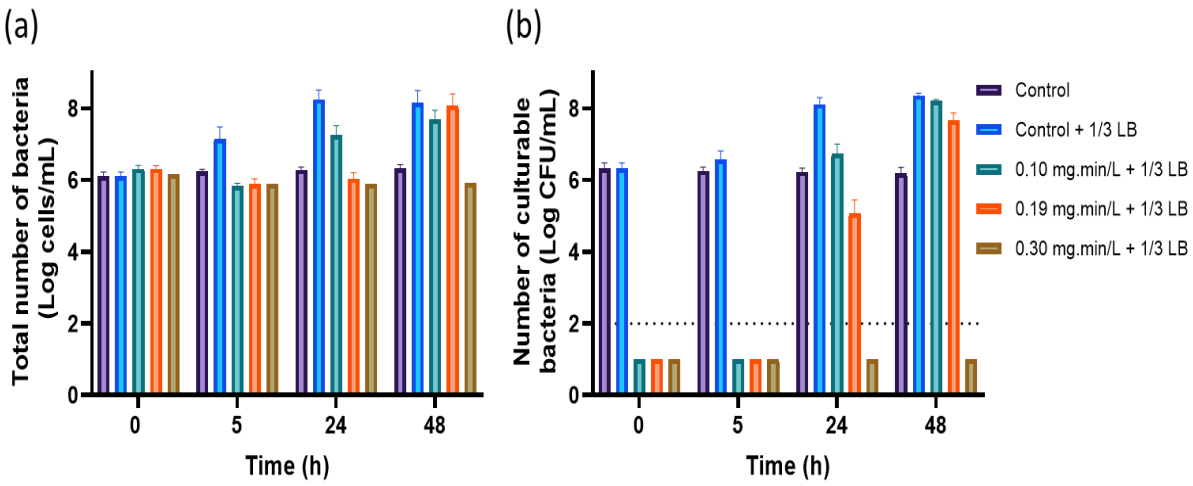


Figure 40 Recovery of *E. coli* after CEC treatment in continuous mode in the presence of 1/3 LB. (a) The number of SYBR stained bacteria and (b) CFU/mL at T= 0 h, 5 h, 24 h and 48 h, respectively. T=0 h refers to samples that were quenched immediately after CEC treatment. T = 5 h, 24 h and 48 h refer to samples that were quenched immediately after CEC, spiked with 1/3 LB and incubated at room temperature for 5 h, 24 h and 48 h, respectively. Control-sample that went through the CEC but did not undergo CEC treatment and was incubated at room temperature for up to 48 hours. Control + 1/3 LB: Control sample spiked with 1/3 LB at T= 0 h and incubated at room temperature for up to 48 hours.

Table 7 shows the increase/decrease in the number of *E. coli* after CEC treatment in the presence of 1/3 LB as determined by SYBR staining and plate counts when exposed to CT values of 0.10 mg.min/L, 0.19 mg.min/L and 0.30 mg.min/L. 1/3rd of LB medium was added at T= 0 h (immediately after CEC) and the samples were incubated at room temperature for 5 h, 24 h and 48 h.

Table 7 Number of *E. coli*.

Sample	Control + LB		0.10 mg.min/L		0.19 mg.min/L		0.30 mg.min/L	
Time (h)	Total Bacteria (cells/mL/h)	Culturable Bacteria (CFU/mL/h)	Total Bacteria (cells/mL/h)	Culturable Bacteria (CFU/mL/h)	Total Bacteria (cells/mL/h)	Culturable Bacteria (CFU/mL/h)	Total Bacteria (cells/mL/h)	Culturable Bacteria (CFU/mL/h)
0 - 5	$+2.61\times10^{6}$	$+3.12\times10^{5}$	0	0	0	0	-1.35×10^{5}	0
5 - 24	$+8.74\times10^{6}$	$+6.71\times10^{6}$	$+9.18\times10^{5}$	$+2.87\times10^{5}$	$+1.71\times10^{4}$	$+6.15\times10^{3}$	$+1.58\times10^{2}$	0
24 - 48	-1.38×10^{6}	$+4.17\times10^{6}$	$+1.33\times10^{6}$	$+6.53\times10^{6}$	$+4.91\times10^{6}$	$+1.34\times10^{6}$	$+1.42\times10^{3}$	0

Recovery of *E. coli* after exposure to stress in the form of oxidants generated by the CEC was observed at CT of 0.10 mg.min/L and 0.19 mg.min/L (**Figure 40a and b**). For the CT of 0.10 mg.min/L and 0.19 mg.min/L, a cellular production (as determined by SYBR staining) of 9.18×10^5 cells/mL/h and 1.71×10^4 cells/mL/h respectively, was observed between 5 h and 24 h (**Table 7**). The higher the CT value (more the cells are stressed), the longer it took for the cells to recover in the presence of nutrients. This recovery is very similar to the VBNC phenomenon. No recovery was observed over 48 hours for *E. coli* exposed to a CT of 0.30 mg.min/L (**Figure 40a and b**) with cell counts (as determined by SYBR staining) remaining stable at about 8×10^5 cells/mL and no culturable cells (as determined by plate counts) for up to 48 hours after nutrient addition. Furthermore, at CT of 0.10 mg.min/L and 0.19mg.min/L, there is a reduction of factor 3 and 2.8, respectively, between the production rate of CFU and cells (**Table 7).** This means that the number of culturable cells produced account for only about 1/3 of the total number of cells stained by SYBR that grow in the sample. This seems to be a VBNC phenomenon considering the high limit of detection (100 CFU/mL).

4.3.2 Resuscitation Test

After 24 hours of recovery in the presence of nutrients, approximately 1.4×10^6 culturable cells were observed. After 24 hours of recovery, a 10X and 100X dilution of the initial sample resulted in approximately 1.1×10^5 CFU/mL and 1.3×10^4 CFU/mL. After 24 hours of recovery, the number of cells in the control sample stayed almost the same (T=0 - 2.3 X 10^6 CFU/mL, T=24 h – 2.2 X 10^6 CFU/mL) since the cells were incubated at room temperature in phosphate buffer only (absence of nutrients to favour growth) (**Figure 41**). It is interesting to note that the final number of culturable cells formed reflects the dilutions applied. This suggests that the cells are recovering from the VBNC state to form culturable cells after 24 hours of recovery in the presence of nutrient.

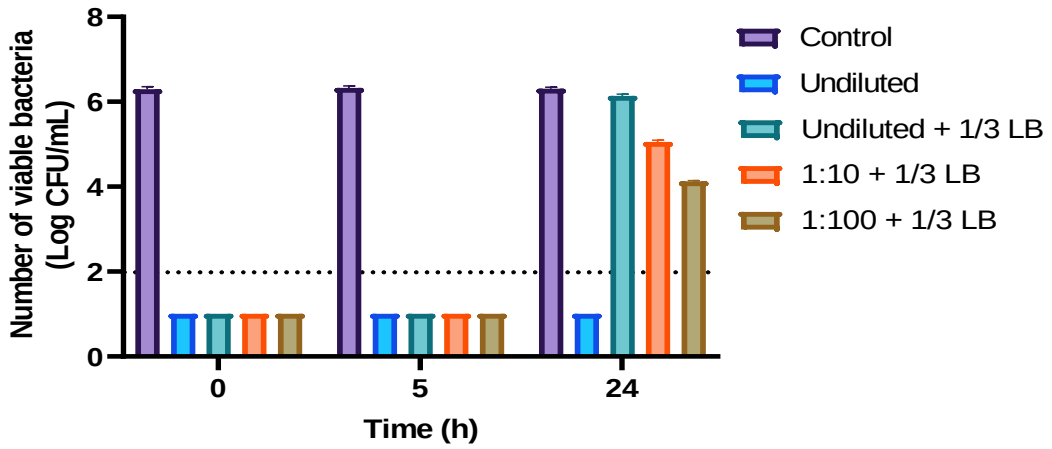


Figure 41 Resuscitation test of *E. coli* exposed to CEC in continuous mode with a CT of 0.09 mg.min/L. The CFU/mL of the samples after CEC treatment when stored at room temperature for 24 hours. Control = non-treated *E. coli* suspension. Undiluted = sample that was quenched immediately after CEC treatment. Undiluted + 1/3 LB = undiluted sample + 1/3 LB at T= 0 h. 1: 10 + 1/3 LB = Undiluted sample diluted 10X + 1/3 LB at T= 0 h. 1:100 + 1/3 LB = undiluted sample diluted 100X + 1/3 LB at T= 0 h.

4.4 Discussion

The oxidants generated by the CEC did not affect the total number of bacterial cells irrespective of the treatment applied (**Figure 39a**). This suggests that there was no measurable cell lysis and no DNA alteration after exposure (CT < 0.19 mg.min/L) to CEC treatment. If CEC treatment would have lysed the cells, we would expect to see a decrease in the total number of cells and a decrease in their fluorescence intensity but this was not the case (Nie et al., 2016; Phe et al., 2007).

The free chlorine concentrations (0.22 mg/L and 1.85 mg/L Cl_2 corresponding to a CT of 0.02 mg.min/L and 0.19 mg.min/L, respectively) generated by the CEC were not enough to cause measurable nucleic acid damage within the very short contact time (6 sec). Phe et al. (2007) have shown that chlorination (a hypochlorite solution, HOCl > 10.5 mg/L for 90 min) can damage nucleic acids (DNA and RNA) in *E. coli* cells using SYBR Green II but the concentrations of chlorine and the contact time applied were much higher than what was generated by the CEC in this study. It is known that hypochlorous acid (HOCl) can react with biological molecules like DNA and RNA (Dennis et al., 1979; Hoyano et al., 1973; Whiteman et al., 1997) in a very specific way that can lead to chemical and structural modifications (Whiteman et al., 1997). For instance, the oxidative damages of HOCl and the formation of abasic sites (as a result of the oxidation of DNA bases) can lead to the dissociation of double-stranded DNA into single strands when the hydrogen bonds are broken (Bernofsky, 1991; Prütz, 1998) and nitrogen-

centred radicals are formed (Hawkins & Davies, 2002). Phe, Dossot, and Block (2004) have also demonstrated that the reaction of HOCl with RNA can damage the tertiary structure of the RNA molecule. For example, the helical structure or folding of the RNA strand is affected when local interactions between the nucleotides are disrupted. Even during the 96 hours of recovery time post-CEC treatment, no measurable nucleic acid damage was observed. This indicates that the concentrations of free chlorine generated under our experimental conditions did not damage the *E. coli* cells to such an extent that we could see a change in the fluorescence intensity of SYBR stained bacteria indicating there were no nucleic acid alterations over the 96 hours of recovery.

On the contrary, the chlorine generated by the CEC decreased the culturability of the *E. coli* cells (**Figure 39b**). This was expected because it is well known that the culturability of the cells will decrease with an increase in the CT value (dose applied). This is because the bacteria that are still alive or metabolically active after being exposed to the stress (increasing chlorine dose), use their energy for repair of lesions and not for growth. The culturable fraction represented 54% of the total number of cells after CEC treatment with CT of 0.02 mg.min/L. No culturable cells (limit of detection 100 CFU/mL) of *E. coli* cells were observed following CEC treatment at the highest dose applied (CT= 0.19 mg.min/L). Wang et al. (2010) showed that *E. coli* cells start to lose their culturability on solid media when exposed to as low as 0.05 mg/L of free chlorine, while the free chlorine used in this study ranged between 0.2 – 1.9 mg/L. Therefore, it is clear that cell culturability is very susceptible to disinfection treatments. The decrease in the number of culturable cells can be due to two main reasons; either the cells are dead or while the cells are still structurally intact they have become non-culturable and some may remain active. The results suggest that in this study the *E. coli* cells may still be intact (as demonstrated above) even though they have lost their culturability. This is consistent with observations in previous studies (Bosshard et al., 2009; Hoefel et al., 2003; Kerwick et al., 2005; Wang et al., 2010).

It is possible that a fraction of the population of *E. coli* has entered into the viable but non-culturable cells (VBNC) state. The VBNC state is a physiological state in which bacteria are no longer culturable on standard bacteriological media but retain certain signs of viability, for instance, virulence, metabolic activity and cellular integrity (Oliver, 2010). According to James D. Oliver (2000), cells enter the VBNC state when they are under natural stress, like starvation, incubation outside the temperature range of growth or when exposed to white light. *E. coli* and other bacteria (such as entero-hemorrhagic *E. coli* and *Legionella pneumophila*) are known to enter the VBNC state when they are under stress from oxidants like chlorine (Howard & Inglis, 2003; Keer & Birch, 2003; Leclerc & Moreau, 2002; Wang et al., 2010). For example, cells enter the VBNC state when they are exposed to chemically and electrochemically dosed chlorine (Wang et al., 2010). Irrespective of the disinfection method (chemical or electrochemical) and contact time (10 min or 40 min), at a free chlorine

concentration of 0.2 mg/L, more than 50 % of the *E. coli* cells enter in the VBNC state. More than 90 % of the *E. coli* cells enter into the VBNC state when exposed to 0.2 mg/L of free chlorine (aqueous sodium hypochlorite) for 10 min or 0.1 mg/L of free chlorine for 40 min (Wang et al., 2010).

During the quite short recovery time, the culturable cells present after CEC treatment in continuous mode (CT= 0.02 mg.min/L) were in an environment that was not as stressful as the CEC treatment. However, these conditions were neither favourable for the recovery of the non-culturable fraction nor the bacterial growth of the culturable fraction over the 96 hours of recovery. As the medium (phosphate buffer) lacked a source of energy for bacterial growth it would be expected there would not be regrowth or recovery of bacteria. According to these results, there are still two questions that need to be answered

- Are the non-culturable cells immediately after CEC dead?
- Are the recovery conditions sufficient to resuscitate the non-culturable cells?

The recovery of *E. coli* over 48 hours in the presence of added nutrients demonstrated the following points; 1) recovery was observed after CEC treatment at CT values of 0.10 mg.min/L and 0.19mg.min/L, 2) no recovery was observed when a CT of 0.30 mg.min/L is applied, 3) the total number of cells produced (measured by flow cytometry) did not match the number of culturable cells recorded.

During the 24 hours of recovery, the number of culturable cells (measured by plate counts) formed did not match that of the total number of SYBR stained cells. It is expected that an increase in the total number of cells would also mean an equivalent increase in the number of culturable cells. But this was not the case (**Figure 40a** and **b**). The number of cells that are able to divide into daughter cells in the liquid medium should also be able to grow on solid agar. However, only 1/3 of the biomass produced are culturable on LB agar (**Table 7**). This suggests that even in the presence of additional nutrient and after 24 hours of recovery, the bacteria may be switching to the VBNC state. So, could it be possible that the cells were not culturable because of: 1) a problem with the agar, 2) the length (24 hours) of incubation time- was it too short? 3) was the liquid environment more favourable for stressed *E. coli* cells to divide than on agar plates? Indeed, previous studies have shown that the addition of ROS scavengers to agar plates reactivated damaged cells (Bang, Drake, & Jaykus, 2007; Calabrese & Bissonnette, 1990; Cuny, Lesbats, & Dukan, 2007; Czechowicz, Santos, & Zottola, 1996; Dukan, Belkin, & Touati, 1999; Gogniat & Dukan, 2007; Lee & Hartman, 1989; Maalej et al., 2004; McDonald, Hackney, & Ray, 1983; Teo, Ziegler, & Knabel, 2001). Furthermore, Ducret, Chabalier, and Dukan (2014) showed that the addition of pyruvate and glutamate to the BCYE agar restored the culturability of VBNC *L. pneumophila* cells.

The resuscitation results suggest that the cells that were recovering and growing to form culturable cells were actually VBNC cells. In the 100X diluted sample, if we hypothesise that one culturable cell is growing and forming culturable cells after 24 hours of recovery in the presence of nutrients. Therefore, with a generation time of 35 min (**Figure 43** in **Appendix**), we should have about 3.8×10^{2} CFU/mL and 2.4×10^{12} CFU/mL after 5 hours and 24 hours of recovery in the presence of nutrients, respectively. But this was not the case. After 5 hours, the number of culturable cells recorded was below the detection limit (100 CFU/mL) and after 24 hours there was about 1.3×10^{4} CFU/mL (**Figure 41**), corresponding to a generation time of approximately 105 min after 24 hours. In addition to that, the factor of recovery corresponding to exactly the factor of dilution of the sample further demonstrates that the cells growing after 24 hours in the presence of nutrients were truly VBNC cells that have resuscitated as opposed to culturable but undetected at T= 0 h (**Figure 41**). Irrespective of the dilution applied, the final number of culturable cells formed never exceeded that in the control sample (untreated sample + 1/3 LB). If culturable but undetected cells were present in the samples tested, the final number of culturable cells should be greater than 10^{8} CFU/mL for the same conditions applied but this was not the case. Hence, this suggests that no culturable but undetected cells were present in the samples immediately after CEC treatment. This implies that the observed culturable cells were actually VBNC cells that have resuscitated after 24 hours of incubation at room temperature and in the presence of nutrients.

4.5 Conclusion

This study aimed to contribute to a better understanding of the potential for bacteria to recover or regrow in the presence or absence of nutrients post electrochlorination using flow cytometry and heterotrophic plate counts.

- No culturable *E. coli* cells were observed immediately after CEC treatment with CT of 0.10, 0.19 and 0.30 mg.min/L. However, flow cytometry showed that the total number of SYBR-stained cells and the mean fluorescence intensity for each cell did not change significantly after treatment. In the absence of any nutrient addition, neither a bacterial growth nor a recovery of culturable cells was observed up to 96h following treatment.

- After 24 hours incubation in the presence of nutrient addition post-CEC treatment, recovery was observed for the cells treated with a CT of 0.10 and 0.19 mg.min/L but not for the cells treated with CT of 0.30 mg.min/L. Cells treated with CT of 0.19 mg.min/L took longer to recover than those cells treated with CT of 0.10 mg.min/L. The number of culturable cells that recovered accounted only for about 1/3 of the total number of cells stained by SYBR. The data shows that flow cytometry has great potential as a diagnostic tool for the monitoring of the

microbiological quality of water by drinking water utilities and can eventually replace the traditional HPC for cell counting. Further studies using a combination of dyes such as SYBR® green I and propidium iodide can be done to determine the viability of cells. ATP analysis is another culture-independent technique that could be used to determine the viable biomass.

- The presence of VBNC after CEC treatment with a CT of 0.09 mg.min/L highlights the need for drinking water utilities to constantly monitor the level of residual disinfectant and the microbiological safety of drinking water from the disinfection point to consumers tap in order to safeguard the public from waterborne diseases. However, further studies are required to confirm the presence of VBNC after CEC treatment. This could have been done by monitoring *E. coli* transcript by RT qPCR or RNAseq or by measuring cell metabolic activity using a combination of dyes (alamarBlue/alamarBlue HS and PrestoBlue/PrestoBlue HS Cell Viability Reagents).

4.6 Appendix

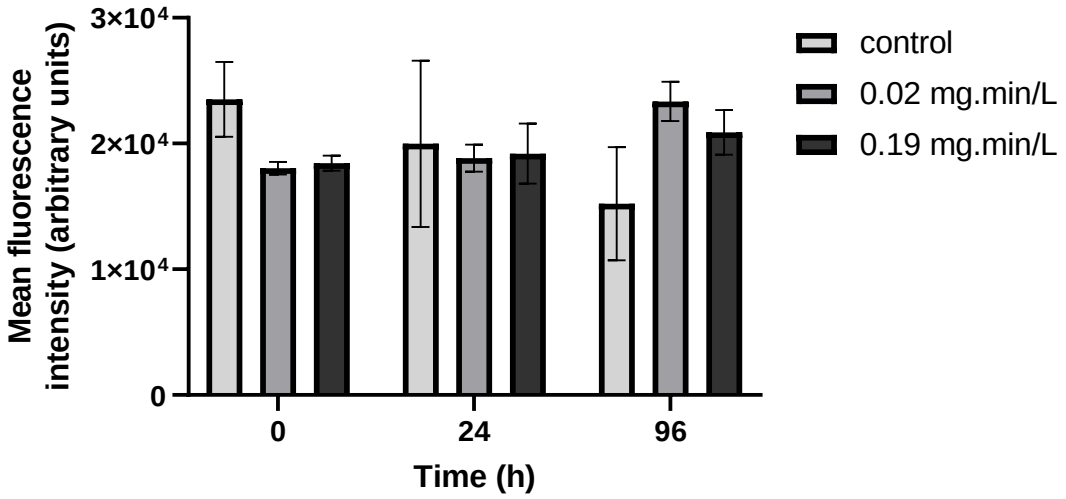


Figure 42 Mean SYBR fluorescence of *E. coli* following CEC treatment in continuous mode and up to 96 hours of recovery time. Samples were taken at T=0 , 24 and 96 hours post-CEC treatment with CT of 0.02 mg.min/L and 0.19 mg.min/L. T=0 h refers to samples that were quenched immediately after CEC treatment. T = 24 h and 96 h refer to samples that were quenched immediately after CEC and then incubated at room temperature for 24 and 96 h, respectively. Control-sample that went through the CEC but did not undergo CEC treatment and was incubated at room temperature for 24 and 96 hours. (Error bars represent SD and n = 3-17)

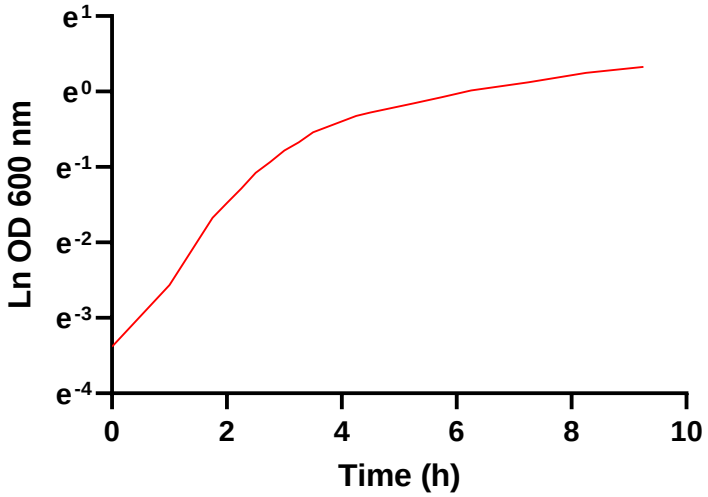


Figure 43 Growth curve of *E. coli B* in 1/3 LB medium.

Chapter 5. Impact of different chemical characteristics of natural waters on the disinfection efficiency of the Pilot CEC unit.

5.1 Introduction

This chapter is focused on the use and application of continuous electrochlorination (CEC) technology as an alternative oxidant for use in ground and surface water. Hydro-Dis® in collaboration with Water Corporation of WA developed a Pilot CEC unit, consisting of a pair of electrolytic cells, designed for sequential and adjustable two-stage water treatment. As previously mentioned in chapter 2 section **2.2**, the pilot unit consisted of four treatment lines (2 cells per treatment line) per stage (**Figure 6**). The first set of electrolytic cells (PUC1) is designed for use as a pre-treatment stage to oxidise common reduced ions found in WA ground waters, such as ferrous (Fe^{2+}) and manganese (Mn^{2+}). The second set of cells (PUC2) provides a secondary treatment stage for disinfection and production of residual chlorine as required. The pilot CEC unit has the capacity to treat up to 30 m^3/h, making it suitable for use in remote communities.

The presence of natural organic matter (NOM) (Ghasemian et al., 2017) and inorganic matter (e.g. bromide ions) in natural waters can significantly impact the disinfection treatment efficiency (Sohn et al., 2004). The performance of the pilot unit as assessed in Chapter 2 (section **2.3.4.3**) demonstrated that CEC technology can efficiently convert naturally occurring chloride found in ground water into chlorine. Moreover, since free chlorine is consumed by dissolved organic carbon (DOC), chlorine concentrations decrease more rapidly in water with high levels of DOC in the presence of increasing concentration of DOC (section **2.3.4.4**). As well as consuming produced chlorine, DOC and inorganic precursors react with chlorine-based oxidants to form DBPs, such as trihalomethanes (THMs) (Sohn et al., 2004). As noted in Section **2.3.4.4**, higher concentrations of adsorbable organic chloride and THMs are produced with increasing DOC concentration.

The objective of this chapter was to assess the inactivation efficiency and DBP (THMs) formation of the pilot CEC unit for 4 ground waters and 2 surface waters with varying characteristics (**Table 8**). Preliminary tests were initially carried out at Gwelup GWTP (Perth metropolitan area) where the pilot CEC unit was first installed to assess its safety and performance (**Chapter 2**). Tests involving raw (untreated water) ground waters were carried out at Gwelup, Australind (high iron and manganese content) and Capel (low chloride content) GWTPs. Pre-treated ground water (water with reduced ions removed) was used at Gwelup GWTP, to investigate the disinfection efficiency of the pilot unit in the presence of increasing DOC concentration. Pre-treated water was used so that the concentration of chlorine generated by CEC was only consumed through inactivation of *E coli* and through reaction with DOC. Two surface waters were tested at Denmark water treatment plant (WTP), Denmark River raw water (high DOC content) and Quickup Dam treated water (water with reduced ions removed and filtered to reduce DOC concentration). Treated water from Quickup Dam was used to investigate the conditions required to inactivate bacteria in a filtered water free from reduced ions (such as Fe^{2+} and

Mn^{2+}). To optimise water treatment at each water treatment site, the treatment by PUC1 and PUC2 were optimised such that near-zero free residual chlorine was produced after PUC1 and a target of 1 mg/L of free residual chlorine was achieved after PUC2 (immediately at the outlet of CEC). This was achieved by varying the feed flowrate and voltages (for both PUC1 and PUC2), the two parameters that has shown to affect chlorine production of the pilot CEC unit. In Chapter 2 (section **2.3.4.1** & **2.3.4.2**), we showed that chlorine production is directly proportional to the applied voltage and is inversely proportional to the feed flowrate.

5.2 Materials and Methods

The different chemical characteristics of the 4 ground waters and 2 surface waters tested to assess the inactivation and DBP (THMs) formation of the pilot unit are shown in **Table 8**.

Table 8 Characteristics of the waters tested in Western Australia.

Water Treatment Site		Units	Ground Waters				Surface Waters	
			Australind	Capel	Gwelup	Gwelup pre-treated water	Denmark River	Quickup treated water
Chloride		mg/L	155	47	140	140	347	181
Bromide		µg/L	229	216	324	180	911	486
DOC		mg/L	2	< 0.1	0.8	1.37	17.3	0.5
Iron	**Filtered**	mg/L	-	4.9	4.55	< LOD	nd	nd
	Total	mg/L	17.5	5.45	5.55	-	0.2 – 0.4	< 0.1
Manganese	**Filtered**	mg/L	-	0.21	0.06	-	nd	nd
	Total	mg/L	2.15	0.218	-	-	0.021	< 0.02
pH			5.99	6.24	6.5	-	6.54	7.2
Conductivity		µS/cm	646	387	806	799	1140/1314	695

** < LOD: less than limit of detection, nd: not detected, Gwelup and Gwelup pre-treated water were tested on different days.*

5.2.1 Experimental Apparatus

The pilot CEC unit with its four sampling points, Raw water (Sample point 4), Raw water and bacteria/Phage (sample point 3), PUC1 – after oxidation by PUC1 (Sample point 3) and PUC2 – after oxidation by PUC2 (Sample point 4), is described in Chapter 2 section 2.2.

5.2.2 Analytical Methods

Chlorine, iron, DOC and THMs analyses were performed as described in Chapter 2 section 2.2.1.

5.2.3 Bacterial culture, growth conditions and enumeration

In this study, the non-pathogenic microorganisms: *E. coli B* and *B. subtilis* ATCC 6633 were used. The culture, growth and washing conditions for each bacteria is described in Chapter 3. A total of 16 L of each bacteria was cultured, washed three times with 10 mM PB (pH 7.2) and resuspended into a final volume of 1 L. The bacterial cultures were then stored at 4 °C. Prior to spiking the Pilot CEC unit with bacteria, the bacterial suspensions were diluted 1 in 10 using Milli-Q® water in a final volume of 10 L. In order to account for the osmotic shock that can kill some of the bacteria, samples were taken before and after dilution into the 10 L container and also at the sampling point on the pilot unit just after the bacterial dosing pump but before PUC1 and PUC2 treatment for bacterial enumeration by plate counts.

The number of culturable bacteria was determined using the drop plate method (Herigstad et al., 2001) by determining the number of CFU/mL. The initial bacterial concentrations in the 10 L of Milli-Q water container were $10^9 – 10^{10}$ CFU/mL and $10^7 – 10^9$ CFU/mL for *E. coli* and *B. subtilis*, respectively.

5.2.4 Amplification and enumeration of bacteriophage T4

The protocol for amplifying and enumeration of phage T4 is described in Chapter 3 section 3.2.2. A phage stock solution was prepared and diluted in a final volume of 10 L of Milli-Q water before injection into the Pilot CEC unit. The initial phage suspensions in the 10 L container were $10^7 – 10^9$ PFU/mL.

5.2.5 Experimental Procedure

Disinfection performance of the pilot CEC unit were initially carried out at Gwelup GWTP (Perth metropolitan area) where the rig was first installed. The pilot CEC unit installed on a trailer was driven to three different sites in Western Australia, Australind, Capel and Denmark. At each water treatment site, known concentrations of bacteria/phage, diluted in a final volume of 10 L of Milli-Q water, were injected into the raw/treated water entering into the Pilot CEC unit at 30 L/h via a dosing pump. Samples were collected from the different sampling points for bacterial/phage enumeration, chlorine, iron, DOC and THMs analysis.

The effect of increasing DOC concentration from 2.48 – 5.40 mg/ on the disinfection efficiency of CEC treatment was investigated by injecting 11 g/L MIEX brine organic matter concentrate together with *E. coli* into the feed water (Gwelup pre-treated water) before PUC1. The number of culturable cells and residual chlorine was analysed before and after PUC2 (PUC1 was turned OFF). THMs were analysed after 15 min of contact time with the residual chlorine to establish the formation potential of chlorination by-products.

5.2.6 Sampling Procedure

Each tap was flushed for at least a minute before sampling water into 50 mL sterile conical centrifuge tubes (Corning™ Falcon, Thermo Fisher). Each tube contained sodium thiosulfate to quench any residual chlorine. Water samples were collected in triplicate. All the samples were kept on ice and transported to the laboratory within 8-10 hours of sampling. The samples were processed as soon as they arrived in the laboratory.

5.3 Results

Disinfection performance tests were performed with 4 ground waters and 2 surface waters. The ground waters tested included 3 raw waters (untreated waters) collected at Gwelup, Australind and Capel ground water treatment plants (GWTPs) and 1 pre-treated water (all reduced ions were oxidised) collected at Gwelup GWTP. The 2 surface waters used were from Denmark water treatment plant (WTP) and included Denmark River raw water and Quickup Dam treated water. The different characteristics of the studied waters are presented in **Table 8**. The 2 main operating conditions (feed flowrate and voltage applied) were optimised to provide near zero residual chlorine content for oxidation of reduced ions present in the waters after PUC1 and achieve a target of 1 mg/L free chlorine residual for disinfection after PUC2 at the different water treatment sites. Overall, for all tested waters, the THMs formed after 15 min and 7 days of contact time were well below the limit of the ADWG of 250 µg/L.

The pilot unit tests involved varying and measuring multiple parameters such as voltage, current, flowrates, free chlorine, total chlorine, non-purgeable organic carbon (NPOC) at each of the four sampling point on the pilot unit. In some instances, while the voltage applied was constant, the flowrate of the raw water entering the pilot unit fluctuated and this affected the current measured at PUC1 and PUC2. All the operational parameters applied on the different natural waters can be found in **Table 15**,**Table 16**,**Table 17**,**Table 18**,**Table 19** and **Table 20** in section **5.6 Appendix.**

5.3.1 Disinfection performance of CEC on ground water

Gwelup ground water (**Table 9**) exhibited high chloride content (140 mg/L), low dissolved iron content (5.55 mg/L) and a low presence of dissolved organic matter (0.8 mg/L). The feed flowrate was set at 8 m^3/L and PUC1 and PUC2 voltages were varied until the residual chlorine target set by Water Corporation was achieved. PUC1 and PUC2 applied voltages were set at 28.3 – 31.4 V and 38.6 V, and 49.6 A, respectively. PUC1 and PUC2 current measured were 36.4 – 40.3 A and 49.6 A, respectively. The concentrations of microorganisms before treatment were: 3.32 ± 0.05 × 10^6 CFU/mL, 3.50 ± 0.45 × 10^6 CFU/mL and 1.01 ± 0.04 × 10^4 PFU/mL for *E. coli*, *B. subtilis* and phage T4, respectively.

After PUC1, no measurable free residual chlorine was recorded but the total residual chlorine detected was 0.01 – 0.02 mg/L. The concentration of ferrous (Fe^{2+}) decreased from 5.55 mg/L to 1.25 - 1.53 mg/L. Following PUC1 treatment, 0.44 – 0.89 log of each microorganism was removed.

After PUC2, 1.21 – 1.47 mg/L of free chlorine was generated. Almost all of the Fe^{2+} were oxidised (0.04 – 0.08 mg/L). Following PUC2 treatment, no culturable microorganisms were detected for any of the samples, indicating at least a 4.3 – 6.1 log reduction in microorganisms had occurred. Therefore the pilot CEC unit both removed all microorganisms and produced the target free chlorine of 1 mg/L.

Table 9 Disinfection efficiency test carried out at Gwelup GWTP

Microorganism	Sample[1]	Flowrate (m^3/h)	Free Cl_2 (mg/L)	Total Cl_2 (mg/L)	NPOC[2] (mg/L)	Number of culturable cells (CFU/mL)	Log removal value (LRV)
E. coli	PUC1	8.63	0	0.02	0.8	1.22 ± 0.02 × 10^6	0.44
	PUC2		1.21	1.60	0.7	< LOD	6.08
B. subtilis	PUC1	8.32	0	0.01	0.8	1.25 ± 0.55 × 10^6	0.77
	PUC2		1.31	1.71	0.8	< LOD	6.10
Phage T4	PUC1	8.04	0	0.02	0.7	2.04 ± 0.12 × 10^4	0.89
	PUC2		1.47	1.73	0.7	< LOD	4.31

[1]Sample: PUC1 and PUC2 represent oxidation by the first and second set of electrolytic cells of the pilot unit, respectively. [2]NPOC: non-purgeable organic carbon.

The DBPs trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane were measured 15 min and 7 days following CEC treatment (**Figure 44**). Initial concentrations of all THMs were less than ~ 5 µg/L after 15 min. However, following 7 days of contact time with residual chlorine, these all increased with dibromochloromethane reaching a concentration of 22 µg/L.

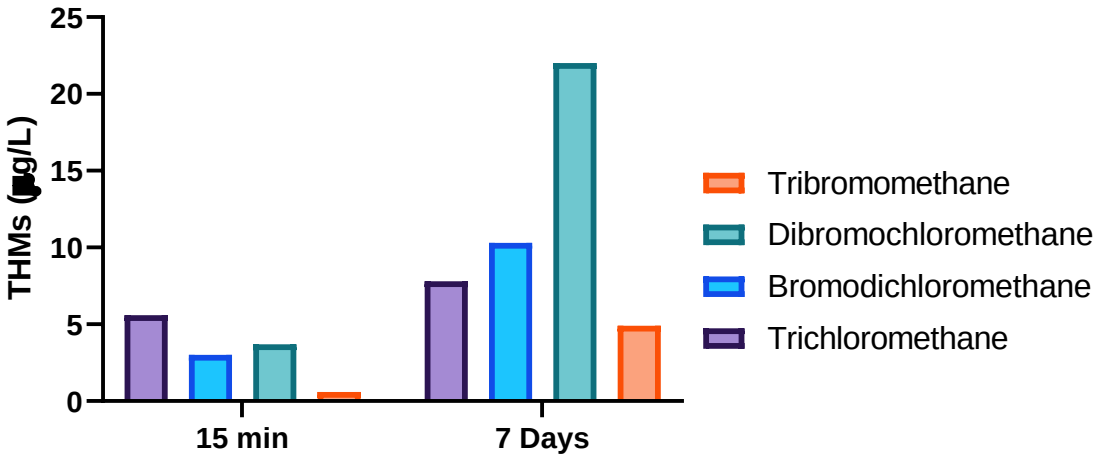


Figure 44 Trihalomethanes production following CEC treatment in continuous mode. Average trihalomethanes formation after 15 minutes and 7 days measured after CEC treatment of ground water from Gwelup GWTP. The free chlorine residual after 15 min and 7 days were 1.01 mg/L and 0.57 mg/L, respectively.

Australind ground water (**Table 10**) exhibited high chloride content (155 mg/L), high reduced iron (17.5 mg/L) and manganese (2.15 mg/L) concentration and the presence of dissolved organic matter (2 mg/L). The feed flowrate and PUC1 and PUC2 voltages were adjusted until the residual chlorine target of 1.0 mg/L was achieved. Due to the high concentration of reduced iron and manganese, a lower feed flowrate (7 m^3/h) and higher voltages (for both PUC1 and PUC2) was applied to generate enough chlorine to meet the residual chlorine target, compared to the test using Gwelup raw ground water (lower concentration of reduced iron and manganese). The final PUC1 and PUC2 voltage applied and current measured were 43 V and 43.4 V, 87.7 A and 100 A, respectively. The initial microorganism concentrations before PUC1 were: 4.67 ± 1.53 × 10^4 CFU/mL, 3.0 ± 1.0 × 10^3 CFU/mL and 3.87 ± 0.87 × 10^4 PFU/mL for *E. coli*, *B. subtilis* and phage T4, respectively.

After PUC1, samples contained a measured free chlorine of 0.02 - 0.05 mg/L. Ninety-nine percent of the ferrous ions (17.5 mg/L) and 75 % of the manganese ions (2.15 mg/L) present in the water were oxidised to ferric ions (Fe^{3+}, 17.4 mg/L) and manganese dioxide (Mn^{4+}, 0.53 mg/L), respectively. The residual chlorine generated removed 0.07 – 0.89 log of each microorganism tested. After PUC2, 1.11 - 1.70 mg/L of free chlorine was recorded. Remaining ferrous and manganese ions were further oxidised to ferric ions (17.45 mg/L) and manganese dioxide (0.62 mg/L). All microorganism were inactivated, resulting in at least 3.5 – 4.7 log reduction. The treatment failed to oxidise all the manganese present in the water with the applied settings.

Table 10 Disinfection efficiency test carried out at Australind GWTP.

Microorganism	Sample[1]	Flowrate (m^3/h)	Free Cl_2 (mg/L)	Total Cl_2 (mg/L)	NPOC[2] (mg/L)	Number of culturable cells (CFU/mL)	Log removal value (LRV)
E. coli	PUC1	7.08	0.02	0.03	2.8	$4.0 \pm 1.0 \times 10^4$	0.07
	PUC2		1.26	1.34		< LOD	4.67
B. subtilis	PUC1	6.84	0.05	0.07	3.3	$2.33 \pm 0.58 \times 10^3$	0.11
	PUC2		1.11	1.16		< LOD	3.48
Phage T4	PUC1	6.79	0.02	0.06	3.1	$5.60 \pm 1.68 \times 10^3$	0.84
	PUC2		1.70	1.88		< LOD	4.59

[1]*Sample: PUC1 and PUC2 represent oxidation by the first and second set of electrolytic cells of the pilot unit, respectively.* [2]*NPOC: non-purgeable organic carbon.*

THMs formation was analysed after 15 min and 7 days of contact time with the residual chlorine (**Figure 45**). When quenched after 15 min, 0.1 - 2.19 µg/L of THMs were measured. After 7 days of contact time, all the THMs measured after 15 minutes increased. Dibromochloromethane was the highest concentration of THMs formed followed by bromodichloromethane, trichloromethane and tribromomethane.

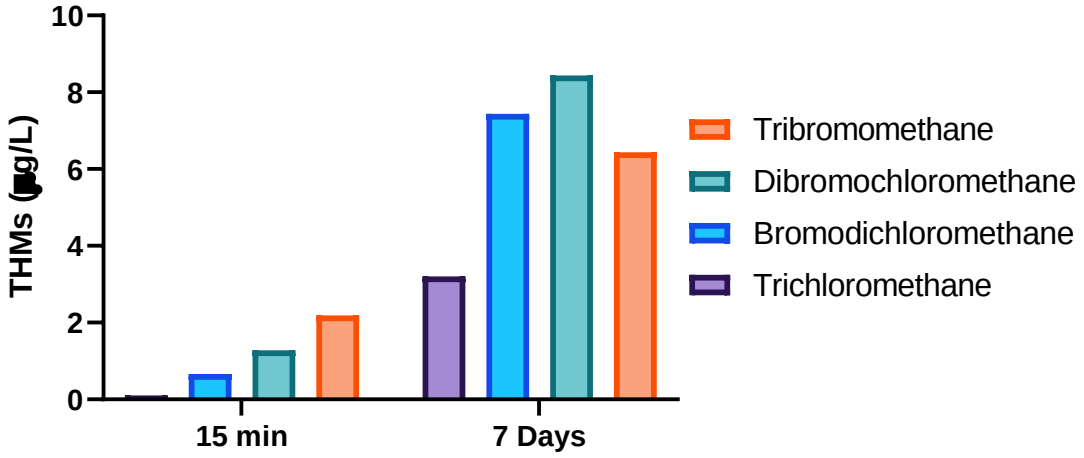


Figure 45 Average trihalomethanes (THMs) formation after 15 minutes and 7 days measured from the outlet of the CEC (after PUC2) following the electrolysis of ground water from Australind GWTP. The free chlorine residual after 15 min was 0.65 – 0.95 mg/L (free residual chlorine was not measured after 7 days but it is most likely to be < LOD considering the non-purgeable organic carbon (NPOC) of the water was 2 – 3 mg/L).

Capel ground water (**Table 11**) is characterised by a lower chloride content (47 mg/L) and is less enriched in iron (5.45 mg/L), manganese (0.22 mg/L) and dissolved organic matter (< 0.1 mg/L) compared to the Australind GW. Due to the low chloride content and the presence of ferrous and manganese ions in Capel ground water, a low feed flowrate (4 m^3/h) and relatively high voltage (for both PUC1 and PUC2) were applied to generate enough chlorine to meet the free chlorine residuals target set by Water Corporation. The final PUC1 and PUC2 voltages applied and current measured were 45.6 V and 31.9 V, 57 A and 41 A, respectively. The initial microorganism concentrations before PUC1 were: $3.67 \pm 1.2 \times 10^6$ CFU/mL, $8.67 \pm 1.5 \times 10^4$ CFU/mL and $3.12 \pm 1.04 \times 10^6$ PFU/mL for *E. coli*, *B. subtilis* and phage T4, respectively.

After PUC1, free chlorine was present between 0.02 and 0.04 mg/L. The concentration of Fe^{2+} and Mn^{2+} ions decreased from 5.45 mg/L to < 0.05 mg/L and 0.22 mg/L to 0.17 mg/L, respectively. The residual chlorine removed 1 – 1.5 log of each of the microorganisms tested.

After PUC2, the free chlorine residual recorded was 0.45 – 0.80 mg/L. Almost all of the reduced iron was oxidised (Fe^2 < 0.05 mg/L) but that was not the case for reduced manganese (Mn^{2+}: 0.086 mg/L). The residual chlorine generated removed 4.94 - 6.56 log of the microorganism tested. Overall, the CEC rig was able to generate enough free chlorine residual from a low chloride-containing water to inactivate all the microorganism tested but was unable to oxidise all the manganese present in the water under the conditions applied.

Table 11 Disinfection efficiency test carried out at Capel GWTP.

Microorganism	Sample[1]	Flowrate (m^3/h)	Free Cl_2 (mg/L)	Total Cl_2 (mg/L)	NPOC[2] (mg/L)	Number of culturable cells (CFU/mL)	Log removal value (LRV)
E. coli	PUC1	4.64	0.04	0.11	1.36	$1.27 \pm 0.2 \times 10^5$	1.46
	PUC2		0.49	1.07		< LOD	6.56
B. subtilis	PUC1	4.78	0.02	0.02	0.37	$9.0 \pm 2.1 \times 10^3$	0.98
	PUC2		0.80	0.95		< LOD	4.94
Phage T4	PUC1	4.78	0.02	0.02	0.01	$3.44 \pm 0.5 \times 10^5$	0.96
	PUC2		0.45	0.88		< LOD	6.49

[1]*Sample: PUC1 and PUC2 represent oxidation by the first and second set of electrolytic cells of the pilot unit, respectively. NPOC: non-purgeable organic carbon.*

THM formation was analysed after 15 min and 7 days of contact time with the residual chlorine (**Figure 46**). When quenched after 15 min, 0.13 – 1.23 µg/L of THMs were measured. Similar to Gwelup and Australind GW tests, after 7 days of contact time, the concentration of THMs increased. However, with Capel ground water, tribromomethane was the highest concentration of THMs formed followed by dibromochloromethane, trichloromethane and bromodichloromethane.

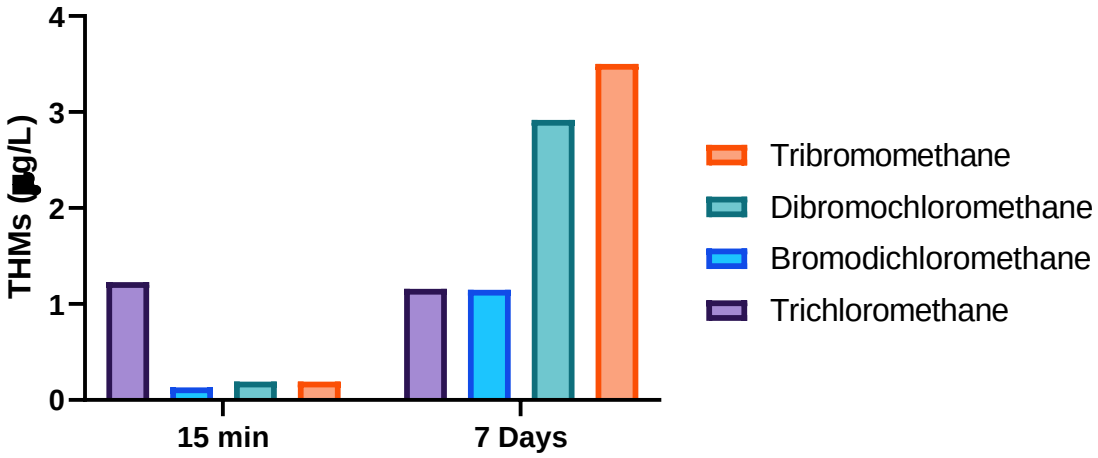


Figure 46 Average trihalomethanes (THMs) formation after 15 minutes and 7 days measured from the outlet of the CEC (after PUC2) following the electrolysis of ground water from Capel GWTP. The free chlorine residual after 15 min and 7 days were 0.15 – 0.35 mg/L and < LOD, respectively.

Gwelup pre-treated water was used to assess the inactivation performance of CEC on *E. coli* in the presence of varying concentrations of NOM (NPOC: 1.39 – 5.4 mg/L). During this study, PUC1 was turned OFF. Two dosing pumps were used to pump *E. coli* at a flowrate of 30 L/h and MIEX brine concentrate at flowrate 1 – 3 L/h. The disinfection efficiency of the pilot unit was assessed based only on the performance of PUC2 since the water had reduced ions previously removed. The feed water flowrate applied was set to 2.5 m^3/L and PUC2 voltage was varied until the target of 1.0 mg/L free residual was achieved. The optimised PUC2 voltage applied and current measured were 24.6 – 24.7 V and 17 – 17.5 A, respectively. The initial *E. coli* concentration before injection into the feed water was 1.13 ± 0.3 × 10^9 CFU/mL (**Table 12**).

After the feed water + microorganism sampling points (No $Treat^t$ - before PUC1 and PUC2 treatment), the free chlorine residual measured was 0.02 mg/L. However, no measurable log removal of any microorganism was measured. After PUC2, the free chlorine residual recorded was 0.74 – 1.11 mg/L. The residual chlorine generated was able to remove 6 - 7 log of *E. coli* in the presence of 1.39 - 5.4

mg/L of NPOC. Overall, CEC was able to achieve the target free chlorine residual of 1.0 mg/L and also inactivate all the *E. coli* tested in the presence of high NOM-containing water.

Table 12 Disinfection efficiency test carried out at Gwelup GWTP using pre-treated water.

Microorganism + NOM	Sample[1]	Flowrate (m^3/h)	Free Cl_2 (mg/L)	Total Cl_2 (mg/L)	NPOC[2] (mg/L)	Number of culturable cells (CFU/mL)	Log removal value (LRV)
E. coli	Feed water + *E. coli*	2.5	0.02	0.08	1.39	$1.03 \pm 0.32 \times 10^7$	-
	PUC2		0.9	1.17		< LOD	7.01
E. coli + 1 L/h NOM	Feed water + *E. coli*	2.5	0.02	0.08	2.48	$1.40 \pm 0.20 \times 10^7$	-
	PUC2		1.11	1.2		< LOD	7.15
E. coli + 2 L/h NOM	Feed water + *E. coli*	2.5	0.02	0.08	4.6	$1.47 \pm 0.20 \times 10^6$	-
	PUC2		1.16	1.25		< LOD	6.17
E. coli + 3 L/h NOM	Feed water + *E. coli*	2.5	0.02	0.08	5.4	$1.17 \pm 0.21 \times 10^7$	-
	PUC2		0.74	1.04		< LOD	7.07

[1]Sample: Feed water + E. coli represents Gwelup pre-treated water + E. coli before PUC2 treatment, and PUC2 represent oxidation by the second set of electrolytic cells of the pilot unit. [2]NPOC: non-purgeable organic carbon.

THM formation was analysed only after 15 min of contact time with the residual chlorine generated by the CEC (**Figure 47**). In the presence of 2.48 mg/L of NPOC (*E. coli* + 1 L/h NOM), dibromochloromethane was the highest concentration of THMs recorded followed by bromodichloromethane, trichloromethane and tribromomethane. However, in the presence of higher NPOC concentrations (4.6 and 5.4 mg/L), trichloromethane was highest followed by bromodichloromethane, dibromochloromethane and tribromomethane.

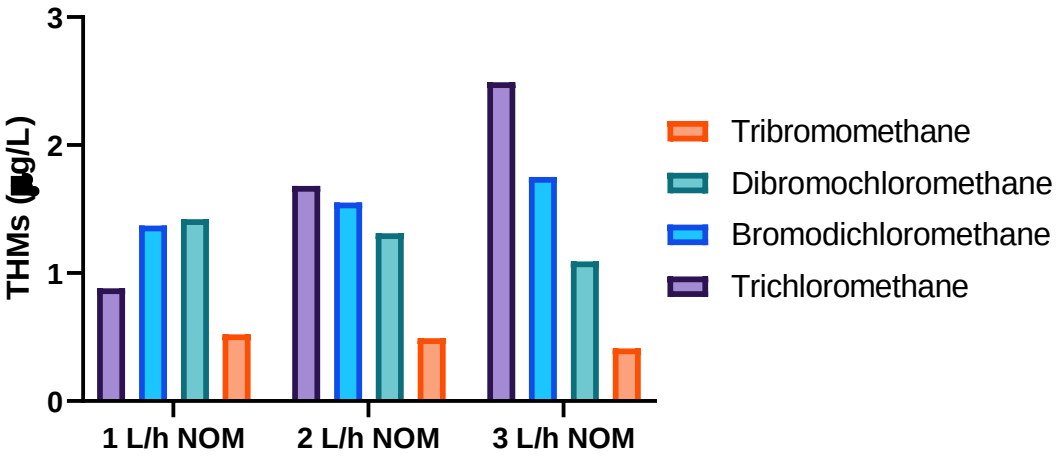


Figure 47 Average trihalomethanes (THMs) formation 15 minutes post-CEC treatment, following the electrolysis of pre-treated ground water from Gwelup GWTP. The free chlorine residuals after 15 min were 0.45 – 0.54 mg/L, 0.15 – 0.22 mg/L and < LOD, for 1, 2 and 3 L/h of natural organic matter (NOM), respectively.

5.3.2 Disinfection performance of CEC on surface waters.

Denmark River water is characterised by high chloride (347 mg/L) and high DOC (14.7 mg/L) content. A high chloride content indicated that a low voltage or a high flowrate can be applied. However, due to the presence of a high DOC content (chlorine consumers), the flowrate was set at 17 m^3/h and the voltages applied were adjusted until the target set by Water Corporation was achieved. The final PUC1 and PUC2 voltages applied and current measured were 16.5 V and 20.2 V and 42.5 A and 64.8 A, respectively. The initial microorganism concentrations before CEC treatment were: 5.67 ± 1.2 ×10^6 CFU/mL, 3.33 ± 0.6 ×10^5 CFU/mL and 4.40 ± 0.6 ×10^4 PFU/mL for *E. coli*, *B. subtilis* and phage T4, respectively **(Table 13**).

After PUC1, free chlorine residuals of 0.06 - 0.08 mg/L were measured. In the absence of reduced ions (surface water), the residual chlorine generated removed all (4.64 – 6.75 log) of the microorganism tested. The inactivation of all the microorganism in the presence of such low levels of measured free chlorine residuals could be a result of possible underestimation of the free residual chlorine present or the interference of NOM with reagents, or a high reaction rate leading to significant chlorine consumption between the sampling time and measurement (few seconds). Nevertheless, the CEC proved to be efficient at inactivating microorganism in a high oxidant demanding water where there was competition for free chlorine between organics and microorganisms.

Table 13 Disinfection efficiency test carried out at Denmark WTP using Denmark River water.

Microorganism	Sample[1]	Flowrate (m3/h)	Free Cl_2 (mg/L)	Total Cl_2 (mg/L)	NPOC[2] (mg/L)	Number of culturable cells (CFU/mL)	Log removal value (LRV)
E. coli	PUC1	17	0.08	0.24	16.44	< LOD	6.75
	PUC2		0.90	1.10		< LOD	0
B. subtilis	PUC1	17	0.06	0.1	17.19	< LOD	5.52
	PUC2		0.76	1.11		< LOD	0
Phage T4	PUC1	17	0.09	0.24	16.41	< LOD	4.64
	PUC2		0.70	1.20		< LOD	0

[1]Sample: PUC1 and PUC2 represent oxidation by the first and second set of electrolytic cells of the pilot unit, respectively. [2]NPOC: non-purgeable organic carbon.

THM formation was analysed after 15 min and 7 days of contact time with residual chlorine (**Figure 48**). When quenched after 15 min, 1.43 - 21.12 µg/L of THMs were measured. After 7 days of contact time, all the THMs, except trichloromethane, increased. Compared to Gwelup and Australind GW tests, after 7 days of contact time, bromodichloromethane was the highest concentration of THMs formed followed by dibromochloromethane, trichloromethane and tribromomethane.

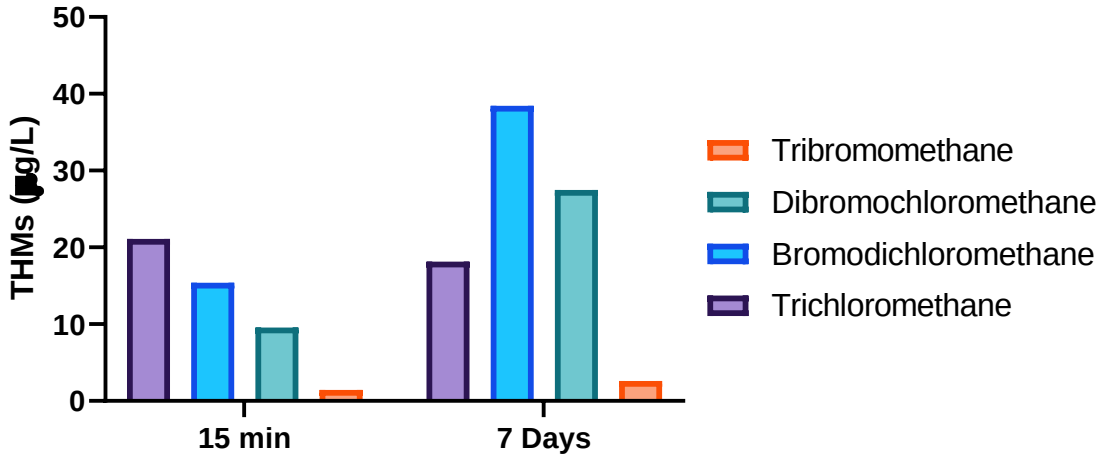


Figure 48 Average trihalomethanes (THMs) formation after 15 minutes and 7 days of contact time with chlorine generated by PUC2 using surface water from Denmark River. The free chlorine residual after 15 min and 7 days were 0.05 – 0.15 mg/L and < LOD, respectively.

Quickup Dam treated water is characterised by a high chloride (181 mg/L), high bromide (486 µg/L) and low DOC (< 0.1 mg/L) content. Given the absence of significant chlorine consumers (reduced ions and high DOC), the feed flowrate was set at 10.5 m^3/L and the voltages applied (for both PUC1 and PUC2) were adjusted until the free chlorine residual target was achieved. The optimised PUC1 and PUC2 voltages applied and current measured were 11.7 V and 20.2 V and 16.9 A and 64.8 A, respectively. The initial microorganism concentrations before CEC treatment were: 1.03 ± 0.15 ×10^7 CFU/mL, 1.10 ± 0.10 ×10^6 CFU/mL and 5.72 ± 0.76 ×10^4 PFU/mL for *E. coli*, *B. subtilis* and phage T4, respectively (**Table 14**).

After PUC1, 0.08 – 0.13 mg/L of free chlorine residual was recorded. In the absence of reduced ions, the residual chlorine generated removed 0.73 – 2.14 log of the microorganism tested. After PUC2, the CEC was able to achieve 2.89 – 3.0 mg/L of free residual chlorine at the outlet. The residual chlorine generated removed 3.90 – 5.46 log of the microorganism tested. Overall, the CEC unit was able to meet the target of 1 mg/L of free residual chlorine after PUC2 and also remove all the microorganism tested from a high chloride, high bromide and low DOC treated water.

Table 14 Disinfection efficiency test carried out at Denmark WTP using Quickup Dam treated water.

Microorganism	Sample[1]	Flowrate (m3/h)	Free Cl_2 (mg/L)	Total Cl_2 (mg/L)	NPOC[2] (mg/L)	Number of culturable cells (CFU/mL)	Log removal value (LRV)
E. coli	PUC1	10.5	0.13	0.21	0.73	2.90 ± 0.10 ×10^5	1.55
	PUC2		2.6	2.98		< LOD	5.46
B. subtilis	PUC1	10.5	0.08	0.19	0.51	8.00 ± 3.00 ×10^3	2.14
	PUC2		2.55	3		< LOD	3.90
Phage T4	PUC1	10.5	0.09	0.21	0.51	1.07 ± 0.08 ×10^4	0.73
	PUC2		2.56	2.89		< LOD	4.03

[1]Sample: PUC1 and PUC2 represent oxidation by the first and second set of electrolytic cells of the pilot unit, respectively. [2]NPOC: non-purgeable organic carbon.

THM formation was analysed after 15 min and 7 days of contact time with residual chlorine (**Figure 49**). When quenched after 15 min, 0.15 - 5.23 µg/L of THMs were measured. As with the previously tested waters, the concentration of THMs increased after 7 days of contact time. Compared to Denmark River water (high chloride and high bromide but higher DOC content), after 7 days of contact time, tribromomethane was the highest concentration of THMs formed followed by dibromochloromethane, bromodichloromethane and trichloromethane.

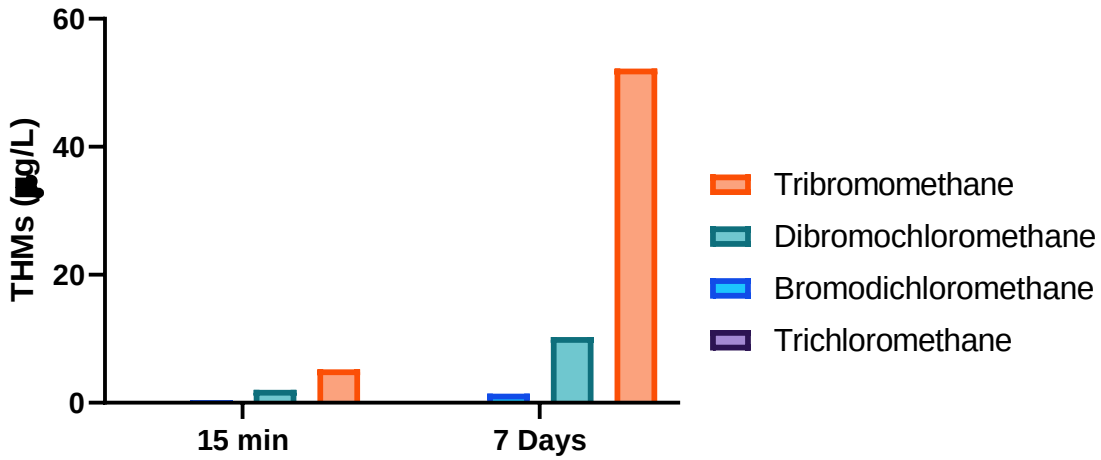


Figure 49 Average trihalomethanes (THMs) formation after 15 minutes and 7 days of contact time with chlorine generated by PUC2 using treated surface water from Quickup Dam. The free chlorine residual after 15 min and 7 days were 2.20 – 2.30 mg/L and 1.71 – 1.82 mg/L, respectively.

Among all of the studied waters (except Gwelup treated water), after 15 min of contact time, the highest total concentration of THMs recorded was from Denmark River water (47.50 µg/L) followed by Gwelup ground water, Quickup treated water, Australind ground water and Capel ground water (**Figure 50**). After 7 days of contact time, Denmark river water had the highest Total THM concentration followed by Quickup treated water, Gwelup ground water, Australind ground water and Capel ground water. Overall, the total THMs, after 15 min and 7 days of contact time, for all studied waters were below the ADWG limit.

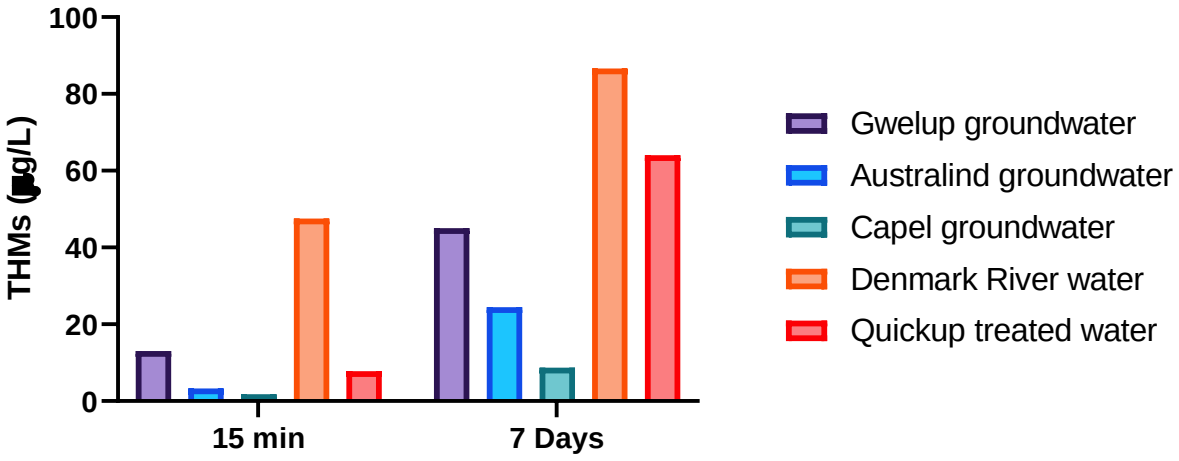


Figure 50 **Total concentration of trihalomethanes (THMs) recorded after 15 minutes and 7 days of contact time for the different studied waters. Total THMs were calculated by adding the concentration of trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane obtained from each of the studied water after 15 min and 7 days of contact time.**

5.4 Discussion

The pilot CEC unit was designed as a two-stage treatment unit, pre-treatment stage (pre-oxidation) by PUC1 and post-treatment (post-oxidation/disinfection) stage by PUC2. The aim of this study was to optimize the CEC settings (PUC1 and PUC2 voltages and feed water flow rates) on such that near zero free residual chlorine is produced after PUC1 and a target of 1 mg/L of free residual chlorine is achieved after PUC2. The disinfection performance of the pilot unit was tested on 4 ground and 2 surface waters, each with different physical chemical characteristics, in Western Australia.

The pilot CEC unit was able to generate near zero free residual chlorine and remove a very low number of bacteria/phage after PUC1 from the disinfection of raw ground waters (Gwelup, Australind and Capel) (**Table 9**, **Table 10** & **Table 11**). Chlorine oxidises Fe^{2+} to Fe^{3+} and Mn^{2+} to Mn^{4+}.Therefore the presence of reduced iron (Fe^{2+}) and manganese (Mn^{2+}) in all the raw ground waters tested likely consumed most free chlorine after PUC1, making it unavailable for disinfection of microorganisms. To satisfy the chlorine demand related to the presence of reduced iron and manganese (i.e., fast chlorine consumers), for pre-treatment by PUC1, much higher voltages (31 – 45V) had to be applied for raw ground waters compared to raw surface water (Denmark River water: 16.5 V) that did not have reduced iron and manganese. The highest PUC1 voltage (45.6 V) was applied at Capel GWTP (**Table 11).** In addition to the presence of reduced iron and manganese, Capel ground water had the lowest chloride content/lowest conductivity among the ground waters tested. Therefore, a higher voltage was required to compensate for the low chloride/low conductivity content of the water. Overall, after

PUC1 treatment, the CEC generated low concentrations of free residual chlorine and consequently very low number of microorganism were removed.

After PUC2 treatment of raw ground waters, the target of 1.0 mg/L of free residual chlorine at the outlet was achieved and all microorganisms injected into the system were killed (**Table 9**, **Table 10** & **Table 11**). The voltages applied on PUC2 were very similar to those on PUC1. However, most of the reduced iron and manganese present in the waters were oxidised by the chlorine generated by PUC1. Therefore, under the conditions applied, the chlorine generated by PUC2 was enough to remove 6 log of each bacteria and phage tested and was also to oxidise any remaining reduced iron and manganese, except in the case of Capel ground water where some reduced manganese (0.086 mg/L) remained. Higher voltages (PUC1: 55.8 V and PUC2: 44.7 V) had to be applied to remove all of the reduced manganese (data not shown). Nevertheless, the pilot unit was able to achieve the target of 1.0 mg/L of free residual chlorine at the outlet (after PUC2) and inactivate 6 log of *E.coli. B. subtilis* and phage T4 for waters with high reduced iron and manganese (Australind ground water), low (Capel ground water) and high (Gwelup ground water) chloride content.

Denmark river water (raw surface water) results show that PUC1 was able to remove 6.75, 5.52 and 4.64 log of *E. coli*, *B. subtilis* and phage T4, respectively (**Table 13**). Even though the voltages applied on both PUC1 and PUC2 were lower than those applied on ground waters, CEC treatment of Denmark river water (high DOC: 17.3 mg/L) was successfully able to generate near zero free residual chlorine concentrations after PUC1 and also achieve the target of 1.0 mg/L free residual chlorine after PUC2. The inactivation of all the microorganisms after PUC1 is surprising considering the high DOC content of the Denmark River water and the very low free chlorine residual measured at the outlet of PUC2. A possible explanation is that we underestimated the free residual chlorine present and requires further studies. Nevertheless, the CEC proved to be efficient at inactivating microorganisms in a high oxidant demanding water where there was competition for free chlorine between organics and microorganisms.

Gwelup pre-treated ground water disinfection performance results show that the pilot unit was able to inactivate 6 – 7 log of *E. coli* in waters with added NOM (DOC: 2.48 – 5.49 mg/L) (**Table 12**). Since the water used in this study was pre-treated (pre-oxidised to remove any reduced iron and manganese), PUC1 was switched OFF and the disinfection efficiency was assessed based on the performance of PUC2 only. Increasing the DOC content of the feed water had a slight impact on the free residual chlorine analysed after PUC2 when operated under the same condition i.e., 24.7 V. The lowest free chlorine residual content (0.74 mg/L Cl_2) was observed for the highest DOC content water (5.40 mg/L DOC). The fact that the voltage (24.7 V) applied stayed the same for all NOM-containing

waters tested, suggest that the lower free residual chlorine observed is due to the chlorine demand from the extra DOC (0.89 mg/L of C). However, no inhibitory effect of DOC on *E. coli* inactivation was observed since the chlorine generated was enough to inactivate all the *E. coli* injected into the system. Therefore, similar to the test with Denmark river water, CEC proved to be efficient at inactivating microorganism in high oxidant demanding water.

The pilot unit was able to remove all the microorganisms injected into the feed water from Quickup Dam treated water (**Table 14**). 0.08 – 0.13 mg/L and 2.5 – 2.6 mg/L of free residual chlorine were generated after PUC1 and PUC2, respectively. The free residual chlorine measured were much higher than the targeted 0 mg/L of free residual chlorine after PUC1 and 1.0 mg/L of free residual chlorine after PUC2. In order to meet the targeted free residual chlorine after PUC1 and PUC2, lower voltages (< 11.7 V for PUC1 and < 20.2 V for PUC2) or a higher flowrate (> 10.5 m^3/h) should have been applied since chlorine production is directly proportional to the voltage applied but inversely proportional to flowrate (sections 2.3.4.1 & 2.3.4.2). Among all the tested waters (except Denmark River water), Quickup Dam treated water had the highest log removal of bacteria after PUC1. This is due to the highest concentration of free chlorine produced after PUC1. Furthermore, 2.89 – 3.0 mg/L of free residual chlorine after PUC2 was more than enough to remove all the remaining bacteria/phage, 5.46, 3.90 and 4.03 log of *E. coli*, *B. subtilis* and phage T4, respectively. Overall, even though the pilot unit did not achieve the targeted free residual chlorine, CEC treatment was successfully able to remove at least 6 log of each microorganism tested from a treated surface water.

The total trihalomethanes (THMs) recorded from highest to lowest was obtained using Denmark River water, Quickup treated water or Gwelup ground water, Australind ground water and Capel ground water after 15 min and 7 days of contact time with the residual chlorine (Figure **50**). The concentration of all THMs formed increased when the contact time increased from 15 min to 7 days. NOM and bromide ions are commonly found DBP precursors in naturally occurring water sources such as ground waters (Agus et al., 2009). Chlorinated/brominated carbonaceous DBPs such as trihalomethanes, haloacetic acids and haloacetonitriles are produced following reactions between chlorine and NOM involving: oxidation, addition to unsaturated bonds, and electrophilic substitution (Golea et al., 2017; Yee et al., 2006). Chlorine is known to produce the highest concentration of halogenated DBPs, with THMs as one of the largest group of DBPs identified in chlorinated water treatment plants (Krasner, 1999). Increasing the contact time allowed the residual chlorine to react with DOC to form more THMs. More brominated THMs were formed after 7 days of contact time compared to 15 min. After 7 days of contact time, the bromide content (from highest to lowest: Denmark river water, Quickup treated water, Gwelup ground water, Australind ground water and Capel ground water) of each studied water seems to dictate the total THMs formation as a consequence of higher production of brominated

THMs (mainly dibromochloromethane and bromodichloromethane) (**Figure 44**,**Figure** 45Figure 46, Figure 48 &Figure 49). While the targeted free residual chlorine was 1.0 mg/L after PUC2 for all studied waters, Denmark River water contained the highest concentration of total THMs because the source water had the highest DOC (17.3 mg/L) and bromide ion (911 µg/L) content among all the studied waters. Capel GW contained the lowest concentration of THMs since the source water had the lowest DOC (< 0.1 mg/L) and bromide ions (216 µg/L) content. As previously mentioned in Chapter 2, the disinfection of bromide-containing waters with chlorine produces hypobromous acid and hypobromite (collectively known as aqueous bromine). Aqueous bromine is well recognised to be a better substituting agent and more reactive than chlorine (hypochlorous acid and hypochlorite ion) (Heeb et al., 2014) when reacting with NOM to generate brominated DBPs via electrophilic substitution and addition (Criquet et al., 2015). Furthermore, a high bromide: DOC ratio is known to favour the formation of brominated-DBPs Hence the highest concentration of brominated-DBPs when disinfecting Denmark River water. Overall, the total THMs, recorded after 15 min and 7 days of contact time with the residual chlorine of the different studied waters, were well below the limit of the ADWG of 250 µg/L. The higher the contact time (15 minutes v/s 7-days), the higher the THMs formation and the relative abundance of brominated THMs, and the higher the DOC (surface water v/s ground water), the higher the THMs formation.

5.5 Conclusion

Using the pilot unit we achieved the target of ~1.0 mg/L of free residual chlorine at the outlet (after PUC2) for ground and surface waters that contained low chloride (47 mg/L), high DOC (17.4 mg/L), high iron (17.5 mg/L) and high manganese (2.5 mg/L). The Pilot unit PUC1 cell voltage and flowrates were adjusted to produce a low concentration of free residual chlorine. The chlorine generated by PUC1 was used to oxidise reduced iron and manganese when present. In the presence of reduced ions, low to no bacterial inactivation was observed after pre-oxidation by PUC1 due to the fast reaction of chlorine with Fe^{2+} and Mn^{2+} ions. In the presence of high DOC content, pre-oxidation by PUC1 remained efficient for microbial inactivation however the possible interference of high DOC water with DPD measurement requires further studies. Following oxidation of Fe and Mn by PUC1, PUC2 voltage was then adjusted to generate 1.0 mg/L of free residual chlorine. The oxidants generated were able to inactivate at least 6 log of each microorganism. The DOC content of the source water determined the THM formation following treatment. Increasing the contact time of CEC-treated water also increased THM formation and the relative abundance of brominated THMs.

5.6 Appendix

Table 15 Operational parameters for the disinfection efficiency test carried out at Gwelup GWTP using raw ground water.

PUC1 ON PUC2 ON without bacteria											
Collected Sample[1]	Flowrate (L/h)	PUC1 voltage (V)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	Fe (filtered 0.45 µm) (mg/L)	Total Fe (mg/L)	NPOC/TN (mg/L)	
Raw water	9470	35.6	38.6	49.6	0			4.55	5.55	0.8/3.2	
PUC1	10000					0.00	0.02	1.53	5.58	0.7/3.2	
PUC2	9620					1.15	1.32	0.06	5.45	0.7/3.1	
PUC1 ON PUC2 ON *E. coli B* (Initial concentration in 10 L[2]: 1.25 ± 0.05 × 10^9 cfu/mL)											
Collected Sample[1]	Flowrate (L/h)	PUC1 voltage (V)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	Fe (filtered 0.45 µm) (mg/L)	Total Fe (mg/L)	NPOC/TN (mg/L)	Log removal value (LRV)
PUC1	8630	28.3	38.6	49.6	30	0	0.02			0.8/3.1	0.44
PUC2						1.21	1.6	0.08	5.55	0.7/3.1	6.08
PUC1 ON PUC2 ON *B. subtilis* (Initial concentration in 10 L[2]: 2.04 ± 0.34 × 10^9 cfu/mL)											
Collected Sample[1]	Flowrate (L/h)	PUC1 voltage (V)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	Fe (filtered 0.45 µm) (mg/L)	Total Fe (mg/L)	NPOC/TN (mg/L)	Log removal value (LRV)
PUC1	8320	31.4	38.7	49.6	30	0	0.01			0.8/3.1	0.77
PUC2						1.31	1.71	0.07	5.55		6.10
PUC1 ON PUC2 ON Bacteriophage T4 (Initial concentration in 10 L[2]: 4.25 ± 0.55 × 10^7 pfu/mL)											
Collected Sample[1]	Flowrate (L/h)	PUC1 voltage (V)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	Fe (filtered 0.45 µm) (mg/L)	Total Fe (mg/L)	NPOC/TN (mg/L)	Log removal value (LRV)
PUC1	8040	31.4	38.7	49.6	30	0	0.02			0.7/3.1	0.89
PUC2						1.47	1.73	0.04	5.55	0.7/3.1	4.31

[1] Samples were collected from 4 sampling points namely Raw water, Raw water + Bacteria, PUC1 (after pre-treatment cells) and PUC2 (after Post-treatment cells). [2] Initial concentration of microorganism in 10 L refers to the concentration of microorganism being dosed into the pilot unit via the dosing pump.

Table 16 Operational parameters for the disinfection efficiency test carried out at Australind GWTP using raw ground water.

PUC1 ON PUC2 ON *E. coli B (Initial concentration in 10L: 2.63 ± 0.55 × 10^8 cfu/mL)*											
Collected Sample	Flowrate (L/h)	PUC1 voltage (V)	PUC1 current (A)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	NPOC (mg/L)	Initial *E. coli* (cfu/mL)	Log removal value (LRV)
PUC1	7.08	43	87.7	43.4	100	30	0.02	0.03	2.8	4.67 ± 1.53 ×10^4	0.07
PUC2							1.26	1.34		4.0 ± 1.0 × 10^4	4.67
PUC1 ON PUC2 ON *B. subtilis (Initial concentration in 10L: 4.00 ± 1.0 × 10^7 cfu/mL)*											
Collected Sample	Flowrate (L/h)	PUC1 voltage (V)	PUC1 current (A)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	NPOC (mg/L)	Initial *B. subtilis* (cfu/mL)	Log removal value (LRV)
PUC1	6.84	43	87.7	43.4	100	30	0.05	0.07	3.3	3.0 ± 1.0 × 10^3	0.11
PUC2							1.11	1.16		2.33 ± 0.58 ×10^3	3.48
PUC1 ON PUC2 ON *Bacteriophage T4 (Initial concentration in 10L: 2.65 ± 0.23 × 10^7 pfu/mL)*											
Collected Sample	Flowrate (L/h)	PUC1 voltage (V)	PUC1 current (A)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	NPOC (mg/L)	Initial Phage T4 (pfu/mL)	Log removal value (LRV)
PUC1	6.79	43	87.7	43.4	100	30	0.02	0.06	3.1	3.87 ± 0.87 × 10^4	0.84
PUC2							1.70	1.88		5.60 ± 1.68 × 10^3	4.59
**all free and total chlorine analysis were filtered through a 0.45 µm filter before adding DPD reagents*											

Table 17 Operational parameters for the disinfection efficiency test carried out at Capel GWTP using raw ground water.

PUC1 ON PUC2 ON *E. coli B* (Initial concentration in 10L: $1.63 \pm 0.3 \times 10^9$ (cfu/mL))											
Collected Sample	Flowrate (L/h)	PUC1 voltage (V)	PUC1 current (A)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	NPOC/TN (mg/L)	Initial *E. coli* (cfu/mL)	Log removal value (LRV)
PUC1	4640	45.3	57.3	35	41	30	0.04	0.11	1.36/0.36	$3.67 \pm 1.2 \times 10^6$	1.46
PUC2							0.49	1.07		$1.27 \pm 0.2 \times 10^5$	6.56
PUC1 ON PUC2 ON *B. subtilis* (Initial concentration in 10L: $4.33 \pm 1.50 \times 10^7$ (cfu/mL))											
Collected Sample	Flowrate (L/h)	PUC1 voltage (V)	PUC1 current (A)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	NPOC/TN (mg/L)	Initial *B. subtilis* (cfu/mL)	Log removal value (LRV)
PUC1	4780	45.6	57	31.9	41	30	0.02	0.02	0.37/0.08	$8.67 \pm 1.5 \times 10^4$	0.98
PUC2							0.8	0.95		$9.0 \pm 2.1 \times 10^3$	4.94
PUC1 ON PUC2 ON Bacteriophage T4 (Initial concentration in 10L: $1.30 \pm 0.43 \times 10^9$ (pfu/mL))											
Collected Sample	Flowrate (L/h)	PUC1 voltage (V)	PUC1 current (A)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	NPOC/TN (mg/L)	Initial Phage T4 (pfu/mL)	Log removal value (LRV)
PUC1	4780	45.6	57	31.9	41	30	0.02	0.02	0.01/0.00	$3.12 \pm 1.0 \times 10^6$	0.96
PUC2							0.45	0.88		$3.44 \pm 0.5 \times 10^5$	6.49

Table 18 Operational parameter for the disinfection efficiency test carried out at Gwelup GWTP using pre-treated water.

PUC1 OFF PUC2 ON *E. coli* B & 1.39 mg/L DOC (Initial concentration in 10L: 1.13 ± 0.3 × 10^9 cfu/mL)									
Collected Sample	Flowrate (L/h)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	NPOC (mg/L)	Initial *E. coli* (cfu/mL)	Log removal value (LRV)
Raw water	2500	24.6	16.1	30	0.02	0.08	1.39	0	0
PUC2					0.9	1.17		1.03 ± 0.32 ×10^7	7.01
PUC1 OFF PUC2 ON *E. coli* B & 2.48 mg/L DOC (Initial concentration in 10L: 1.13 ± 0.3 × 10^9 cfu/mL)									
Collected Sample	Flowrate (L/h)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	NPOC (mg/L)	Initial *E. coli* (cfu/mL)	Log removal value (LRV)
Raw water	2500	24.6	17	30	0.02	0.08	2.48	0	0
PUC2					1.11	1.2		1.40 ± 0.20 ×10^7	7.15
PUC1 OFF PUC2 ON *E. coli* B & 4.60 mg/L DOC (Initial concentration in 10L: 1.13 ± 0.3 × 10^9 cfu/mL)									
Collected Sample	Flowrate (L/h)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	NPOC (mg/L)	Initial *E. coli* (cfu/mL)	Log removal value (LRV)
Raw water	2500	24.7	17.5	30	0.02	0.08	4.60	0	0
PUC2					1.16	1.25		1.47 ± 0.20 ×10^6	6.17
PUC1 OFF PUC2 ON *E. coli* B & 5.40 mg/L DOC (Initial concentration in 10L: 1.13 ± 0.3 × 10^9 cfu/mL)									
Collected Sample	Flowrate (L/h)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	NPOC (mg/L)	Initial *E. coli* (cfu/mL)	Log removal value (LRV)
Raw water	2500	24.6	17.3	30	0.02	0.08	5.40	0	0
PUC2					0.74	1.04		1.17 ± 0.21 ×10^7	7.07

Table 19 Operational parameters for the disinfection efficiency test carried out at *Denmark WTP using Denmark River water.*

PUC1 ON PUC2 ON *E. coli B* (initial concentration in 10 L: $1.47 \pm 0.32 \times 10^{10}$ cfu/mL)											
Sample collected	Flowrate (L/h)	PUC1 voltage (V)	PUC1 current (A)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	NPOC/TN (mg/L)	Initial *E. coli* cfu/mL	Log removal value (LRV)
PUC1	17000	16.5	42.5	20.2	64.8	30	0.08	0.24	16.44/0.99	$5.67 \pm 1.2 \times 10^6$	6.75
PUC2							0.9	1.1		0	0
PUC1 ON PUC2 ON *B. subtilis* (initial concentration in 10 L: $1.37 \pm 0.38 \times 10^8$ cfu/mL)											
Sample collected	Flowrate (L/h)	PUC1 voltage (V)	PUC1 current (A)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	NPOC/TN (mg/L)	Initial *B. subtilis* cfu/mL	Log removal value (LRV)
PUC1	17000	16.5	42.5	20.2	64.8	30	0.06	0.1	17.19/1.00	$3.33 \pm 0.6 \times 10^5$	5.52
PUC2							0.76	1.11		0	0
PUC1 ON PUC2 ON Bacteriophage T4 (initial concentration in 10 L: $2.98 \pm 0.33 \times 10^7$ pfu/mL)											
Sample collected	Flowrate (L/h)	PUC1 voltage (V)	PUC1 current (A)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	NPOC/TN (mg/L)	Initial Phage T4 pfu/mL	Log removal value (LRV)
PUC1	17000	16.5	42.5	20.2	64.8	30	0.09	0.24	16.41/0.83	$4.40 \pm 0.6 \times 10^4$	4.64
PUC2							0.7	1.2		0	0.00

Table 20 Operational parameters for the disinfection efficiency test carried out at Denmark WTP using Quickup Dam treated water.

PUC1 ON PUC2 ON *E. coli B (initial concentration in 10 L: 2.07 ± 0.21 × 10^9 cfu/mL)*											
Sample collected	Flowrate (L/h)	PUC1 voltage (V)	PUC1 current (A)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	NPOC/TN (mg/L)	Initial *E. coli* cfu/mL	Log removal value (LRV)
PUC1	10500	11.7	16.9	20.2	64.8	30	0.13	0.21	0.73/0.20	1.03 ± 0.15 ×10^7	1.55
PUC2							2.6	2.98		2.90 ± 0.10 ×10^5	7.01
PUC1 ON PUC2 ON *B. subtilis (initial concentration in 10 L: 3.33 ± 1.53 × 10^8 cfu/mL)*											
Sample collected	Flowrate (L/h)	PUC1 voltage (V)	PUC1 current (A)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	NPOC/TN (mg/L)	Initial *B. subtilis* cfu/mL	Log removal value (LRV)
PUC1	10500	11.7	16.9	20.2	64.8	30	0.08	0.19	0.51/0.14	1.10 ± 0.10 ×10^6	2.14
PUC2							2.55	3		8.00 ± 3.00 ×10^3	6.04
PUC1 ON PUC2 ON Bacteriophage T4 (initial concentration in 10 L: 1.86 ± 0.36 × 10^7 pfu/mL											
Sample collected	Flowrate (L/h)	PUC1 voltage (V)	PUC1 current (A)	PUC2 voltage (V)	PUC2 current (A)	Dosing pump flowrate (L/h)	Free chlorine (mg/L)	Total chlorine (mg/L)	NPOC/TN (mg/L)	Initial Phage T4 pfu/mL	Log removal value (LRV)
PUC1	10500	11.7	16.9	20.2	64.8	30	0.09	0.21	0.51/0.13	5.72 ± 0.76 ×10^4	0.73
PUC2							2.56	2.89		1.07 ± 0.08 ×10^4	4.76

Chapter 6. General Discussion, Conclusion and Future Considerations

6.1 General overview of the research

The overarching objective of this study was to investigate the disinfection performance of continuous electrochlorination (CEC) using both a pilot unit and laboratory units (EC1 & EC2) on natural and synthetic waters. We examined four main aspects of the CEC process, namely (i) characterisation of CEC in terms of chlorine production, inorganic by-product and disinfection by-product formation (for both pilot and laboratory units), (ii) inactivation efficiency of the laboratory unit on bacteria and bacteriophage, (iii) assessment of the microbiological quality of water after CEC treatment (using laboratory unit) with and without nutrient addition, and (iv) inactivation efficiency of the pilot unit using ground and surface waters in Western Australia.

6.2 General Discussion

The first objective of this study was to characterise the performance of lab and pilot units made from Titanium-based electrodes coated with a MMO layer of Ti/Rh/Ru, based on chlorine production, inorganic by-product and disinfection by-product formation before carrying any microbiological work. The findings indicated that voltage and NaCl concentration was directly proportional to chlorine production while chlorine production was inversely proportional to flowrate for both the lab and pilot units (**Chapter 2**).

For the laboratory unit, chlorine production was directly proportional to voltage applied and NaCl concentration but inversely proportional to flowrate. For a given voltage and NaCl concentration, increasing the flowrate decreased chlorine production, due to the reduced contact time with the electrodes of the CEC. For a given flowrate, a linear increase in chlorine production was observed with an increase in voltage and NaCl concentration. Increasing the voltage applied increased the current generated (Ohm's Law), which in turn increased chlorine production. A higher NaCl concentration increased the conductivity of the solution, thereby generating a higher current. Consequently, with a greater current, more chloride was oxidised to chlorine. The electrolysis of NaCl together with similar molar concentrations of sodium sulfate or sodium bicarbonate together generated higher currents than a solution containing only NaCl. Generally, chlorine production increases with higher current. Chlorine production increased in the presence of sodium bicarbonate by 41 % but decreased by 29 % in the presence of sulfate. The decrease in chlorine production in the presence of sodium sulfate was likely due to the complexing of sulfate ions on the surface of the anode thereby inhibiting the direct oxidation of chloride ions to chlorine.

The chlorine production of the pilot unit followed the same trends as observed with the lab-scale unit except that chlorine production was unaffected by flowrates, indicating the electrolysis of sodium chloride was mass transfer limited and not diffusion limited. The production of chlorine (g/h) was

proportional to the applied voltage and NaCl concentration. However, increasing the flowrate diluted chlorine produced and thus final chlorine concentration was proportional to flowrate.

Inorganic by-product formation (chlorate) was proportional to voltage and NaCl concentration but inversely proportional to flowrate for both the pilot and lab units. This was expected since an increase in chlorine production is usually accompanied by an increase in chlorate formation. The chlorate production was directly proportional to chloride concentration using the lab unit, however, for the pilot unit only a small fraction of the added chloride was converted to chlorate regardless of the chloride concentration. Furthermore, for a given flowrate on the lab unit, increasing the applied voltage increased chlorate production exponentially and increased the ratio of chlorate to free chlorine. This indicates that the voltages should be minimised to maintain sufficient chorine production while minimising chlorate production. While chlorate increased following CEC treatment, concentrations detected in this study are well below the WHO guidelines (700 µg/L). Bromate and chlorite were below the detection limit of the ion chromatography analysis, therefore were also well below the limit of the Australian Drinking Water Guidelines (0.80 mg/L and 0.02 mg/L, respectively).

Increases in dissolved organic carbon (2.35 – 4.25 mg/L) during treatment with the pilot unit led to increased disinfection by-products including adsorbable organic chloride, trihalomethanes and haloacetonitriles. When bromide ions (180 µg/L) were present in the water, adsorbable organic bromide was also produced but in lower concentration than adsorbable organic chloride and remained relatively constant with increasing DOC concentrations. This is because aqueous bromine (hypobromous acid and hypobromite ion) is a better substituting agent and more reactive than chlorine (Heeb et al., 2014) when reacting with NOM to generate brominated DBPs via electrophilic substitution and addition (Criquet et al., 2015). Furthermore, with increasing DOC concentration, the chorine containing species, trichloromethane, bromodichloromethane, dichloroacetonitrile and bromochloroacetonitrile increased. The predominant chlorine-containing species formed were trichloromethanes. On average, THMs incorporated 50% of the adsorbable organic chloride and 70% of the adsorbable organic bromide generated, a result that is commonly observed from the chlorination of bromide containing surface waters.

Following the characterisation of the CEC units (Chapter 2), the lab-scale CEC settings were optimised to examine the effects of the active chlorine species generated on common waterborne pathogens in a chloride-containing solution using plate counts and flow cytometry, in parallel (**Chapter 3**). The bacterial/phage suspensions tested consisted of 20 mg/L NaCl, 10 mM PB, pH 7.2 and the flowrate applied was set at 200 mL/min. Plate counts and plaque assays were performed before and after CEC treatment to assess the disinfection efficiency of CEC. Under the conditions applied in this study, CEC

was successfully able to inactivate all of the microorganisms tested (*E. coli*, *B. subtilis* and *S. maltophilia*, phage T4) except for *L. pneumophila* and MS2 phage, in a very short contact time (4.5 seconds). For a given CT value, *B. subtilis* was the most sensitive bacterium followed by *E. coli, S. maltophilia and L. pneumophila* (most resistant). For bacteriophage, Phage T4 was more sensitive than MS2 phage to the chlorine generated under the conditions applied in this study. Interestingly, *B. subtilis* (in the vegetative form in this study), showed the fastest inactivation rate when exposed to the oxidants generated by CEC. The disinfection results corroborated with previously reported observations that *B. atrophaeus* (previously reported as *B. subtilis* var. *niger*) is more sensitive (Bruguera-Casamada et al., 2016) than *Legionella* to free chlorine generated from a hypochlorite solution (Cervero-Aragó et al., 2015; Kuchta et al., 1983).

Irrespective of the CT applied, the total number of bacterial cells (both intact and damaged - *E. coli*, *S. maltophilia* and *B. subtilis*) measured by SYBR Green I, was not affected by the free chlorine generated by CEC under the same experimental conditions. The findings suggested that there was no immediate measurable cell lysis measurable by flow cytometry after exposure to CEC treatment in continuous mode. When exposed to a low CT (0 - 0.10 mg.min/L), the free chlorine (0 – 1.0 mg/L) generated was likely high enough to cause cellular membrane damage and enter the cytoplasm of the cell. Whereas at high CT (0.10 - 0.27 mg.min/L), the free chlorine (1.0 – 2.7 mg/L) generated was likely to cause more extensive cellular membrane damage and altered the nucleic acid but not enough to cause conformational changes to the binding sites of SYBR Green I and SYTOX Orange since the number of cells stained with SYTOX and their mean SYTOX fluorescence intensity increased. This was likely due to the very short contact time of 4.5 seconds and the low concentrations of free chlorine (0.2 - 2.7 mg/L) generated by CEC on the bacterial cells under the conditions applied. DNA and RNA damage occur when cells are treated with a hypochlorite solution (HOCl > 10.5 mg/L) for a 90 min contact time (Phe et al., 2007).

While the total number of cells measured by SYBR Green I remained the same following CEC treatment, the HPC results showed that the free chlorine generated by the CEC decreased the culturability of the *E. coli* cells when exposed to low CTs (0 - 0.10 mg.min/L, free chlorine 0 – 1.0 mg/L). The results suggested that either the cells are dead or the cells have entered into a VBNC state where they would no longer be detected by plate counts.

We demonstrated that even in the absence of chloride ions, CEC was able to inactivate 10^6 cells of *E. coli* when the unit was used in a recirculating mode for 10 min. A possible cause of this 10^6 cells reduction in the absence of chlorine production was the production of free radicals by the CEC electrodes. However, recent studies of a CEC cell made of the same electrode material as those used

here has revealed that radicals are unlikely to be produced during electrolysis (Curtin Water Quality research Centre, personal communication, unpublished data). Moreover, we observed no significant difference between the levels of *E. coli* inactivation cause by CEC-treated water (in the presence of chloride ions) and conventionally chlorinated (sodium hypochlorite) water for the same contact time, suggesting that there were no additional microbe-inhibiting compounds produced by the CEC. A more likely explanation is that the 6 log inactivation of *E. coli* observed in the absence of chloride ions was attributable to the direct oxidation of the *E. coli* cells on the surface of the electrodes. These experiments suggested that some direct killing of microbes might occur on electrodes, however, there might be limited practicality (time and running cost) for water treatment in operating the CEC unit in recirculation mode.

In order to assess the microbiological quality of water after disinfection, the potential for *E. coli* to recover or regrow following CEC treatment of a chloride-containing solution was investigated using the lab-scale CEC unit (**Chapter 4**). The ability of *E. coli* cells to recover in the presence or absence of nutrient addition following CEC treatment was analysed using flow cytometry and heterotrophic plate counts (HPC) in parallel. The results indicated that no culturable cells were detected immediately after CEC treatment with CT ≥ 0.10 mg.min/L as previously shown in chapter 3. However, the flow cytometry results showed that the total number of SYBR-stained cells and their mean fluorescence intensity for each cell did not change significantly after treatment. In the absence of any nutrient addition (only phosphate buffer), no bacterial growth and no recovery of culturable cells were observed during the 96 hours of incubation after CEC treatment. When nutrients (diluted LB, TOC: 2.2 mg/L) were added after CEC treatment, cell recovery was observed after 24 hours. Recovery of cells only occurred for those treated with a CT 0.19 mg.min/L or less. No recovery was observed for the cells treated with CT of 0.30 mg.min/L. The rate of recovery was found to be slower for cells exposed to higher CT (0.19 mg.min/L), suggesting more membrane damage occurred at higher CT.

Since the number of recovered cells following CEC treatment and nutrient addition represented about 1/3 of the total number of cells stained by SYBR Green I, it seemed likely that the recovering cells were previously injured and unculturable by the standard plate counts (VBNC) or were undetected immediately after CEC treatment (detection limit of 100 CFU/mL). Ninety-six bacterial species (including *E. coli*) are known to enter into the VBNC state. The VBNC state is a physiological state in which bacteria are no longer culturable on standard bacteriological media but retain certain signs of viability, for instance, virulence, metabolic activity and cellular integrity (Oliver, 2010). VBNC cells are known to recover and become culturable cells when the stress applied is removed and/or the cells are supplied with nutrient rich media (Kan et al., 2019). A resuscitation test was performed to confirm if

the cells that recover were previously injured cells (VBNC) or were undetected immediately after CEC treatment by plate counts. CEC treated samples were diluted 10 and 100 times and nutrient in the form of diluted LB was added to the diluted samples. An *E. coli* suspension without CEC treatment and an undiluted CEC-treated sample without LB addition were used as controls. After 24 hours of recovery at room temperature (22 ^{o}C - 24 ^{o}C) in the presence of nutrients, culturable cells were observed for CEC treated samples to which nutrients were added.

An alternative explanation for the apparent resuscitation of VBNC cells following CEC treatment was that the initial number of living cells in the CEC-treated samples was beyond the limit of detection for HPC and that following addition of LB an initially undetectable number of cells grew exponentially during the 24-hours incubation period. The doubling time for *E. coli* in LB is ~20 minutes (Sezonov, Joseleau-Petit, & Ari, 2007) so a single *E. coli* cell takes approximately 10 hours (31 doublings) to reach a typical maximum stationary-phase density of 1 x 10^9. In our experiments the initial concentration of 1.5 × 10^6 CFU/mL of cells were completely undetectable following treatment with 0.19 mg.min/L chlorine. The HPC method to detect these cells following CEC treatment assayed 10 µL samples of culture and therefore had a minimum limit of detection of 1 x 10^2 CFU/mL. Therefore, it was possible that a very small number of cells (less than 100) were indeed active (not VBNC) in the initial samples but were not detected by HPC. However, when the CEC-treated cell suspensions were diluted 1 in 10, and 1 in 100, prior to supplementation with LB, the number of culturable cells that formed following 24-hour incubation contained approximately 10- and 100-times less culturable cells than the undiluted samples. For two reasons this result implies that it was unlikely that a small number of normal and actively growth cells were missed by HPC counts. Firstly, if the number of initial viable cells present were indeed less than 100 CFU/mL, then there would be only 10 and 1- cell per mL in these diluted samples (respectively) and therefore the variance between the triplicates for each dilution would be very large (Luria & Delbrück, 1943). Indeed, in a 10 µL sample tested by HPC only one in ten replicates would be expected to contain any cells. Secondly, if an initially undetectable, but actively growing number of cells replicated exponentially for 24 h, all dilutions should have reached the same stationary-phase density within this period, but this did not occur. These observations support the hypothesis that the observed culturable cells were indeed VBNC cells that slowly recovered in the presence of nutrients. Therefore, the results of this study emphasised the importance of drinking water utilities to routinely monitor the level of residual disinfection, the microbiological quality of water after the point of disinfection and implement flow cytometry as a diagnostic tool in addition to HPC in order to safeguard the public from waterborne diseases undetectable by HPC.

The inactivation efficiency and the DBP (primarily THMs) formation of the pilot unit were investigated on ground and surface waters in Western Australia (**Chapter 5**). Five different waters with different chemical characteristics were tested for disinfection by injecting the pilot unit with known concentrations of common waterborne pathogens, *E. coli*, *B. subtilis* and bacteriophage T4, and the number of viable cells were analysed both before and after CEC treatment. The pilot unit consisted of two sets of cells, pilot unit cell 1 (PUC1) and pilot unit cell 2 (PUC2). PUC1 was designed for oxidation of reduced ions present in the waters and PUC2 was designed for disinfection. The pilot unit flowrate and voltage settings were optimized such that near zero free residual chlorine (0 – 0.13 mg/L) was produced after PUC1 and a target of 1 mg/L of free residual chlorine after PUC2 (immediately at the outlet of CEC). The chlorine generated by PUC1 was used to oxidise mainly reduced iron (Fe^{2+}) and manganese (Mn^{2+}) present in the ground waters to Fe^{3+} and Mn^{4+}, respectively. In the presence of reduced ions, low to no bacterial inactivation was observed after PUC1 due to the fast reaction of chlorine with Fe^{2+} and Mn^{2+}. In the presence of the high DOC-containing water (Denmark River water-surface water), no reduced ions were detected. Even though low levels of free residual chlorine (0.06 – 0.09 mg/L) were generated by PUC1, all the microorganism injected into the pilot unit were inactivated. This inconsistency could be due to interference of high DOC water with DPD measurements, however, this possibility was not confirmed in this work.

In summary, the pilot unit was able to meet the target of 1.0 mg/L of free residual chlorine after PUC2 for waters that contained low chloride, high DOC, high iron and high manganese. The chlorine produced by PUC2 was able to oxidise any remaining reduced ions and inactivate at least 6 log of each microorganism that were injected into the pilot unit. Therefore, the pilot unit was effective for both pre-oxidation and disinfection.

THMs formation increased with increasing DOC in the source water. The highest total THM (86.64 µg/L) formation was recorded following treatment of Denmark River water (surface water) after 7 days of contact time with the residual chlorine, since the water contained the highest DOC concentration (17.3 mg/L). Moreover, the concentration of all THMs formed increased when the contact time increased from 15 min to 7 days. Since all the tested waters contained bromide ions, the relative abundance of brominated THMs also increased with an increase in contact time. Nevertheless, the total THMs formed after 15 min and 7 days of contact time with the residual chlorine for all the studied waters were well below the ADWG limit of 250 µg/L.

6.3 Future Considerations

Water Corporation of WA has successfully commissioned the first large scale CEC treatment plant at Horrocks (Western Australia). CEC has proved to be an efficient technology for pre-treatment (improve aesthetics of water) and disinfection of ground waters. CEC has shown to be safe by eliminating manual handling of hazardous chemicals (i.e. chlorine gas, sodium hypochlorite, calcium hypochlorite). CEC is a green technology reducing reliance on existing source water supply (for e.g. CEC can replace conventional chlorination involved in brackish and wastewater reuse), and lowering logistical impacts (fuel for the transport of chemicals such as chlorine gas) and the CO_2 emission from the CEC units were low (< 4 % CO_2). Furthermore, the hydrogen gas generated at the cathode (more details in **2.3.4.5**) can be recovered as a resource for further technology development. The hydrogen can be used as a source of energy (fuel cell) to provide power to operate CEC, even on an industrial scale. Thereby, decreasing the carbon footprint since the combustion of hydrogen only emits water. The recovered hydrogen gas can also be stored and used as an emergency backup power supply. CEC has a lower capital expenditure and operational expenses for pre-treatment and disinfection since salt is cheap and it is easy to use and operate (Water Corporation of WA findings). As such, CEC is now part of Water Corporation's treatment assets fleet and will soon be replacing conventional chlorination on water treatment sites in Western Australia.

The transfer of DNA between bacteria is of critical importance to our healthcare systems due to the increasing rise of antibiotic resistance genes in bacterial pathogens. Further studies are required to determine if chlorination is able to reduce "genetic pollution" by destroying or mutating free DNA or bacterial viruses also known as bacteriophage (or phage), which could potentially facilitate transfer of antibiotic-resistance genes. Purified DNA and bacteriophage particles could be treated using CEC, re-isolated, sequenced and analysed for DNA lesions and mutations. Identified mutations would provide insight into the chemical modifications induced by CEC, which would lead to hypotheses that can be tested *in vitro* using purified nucleotides, mass spectrometry and NMR. The results would contribute to assess the effectiveness of chlorination using CEC on preventing the spread of antibiotic-resistance genes in the environment.

A worrying observation from this work was that a proportion of *E. coli* cells appeared to recover following CEC treatment, suggesting they had entered a VBNC state and subsequently recovered. Further studies using a combination of dyes such as SYBR® green I and propidium iodide can be done to confirm the viability of cells. ATP analysis is another culture-independent technique that could be used to determine the viable biomass. This would help to elucidate the inactivation mechanism of CEC on bacteria.

Chapter 7. References

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