THE DEVELOPMENT OF POINT-OF-USE TREATMENT TECHNOLOGIES FOR THE PRODUCTION OF DRINKING WATER

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Authors Declaration

Abstract

An estimated 884 million people worldwide lack access to drinking water from improved water sources. With the global population expected to reach eleven billion by the end of the 21st Century, stress on water and energy resources will be exacerbated. The development and implementation of innovative drinking water treatment technologies ensuring safe, sustainable drinking water provision is required. The overall aim of this project was to develop point-of-use [POU] water treatment technologies for the production of chemically and biologically safe drinking water.

A proof-of-concept decentralised drinking water treatment system [DWTS] investigated whether Drinking Water Inspectorate [DWI] standard drinking water could be produced by combining multi-step filtration processes, including ultrafiltration [UF] membrane columns, and low dosing of electrochemically activated solution [ECAS] dosing pre- and post-UF column membrane (total 1% [v/v]). The ECAS dosing regimen produce DWI standard drinking water, whilst the treated water produced throughout the control (no ECAS dosing) field trial was not biologically safe. The field trials brought to light the need for further investigations regarding the effect ECAS has on producing chemically (e.g. in terms of trihalomethanes [THMs]) and biologically safe water, as well as managing biofilm formation to minimise biofouling. THMs are regulated disinfection products [DBPs], and form through chlorine-based disinfectants reacting with organic matter. Comparing THM formation in water when treated with three disinfectants (ECAS, NaOCl and HOCl) as a function of contact time and free chlorine resulted in NaOCl producing significantly higher concentrations compared to HOCl and ECAS.

Chlorination processes in drinking water treatment ensure the production of biologically safe water. The comparative antimicrobial activity of ECAS against NaOCl and HOCl against standard microbial challenges in planktonic phase, and as biofilms, was determined. Throughout all standard chemical bactericidal assays against planktonic *Escherichia coli* ATCC 10536, neutral (HOCl) and acidic (ECAS) disinfectants exhibited significantly greater antimicrobial activity ($p < 0.01$) in comparison to NaOCl the alkaline disinfectant, except at the highest organic load. Increasing organic load resulted in significantly reduced antimicrobial activity for all disinfectants tested, except for HOCl at free chlorine concentrations > 50 mg L^{-1}. The antimicrobial activity of all disinfectants decreased against a mature *Pseudomonas aeruginosa* ATCC 15422 biofilm. ECAS demonstrated the greatest reduction in biofilm density compared to NaOCl and HOCl at free chlorine concentrations ≥ 50 mg L^{-1}. *In-situ* disinfectant dosing biofilm models to represent disinfection processes in water treatment were developed. Preliminary experiments demonstrated an inhibitory effect against biofilm formation through *in-situ* dosing, however, further model development and experimentation is required.

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Abbreviations

Abbreviation	Definition
AC	Activated Carbon
ATCC	American Type Culture Collection, USA
BDCM	Bromodichloromethane
BSA	Bovine serum albumen
CAR/PDMS	Carboxen/polydimethylsiloxane
CDC	Centre for Disease Control
CDI	Capacitive deionisation
CFU	Colony forming units
Coag	Coagulation
DBCM	Dibromochlotomethane
DI	Deionised water
DWI	Drinking Water Inspectorate
DWTS	Drinking water treatment system
DBP	Disinfection by-products
DPD	N, N-diethyl-p-phenylenediamine sulphate
ECAS	Electrochemically activated solutions
EDCs	Endrocrine disrupting chemicals
EPS	Extracellular polymeric substances
Floc	Flocculants
FTU	Formazin Turbidity Unit
GAC	Granular activated carbon
GC/MS	Gas chromatography mass spectrometry
GNI	Gross National Income
HAAs	Haloacetic acids
HOCl	Hypochlorous acid

Abbreviation	Definition
HS-SPME	Headspace solid-phase micro extraction
IHSS	International Humic Substance Society
LIC	Low income country
LMC	Low/middle income country
LoD	Limit of detection
MDGs	Millennium Development Goals
MF	Microfiltration
MLGA	Membrane lactose glucuronide *agar*
MPs	Microplastics
NaDCC	Sodium dichloroisocyanurate
NaOCl	Sodium hypochlorite
NOM	Natural organic matter
OD	Optical density
ORP	Oxidation reduction potential
PC	Polycarbonate
PES	Polyethersulfone
PET	Polyethylene terephthalate
POU	Point-of-use
PV	Photovoltaic
R2A agar	Reasoners 2A agar
RO	Reverse osmosis
RSF	Rapid sand filter
SDGs	Sustainable Development Goals
SODIS	Solar disinfection
SRHA	Suwannee River humic acid
THMs	Trihalomethanes

Abbreviation	Definition
TSA	Tryptone soya agar
TSB	Tryptone soya both
tTHMs	Total trihalomethanes
UF	Ultrafiltration
UK	United Kingdom
USA	United States of America
VBNC	Viable but nonculturable
WASH	Waster, Sanitation and Health
WHO	World Health Organization

Chapter 1. Introduction and Literature Review

1.1 Overview

Fresh water has been an integral part of human civilisations for thousands of years (Hesse and McDonald, 1974). However, uneven fresh water distribution, industrialisation and rapid increases in global population has increased pressure on the quantity and quality of fresh water sources accessible to humans (Carpenter, Stanley and Vander Zanden, 2011). An estimated 884 million people worldwide lack access to drinking water from improved water sources (Corcoran *et al.*, 2010; World Health Organization, 2016b), and this is expected to increase with the predicted global population expected to reach 11 billion by the end of the 21st Century (United Nations, 2004). Therefore, the development and implementation of alternative drinking water treatment technologies to ensure safe, sustainable drinking water provision is required.

The overall aim of this thesis was to develop point-of-use [POU] water treatment technologies for the production of chemically (i.e. trihalomethanes) and biologically safe drinking water. To achieve this, the specific aims of this thesis were four-fold:

1. To demonstrate the production of drinking water from a raw water source to Drinking Water Inspectorate (DWI) standards, using a decentralised drinking water production system.
2. To investigate the comparative formation of total trihalomethanes (tTHM) in water when treated with three chlorine-based disinfectants.
3. To compare the antimicrobial activity of three chlorine-based disinfectants for point-of-use drinking water treatment applications.
4. To investigate the inhibitory effect of three chlorine-based disinfectants against biofilms, through *in-situ* disinfectant dosing.

This thesis is comprised of seven chapters. Chapter 1 provides an overview to the problems faced in providing safe drinking water, including fresh water quality and quantity. Chapter 2 describes experimental methods used throughout this project. Chapter 3 demonstrates the capability of a novel drinking water treatment system to produce biologically safe drinking water from a raw water source to DWI standards. Chapter 4 compares tTHM formation in a laboratory model, when treated with three chlorine-based disinfectants as a function of contact time and free chlorine. Chapter 5 compares the antimicrobial activity of three chlorine-based disinfectants against standard microbiological challenges in planktonic phase (*Escherichia coli* ATCC 10536), and as biofilms (*Pseudomonas aeruginosa* ATCC 15422). Chapter 6 assesses the effect of *in-situ* disinfectant dosing at managing the formation of single species *Pseudomonas aeruginosa* ATCC 15422, and environmental multispecies biofilms. Finally, Chapter 7 provides a summary discussion of the data presented in Chapters 3, 4, 5 and 6, as well as overall conclusions and recommendations for future work.

1.2 Fresh Water Resources

1.2.1 The importance of water

"Water is life" and fresh water systems have been an integral part of human history for thousands of years (Hesse and McDonald, 1974). Fresh water has provided water for drinking, cooking, cleaning as well as provision for livestock, agricultural practices, and more recently as part of industrial development. Industrialisation, urbanisation and population growth has resulted in almost all fresh water systems becoming contaminated (i.e. agricultural, industrial and human waste), causing fresh water to be unsafe for human consumption without treatment. Intensive agricultural practices and industrial processes have caused emerging contaminants and pollutants such as antibiotics (Xi *et al.*, 2009; Schwartz *et al.*, 2003; Armstrong *et al.*, 1981), endocrine disrupting chemicals [EDCs] (World Health Organization, 2016a; Snyder and Benotti, 2010) and microplastics [MPs] (Mintenig *et al.*, 2019; Pivokonsky

et al., 2018) to contaminate virtually all water sources (Knobeloch *et al.*, 2000; Fawell and Nieuwenhuijsen, 2003; Carpenter, Stanley and Vander Zanden, 2011). The needs of a growing population with an expectation of a high quality of life has increased global resource demand, including water; increasing water stress to vast portions of the global population.

1.2.2 Water abundance and distribution

Approximately 70% of the earth's surface is covered by water (1,386,000,000 km^3), however, only 3% of this is fresh, approximately 35,029,000 km^3 (Shiklomanov, 1993), see Figure 1.1 (United States Geological Society, 2015). Of that, only 0.3% of fresh water is available in the form of surface waters such as lakes (91,000 km^3) and rivers (2120 km^3). The remainder of fresh water is stored in ice caps and glaciers (69.7% [24,064,000 km^3]), ground water (30.1% [10,530,000 km^3]), or other sources such as atmospheric and biological waters (0.9%) (Carpenter, Stanley and Vander Zanden, 2011). However, fresh water is not equally distributed, and typically, the greatest population densities occurred in regions where fresh water is plentiful, (i.e. temperate or tropical regions [Figure 1.2]). Industrialisation and technological advancements in recent years has resulted in population density increasing in regions where fresh water is not readily accessible. For example, the population of sub-Saharan Africa has increased from 228.5 million to 1.06 billion between 1960 and 2017 (The World Bank, 2019).

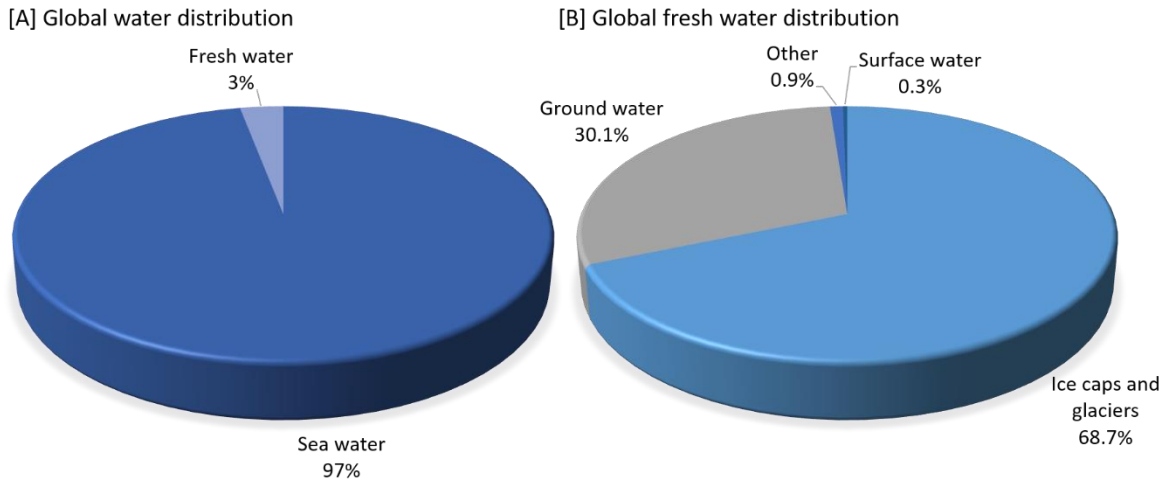


Figure 1.1: Distribution of Earth's water [A] and fresh water [B]. Data adapted from Shiklomanov, 1993.

Increases in global populations have added greater stress on fresh water resources, causing millions of people to experience water scarcity. Mekonnen and Hoekstra (2016) estimated that 4 billion people live under severe water scarcity for at least one month per year. Areas most affected by water scarcity are India, China, Bangladesh, western states of the USA, Pakistan, Nigeria and Mexico ((Figure 1.2) Mekonnen and Hoekstra, 2016),all of which are eligible for official development assistance, except for the USA, according to the Organisation for Economic Co-operation and Development (OECD, 2017).

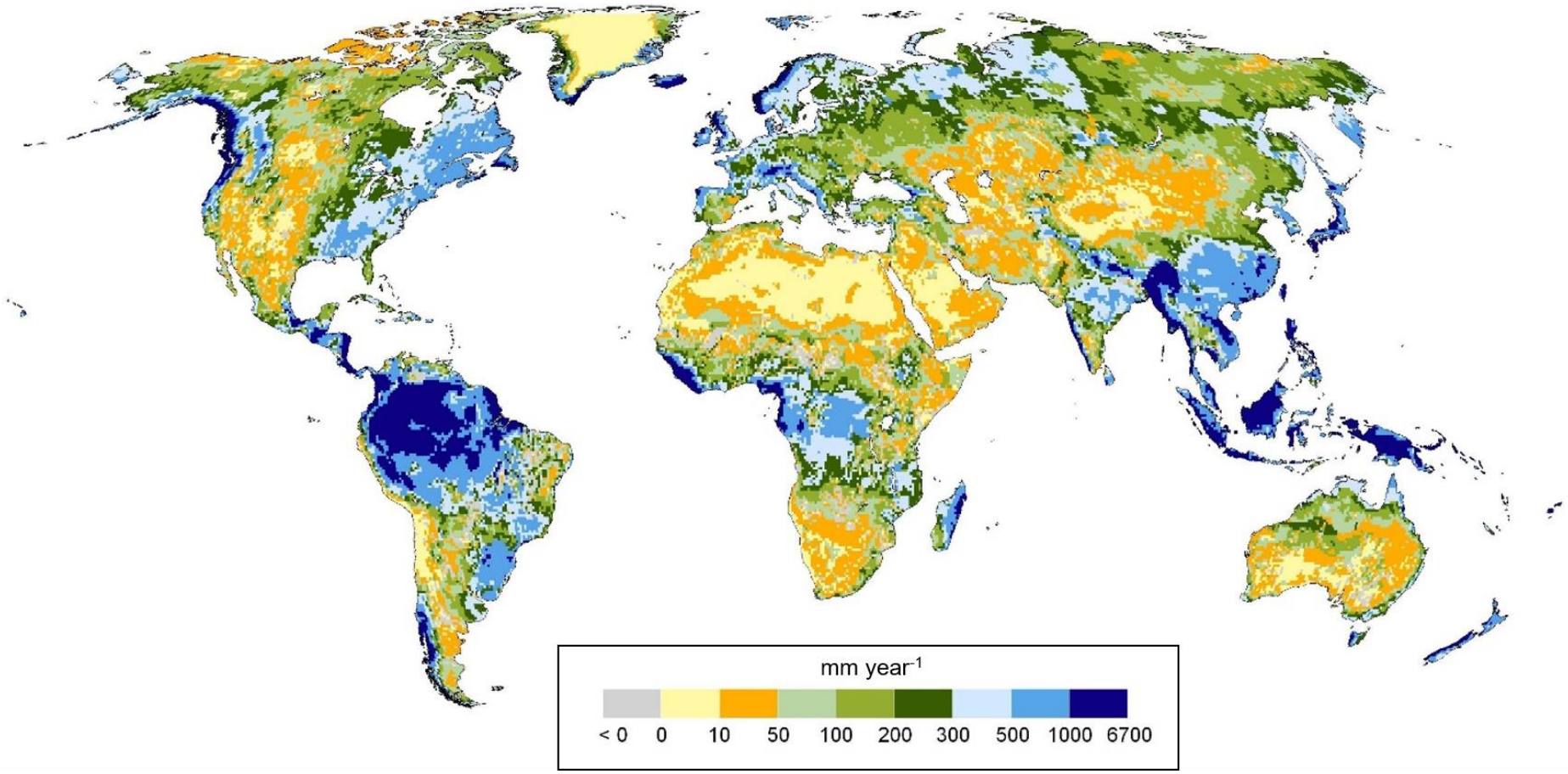


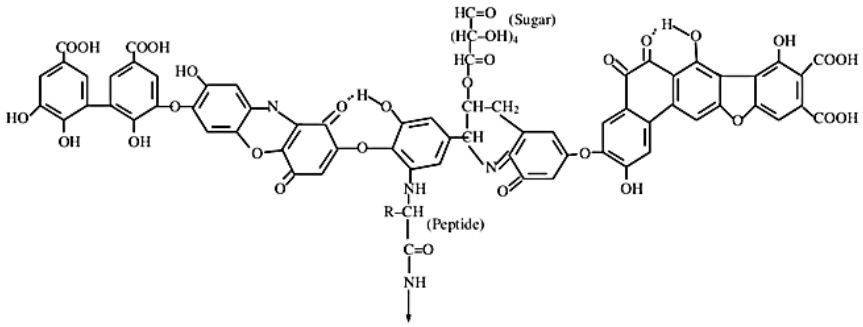
Figure 1.2: Long-term average of total renewable fresh water resources from land and open waters, between 1961 – 1990, in mm year^{-1}. Taken from Döll and Fielder (2008) CC BY-SA 3.0.

1.3 Drinking Water Quality

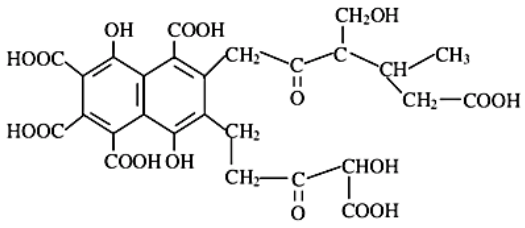
1.3.1 Chemical quality

1.3.1.1 THMs in drinking water

Natural organic matter [NOM] is a broad term for naturally occurring organic substances, encompassing dissolved, suspended, particulate organic carbon or matter which occur in aquatic systems (Demiral, Bekbolet and Swietlik, 2006). Chlorination of water containing NOM, such as humic or fulvic acid (Figure 1.3), can result in the formation of disinfection by-products [DBPs] (World Health Organization, 2000). The most common disinfection by-products formed if chlorination occurs are haloacetonitriles (HANs), haloacetic acids (HAAs), and trihalomethanes (THMs) (World Health Organization, 2011). HANs consist of dichloroacetonitrile, dibromoacetonitrile, bromochloroacetonitrile and trichloroacetonitrile. Dichloroacetonitrile and dibromoacetonitrile have regulated guideline limits of 20 μg L^{-1} and 70 μg L^{-1}, respectively (World Health Organization, 2011; United States Environmental Protection Agency, 2006; Canada Health, 2017).



(a) Humic Acid (Stevenson 1982)



(b) Fulvic Acid (Buffle 1977)

Figure 1.3:Examples of natural organic matter (NOM), proposed structures of [A] humic acid and [B] fulvic acid. Structures cited in Wang, S. and Mulligan, C.N. (2006) Effect of natural organic matter on arsenic release from soils and sediments into groundwater. *Environmental Geochemistry and Health* [Figure 3]. 28 (3), pp. 197–214. Reproduced with permission from Springer Nature under License Number 4701831109870.

HAAs are the second most common DBP after THMs and, yet, are not regulated as stringently as THMs. Five HAAs are regulated: trichloroacetic acid, dichloroacteic acid, monochloroacetic acid, monobromoacetic acid and dibromoacetic acid. In the US and Canada maximum concentrations in drinking water are 60 μg L^{-1} and 80 μg L^{-1}, respectively

1.3.1.1.1 Disinfection by-products, including trihalomethanes (THMs)

Trihalomethanes are formed when hydrogen in methane (CH_4) is replaced with a halogen, most commonly chlorine or bromine. The presence of iodine can result in iodinated-THMs (Pantelaki and Voutsa, 2018; Richardson, 2003), however, these are less common in drinking water treatment processes. Four regulated THMs within drinking water are chloroform, bromodichloromethane, dibromochloromethane and bromoform (Figure 1.4) (World Health Organization, 2011; Drinking Water Directive, 1998). THMs form when natural organic material (NOM) reacts with chlorine based disinfectants over time, see Figure 1.5 (Grunwald *et al.*, 2002; Di Cristo, Esposito and Leopardi, 2013; Chowdhury, 2013; Brown, Bridgeman and West, 2011a).

Chloroform | Bromodichloro-methane | Dibromochloro-methane | Bromoform

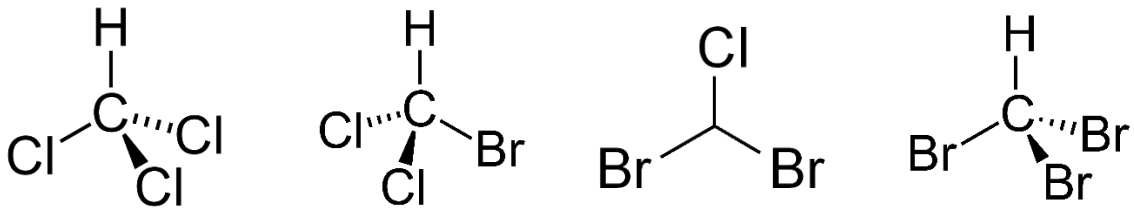


Figure 1.4: Four regulated trihalomethanes (THMs) referred to as total trihalomethanes (tTHMs). From left to right: chloroform, bromodichloromethane [BDCM], dibromochloromethane [DBCM] and bromoform. Taken from ChemSpider database, Royal Society of Chemistry, UK.

The consumption of water containing THMs is suspected to cause serious health concerns such as cancer, liver and kidney damage, miscarriages and birth defects (King, Dodds and Allen, 2000; Dodds and King, 2001; Chowdhury, Rodriguez and Sadiq, 2011), see Section 1.2.1.1.3. There is strong evidence that THMs exhibit carcinogenic and mutagenic properties in humans when consumed in water over long periods of time (Llopis-González *et al.*, 2010; Chowdhury, Rodriguez and Sadiq, 2011). Health implications associated with THMs are discussed further in Section 1.2.1.1.3.

1.3.1.1.2 What affects THM Formation?

Figure 1.5 depicts THM formation as a function of several factors including: NOM type (i.e. humic or fulvic acid, Figure 1.3) and concentration; source water pH and temperature; as well as chlorine type, contact time and concentration (Chowdhury, Rodriguez and Sadiq, 2011; Chowdhury, 2013; Di Cristo, Esposito and Leopardi, 2013; Brown *et al.*, 2010; Brown, Bridgeman and West, 2011b).

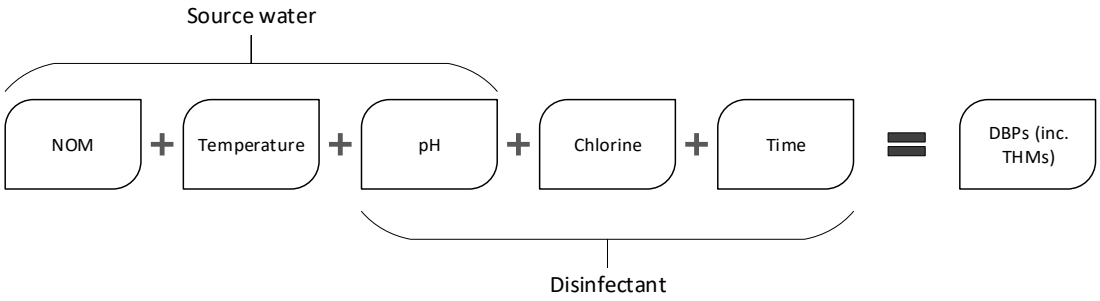


Figure 1.5: Factors affecting the formation of disinfection by-products (DBPs) and THMs formation throughout drinking water treatment.

The four main THMs that contribute to total THMs (tTHMs) are; chloroform ($CHCl_3$), bromodichloromethane ($CHBrCl_2$), dibromochloromethane ($CHClBr_2$) and bromoform ($CHBr_3$). Chloroform is usually the most abundant and has been shown to form within samples

of higher temperatures, alkaline pH levels and in the presence of greater free chlorine concentrations. Bromoform forms at lower temperatures, acidic pH and lower free chlorine availability with bromide being a prerequisite within the water source (Chowdhury, 2013).

Typically in summer and autumn months, concentrations of THMs created after chlorination are increased compared to cooler seasons with lower temperatures (Elshorbagy, 2000; Toroz and Uyak, 2005; Brown, Bridgeman and West, 2011a; Summerhayes *et al.*, 2011; Kumari and Gupta, 2015). This is due to an increased reaction rate combined with greater concentrations and differing varieties of NOM (i.e. humic acid, fulvic acid, dissolved organic carbon) in warmer months (Chowdhury, Rodriguez and Sadiq, 2011). This phenomenon of increased THM formation at increased temperatures has been observed frequently (Kavanaugh *et al.*, 1980; Brown *et al.*, 2010; Brown, Bridgeman and West, 2011b, 2011a; Toroz and Uyak, 2005). It has been evidenced that the rate constant doubles for every 10°C temperature increase (3°C, 20°C and 40°C), whilst a threefold rate constant increase was observed for each pH unit (pH 7 – pH 10) increase (Kavanaugh *et al.*, 1980).

Predicting and modelling tTHM formation is challenging due to the number of interdependent components that affect formation (see Figure 1.5 and Equation 1-1). Several non-linear and log-log mathematical models have been developed to model trihalomethane formation potential, including that of Gary Amy (1987; 1998). Equation 1-1 demonstrates how the importance of THM precursors changes between short (2 hours) and long (96 hours) term reaction times (Amy, Chadik and Chowdhury, 1987):

$$[1]\ THMFP(\text{short term [2 hours]}) = Time > Temp > TOC > pH > Cl_2\ dose > Br$$

$$[2]\ THMFP(\text{long term [96 hours]}) = Time > Cl_2\ dose > pH > TOC > Temp > Br$$

$$[3]\ THMFP = Temp > Cl_2\ dose > pH > TOC > (Br + 1)$$

Equation 1-1

Over short reaction times (Equation 1-1 [1]), temperature is a more influential precursor, compared to pH and chlorine dose, whilst, chlorine dose and pH are more significant THM precursor parameters compared to temperature in long term reaction times (Equation 1-1 [2]). Sensitivity analysis demonstrated that reaction temperature, chlorine dose and pH were the most important THM formation precursors (Equation 1-1 [3]).

A positive association between waters with a higher pH and chlorine concentration, and THM formation has been frequently observed (Liang and Singer, 2003; Brown, Bridgeman and West, 2011a; Ghebremichael *et al.*, 2011; Hua and Reckhow, 2008; Kavanaugh *et al.*, 1980; Clayton, Thorn and Reynolds, 2019a). Hua and Reckhow (2008) observed almost a threefold increase in THM concentration between pH 5 and pH 10 after a 72 hour contact time, whilst Stevens *et al.* (1976) reported a twofold increase in THMs between pH 6.7 and pH 9.2 over a 96 hour reaction period. Surface waters naturally vary in pH (i.e. approximately 6.5 – 8.5 (Baird and Cann, 2012)) due to bedrock geology and NOM concentration and type [i.e. dissolved organic carbon, particulate organic carbon or total organic carbon] (Demiral, Bekbolet and Swietlik, 2006). An increase in NOM concentration within natural waters will provide a greater THM formation potential, if not sufficiently removed before chlorination occurs as part of the disinfection process in drinking water treatment. The pH of chlorine solutions will affect the formation of THMs, whereby more alkaline solutions (i.e. NaOCl; pH > 8), have a greater affinity to generate THMs, compared to neutral (i.e. HOCl; pH ~6) or acidic (i.e. ECAS; pH < 3.5) disinfectants (Liang and Singer, 2003). THM distribution is affected by the type (i.e. humic acid, fulvic acid, dissolved organic carbon) and concentration

of NOM (Liang and Singer, 2003). Both hydrophobic and hydrophilic portions of NOM have shown to act as THM precursors (Liang and Singer, 2003; Gang, Clevenger and Banerji, 2003). Functional groups within NOM can affect the formation of DBPs, for example, if NOM has a high concentration of bromide, then brominated THM species, such as dibromochloromethane and bromoform, may be more prominent.

THM formation is reliant upon NOM interacting with free chlorine, therefore an increase in free chlorine concentration should increase the formation potential (Brown, Bridgeman and West, 2011a; Di Cristo, Esposito and Leopardi, 2013; Liang and Singer, 2003). The relationship between the rate at which THMs form includes NOM concentration and free available chlorine (Brown, Bridgeman and West, 2011a). Therefore, conventional chlorination can result in increased THM formation, as residual chlorine is required in distribution systems, therefore providing an extended contact time.

1.3.1.1.3 Health implications from THMs

There is evidence which indicates that exposure to THMs through dermal contact or through consumption can have mutagenic and carcinogenic effects (Summerhayes *et al.*, 2011; Farghaly *et al.*, 2013; Mohamadshafiee and Taghavi, 2012). Once ingested, THMs are unable to degrade, and so compounds are stored within tissues. Carcinogens can result in DNA mutations, interfering with the immune system and disrupting cell growth (Mohamadshafiee and Taghavi, 2012). There are various modes of exposure to THMs, including consumption (i.e. drinking or cooking), showering (Grazuleviciene *et al.*, 2013; Chowdhury, Rodriguez and Sadiq, 2011) and swimming (Lee *et al.*, 2010; Stack *et al.*, 2000). Exposure to THMs when showering or bathing occurs through dermal contact, as well as inhalation. Raised water temperatures increase the mass transfer of the volatile compounds into a person via dermal contact and inhalation (Chowdhury, Rodriguez and Sadiq, 2011). Exposure in swimming pools or recreational waters is generally through dermal contact, with the potential for consumption.

Many studies have investigated the potential hazards to humans associated with THMs through long-term consumption of drinking water which have detectable concentrations (Hildesheim *et al.*, 1998; King, Marrett and Woolcott, 2000; Nieuwenhuijsen *et al.*, 2000; Wang, Deng and Lin, 2007; Rahman *et al.*, 2014, 2010). Frequently results are inconclusive, or insufficient evidence is available to confidently determine the extent of THMs being a direct cause of cancer development (Hildesheim *et al.*, 1998; Rahman *et al.*, 2014; Wang, Deng and Lin, 2007; King, Marrett and Woolcott, 2000; Chowdhuryf, Rodriguez and Sadiq, 2011; Bove, Rogerson and Vena, 2007; Rahman *et al.*, 2010; Madabhushi, 1999), or adverse reproductive and developmental effects (Nieuwenhuijsen *et al.*, 2000; Bielmeier *et al.*, 2001; Dodds and King, 2001; Wright, Schwartz and Dockery, 2004; Grazuleviciene *et al.*, 2013; Cao *et al.*, 2016). Many factors contribute towards THMs' potential effect on an individual including; lifestyle, metabolism and immune status (Cao *et al.*, 2016; World Health Organization, 2005). The variation in THMs concentration within drinking water is also a result of seasonal and spatial fluctuations (Toroz and Uyak, 2005; Ghernaout, Naceur and Aouabed, 2011; Saidan, Rawajfeh and Fayyad, 2015, 2013). The potential health implications from THMs do not provide immediate risks to humans when compared to biological pathogens. For example, *Escherichia coli* O157:H7 which can result in haemolytic uremic syndrome has an incubation time of 3 -5 days from exposure to symptoms presenting themselves (Nauschuetz, 1998; World Health Organization, 2018), whilst the incubation period for *Salmonella enterica sv. typhi,* which can result in typhoid fever, is between 8 and 21 days (Olsen *et al.*, 2003). Generally, any adverse effects through the consumption of drinking water containing THMs may present themselves after many years of consumption (Villanueva *et al.*, 2006; Nieuwenhuijsen *et al.*, 2009).

1.3.1.1.4 Preventing or reducing THM formation

Preventing DBP and THM formation is possible through controlling one or several factors that contribute to formation (Figure 1.5). An integral factor in THM formation is NOM (Brown *et al.*, 2010; Di Cristo, Esposito and Leopardi, 2013; Liang and Singer, 2003), which is typically

removed in drinking water treatment systems through filtration, flocculation or coagulation processes (Thames Water, 2016). Physically removing NOM will therefore limit its availability to react with chlorine throughout the disinfection stage of water treatment. Filtration typically uses membranes and is a viable option in remote locations as filtration membranes can be gravity driven or require relatively low pressures to remove a high proportion of particulate matter. In contrast, flocculants and coagulants allow for particulate matter to coagulate to form larger aggregates which then settle, via sedimentation (Vigneswaran and Visvanathan, 1995). Such chemicals (i.e. aluminium sulphate) or polymers (i.e. ferric chloride) may be difficult, or expensive, to purchase for use in remote or rural locations and so are unfeasible within decentralised water treatment systems (Vigneswaran and Visvanathan, 1995). There are studies which utilise flocculation/coagulation as a pre-treatment as part of point-of-use drinking water treatment systems (Wendt *et al.*, 2015; Zhu *et al.*, 2014).

The four main THMs form through the interaction between organic matter and chlorine, therefore, using alternative disinfection methods could be beneficial in limiting THM formation. The use of ozone in comparison to chlorination has demonstrated a reduction in THMs as dissolved organic carbon (DOC) is transformed from hydrophobic to hydrophilic DOC, lowering the THM formation potential, in natural and model waters (Sadrnourmohamadi and Gorczyca, 2015; Galapate, Baes and Okada, 2001). Utilising ozone will also result in a reduced free chlorine availability which can react with NOM, therefore reducing the THM formation potential (Gang, Clevenger and Banerji, 2003). Ghebremichael *et al.* (2011) demonstrated that using a neutral mixed oxidant solution against model waters containing IHSS Suwannee River Humic Acid (SRHA) as a DOC source, resulted in reduced THM formation, in comparison to sodium hypochlorite (NaOCl). The antimicrobial kinetics of ECAS, or similar products, rely on a high oxidation reduction potential (i.e. ORP >1100 mV), which is a result of reactive oxygen species generated, rather than only free chlorine (Liao, Chen and Xiao, 2007) (Section 1.4.2.2), consequently, potentially lowering the need for high

concentrations of chlorine as a primary disinfection agent. The maximum allowed concentration of THMs at point of consumption in drinking water in the UK is 100 μg L^{-1}, and bromoform concentrations cannot exceed 10 μg L^{-1} (Drinking Water Inspectorate, 2012, 2010; Jackson *et al.*, 2015). However, in other European countries, as well as the USA, total THM concentration within drinking water is regulated to 80 μg L^{-1} (Brown *et al.*, 2010; United States Environmental Protection Agency, 2006).

1.3.1.1.5 THM measuring and monitoring

To quantify THM concentration within samples many studies use liquid-liquid extraction (LLE), purge and trap gas or more recently solid phase micro-extraction (SPME) chromatography/mass spectroscopy (GC/MS) (United States Environmental Protection Agency, 1979a, 1979b; British Standards Institution, 2016). These techniques allow for specific analytes within samples to be accurately measured for quantifying compound concentrations, or can be used to simply determine whether specific compounds are present (Gang, Clevenger and Banerji, 2003; Chowdhury, 2013).

1.3.2 Biological water quality

1.3.2.1 Bacteria in drinking water

In the mid-19th Century, John Snow discovered that contaminated water contributed to the spread of cholera in London (Snow, 1849). This was contradictory to the originally believed miasma, "*some vague atmospheric presence*", or contagion, "*contact with a sick person's body"*, transmission route (Snow, 1849; Newsom, 2006). The use of disinfectants to ensure biologically safe drinking water has been employed in large scale water treatment in the UK since the end of the 19th Century and in the United States of America since the beginning of the 20th Century (McGuire, 2016). Chlorination processes are effective at inactivating indicator species such as *Clostridium perfringens, Escherichia coli*, *Enterococci* and total

coliforms that are frequently found in natural and wastewaters. The World Health Organization recommends 0 CFU 100 mL^{-1} for indicator organisms such as *E. coli, Enterococci* and total coliforms, in treated water due to their potential pathogenic nature (World Health Organization, 2011; Drinking Water Inspectorate, 2012). Monitoring indicator bacterial species can help control the spread of pathogenic bacteria such as *Escherichia coli* O157:H7 (Nauschuetz, 1998; Cabral, 2010).

Where little or no treatment of natural waters occurs, either due to insufficient infrastructure or after the occurrence of natural disasters, providing biologically safe water is a challenge. Approximately 800,000 people die each year as a result of diarrhoeal diseases through consumption of contaminated waters containing sewage and animal waste (Corcoran *et al.*, 2010; World Health Organization, 2016b; Prüss-Ustün *et al.*, 2014).

Centralised drinking water treatment systems are capable of ensuring biological safe drinking water is released into distribution systems through control measures which are informed by drinking water regulations and regular monitoring (Health Canada, 2017; World Health Organization, 2011; Drinking Water Inspectorate, 2012). The majority of drinking water distribution networks contain a chlorine residual of between 0.5 and 5 mg L^{-1} which maintains potable water quality by limiting microbial re-growth (World Health Organization, 2011). There are pathogenic microbial species which are chlorine resistant such as *Aeromonas hydrophila* (Fernandez *et al.*, 2000) and *Mycobacterium Avium Complex* (Cabral, 2010), which have been isolated from treated drinking water. Other bacterial species, such as heterotrophic bacteria, can tolerate chlorination or residual chlorine through forming biofilms (Section 1.2.2.2), which can result in operational failures due to biofouling (Bachmann and Edyvean, 2005; Flemming, 2002). Biofouling and the formation of biofilms in water systems have been described as "*undesired development of microbial layers on a surface"* (Flemming, Percival and Walker, 2002). This can result in corrosion of materials (Lehtola *et al.*, 2004;

Beech and Sunner, 2004), or the blocking of filters (i.e. ultrafiltration) or pipe work (i.e. distribution networks) (Renner and Weibel, 2011).

1.3.2.2 Biofilm formation

Biofilms have been described as communities of microorganisms, including bacteria, algae, fungi and protozoa, attached to a surface encased in an extracellular polymeric substance [EPS] (O'Toole, Kaplan and Kolter, 2000; Vu *et al.*, 2009; Flemming *et al.*, 2016; Yang *et al.*, 2012). The successive stages of biofilm formation upon a surface have been well characterised (Satpathy *et al.*, 2016; Renner and Weibel, 2011; Bernstein *et al.*, 2014; Stoodley *et al.*, 2002) and are represented in Figure 1.6. The first stage is the reversible attachment of a cell to a surface, therefore the management and inhibition of bacterial attachment to a surface, and ultimately, biofilm formation at this stage is critical (Stoodley *et al.*, 2002). Surfaces within water treatment systems are fully, or partially, immersed with fluids that contain properties that allow for the survival of the cells. Cells irreversibly attach to a surface through secretion of EPS in the second stage (Flemming and Wingender, 2010), which can occur over a period of seconds/minutes. Cells within the community replicate and form microcolonies whilst continuing to secrete EPS (hours/days), acting as a protective layer between the microbial community and the bulk water. Microcolonies mature into a three dimensional biofilm through replication and the accumulation of EPS which now protects the biofilm from external stresses (Davies *et al.*, 1998). During maturation, as the biofilm grows cells can detach into the bulk water as a result of mechanical damage (i.e. scrubbing) and chemical inhibitors within the bulk water. In the final stage, cells will detach from the main biofilm to be dispersed and transported back into the bulk water (Stoodley *et al.*, 2001). This detachment allows for the attachment of cells in new niche environments, and for the formation of new biofilm communities.

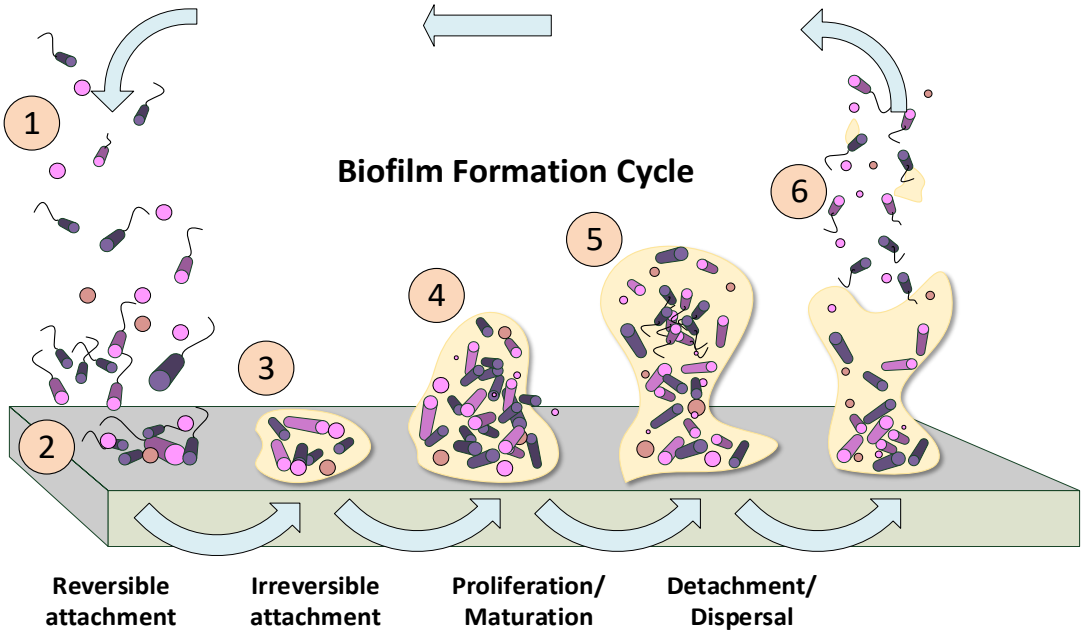


Figure 1.6: Stages involved in biofilm formation (Microsoft Visio Professional 2016). Planktonic bacteria [1] reversibly attach to a surface [2], after seconds/minutes initial colonisers irreversibly attach [3]. Cells proliferate and mature over a period of hours or days [4], encouraging EPS production [5], before cells disperse into the planktonic phase [6] over periods in excess of days.

1.3.2.2.1 Biofilms in Drinking Water

Microbial biofilms are ubiquitous in nature through nutrient cycling (Paul, Duthie and Taylor, 1991; Lyon and Ziegler, 2009; Beveridge *et al.*, 1997), and contributing to maintaining healthy microbiomes in humans (Vos, 2004). Despite this, biofilms can be hazardous as pathogenic species can proliferate within biofilms, resulting in infections (Percival, Suleman and Donelli, 2015) or hazards in contaminated food (Galié *et al.*, 2018; Hao *et al.*, 2012; Simões, Simões and Vieira, 2010; Liao *et al.*, 2017) and water (Douterelo *et al.*, 2016; Flemming, 2002; Mathieu *et al.*, 2014; Lehtola *et al.*, 2004; Wingender and Flemming, 2011, 2004). Biofilms can also result in biofouling, which has been described as "*the undesired development of microbial layers on surfaces*" (Flemming, 2002), which can result in blockages of pipes or filters, corrosion of surfaces leading to operational failure, ultimately, impeding technical or operational requirements throughout treatment systems (Shi *et al.*, 2014; Bachmann and

Edyvean, 2005; Flemming, 2002; Vargas *et al.*, 2014). Naturally occurring, or environmental biofilms, are typically multispecies polymicrobial communities encased in EPS (Percival and Walker, 1999; Schwering *et al.*, 2013). The EPS is vital in contributing towards a biofilm's resistance to external stresses, such as disinfectants and environmental toxins (Campanac *et al.*, 2002; Schwering *et al.*, 2013; Mulamattathil, Bezuidenhout and Mbewe, 2014; Bernstein *et al.*, 2014).

Conventional disinfectants, such as chlorine, can be ineffective in penetrating biofilm EPS at chlorine concentrations greater than what are typically used within water treatment, allowing cells contained within the biofilm to remain viable (De Beer, Srinivasan and Stewart, 1994; Stewart *et al.*, 2001; Chen and Stewart, 1996; Singh *et al.*, 2017). This is a potential risk to consumers as it has been estimated that up to 95% of bacteria in drinking water treatment systems are attached to infrastructure surfaces as biofilms (Flemming, Percival and Walker, 2002; Lehtola *et al.*, 2004). Biofilms can form on a wide variety of material surfaces, including those used to manufacture water pipes and membrane filters (Römling and Balsalobre, 2012; Farkas *et al.*, 2013) and can obtain nutrients from the material surface, as well as from the bulk water flow (Farkas *et al.*, 2013; Mulamattathil, Bezuidenhout and Mbewe, 2014). Potential pathogens within water bodies may form or attach to existing biofilm, acting as a reservoir and source of pathogenic organisms within the water body (Wingender and Flemming, 2011). If pathogenic bacterial species are present in detached biofilm fragments within the bulk water, then potential risks to end users and consumers arise (Lechevallier, Cawthon and Lee, 1988; Wingender and Flemming, 2004).

1.3.2.2.2 Biofilms and disinfectants

Centralised drinking water treatment systems typically have a final disinfection stage to ensure biologically safe water is distributed throughout networks (Drinking Water Inspectorate, 2012; World Health Organization, 2011). This disinfection stage requires a free chlorine

residual concentration of between 0.5 – 5.0 mg L^{-1} to be maintained throughout the distribution network through to consumer's taps to ensure microbial water quality (World Health Organization, 2011). Residual chlorination should maintain water quality, minimising the potential hazards in the treated water, however, the chlorine residual concentration may not be sufficient to penetrate the EPS of a biofilm formed after the disinfection stage of treatment on distribution network infrastructure (De Beer, Srinivasan and Stewart, 1994; Chen and Stewart, 1996; Stewart *et al.*, 2001; Singh *et al.*, 2017; Mah and O'Toole, 2001). Increasing the residual concentration can have negative effects on the perception of water quality by the consumer, resulting in unwanted chlorine taste and odours (Fawell and Nieuwenhuijsen, 2003), as well as result in the formation of DBPs. Alternative disinfectants are used in centralised drinking water treatment systems such as chloramination (Mi *et al.*, 2015), ozonation (Zhu *et al.*, 2014) or ultra-violet disinfection (Carratalà *et al.*, 2016), to enhance disinfection efficacy as well as minimise DBP formation.

Point of use decentralised water treatment systems do not require residual chlorine, as the drinking water produced should be used promptly, and would not require extensive distribution networks. The use of alternative disinfectants, which can be generated on-site or *in-situ*, to reduce, or manage, biofilm formation on water treatment system infrastructure should be investigated.

1.3.2.3 Viruses in drinking water

Waterborne viruses are challenging to manage throughout water treatment systems. The physical removal of enteric viruses (i.e. norovirus, rotavirus or hepatitis A virus) cannot occur as part of water treatment as conventional filters, such as ultrafiltration (Gall *et al.*, 2015), are unable to remove them from bulk water. Viruses can be highly resistant to disinfectants and can survive in a wide pH range (Lester and Birket, 1998), therefore are unlikely to be inactivated as part of disinfection stages. Viruses are typically transmitted person to person

(Ashbolt, 2015), or through the faecal-oral route (Lester and Birket, 1998), causing infections in the respiratory or gastro-intestinal tracts. Frequently the resulting infections causes fever and diarrhoea (Lester and Birket, 1998), which to most is not fatal. However, for people living in low income countries (GNI per capita <$1025) where sanitation and hygiene conditions are basic, such infections can be fatal, especially in young children, the elderly and the immunocompromised (World Health Organization, 2011).

1.3.2.4 Eukaryotes in drinking water

Eukaryotes can harbour opportunistic pathogens such as *Aeromonas* and *Pseudomonas* species which occur naturally in many fresh water sources, (Belila *et al.*, 2017). Eukaryotes, such as *Cryptosporidium* spp.*,* can be effectively removed from bulk water through standard drinking water treatment practices such as coagulation-flocculation, sedimentation (Medema *et al.*, 1998) and granular media filtration (Betancourt and Rose, 2004), as the average oocyst size is 5µm (Medema *et al.*, 1998). However, conventional drinking water disinfectants (i.e. chlorine or chloramine) can be ineffective in inactivating *Cryptosporidium* spp. (Rasmussen *et al.*, 1995; Venczel *et al.*, 1997). Pollution events from animal or human waste (Percival and Walker, 1999) can result in *Cryptosporidium* spp. be released into water source. *Cryptosporidium* spp. are transmitted person to person or through the faecal-oral route (Bouzid *et al.*, 2013), and can result in diarrhoea, vomiting and fever. However, for those who are immunocompromised cryptosporidiosis can be life threatening (World Health Organization, 2011). As per European Council guidelines, treated water which originates from, or is influenced by, surface waters is required to monitor spores (i.e. C*lostridium perfringens* or *Clostridium* spp.) to ensure safe drinking water is produced. No spores are permitted within treated water, and in the event of non-compliance an investigation must take place to ensure there is no danger to human health (Drinking Water Directive, 1998).

1.4 Water, Sanitation and Hygiene (WASH)

Improving access to and quality of water, sanitation and hygiene [WASH] provisions has been at the forefront of sustainable development models (United Nations, 2015a). Millennium Development Goals [MDGs] were eight overarching goals, and WASH was included as part of Goal 7; to "*ensure environmental sustainability"* (United Nations, 2015b). By 2015 91% of the global population used improved drinking water sources, whilst 2.1 billion people gained access to improved sanitation (United Nations, 2015b). However, after the MDGs period concluded, the Sustainable Development Goals [SDGs] took their place, whereby, 17 targeted goals provide a "*global blueprint for dignity, peace and prosperity for people and the planet, now and in the future"* (United Nations, 2018).

1.4.1 Sustainable Development Goal 6

The goal of SDG 6 is to "*ensure availability and sustainable management of water and sanitation for all"* (United Nations, 2018), and will be achieved through specific targets (Table 1-1). SDG 6 encourages better overall management of water in terms of drinking water [SDG 6.1] and sanitation [6.2], reducing pollutants in source water [6.3], becoming more efficient in water usage [6.4 and 6.5] and to better protect and restore water sources [6.6]. These targets are only achievable through international cooperation [6.A] and local engagement [6.B]. There are several conditions in order to determine the safety of a drinking water source by establishing what service level water is collected from (i.e. safely managed, basic, limited, unimproved or surface water). To be deemed safely managed the water must be from an improved water source (i.e. piped water, borehole, protected spring or delivered water), located on the premises, available when required and biologically and chemically safe (World Health Organization and UNICEF, 2017b). Basic drinking water access requires the collection of improved drinking water to be less than a 30 minute round trip, and limited access is collection of water from improved water sources with a greater than 30 minute round trip.

Table 1-1: Sustainable Development Goal 6: clean water and sanitation targets (United Nations, 2017)

SDG 6 Targets	
6.1	By 2030, achieve universal and equitable access to safe and affordable drinking water for all
6.2	By 2030, achieve access to adequate and equitable sanitation and hygiene for all and end open defecation, paying special attention to the needs of women and girls and those in vulnerable situations
6.3	By 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally
6.4	By 2030, substantially increase water-use efficiency across all sectors and ensure sustainable withdrawals and supply of freshwater to address water scarcity and substantially reduce the number of people suffering from water scarcity
6.5	By 2030, implement integrated water resources management at all levels, including through transboundary cooperation as appropriate
6.6	By 2020, protect and restore water-related ecosystems, including mountains, forests, wetlands, rivers, aquifers and lakes
6.A	By 2030, expand international cooperation and capacity-building support to developing countries in water- and sanitation-related activities and programmes, including water harvesting, desalination, water efficiency, wastewater treatment, recycling and reuse technologies
6.B	Support and strengthen the participation of local communities in improving water and sanitation management

1.4.2 Access to improved drinking water

It is estimated that 884 million people worldwide do not have access to basic, clean potable water from improved water sources (World Health Organization and UNICEF, 2017a, 2017b). Safe drinking water is defined as not presenting "*any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages*" (World Health Organization, 2011). Despite global improvements (19.5%) enabling access to improved water sources increasing between 1990 and 2015, see Figure 1.7 (WHO/UNICEF, 2015), approximately 30% of the global population do not have access to a safely managed drinking water service (World Health Organization and UNICEF, 2017b). Furthermore, approximately 1.2 billion people worldwide are unable to access reliable sources of electricity, and 2.9 billion still rely on wood, coal, charcoal or agricultural waste to heat their homes, or cook meals (United Nations Development Programme, 2014; International Energy Agency, 2016). The global population is expected to increase to approximately eleven billion by the end of the 21st Century (United Nations, 2004), this is therefore likely to increase water and power (gas and electricity) stresses worldwide. To mitigate and reduce stress on fresh water, and increase safe drinking water provisions, innovative drinking water treatment techniques and technologies require development and implementation to help ensure safe drinking water provision which is sustainable (UN Water, 2013), and are one of the United Nations Sustainable Development Goals [SDG]. Whereby, SDG 6 aims to "ensure availability and sustainable management of water and sanitation for all" (United Nations, 2015a).

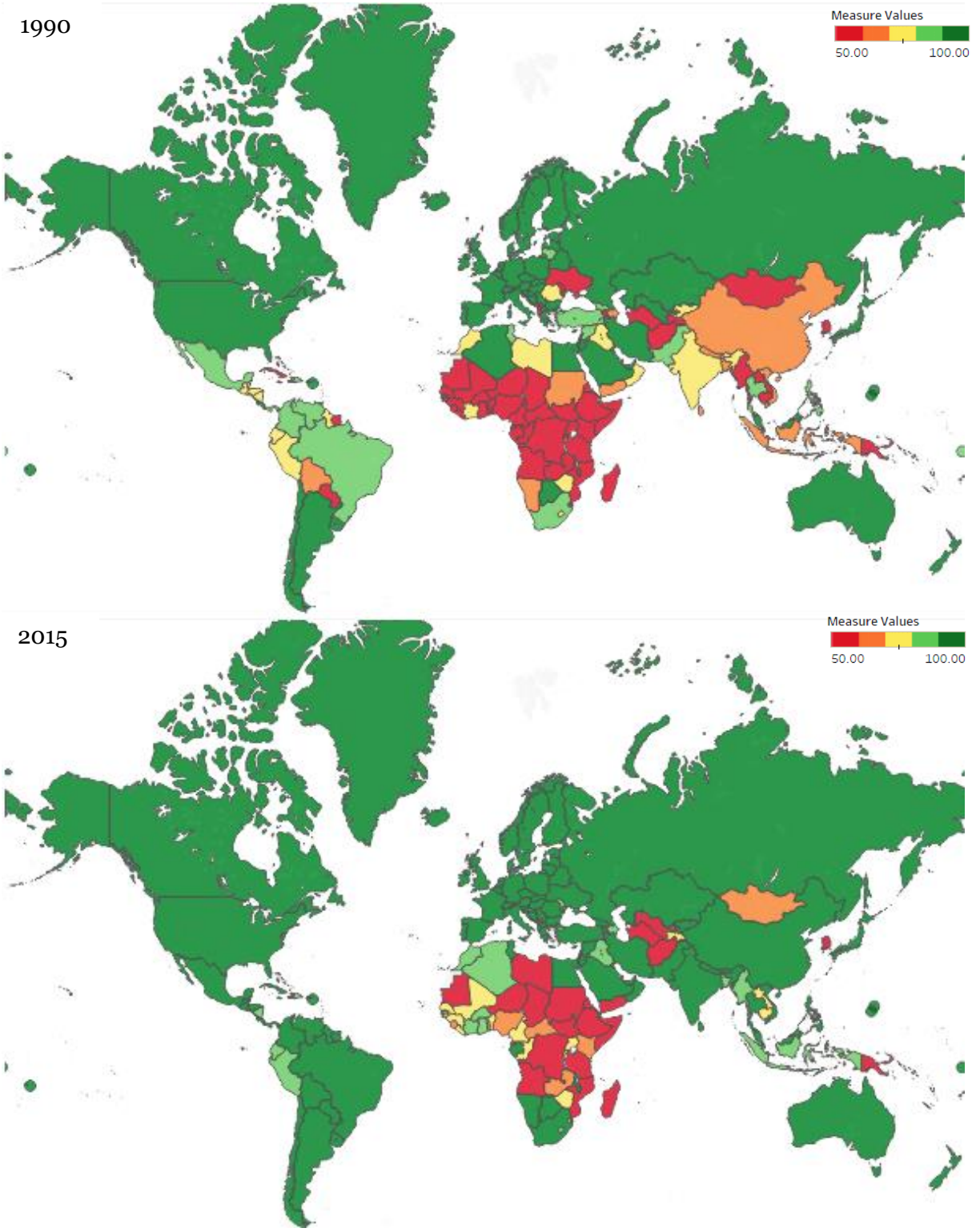


Figure 1.7: Improvement in water access between 1990 and 2015. Measured value colour scale refers to % access to improved water, whereby coloured red if access ≤ 50%. Data adapted from WHO/UNICEF Joint Monitoring Programme (JMP) for Water Supply and Sanitation.(WHO/UNICEF, 2015). [Graphs created using Tableau Desktop Professional Edition 10.5.2].

High income nations (Gross National Income [GNI] per capita >$12,476) can invest in large scale water treatment technologies, such as reverse osmosis (RO) desalination plants to treat brackish ground water or seawater, to increase access to potable water (Hyflux, 2011). Such plants require large upfront investments, and have high running costs, making such technological approaches difficult to implement in low or middle income nations (as defined by GNI per capita <$1025, or $1026 - $4035, respectively) that may be unable to afford such investments.

High income nations (GNI per capita >$12,476) traditionally have large centralised water treatment systems connected to extensive distribution systems which are not only expensive to build, but also expensive to maintain (Gumerman, Culp and Hansen, 1978). Centralised treatment processing of fresh water in the United Kingdom (Figure 1.8 (Thames Water, 2016)) often involves pre-filtration, or screening, which removes large debris within the storage reservoir, followed by coagulation and/or flocculation whereby suspended particles bind, which either settle or are skimmed from the storage tank surface. Small-suspended particles that are present within the water body are then removed through filtration, often involving ultrafiltration that removes particles larger than 0.01μm. To ensure biologically safe water is provided to consumers, water is disinfected in a final treatment stage (Section 1.4.2), most frequently with chlorine based disinfectants such as calcium or sodium hypochlorite, before a final screening. This final screening ensures the biological quality of the treated water and that a sufficient residual chlorine concentration (0.5 and 5.0 mg L^{-1} throughout the distribution network (World Health Organization, 2011, p.334)) has been maintained (Vigneswaran and Visvanathan, 1995; Thames Water, 2016). The final screening of treated waters, which can include in-line monitoring and regular sampling, ensures high quality water feeds distribution networks adhering to local (Drinking Water Inspectorate, 2012) or the World Health Organisation guidelines (World Health Organization, 2011).

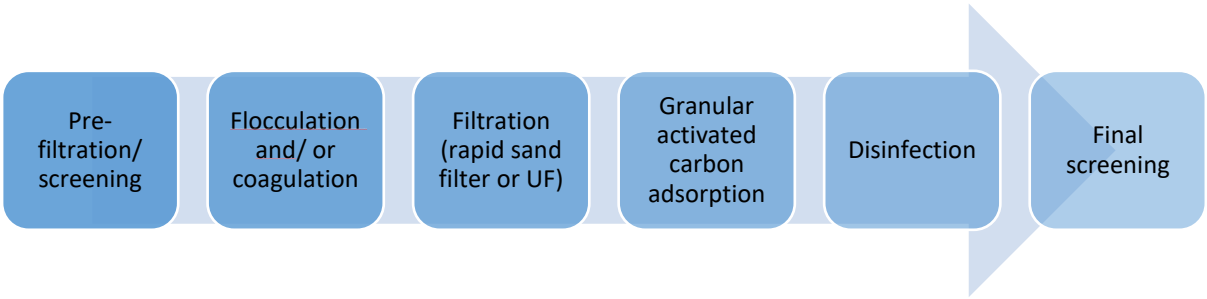


Figure 1.8: Overview of conventional UK drinking water treatment process. Adapted from Thames Water (Thames Water, 2016).

The majority of high income countries have established centralised water, gas and electricity (power) networks, supplying the majority of a country's population with sufficient water and power (United Nations Development Programme, 2014). In 2015 over 95% of populations in high (e.g. UK) and upper middle income countries (i.e. Argentina and South Africa where Gross National Income per capita is $4036 - $12475), had access to improved water sources (Figure 1.9), and over 98% of these populations had access to electricity (The World Bank, 2018b). Access to improved water sources in low income countries ([LIC] < GNI per capita $1025) and low-middle income countries (LMC GNI per capita $ 1026 - $4035) have improved by 41% and 28%, respectively between 1990 and 2015. However, this improvement translates into 65% and 89% of a country's population having access to improved water sources. It is worth noting that improved water sources include "*public taps or standpipes, tube wells or boreholes, protected dug wells, protected springs and rainwater collection*" (WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation, 2008), and not necessarily high quality potable water. High quality drinking water typically involves a multi-process approach, which requires reliable access to electricity. In 2012 it was estimated that 25% of LIC populations had electricity access, which is a 66% increase since 1990 (The World Bank, 2018b).

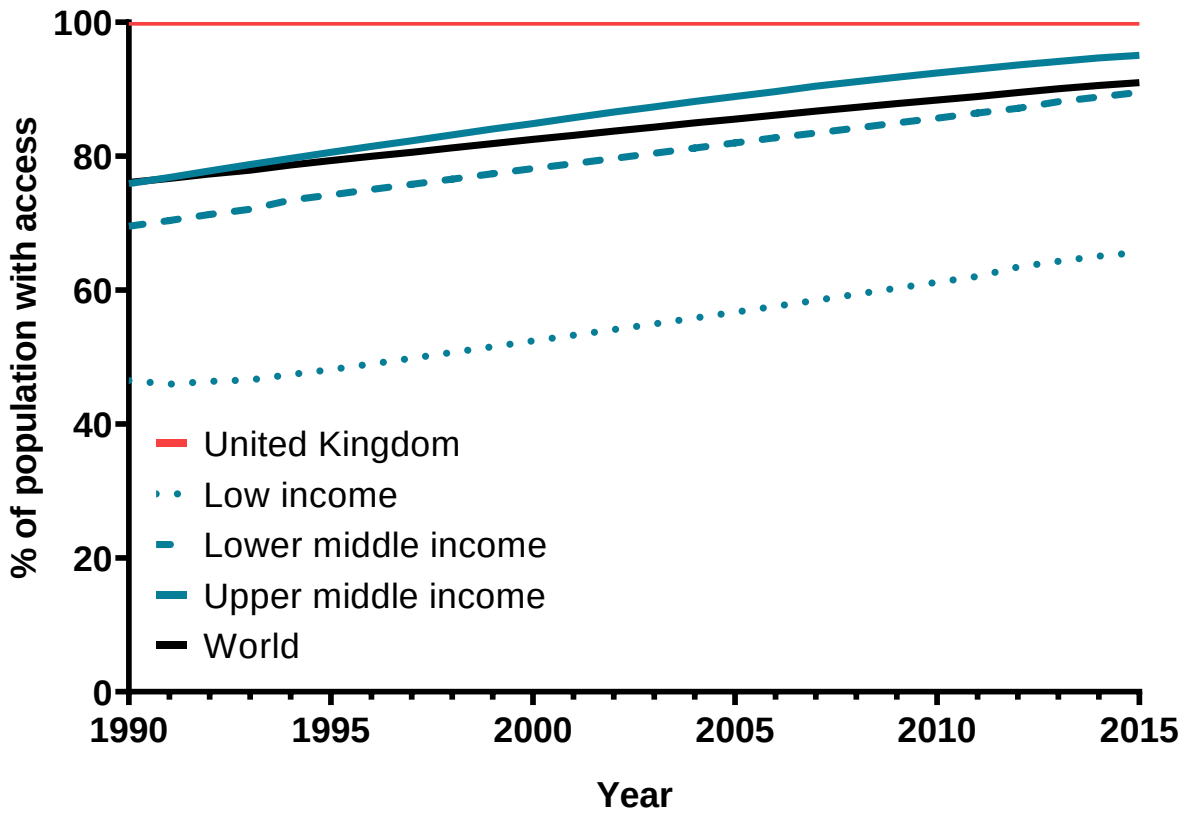


Figure 1.9: Access to improved water sources in terms of percentage population UK [red]; Low income countries (LIC: < $1025 GNI per capita [dotted green]); Lower middle income countires (LMC: GNI per capita $ 1026 - $4035 [dashed green]; Upper middle income countires (UPC: GNI per capita is $4036 - $12475 [solid green]); and World [black]. Data taken from The World Bank (The World Bank, 2018b; WHO/UNICEF, 2015).

Low income [LIC] or low middle-income countries [LMC], for this reason, do not have the same established water and power infrastructure systems. In such LIC or LMC countries, those living in rural areas has decreased, in many instances over 50% of the total countries population live in rural areas (Figure 1.10). Access to reliable and safe power and safe drinking water in such areas is limited or non-existent (United Nations Development Programme, 2014). It has been reported that 824,000 people die worldwide from diarrhoeal diseases (Corcoran *et al.*, 2010; World Health Organization, 2016b), the majority of which were a result of unsafe or contaminated water consumption (Prüss-Ustün *et al.*, 2014).

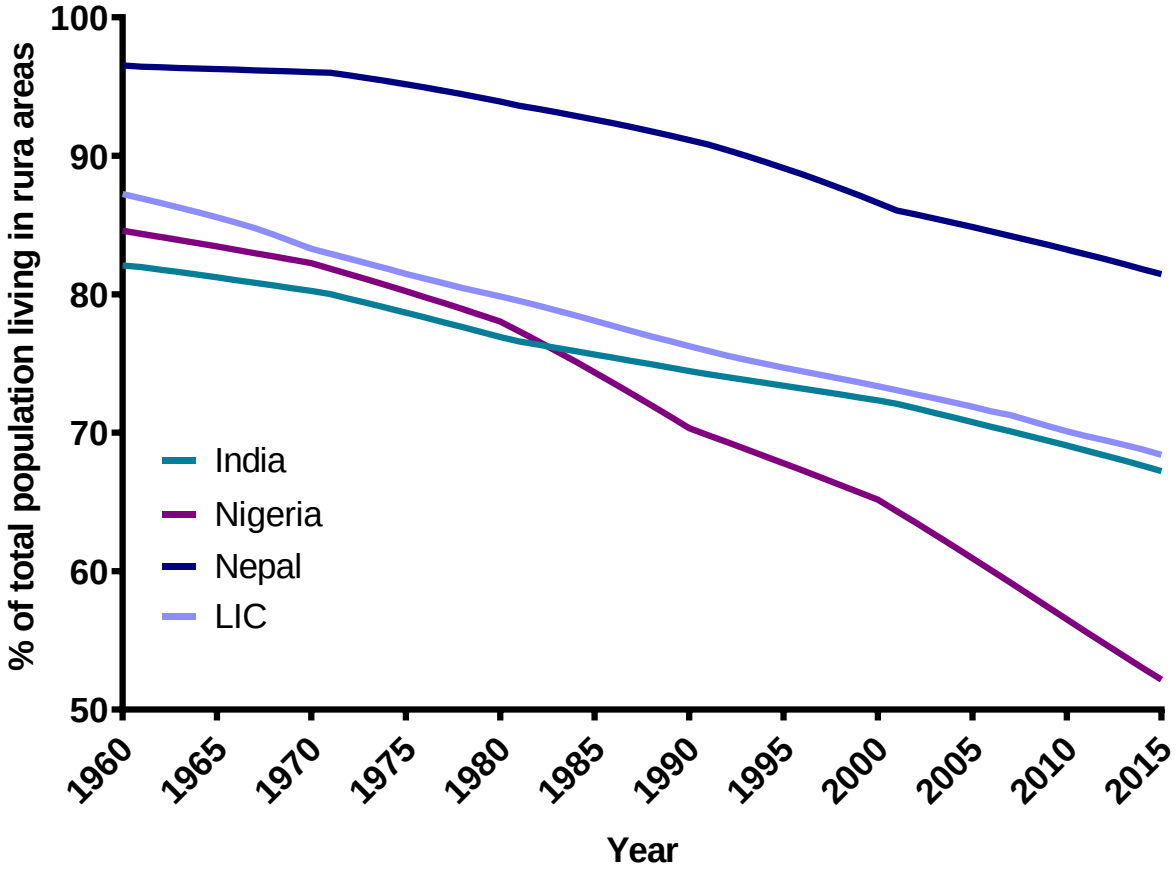


Figure 1.10: Percentage of population living in rural areas. Data taken from World Bank staff estimates based on the United Nations Population Division's World Urbanization Prospects: 2018 Revision (The World Bank, 2018a).

1.5 Technologies for Point-Of-Use Drinking Water Treatment

The two main challenges concerning the production of potable water are quality, both biological and chemical, and quantity; ensuring "*availability and sustainable management of water*" for all (United Nations Development Programme, 2016), for an ever-increasing global population, in a sustainable manner. Research into off-grid, or decentralised, water treatment systems as alternatives to centralised water treatment for the provision of safe drinking water for remote, rural or temporary communities has increased due to unfeasible practicalities of centralised systems (Sima and Elimelech, 2013; Peter-Varbanets *et al.*, 2009; Zhu *et al.*, 2014; Huang, Jacangelo and Schwab, 2011; Bouchekima, 2003). Such impracticalities include increased water scarcity (i.e. uneven global distribution of water (Mekonnen and Hoekstra,

2016) and stress (i.e. water demand exceeds availability (European Environment Agency, no date)), as well as economic cost of building and maintaining such infrastructures (Elimelech, 2006). Decentralised water treatment systems could provide modular capabilities, whereby output volume can be up scaled to cope with increasing populations.

Off-grid treatment systems refer to systems that are self-reliant, whereby they are not connected to centralised power or energy networks, and typically operate from renewable energy sources, such as photovoltaics or hydroelectricity. Decentralised systems refer to treatment systems that may take energy from a centralised power or energy network. Both off-grid and decentralised treatment systems are intended for point-of-use, thus negating the need for distribution networks. Off-grid and decentralised water treatment systems vary in complexity, reliability and resource requirements (Table 1-2) (Pooi and Ng, 2018; Loo *et al.*, 2012; Peter-Varbanets *et al.*, 2009). Treatment methods focus on two main principles: filtration and disinfection. Filtration techniques physically remove particulate matter, as well as bacteria, to help reduce water turbidity, as well as reduce potential microbial load in bulk water. Disinfection typically occurs after filtration stages to ensure biologically safe water is maintained (Arnal *et al.*, 2010), and to minimise reactions between chlorine and natural organic matter (NOM), which can form unwanted disinfection by-products [DBPs] (World Health Organization, 2000).

However, disinfection, and typically chlorination (Martínez-Huitle *et al.*, 2008; Långmark *et al.*, 2005), can be adopted after decentralised filtration e.g. sand bed filter (Mahmood *et al.*, 2011; Ali Baig *et al.*, 2011), or ultrafiltration (Chaidez *et al.*, 2016; Arnal *et al.*, 2010), to ensure biological safety of drinking water. The widespread use of chlorine in centralised drinking water systems is beneficial as it provides residual disinfection throughout the distribution network (Drinking Water Inspectorate, 2012; World Health Organization, 2011). Many decentralised systems are point-of-use, whereby extensive distribution networks are not

required, negating the need for residual chlorination concentrations. Point-of-use treatment systems in remote or rural locations require low cost and effective disinfection.

Table 1-2: An overview of studies investigating decentralised drinking water treatment technologies. Photovoltaic [PV]; Microfiltration [MF]; Ultrafiltration [UF]; Reverse osmosis [RO]; Rapid sand filter [RSF]; Activated carbon [AC]; Coagulation [Coag]; Flocculants [Floc].

Input water	Scale (if specified)	Filtration	Disinfection	Comments	References
Solar Disinfection (SODIS)					
Fresh water	Personal		Solar disinfection	Reliant on strong sun and turbidity effects efficacy	(Carratalà *et al.*, 2016; McGuigan *et al.*, 2012)
Solar Still					
Brackish or sea water	Household or community		Solar disinfection		(Bouchekima, 2003)
Portable UF					
Fresh water	Personal or Household	UF	Optional post-chlorination	Coag. or Floc. can be used as pre-treatment	(Chaidez *et al.*, 2016; Derlon *et al.*, 2013, 2014)
Reverse Osmosis					
Sea water	Community (10 m^3 day^{-1})	RO		Powered by PV, therefore reliant on sunlight	(Espino *et al.*, 2003)
Biofilters					
Fresh or ground water	Household or community	Biofilm filter	Post-chlorination	Floc. as a pre-treatment	(Wendt *et al.*, 2015)

Input water	Scale (if specified)	Filtration	Disinfection	Comments	References
Sand filter					
	Household or community	Gravel (6mm – 12mm); sand; gravel (6mm) gravel (15mm)			(Mahmood *et al.*, 2011; Ali Baig *et al.*, 2011)
Capacitive Deionisation (CDI)					
Brackish or sea water	Household or community	Sand filter (pre-treatment)	CDI		(Mossad and Zou, 2012; Mossad, Zhang and Zou, 2013)
Coagulation-Flocculation					
Fresh water	Personal Household	Straining through cloth	Chlorine-based disinfectant	Alkaline agent and flocculation aids are also included within the Pur® Water Purifier.	(Souter *et al.*, 2003)
Hybrid decentralised					
Fresh water	Household or community	50µm pre-filtration, MF (200nm), ceramic UF membranes (80nm) and AC	Ozone and NaOCl		(Sartor *et al.*, 2008)
Brackish or ground water	Community	Multi-layer sand pyrolusite filter, AC, RO columns	Pre-chlorination (NaOCl); post-RO UV radiation		(Loizidou *et al.*, 2015)

Input water	Scale (if specified)	Filtration	Disinfection	Comments	References
Fresh water		Ceramic UF	Ozonation, GAC and NaOCl	Coag. as pre-treatment	(Zhu *et al.*, 2014)
Fresh water	Community (400m^3 day^{-1})	Centrifuge hydraulic flow, sedimentation/ clarifier, RSF, AC filter	Pre-disinfectant and final stage chlorine disinfection	Powered from a 5kW generator. Coag. and Floc. adopted prior to clarification.	(Garsadi *et al.*, 2009)
Fresh or ground water	Community (2.88 m^3 day^{-1})	115 µm intake pump, 100 µm reverse flushing filter, and 0.02 µm UF membrane columns	Electrochemically activated solution (1% total v/v)	Self-contained unit	(Clayton, Thorn and Reynolds, 2019b)
Other					
	Personal or household		Sodium dichloroiso-cyanurate (NaDCC) tablets		(Clasen and Edmondson, 2006; Jain *et al.*, 2010)

1.5.1 Filtration for drinking water treatment

Filtration is the removal of particulates, colloids or microorganisms through size exclusion (Pooi and Ng, 2018), conventionally through media (i.e. gravel and sand) or pores (Figure 1.11). The type of filtration technique employed is dependent on the complexity of the system, energy requirements and investment available. Sand bed, or bio-sand, filters are low energy filtration systems which typically combine gravel and sand of different particulate sizes (Mahmood *et al.*, 2011; Ali Baig *et al.*, 2011). One study demonstrated >90% reductions in *E. coli* and total coliforms over a 90 day period (Ali Baig *et al.*, 2011). Ultrafiltration [UF] membranes are widely used in large scale and POU drinking water treatment systems as a result of effectively removing particulate matter and bacteria (Chaidez *et al.*, 2016; Álvarez-Arroyo *et al.*, 2015), however are unable to remove salts (Figure 1.11). Nanofiltration [NF] membranes can be incorporated within treatment processes of surface or ground waters to effectively remove viruses (Van der Bruggen and Vandecasteele, 2003), pesticides (Košutić *et al.*, 2005; Plakas and Karabelas, 2012; Van der Bruggen and Vandecasteele, 2003; Van der Bruggen *et al.*, 1998), heavy metals (Košutić *et al.*, 2005), pharmaceuticals (Mohammad *et al.*, 2015) or high salt concentrations (Hilal *et al.*, 2005). Reverse osmosis [RO] has been widely used for desalination of brackish or seawater, but are costly due to the energy intensive high pressures required to pass bulk water through the membranes. In recent years, developments in RO systems enabling them to be powered through renewable energy sources (i.e. photovoltaics or wind) have provided alternatives to lower income countries (Subramani and Jacangelo, 2015; Mathioulakis, Belessiotis and Delyannis, 2007; Li, Goswami and Stefanakos, 2013).

Combining filtration techniques in multi-step processes (i.e. sand bed filter, MF and UF) can allow for more efficient particle removal from bulk water, by reducing the potential of blockages of pores. For example, ultrafiltration pores will not block as quickly if installed

downstream of particulate, or micro filtration (i.e. sand bed filter), which can effectively remove large particulates, compared to if UF membranes are solely installed.

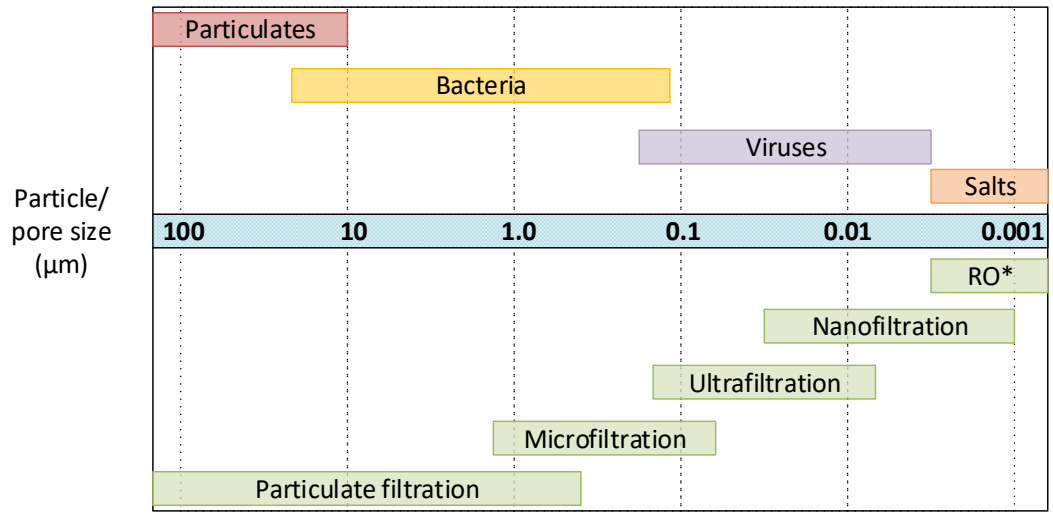


Figure 1.11: How pore size effects the exclusion of particulates and microorganisms. *RO: Reverse osmosis.

1.5.2 Drinking water disinfection

For the past century chlorination has been used extensively in water treatment processes as it is cheap, widely available and efficacious (Farghaly *et al.*, 2013; Kumari and Gupta, 2015; Rodriguez and Sérodes, 2001). Centralised drinking water treatment which feeds distribution networks require a maintained residual free chlorine concentration to ensure that drinking water quality is sustained (Di Cristo, Esposito and Leopardi, 2013). Chlorine disinfectants are proficient at reducing the microbial loads in bulk water, yet these conventional disinfectants are toxic to aquatic environments and can have a detrimental effect on ecosystems' health (Larson *et al.*, 1978). Chlorine disinfectants are successful in the disinfection stage of drinking water treatment, but unwanted DBPs can form as a result of chlorine reacting with NOM, whereby the drinking water may not be chemically safe for consumption, as discussed in Section 1.2.1.1. Chlorine is unable to sufficiently penetrate mature biofilm EPS, causing biofilms to continue to proliferate (De Beer, Srinivasan and Stewart, 1994; Stewart *et al.*, 2001;

Chen and Stewart, 1996; Singh *et al.*, 2017). The effect of chlorination on managing biofilms within water treatment systems is discussed in Section 1.2.2.2.1.

1.5.2.1 Chlorination for drinking water treatment

In centralised drinking water treatment the most commonly used primary, or conventional, disinfectants throughout water treatment are chlorine, chlorine dioxide and ozone (World Health Organization, 2004a). Primary disinfection is described as "*a common component of primary treatment of drinking-water, and important because granular filter media do not remove all microbial pathogens from water*" (World Health Organization, 2004b). Chlorine is the most common disinfectant in water treatment due to its low cost and effective antimicrobial properties (Xiao *et al.*, 2014; Gil *et al.*, 2015). Chlorine can be added in several forms: chlorine dioxide (ClO_2), sodium hypochlorite (NaOCl), calcium hypochlorite ($Ca(OCl)_2$), or chlorine gas (Cl_2). The most commonly used are NaOCl and $Ca(OCl)_2$ as they are easier to transport, and are less hazardous to human health compared to chlorine gas, which is very unstable (Collivignarelli *et al.*, 2017). Equation 1-2 illustrates the resultant products, hypochlorite ions and hypochlorous acid [HOCl], of NaOCl dissolving in water.

$$2NaOCl + 2H_2O \leftrightarrow 2H_2O + 2Na^+ + 2OCl^-$$

$$NaOCl + H_2O = HOCl + NaOH$$

Equation 1-2

Some countries (i.e. the Netherlands) have adopted non chlorine disinfection methods within centralised drinking water treatment, as a direct result of Rook's discovery in 1976 of chlorinated DBPs which form as part of chlorination disinfection stages of drinking water treatment (Rook, 1976). Instead, the Netherlands use high quality feed waters and a combination of oxidation (i.e. ozonation) and granular activated carbon filtration (GAC) as

disinfection processes (Smeets, Medema and Van Dijk, 2009). For the majority of the world, however, chlorination is primarily used throughout disinfection stages of water treatment (World Health Organization, 2003b). Chlorination is an effective disinfectant as it interrupts fundamental bacterial functions by oxidising sulfhydryl groups in enzymes, such as triosephosphate dehydrogenase (Venkobachar, Iyengar and Prabhakara Rao, 1977; Virto *et al.*, 2005; Collivignarelli *et al.*, 2017; Fair *et al.*, 1948; Cho *et al.*, 2010; Knox and Stumpf, 1948; Green and Stumpf, 1946)

The World Health Organisation (WHO) set guidelines which define a residual free chlorine concentration of between 0.5 and 5.0 mg L^{-1} be maintained throughout a water distribution network to ensure effective disinfection throughout the system until it reaches the end user (World Health Organization, 2011, p.334). Residual chlorine ensures water quality is maintained throughout the distribution network, and can manage the potential formation of biofilms within water treatment system systems (Di Cristo, Esposito and Leopardi, 2013). Excessive chlorine, as defined by greater than 5 mg L^{-1} levels throughout drinking water treatment and distribution systems can result in unpleasant tastes and odours (Fawell and Nieuwenhuijsen, 2003) for end users, as well as the formation of DBPs (Grunwald *et al.*, 2002; Chowdhury, 2013). The formation of DBPs, including trihalomethanes (THMs) and haloacetic acids (HAAs), occurs through the reaction between disinfectants, such as chlorine, and organic matter (i.e. humic acid or biofilms). The presence of these disinfection by-products within drinking water can pose a serious danger to human health (King, Dodds and Allen, 2000; Dodds and King, 2001; Chowdhury, Rodriguez and Sadiq, 2011). DBP formation, and specifically THM formation, was previously discussed in Section 1.2.1.1.

1.5.2.2 Electrochemically activated solutions (ECAS)

Electrochemically activated solutions (ECAS) have been widely used in a range of settings; including healthcare (Thorn *et al.*, 2012; Robinson, Thorn and Reynolds, 2013; Selkon, Babbt

and Morris, 1999) and food production (Thorn, Pendred and Reynolds, 2017; Rahman *et al.*, 2012; Park *et al.*, 2008; Huang *et al.*, 2008; Tomás-Callejas *et al.*, 2011; Gómez-López, Gil and Allende, 2016), and are often referred to as 'green biocides' (Rahman, Ding and Oh, 2010). ECAS are known by several terms, the most common being: electrochemically activated water (ECAW), electrolysed water (EW), electrolysed oxidising water (EOW) and mixed oxidant (MIOX) solutions. ECAS is generated by passing a saline solution through an electrochemical cell, whereupon it becomes electrolysed when a direct current is applied (Figure 1.12).

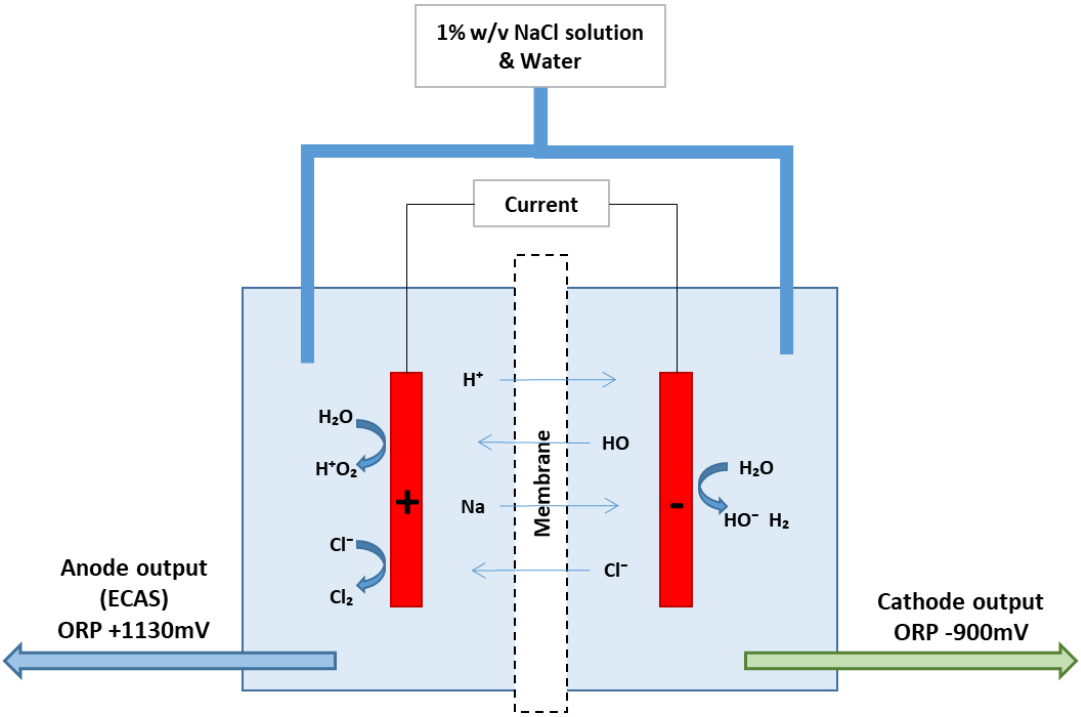


Figure 1.12: Schematic of antimicrobial species formed as part of ECAS generation. A direct current is applied across the positive (anode) and negative (cathode) electrodes that are separated by a semi-permeable ion exchange membrane allowing a constant flow of electrolyte solution (1% w/v NaCl). The anolyte solution (ECAS) is acidic with a high oxidising potential, whilst the catholyte solution is alkaline and highly reductive. ECAS is used as the disinfectant solution.

ECAS generators that have a semi-permeable membrane separating two electrodes, produce two separate solutions; anolyte and catholyte. Catholyte solutions are alkaline and highly reductive with a negative oxidation reduction potential [ORP] (- 800 mV) (Cloete *et al.*, 2009; Helme *et al.*, 2010; Marais and Brözel, 1999; Huang *et al.*, 2008), whilst anolyte solutions,

here referred to as ECAS, are acidic and highly oxidative (+ 800 mV). Reactions which occur at the anodic surface result in chlorine (Cl_2) and oxygen production, as well as hydroxyl radicals and transient oxidative functional groups (e.g. OH-, O_3, H_2O_2 and O_2-(Jeong, Kim and Yoon, 2006; Martínez-Huitle *et al.*, 2008)). The numerous transient reactive species increase the ORP of the anolyte solution, resulting in a pH shift towards the acidic range. This is dependent on redox reactions of strongly adsorbed electro-active water-derived intermediate molecular species (Boggio *et al.*, 1985; Burke and O'Neill, 1979; Erenburg, Krishtalik and Rogozhina, 1984; Trasatti, 1991). Initially, water is decomposed at the anode surface:

$$H_2O_{ad} \rightarrow OH_{ad} + H^+ + e^-$$

Equation 1-3

$$OH_{ad} \rightarrow O_{ad} + H^+ + e^-$$

Equation 1-4

Dissociated chloride ions from NaCl through direct current polarisation are then adsorbed:

$$O_{ad} + Cl^- \rightarrow OCl_{ad} + e^-$$

Equation 1-5

Chlorine gas and oxygen are both then produced from these intermediates:

$$OCl_{ad} + Cl^- + H^+ \rightarrow OH_{ad} + Cl_{2\,(g)}$$

Equation 1-6

$$O_{ad} + O_{ad} \rightarrow O_{2(g)}$$

Equation 1-7

A large scientific body of evidence now exists for the two 1-electron processes shown above in Equation 1-3 and Equation 1-4 (Cai, 2005; Stoner *et al.*, 1982; Trasatti, 1987). The electrochemically generated chlorine then reacts with water producing hypochlorous acid:

$$Cl_2 + H_2O \rightarrow HOCl + HCl$$

Equation 1-8

This reaction is pH dependent, and (according to the Nernst equation) dictates which form of free chlorine is predominant within generated solutions: Cl_2 HOCl or OCl (Sivey, McCullough and Roberts, 2010; Stoner *et al.*, 1982).

The numerous antimicrobial chemical species in ECAS have been shown to be comparatively more efficacious in contrast to disinfectants such as sodium hypochlorite (Robinson, Thorn and Reynolds, 2013). This is thought to be a result of a high ORP environment has been shown to damage and rupture inner and outer microbial membranes, prohibiting microbial functionality, including energy generating mechanisms (Liao, Chen and Xiao, 2007). Combining the high ORP environment with free chlorine present in solutions that interrupts enzyme function (Venkobachar, Iyengar and Prabhakara Rao, 1977).

ECAS has a minimal environmental impact as during chemical relaxation the solution reverts back to weak saline (Petrushanko and Lobyshev, 2001; Huang *et al.*, 2008; Thorn, Robinson and Reynolds, 2013). However, it has been shown that a residual free chlorine concentration [3.2 mg L^{-1} from 5 mg L^{-1}] remains after disinfection [90 minute contact time] (Venczel *et al.*, 2004). To ensure maximum disinfection efficacy, filtration of particulate organic matter prior to disinfection would be most effective, as ECAS is a broad spectrum antimicrobial, therefore, it does not discriminate between different organic matter to react with. ECAS has been proven to be safe to use in a variety of applications including clinical and healthcare environments (Thorn *et al.*, 2012; Selkon, Babbt and Morris, 1999; Kirkpatrick, 2009), as well as in food production (Huang *et al.*, 2008; Robinson *et al.*, 2010; Robinson, Thorn and Reynolds, 2013; Thorn, Pendred and Reynolds, 2017).

1.5.2.3 Solar disinfection for drinking water

Disinfection techniques in decentralised systems often utilise solar disinfection [SODIS] (Bouchekima, 2003; Attisani, 2016; Carratalà *et al.*, 2016), to provide biologically safe water. The combination of increased temperature and UV radiation associated with SODIS results in damage to cells through distorting the DNA helix (Sinha and Hader, 2002). In many low-income countries SODIS occurs through water contained in polyethylene terephthalate [PET] bottles placed on roofs and left throughout the day to inactivate bacteria and viruses (McGuigan *et al.*, 2012; Carratalà *et al.*, 2016). Due to the variability and inconsistency in relying on sunshine to disinfect water, devices like WADI (Helioz, 2019) monitor UV radiation and can help determine when water has been disinfected.

1.5.3 Coagulation and flocculation in drinking water treatment

Coagulation and flocculation are widely used throughout drinking water treatment to reduce turbidity and colour by removing NOM in the form of suspended particles from bulk water (Sillanpää, 2015; Sillanpää *et al.*, 2018). Coagulation destabilises small particles (0.01 – 1 μm) to form larger particles, whilst flocculation results in the formation of flocs from destabilised particles, which can then be removed via settlement or filtration (Tebbutt, 1998; Tchobaniglous *et al.*, 2014; Schroeder, 1977). Removing NOM will therefore reduce DBP formation potential (Volk *et al.*, 2000). Conventionally, coagulant dosing of alum, iron salt or lime requires proper training (Pooi and Ng, 2018), and so may not be feasible in POU technologies as a disinfection stage would also be required. Commercially available POU coagulation-disinfection treatment intended for household use have been developed and evaluated (Souter *et al.*, 2003). Souter *et al.* (2003) reported > 3 log reduction for parasites, viruses (> 4 log) and bacteria (> 7 log), with no *E. coli* or coliforms present in any treated waters taken from natural waters in Guatemala, Kenya, Pakistan, Philippines or South Africa. It was also effective in removing >97% of arsenic in natural waters with low arsenic concentrations (11 - 16 μg L^{-1}), and >99% of arsenic in natural waters with high arsenic

concentrations (> 49 μg L^{-1}). However, there is still a processing time associated with such coagulation-flocculation methods, and the integrated disinfectant will not allow for a separate disinfectant solution for general use, which could be the case for ECAS as it has been used in many applications (Section 1.5.2.2).

1.6 Current Challenges in Drinking Water Treatment

1.6.1 ECAS usage for distribution networks

Distribution networks associated with centralised water treatment systems maintain water quality through residual chlorine concentrations of between 0.5 and 5.0 mg L^{-1} (World Health Organization, 2011; Drinking Water Inspectorate, 2012). However, due to the rapid antimicrobial properties of ECAS (Robinson *et al.*, 2011; Liao, Chen and Xiao, 2007), maintaining residual chlorine concentrations is unlikely throughout vast distribution networks when compared to NaOCl or $Ca(OCl)_3$, see Section 1.4.2.1. Point-of-use drinking water treatment negates the requirement for residual chlorine concentrations, as extensive distribution networks are not required.

1.6.2 Biofouling of UF membranes

Ultrafiltration (UF) membranes are frequently adopted in both centralised and decentralised systems due to their effective removal of both particulate matter and bacteria [Figure 1.13] (Chaidez *et al.*, 2016; Álvarez-Arroyo *et al.*, 2015), with relatively low energy requirements (Chang *et al.*, 2008). UF membranes can be prone to biofouling when high organic loads, such as particulate matter and bacteria, accumulate to form biofilms (Crozes *et al.*, 1997; Sillanpää, 2015). This formation can result in blocked pores, increasing the transmembrane pressure and reducing operational time (Crozes *et al.*, 1997).

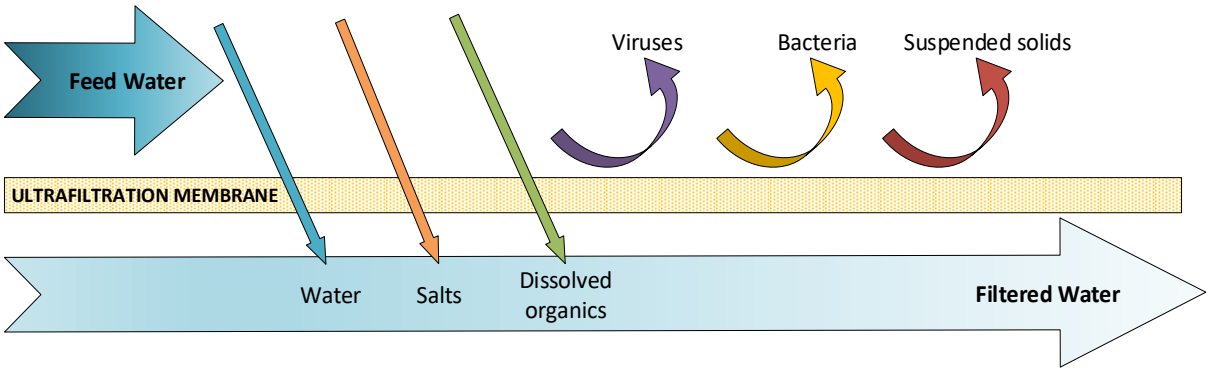


Figure 1.13: Ultrafiltration rejection of organisms and particles > 0.2µm, such as viruses, bacteria and suspended solids, whilst retaining salts and dissolved organics in water.

UF membrane fouling can be inferred through permeability, which is common practice within water industries (Crozes *et al.*, 1997). Unstable permeability can decrease the efficiency of UF membranes, and decrease the time between the intensive chemical cleaning (Clayton, Thorn and Reynolds, 2019b; Mosqueda-Jimenez, Huck and Basu, 2008). Decentralised drinking water treatment systems in remote or rural locations, may not be able to easily access such cleaning chemicals, i.e. sodium hydroxide, sodium hypochlorite and hydrochloric acid (Porcelli and Judd, 2010), which are needed after biofouling of UF membranes (Inge GmbH, 2015). Managing biofilm formation, which can result in biofouling, is beneficial as operational efficiency can be maintained, providing longer periods between routine deep chemical cleans.

It is recommended that ultrafiltration membranes which operate under low pressures do not have chlorine as pre-treatment due to its highly oxidising effect on the membranes (Inge GmbH, 2015). Some centralised drinking water treatment systems include UV or ozone as pre-treatments prior to the disinfection phase to oxidise organics with bulk water, reducing the formation potential of DBPs. This can reduce biofouling on pipework, or infrastructure, as well as begin breaking down particulate matter within the bulk water (Galapate, Baes and Okada, 2001; Hu *et al.*, 1999). The use of ozone or UV in decentralised drinking water treatment systems, may not be realistic as both are energy intensive (Chang *et al.*, 2008). Another

potential limitation for the use of ozone or UV within decentralised systems, would be the inability of having a broad spectrum anti-microbial solution that can be used for numerous applications. ECAS can be generated on-site, only requiring salt, water and some energy (Clayton, Thorn and Reynolds, 2019b; Thorn *et al.*, 2012; Robinson *et al.*, 2011; Thorn, Pendred and Reynolds, 2017). ECAS also have the advantage of being non-selective, broad spectrum disinfectant having been widely investigated for use within healthcare (Thorn *et al.*, 2012; Selkon, Babbt and Morris, 1999; Kirkpatrick, 2009) and food industries (Huang *et al.*, 2008; Robinson *et al.*, 2010; Robinson, Thorn and Reynolds, 2013; Thorn, Pendred and Reynolds, 2017), as described previously in Section 1.4.2.2.

1.7 Study Aims

1.7.1 Proof-of-concept; the development of a point-of-use drinking water treatment system

Increasing numbers of investigations into decentralised drinking water treatment systems [DWTS] have occurred over the past few decades (Table 1-2). The development of robust and reliable decentralised DWTS, which can ensure high quality potable water is produced sustainably, is essential. Utilising disinfection processes which are cost effective and consistent, whilst minimising the formation potential of hazardous by-products to either the consumer or the environment need to be considered. Chapter 3 demonstrates the production of drinking water from a raw water source (artificial water body) to Drinking Water Inspectorate (DWI) standards, using a decentralised drinking water treatment system (DWTS). The DWTS incorporates the use of multi-step filtration processes, with pre- and post-ultrafiltration disinfectant dosing of 1% (v/v) ECAS.

1.7.2 The comparative formation of trihalomethanes using chlorine-based disinfectants within a model system

Trihalomethanes in drinking water are regulated by most countries due to potential carcinogenic and mutagenic hazards. Conventional chlorine-based disinfectants form disinfection by-products, including trihalomethanes, throughout disinfection processes in drinking water treatment. Alternative disinfectants which can be generated on-site, *in-situ* and in volumes required, such as ECAS, have not had thorough investigation regarding their effect in formation THMs. Chapter 4 investigates the comparative formation of THMs in water when treated with three disinfectants (ECAS, NaOCl and HOCl) as a function of contact time and free chlorine.

1.7.3 The comparative antimicrobial efficacy of chlorine-based disinfectants for use in point-of-use drinking water applications

Conventional chlorination is effective in centralised drinking water treatment systems in providing potable water. Decentralised drinking water treatment systems, do not necessarily require residual disinfection that is essential for drinking water distribution networks. Sodium hypochlorite (NaOCl) is widely used in centralised drinking water treatment, whereas hypochlorous acid (HOCl) is the active chlorine agent in drinking water purification tablets, which are often offered as part of disaster relief, and for transient communities (Cotruvo *et al.*, 2007). Decentralised water treatment systems require fast-acting, reliable disinfectants that can effectively reduce microbial loads in water, and provide high quality drinking water for rural communities, as well as part of disaster relief efforts. Chapter 5 investigates the efficacy of ECAS against *Escherichia coli* ATCC 10536 compared to NaOCl and HOCl, using standard chemical disinfectant assays against standard pathogenic strains, through equivalent free chlorine concentrations. This chapter also investigates the efficacy of NaOCl, HOCl and ECAS at reducing mature biofilm density (48 hours) of *Pseudomonas aeruginosa* ATCC 15442 through direct disinfection.

1.7.4 Biofilm management; investigating the inhibitory effects of chlorine-based disinfectants on biofilms

Standard disinfection assays are effective for determining the efficacy of a disinfectant against laboratory bacterial strains and interfering substances. The development of representative models is beneficial as real-world situations are far more complex than laboratory experiments. Chapter 6, investigates the efficacy of ECAS, compared to NaOCl and HOCl, against planktonic bacteria (*Escherichia coli* ATCC 10536) using environmental water as an interfering substance. This chapter also determines whether NaOCl, HOCl and ECAS manage environmental bacterial biofilm formation on polyethersulfone material in a modified *in-situ* dosing biofilm reactor model.

Chapter 2. Materials and Methods

2.1 Disinfectant Solutions

Three disinfectants were used throughout the studies in the project. Sodium hypochlorite (NaOCl) which is conventionally used in centralised drinking water treatment. Hypochlorous acid (HOCl) which is frequently used in small-volume disinfection, for example chlorine release tablets that produce approximately 2500 mg L^{-1} free chlorine per tablet (Haz-Tab, Guest Medical, UK). Similar tablets are advised to be used in water bottles where sterilisation cannot occur. The final disinfectant is electrochemically activated solutions (ECAS), which can be generated *in-situ* (see Section 1.4.2.2).

Hypochlorous acid stock solution was produced through the dissolution of sodium dichloroisocyanurate (NaDCC) within 1 litre of deionised water producing a free chlorine concentration of 201 ± 13.55 mg L^{-1}, with a pH of 5.6 ± 0.25, and a mean ORP of + 958 ± 18.98 mV. Stock solution of sodium hypochlorite was prepared by diluting a commercial bleach (Pattersons bleach; Pattersons Ltd., Bristol, UK) in deionised water to a final free chlorine concentration of 508 ± 18.19 mg L^{-1}, with a mean pH of 11.4 ± 0.1, and a mean ORP of + 588 ± 0.95 mV. Electrochemically activated solutions were generated using an electrochemical cell supplied by Bridge Biotechnology Ltd (Fife, Scotland, UK), see Section 2.1.1. Solutions of ECAS containing free chlorine concentrations of 1158.63 ± 18.66 mg L^{-1}, with mean pH of 3.3 ± 0.16 and ORP of + 1134 ± 3.26 mV were generated and stored at 4°C in the dark, and used within 5 days of production (Robinson *et al.*, 2011). Disinfectant solutions were diluted using deionised water to produce equal concentrations of free chlorine (1 – 5 mg L^{-1}) as determined using the N, N-diethyl-p-phenylenediamine sulphate (DPD) no. 1 Palintest test (Palintest Ltd., Gateshead, UK). The pH and ORP of solutions were measured using an Orion Dual Star (Fisher Scientific, UK). **Generation of Electrochemically activated solutions (ECAS)**

A bespoke ESOL™ generator (Bridge Biotechnology Ltd, Dunfermline, Scotland, UK (Figure 2.1 [i])), was used to produce electrochemically activated solutions (ECAS). ECAS was generated by passing a mixture of softened tap water [ii] (CalSoft Non-Electrical, CalMag Ltd, Keighley UK) and saline (1% NaCl w/v [iii]) through an electrochemical cell [vi]. The electrochemical cell current and the catholyte/anolyte flow ratio is controlled through a screen to the front of the generator [iv] and can be controlled to vary the type of solution generated [v]. The electrochemical cell [vi] has two electrodes divided by a semi permeable membrane (Figure 1.12). The anodic electrode is composed of titanium with a mixed metal oxide layer, which improves stability, corrosive resistance and the electrode lifetime (Montgomery, 2018), whilst the cathode is composed of pure titanium (Montgomery, 2018). Softened tap water (Figure 2.1) mixes with weak saline before reaching the cathode and anode, generating catholyte and anolyte solutions. ECAS refers to the anolyte solution.

Cell current affects the physicochemical properties of the ECAS solutions generated (Figure 2.2). Inverse relationships are observed between pH and ORP (Figure 2.2 A), as well as between pH and free chlorine (Figure 2.2 B). There is no difference between the concentration of free and total chlorine of generated ECAS (Figure 2.2 C).

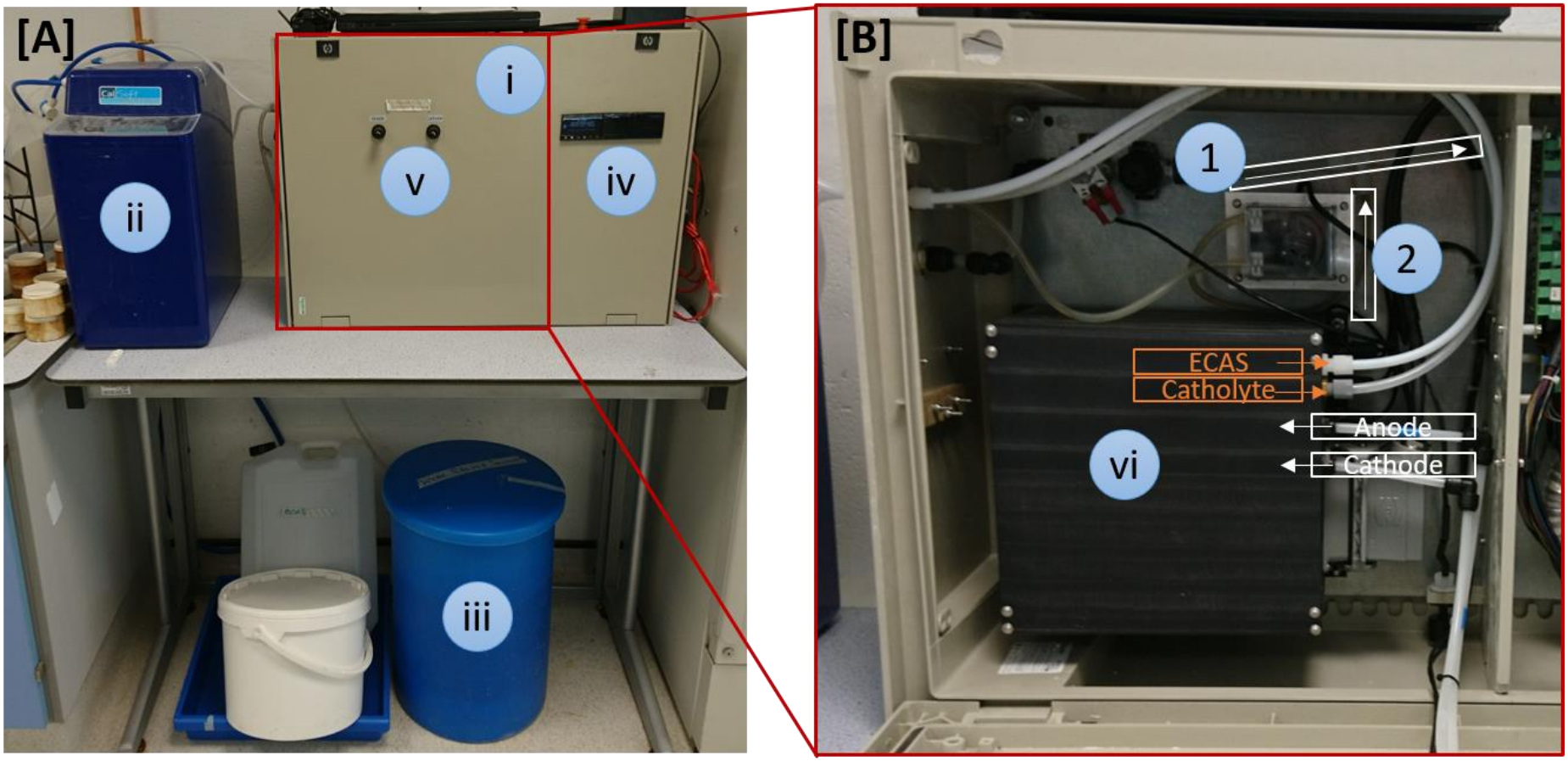


Figure 2.1: Bridge Biotechnology 60-021-11-16 ESOL™ Generator. [A]: ECAS generator [i], which is fed with softened tap water [ii], and a weak saline solution [iii]. Cell current is controlled through the screen at the front of the generator [iv], where the catholyte/anolyte flow ratio are also controlled [v]. [B]: The electrochemical cell [vi] electrolyses softened tap water [1] and saline [2] flowing to the negative cathode and positive anode, and generated cathodic and anodic (ECAS) solutions are produced. (See Figure 1.12).

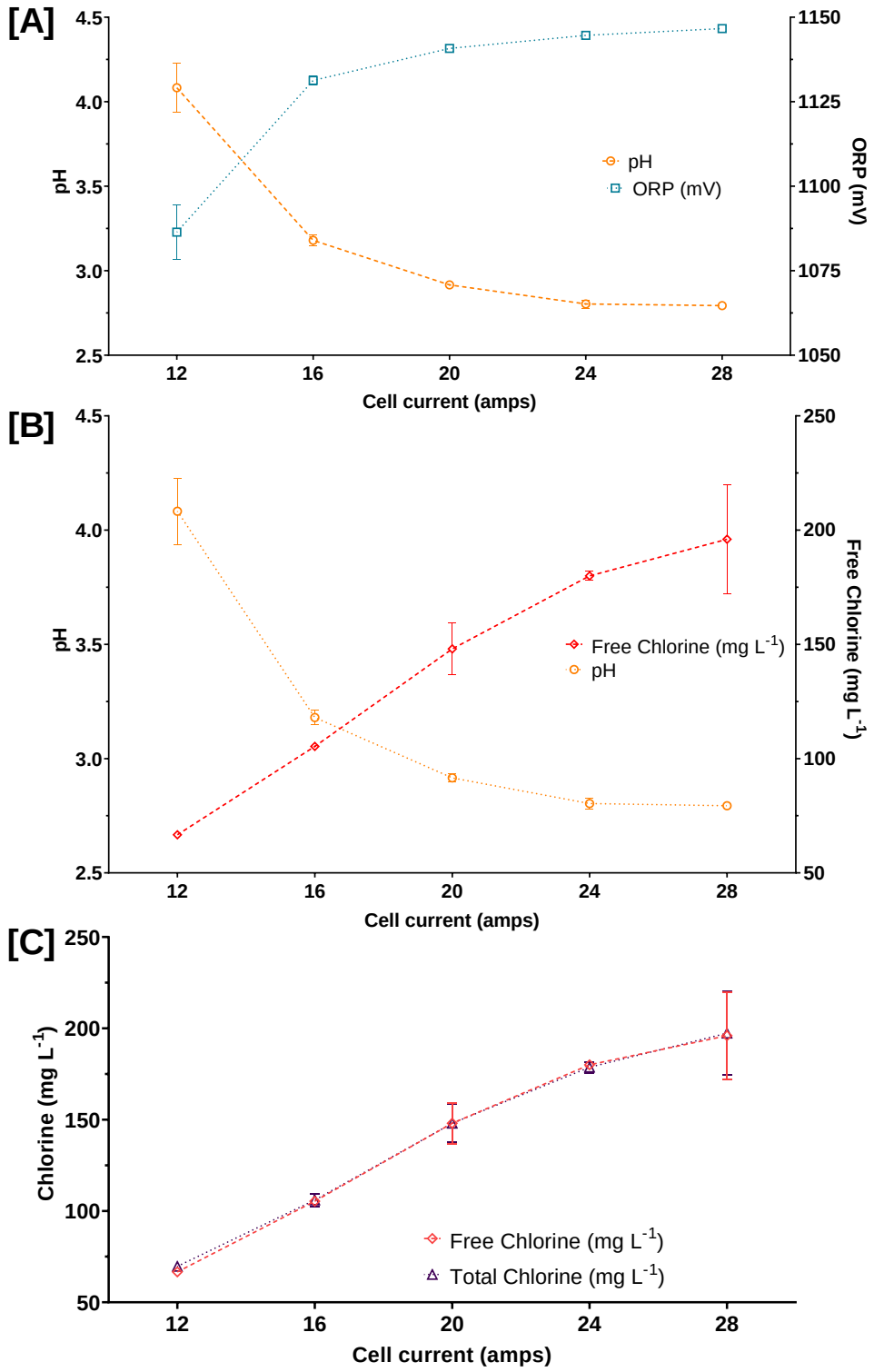


Figure 2.2: [A] The effect of cell current on pH (○) and oxidation reduction potential (□) of ECAS generated; [B] the effect of cell current on pH (○) and free chlorine [◇] concentration; and [C] the effect of cell current on free [◇] and total [△] chlorine concentration. All solutions were generated at a cell flow rates of 1160 mL min^{-1} (anode) and 550 mL min^{-1} (cathode). n = 3 (± SD).

2.2 Point-Of-Use Drinking Water Treatment System

A point-of-use drinking water treatment system (DWTS) has been developed and built on Frenchay Campus UWE, Bristol (Figure 2.3). Input raw environmental water is taken from a modified artificial water body, an urban drainage pond located on the south east area of Frenchay Campus, UWE, Bristol [N51°29′56″, W2°32′39″] (Figure 2.3). A schematic of the decentralised drinking water treatment system (DWTS) is shown in Figure 2.4. This comprises of a submersible filter pump (115 µm (Idrogo 4006-16, Rotorflush, Dorset, UK)) feeds into a reverse flushing filter (100 µm (F76S Honeywell, Bracknell, UK)) and into a particulate settle tank. Water is then drawn towards the ultrafiltration [UF] membrane columns (0.02 µm (LineGuard UF-100, Pentair, Netherlands)). ECAS is generated as described in Section 2.1.1 (Figure 1.12) and stored in a 100L tank and automatically dosed directly into the DWTS pipework immediately before and after the UF membranes. Data logging pressure gauges are installed pre- and post-UF membranes to monitor UF membrane health [PG] and a telemetry sensor network monitors the water quality after treatment before reaching the treated water tank.

Two real time *in-situ* telemetry networks are associated with the DWTS. The first sensor network monitors the raw artificial water body using a Eureka Manta2 sensor (Texas, USA) and is connected to the Adcon Addit4 radio telemetry unit (Adcon Telemetry Group, Austria), where data is collected in 15-minute intervals. Current data is viewed and historical data is downloaded through the advantage Pro 6.6 (Adcon Telemetry Group, Austria). The Manta2 records the pH, conductivity (µS cm^{-1}), dissolved oxygen [DO (mg L^{-1})] and temperature (°C) of the artificial water body. The second sensor network monitors treated water quality through the WebMaster sensor network (Walchem, Holliston, USA). Treated water quality parameters monitored are conductivity (µS cm^{-1}), oxidation reduction potential [ORP (mV)], pH, dissolved oxygen (mg L^{-1}) and free chlorine (mg L^{-1}). Pre- and post- membrane pressures are

also recorded (mbar), to enable the determination of permeability (see Equation 2-1, Equation 2-2 and Equation 2-3).

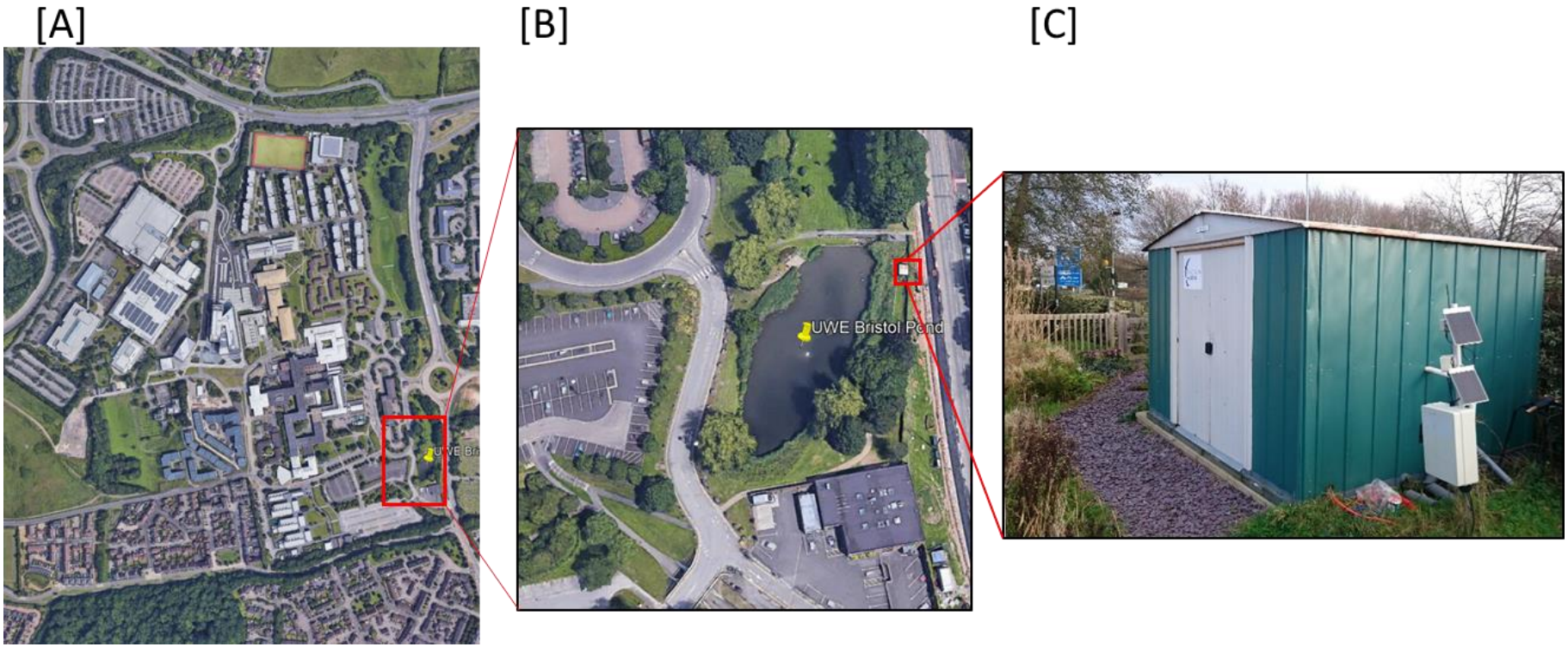


Figure 2.3: The location of the decentralised drinking water treatment system based on UWE, Bristol's Frenchay Campus [A]. The artificial water body [B], which feeds the decentralised drinking water treatment system [C], is located at the southeastern edge of campus. [N51°29′56″, W2°32′39″]. Maps [A] and [B] were adapted from Map Data © 2019 Google.

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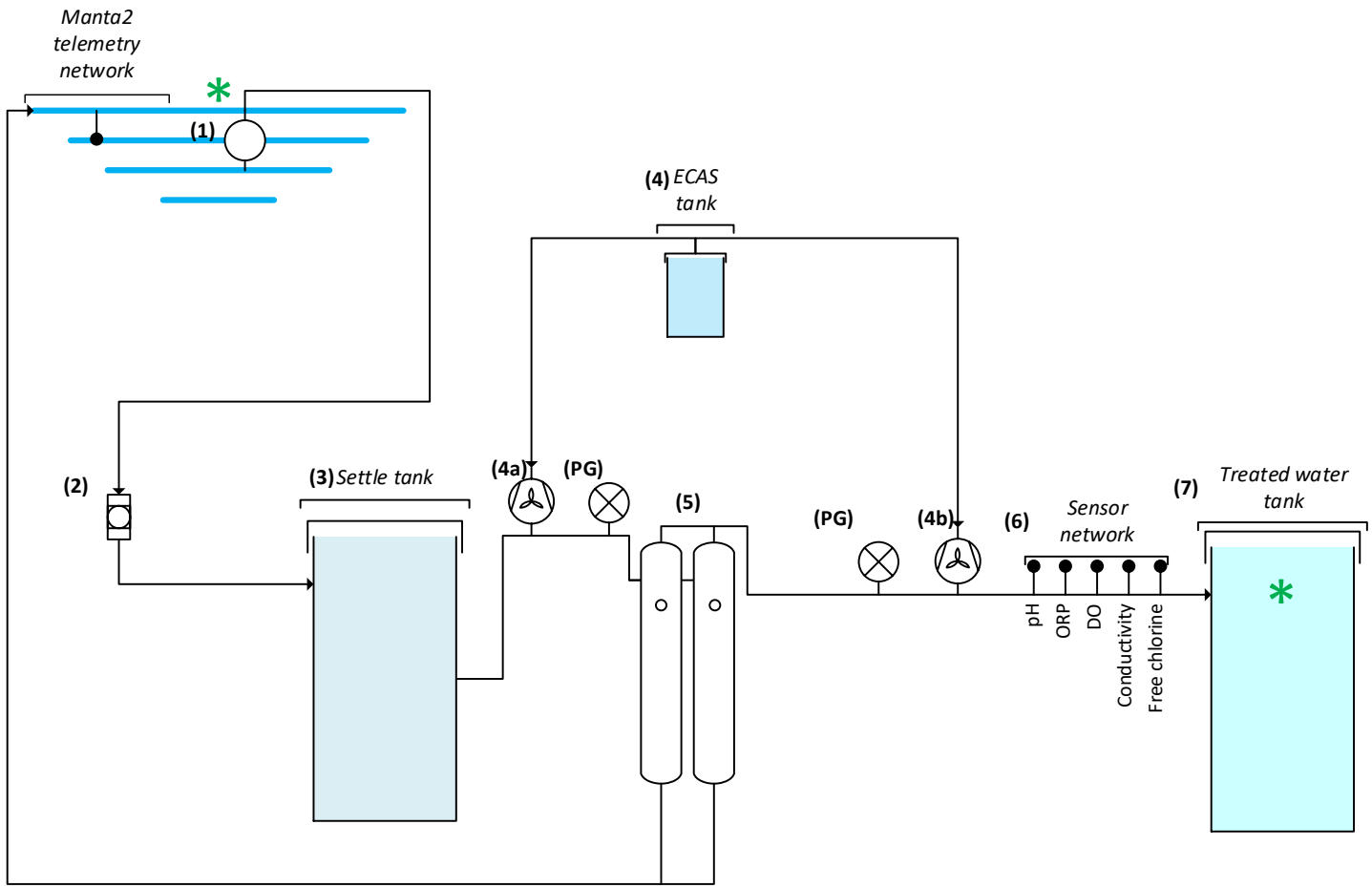

Figure 2.4: Technical schematic of the off-grid drinking water treatment system. Direction of arrows refer to water flow direction. (1) Submersible filter pump (115 µm); (2) Reverse flushing filter (100 µm); (3) Particulate settle tank; (4) 100L ECAS storage tank for ECAS generated outside of the DWTS; (4a/b) ECAS peristaltic dosing pump positions for delivering ECAS into the bulk treated water stream; (5) UF membrane columns (0.02 µm); (6) telemetry network monitoring water quality; (7) Treated water tank. (PG) Pressure gauges measure pressure across UF membranes. Raw and treated water sampling points are represented by [*]

2.2.1 Field trials

Two field trials were performed; Field trial 1 dosed 0.5% (v/v) ECAS pre- and post-UF membranes, therefore a total of 1% (v/v) was directly dosed into the DWTS pipework, equivalent to a final free chlorine concentration of 1.5 mg L^{-1}. As a control, Field trial 2 had no ECAS dosed into the DWTS pipework pre- and post- UF membranes. Both field trials were conducted over 18 operational days, with 18 days between the end of one trial and the start of the next. In this non-operational period, the UF membranes were thoroughly cleaned by alkaline and acid washes using, sodium hypochlorite, sodium hydroxide and hydrochloric acid (Appendix I).

2.2.2 Water quality analysis

Six samples of raw and treated water samples were collected for analysis throughout each field trial. Samples were collected directly from the raw water source (modified artificial water body), and the treated water outlet within the DWTS. The samples were then immediately transported to an independent ISO 17025 accredited laboratory for a standard suite of drinking water analysis. Tables (Table 2-1) provide a full list of parameters tested as part of the standard suite analysis.

Significant differences were determined between the raw and treated water samples throughout Field trial 1 and 2 *t-tests* were performed for each parameter listed in Table 2-1, and a P value of <0.05 was considered statistically significant. Graph construction and statistical analysis was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA), and Microsoft Excel 2013 for Windows (Microsoft Corporation, Redmond, WA).

Table 2-1: Parameters tested as part of the standard suite drinking water analysis in Chapter 3. Analysis was conducted at an independent ISO 17025 accredited laboratory. No standard is represented by NS

Water analysis parameter	**Unit**	***DWI Limit***
Biological		
Plate count (2 day @ 37°C)	mL^{-1}	*No abnormal change*
Plate count (3 day @ 22°C)	mL^{-1}	*No abnormal change*
Non-lactose fermenters	100 mL^{-1}	
Presumptive coliform bacteria	100 mL^{-1}	*0*
Coliform bacteria	100 mL^{-1}	*0*
Presumptive *E. coli*	100 mL^{-1}	*0*
Escherichia coli	100 mL^{-1}	*0*
Clostridium perfringens	100 mL^{-1}	*0*
Enterococci	100 mL^{-1}	*0*
Basic water parameters		
Alkalinity		
Colour (spectrophotometer)	mg L^{-1} Pt/Co	*20*
Colour estimated	Deg Hazen	
Conductivity	µS cm^{-1} @ 20°C	*2500*
pH		*6.5 - 10*
Total hardness	Mg Ca L^{-1}	*NS*
Turbidity	FTU	*4*
Chemical analysis		
Ammonium	mg L^{-1}	*0.5*
Chloride	mg L^{-1}	*250*
Nitrate	mg L^{-1}	*50*
Nitrite	mg L^{-1}	*0.5*
Orthophosphate	mg L^{-1}	
Silica	mg L^{-1}	
Sulphate	mg L^{-1}	*250*
Metal analysis		
Aluminium	µg L^{-1}	*200*
Cadmium	µg L^{-1}	*5*
Calcium	mg L^{-1}	*NS*
Copper	mg L^{-1}	*2*
Iron	µg L^{-1}	*200*
Lead	µg L^{-1}	*25*
Magnesium	mg L^{-1}	*NS*
Manganese	µg L^{-1}	*50*
Nickel	µg L^{-1}	*20*
Potassium	mg L^{-1}	
Sodium	mg L^{-1}	*200*
Zinc	mg L^{-1}	*3*

2.2.3 Calculating the permeability of UF membranes

UF membrane health was determined by calculating the filtration flux and pressure differential across the UF membrane column module (Equation 2-1 and Equation 2-2, respectively), and converting this to membrane permeability, the industry standard for membrane health (Equation 2-3).

$$\text{Filtration flux}\left(\text{L}\ \ \text{m}^{-2}\text{h}^{-1}\right) = \frac{\text{Feedflow}\ [\text{m}^3\text{h}^{-1}] \times 1000}{\text{A} \times \text{B}\ [\text{m}^2]}$$

Equation 2-1

Whereby; UF feed flow is measured on the module, A = Number of membrane housings, and B = Membrane area per membrane housing [m²].

$$\text{Transmembrane Pressure (TMP) filtration [bar]} = \text{PT}_{\text{feed}} - \text{PT}_{\text{permeate}}$$

Equation 2-2

$$\text{Permeability UF module}\left[\text{L}\ \ \text{m}^2\text{h}^{-1}bar^{-1}\right] = \frac{\text{Filtration flux}\left(\text{L}\ \ \text{m}^{-2}\ \text{h}^{-1}\right)}{\text{Transmembrane Pressure (TMP) filtration [bar]}}$$

Equation 2-3

2.3 Trihalomethane Formation and Analysis

2.3.1 Reagents

Ultrapure water with a resistivity output of 18.2 MΩ, was used for preparation of humic acid solutions (Purite Water Purification Solutions, United Kingdom). Calibration and internal standard THM solutions, Fluorobenzene, (Sigma Aldrich, Dorset, United Kingdom) were prepared using high grade (HPLC) methanol (Fisher Scientific, United Kingdom).

2.3.2 Disinfectant solutions

Disinfection solutions were produced as per Section 2.1. The pH and ORP of disinfectants were measured over the 10-minute reaction time, with pH (Figure 4.4) and ORP (Figure 4.6) measured in 1-minute intervals. Free and total chlorine measured at 1, 5 and 10 minutes (Figure 4.5).

2.3.3 Preparation of THM and NOM standard solutions

THM standard solutions containing chloroform ($CHCl_3$), bromodichloromethane ($CHCl_2Br$), dibromochloromethane ($CHClBr_2$) and bromoform ($CHBr_3$) each at 20 μg mL^{-1} were prepared from a standard THM stock solution (200 μg mL^{-1}) and HPLC grade methanol, both supplied by Sigma Aldrich (Dorset, UK).

A NOM stock solution was prepared by dissolving 4 mg of IHSS Suwannee River humic acid [SRHA] (International Humic Substances Society, St Paul, MN, USA), in 100 mL of ultrapure water (overnight) to achieve a concentration of 40 mg L^{-1} (Gadad *et al.*, 2007). From this, standard NOM solutions of 4 mg L^{-1} were prepared. This resulted in a SRHA solution with a mean pH of 4.65 ± 0.16 and an ORP of 383 ± 7.96 mV.

2.3.4 Gas chromatography and mass spectrometry analysis

The quantification of THMs in pre and post disinfected water samples were determined using the standard method (BS EN ISO 17943, British Standards Institution, 2016), which incorporates headspace solid-phase microextraction (HS-SPME) gas chromatography and mass spectrometry (GC/MS). GC/MS was carried out using an Agilent 7820A GC System with an Agilent 5977B high efficiency source with Mass Selective Detection, and a phenyl methyl silox capillary column (Agilent Technologies, Santa Clara, California, US), see Table 2-2 (British Standards Institution, 2016). An internal standard (IS) of fluorobenzene (British Standards Institution, 2016), was diluted to a working concentration of 20 μg mL^{-1} in HPLC grade methanol from a solution of 2000 μg mL^{-1} (Sigma Aldrich, Dorset, UK).

Table 2-2: Operational conditions for GC/MS analysis

Parameter	Conditions
Capillary column, dimensions	Phenyl methyl silox; 60°C - 325°C 30m x 250μm x 0.25μm
Carrier gas	Helium 1.2 mL min^{-1}
GC equipment	Agilent 7820a GC system
MS detector	Agilent 5977b MSD
Selected ion monitoring (SIM) ions used (dwell time 100 ms)	82.9, 84.9, 96, 128.8, 207.8, 251.7
Temperature programme	35°C, 5 minutes; 20°C/minute to 250 °C; 5 minutes hold at 250 °C

2.3.5 HS-SPME experimental procedure

A Supelco solid-phase microextraction (SPME) fiber holder for manual sampling was fitted with an 85 µm carboxen/polydimethylsiloxane (CAR/PDMS) fibre (Sigma Aldrich, Dorset, United Kingdom). The fibre was conditioned at 280°C for two hours.

A working calibration curve for HS-SPME extracted THMs from water samples was constructed by dissolving mixed THM standard solutions (each at 20 µg mL^{-1}) in ultrapure water containing 6g NaCl, to produce solutions at 10, 20, 40, 60, 80 and 100 µg L^{-1}, respectively. An example chromatograph is shown in Figure 2.5. Regression analysis (r^2 values) and mean retention times for each individual THM(s) extracted from the standard mixed solution are shown in Table 2-3. The total THMs (tTHMs) extracted from standard mixed solutions (i.e. the sum of $CHCl_3$, $CHCl_2Br$, $CHClBr_2$ and $CHBr_3$) is also shown in Table 2-3.

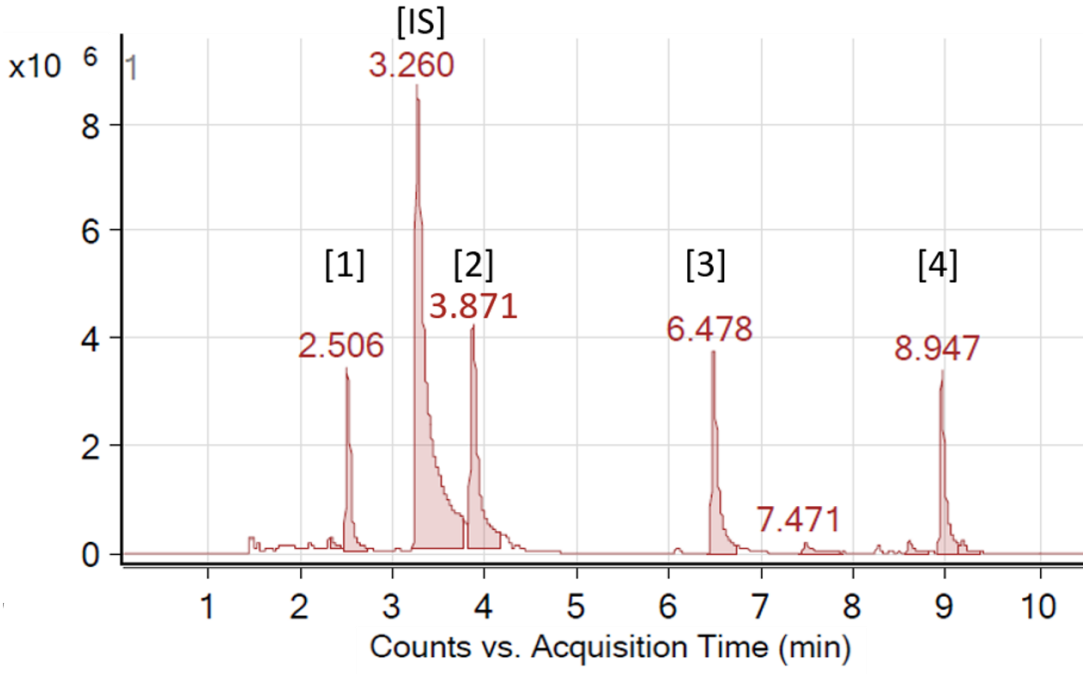

Figure 2.5: Example chromatograph of 60 µg L^{-1} per THM species (chloroform [1]; bromodichloromethane [2]; dibromochloromethane [3] and bromoform [4]), and 100 µg L^{-1} internal standard [IS] n = 3 (± SD).

Table 2-3: THM calibration regression values for THM calibration mix with dilutions of 10, 20, 40, 60 80 and 100 µg L^{-1} (n = 3). Mean retention time across all THM calibration mix dilutions (n = 18 ± SD)

Compound	***r²* (linear)**	**Mean retention time (minutes) [± SD]**
	10 – 100 µg L^{-1}	
Chloroform	0.7453	2.52 ± 0.0075
Bromodichloromethane	0.8555	3.87 ± 0.0068
Dibromochloromethane	0.9657	6.48 ± 0.0054
Bromoform	0.9883	8.95 ± 0.0059
tTHM	*0.9404*	

2.3.6 Preparation of test samples

For the reaction vials (sterile glass universals with solid high-density polyethylene screw caps), disinfectants were added to standard NOM solution (4 mg L^{-1} IHSS Humic acid), maintaining a total reaction volume of 30 mL, to achieve free chlorine concentrations of 0, 1, 3 and 5 mg L^{-1}. Reaction times (1, 5 or 10 minutes) were controlled by taking a 20 mL sample from the test reaction vial, and injected into a test extraction vial. Test extraction vials (30 mL sterile extraction glass universals with high density polyethylene screw cap with silicone septum) contained 6 g laboratory grade NaCl, 5 g L^{-1} sodium thiosulphate (Environmental Protection Agency, 2011; British Standards Institution, 2005a; Antoniou, Koukouraki and Diamadopoulos, 2006) and the internal standard, fluorobenzene, at a final concentration of 100 µg L^{-1}. Prior to headspace extraction, all samples were incubated at 40 °C for 20 minutes, inclusive of 10 minutes headspace extraction (fiber exposed). During SPME fiber exposure the manual SPME holder was injected through the septum into the headspace of the sample vial, exposing the CAR/PDMS fiber. After the 10-minute fiber exposure period, care was taken to ensure the CAR/PDMS fiber was retracted into the manual SPME holder and inserted into the GC/MS inlet (<30 seconds), minimising extraneous exposure of the fiber. All sample fibers had a desorption period of 2 minutes prior to analysis.

2.3.7 Data analysis

Individual THM concentrations were calculated using Agilent Mass Hunter Enhanced Data Analysis Software (Santa Clara, California, United States). tTHMs were calculated from the sum of $CHCl_3$, $CHCl_2Br$, $CHClBr_2$ and $CHBr_3$. Values reported were blank corrected, and a limit of detection (LoD) of 0.86 µg L-1 for all samples was determined experimentally. Where analysis is below the LoD, then data values are represented by an asterisk (*).

Comparative statistical analysis of THM concentrations (between experimental variables) was performed using a two-way ANOVA with Bonferroni post-hoc test (GraphPad Prism version 5.00 for Windows, San Diego, CA). A P value of <0.05 was regarded as significant.

2.4 Bacterial Strains

Escherichia coli (*E. coli*) American Type Culture Collection [ATCC] 10536 and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 15422 were grown on Tryptone Soya Agar (TSA [CM0131; Oxoid, Thermo Scientific, UK]) for 24 hours at 37°C from frozen stocks stored at - 80°C. *P. aeruginosa* was cultured in 100 mL Duran bottles containing 100 mL of 100 mg L^{-1} tryptone soya broth (TSB [CM0129; Oxoid, Thermo Scientific, UK]) within a shaking incubator for 24 hours at 35°C at 150 rpm, resulting in a final microbial density of $1.43 \times 10^8 \pm 1.29 \times 10^8$ CFU mL^{-1} ($n = 23$).

2.5 Planktonic Assays for Bactericidal Activity of Chemical Disinfectants

2.5.1 Minimum Inhibitory Concentration (MIC) / Minimum Bactericidal Concentration (MBC) assays

The MIC and MBC for NaOCl, HOCl and ECAS against *E. coli* ATCC 10536 and *P. aeruginosa* ATCC 15442 were determined through an amended 96 well microtiter plate serial dilution method (Elshikh *et al.*, 2016). *E. coli* and *P. aeruginosa* were cultured onto TSA for 24 hours at 37°C from frozen stocks stored at - 80°C. Colonies taken from *E. coli* and *P. aeruginosa* plates were emulsified in 10mL of 100 mg L^{-1} TSB in sterile universals. Optical densities were determined for *P. aeruginosa* and *E. coli* at 0.118 ± 0.007 OD and 0.243 ± 0.004 OD, respectively. Positive and negative controls were, inoculated TSB media (30g L^{-1}) with either *P. aeruginosa* or *E. coli*, and 30 g L^{-1} TSB, respectively.

The maximum free chlorine concentrations for each of the disinfectants were 110 mg L^{-1} (NaOCl), 75 mg L^{-1} (HOCl) and 94 mg L^{-1} (ECAS). Serial dilutions (1:2) were then carried out until a free chlorine concentration 1.71 mg L^{-1} (NaOCl), 1.15 mg L^{-1} (HOCl) and 1.46 mg L^{-1} (ECAS) was achieved. Inoculated TSB media was diluted to approximately 10^5 CFU mL^{-1}, and 50µl was pipetted into each treatment well. The 96 well microtiter plates were incubated at 37°C for 24 hours.

After 24 hours the optical density at 595nm of the 96 well microtiter plates were taken (TECAN Infinite F200 pro, Tecan Trading AG, Switzerland). MIC was determined as the point at which bacterial growth was inhibited. The MBC was determined by plating 50 µL samples from microtiter wells directly onto TSA, taken from treatment wells which were immediately before and after the proposed MIC, and plated onto TSA and incubated at 37°C for 24 hours. The

MBC was determined as the concentration where no bacterial growth was observed on TSA plates.

2.5.2 Quantitative suspension assays evaluating the bactericidal activity of chemical disinfectants

Standard suspension assays for determining bactericidal activity against *E. coli* ATCC 10536 were carried out according to BS EN 1040 (British Standards Institution, 2005) and BS EN 1276 (British Standards Institution, 2009). A diluent solution consisting of 1 g L^{-1} tryptone (LP0042; Oxoid, Fisher Scientific) and 8.5 g L^{-1} of laboratory grade NaCl (Fisher Scientific) in DI water, was sterilised through autoclaving. A neutralising solution of 5 g L^{-1} sodium thiosulphate and 25.7 g L^{-1} Letheen Broth (BD 263010; BD Difco, Becton Dickinson) was suspended in DI and sterilised through autoclaving.

The BS EN 1040 assay is conducted in the absence of an interfering solution, whilst inhibitory solutions for BS EN 1276 were bovine serum albumen ([BSA] 268130100; Acros Organics, Fisher Scientific, UK) at two concentrations. These were prepared through dissolving 0.3 g 100 mL^{-1}, for clean BSA, or 3.0 g 100 mL^{-1}, for dirty BSA, then filter sterilised through 0.45 µm syringe filters to obtain final concentrations of 0.3 g L^{-1} and 3.0 g L^{-1}, respectively. A modified BS EN 1276 assay was undertaken using environmental waters taken from an artificial water body (an urban drainage holding pond, University of the West of England, Bristol, UK [N 51°29′56″, W 2°32′392) as the interfering solution.

2.6 Assays for Determining the Antimicrobial Efficacy of Chemical Disinfectants Against Biofilms

2.6.1 CDC biofilm reactor set up

The Centre for Disease Control (CDC) biofilm reactor allows for biofilms to be grown under high shear conditions and continuous media flow. A CDC biofilm reactor consists of a litre glass vessel with an effluent spout, at 330 mL volume(see Figure 2.6). The Teflon stir blade with magnetic baffle allows for a constant stir rate to be set to provide shear stress against 8 polypropylene coupon, or blank, rods. Each coupon rod can hold three coupons that are 12.7 mm in diameter and 3.0 mm deep, providing a total of 24 coupons per reactor. Standard coupons are polycarbonate (Figure 2.6 [B]), however alternative materials can be used, depending on application. Custom polyethersulphone coupons (Figure 2.6 [C]) provided a representative surface material for ultrafiltration membranes.

A schematic of the complete standard set up for a CDC biofilm reactor (BioSurface Technologies Corp., no date), is shown in Figure 2.6. Briefly, sterile input media (i.e. 100 mg L^{-1} TSB) is held in sterile 20 L Thermo Scientific™ Nalgene™ autoclavable carboys (2250-0050; Fisher Scientific, UK). A calibrated single channel peristaltic pump (Watson-Marlow 120S, Watson-Marlow Ltd, Falmouth, UK) draws input media towards the CDC biofilm reactor [3] at a set flow rate (i.e. 12 mL min^{-1}). Waste media from the CDC biofilm reactor is removed via the effluent spout into a sterile Nalgene waste carboy.

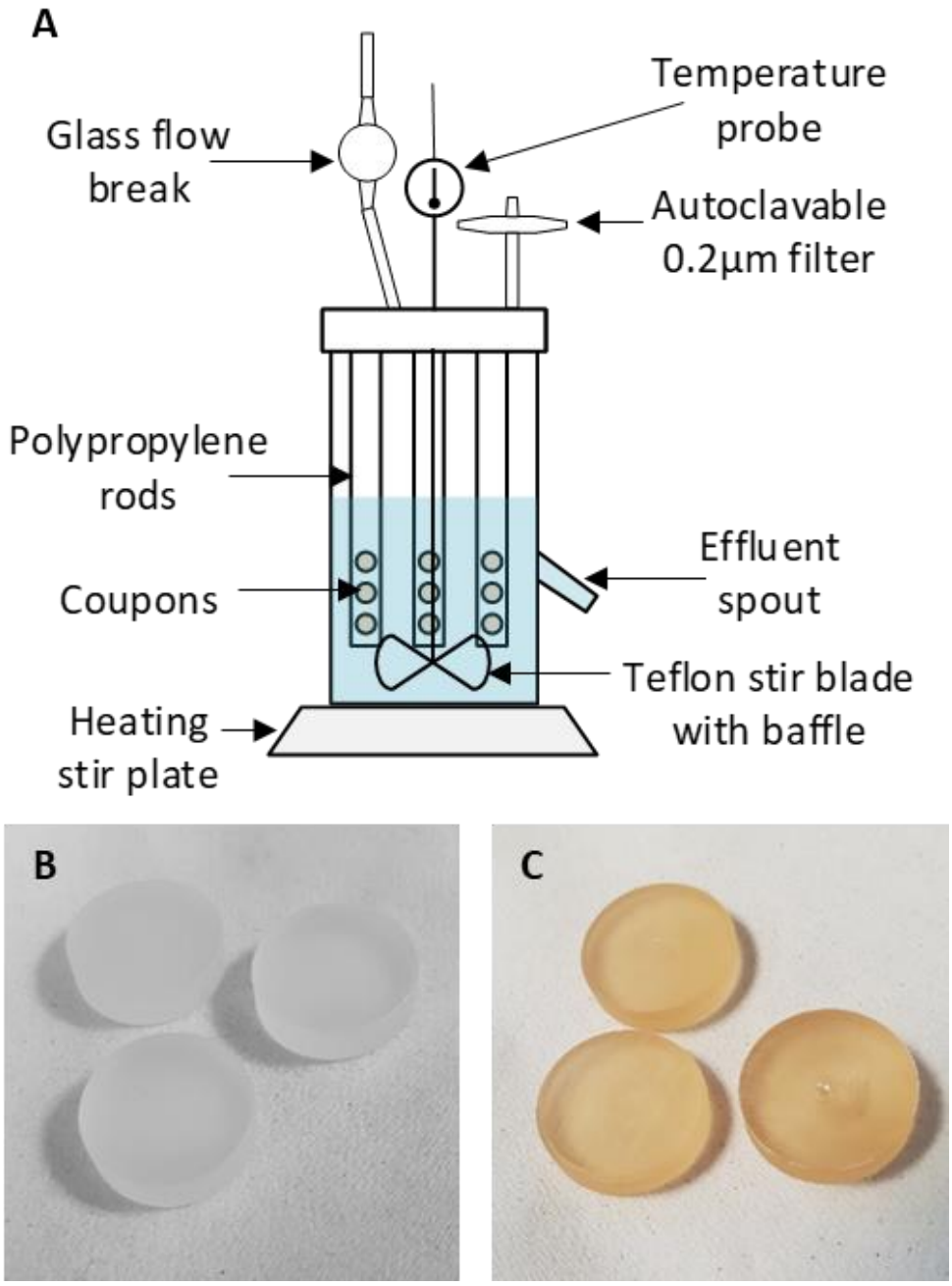

Figure 2.6: [A] The set up for a Centre for Disease Control Biofilm Reactor. Media enters the reactor though a glass flow break, flow rate and reactor temperature is set by a heating stir plate and monitored by a temperature probe, an autoclavable 0.2 µm filter (bacterial air vent) allows the reactor to exhaust whilst autoclaving and throughout the run time. Eight polypropylene rods, each containing three 12.7mm (diameter) x 3.0mm (deep) coupons, ensure one side of each coupons faces a Teflon stir blade held together by a magnetic stir bar. Standard polycarbonate coupons [B] and custom made polyethersulphone coupons [C].

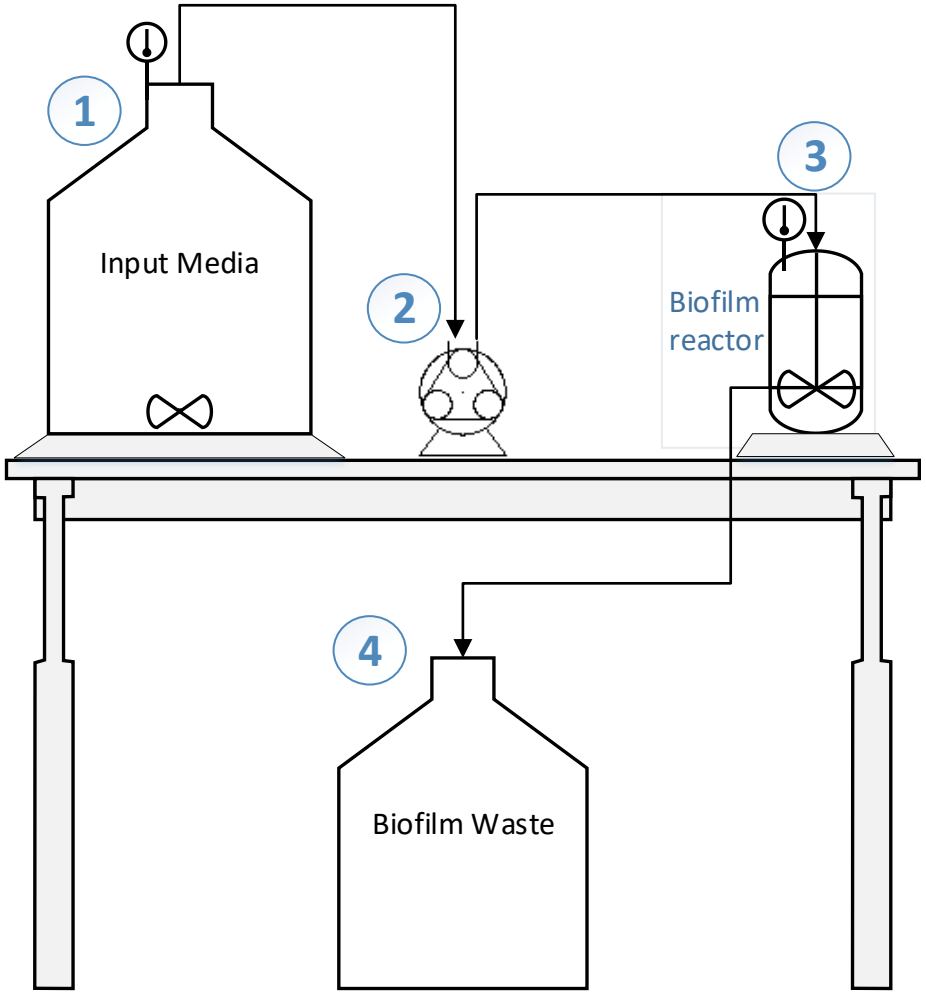

Figure 2.7: Standard experimental set up of CDC biofilm reactor. Sterile input media (i.e. tryptone soya broth) is held within a 20 L Nalgene carboy [1], and drawn through a single channel peristaltic pump [2] towards the CDC biofilm reactor [3]. A sterile Nalgene waste carboy collects CDC biofilm reactor waste.

2.6.2 CDC biofilm reactor operation

Batch phase (24 hours) for the CDC biofilm reactor, allows bacteria to attach to coupons and establish a biofilm. Continuous phase (24 hours) is where a continuous flow of media allows for the established biofilm to mature and increase in density through the introduction of fresh media. The total experimental duration for the standard CDC biofilm reactor method is 48 hours.

2.6.2.1 Batch phase

The CDC biofilm reactor consists of eight polypropylene coupon rods with secured polycarbonate [PC] coupons, stir blade, baffle, and clamped effluent spout (Figure 2.6). Foil securely covered all tubing connections, and the CDC reactor vessel contained 500 mL TSB (30 mg L^{-1}). The whole reactor was sterilised through autoclaving. The reactor was placed on a heating stir plate, with the temperature controlled by an *in-situ* probe located within the reactor vessel and continuously measured on the stir plate. The bacterial vent was opened and inoculated with 1 mL of an overnight culture of *P. aeruginosa* ATCC 15422 at a concentration of 7.79 ± 0.17 Log_{10} CFU mL^{-1} (Section 2.4). The reactor with inoculated medium was then constantly stirred for 24 hours at 125 rpm, and kept at a temperature of 22.5°C ± 1°C (Montana State Univeristy, 2016).

2.6.2.2 Continuous phase

Prior to beginning the continuous flow of media (100 mg L^{-1} TSB) the flow rate was calculated by:

$$Flow\ rate\ (mL\ min^{-1}) = Reactor\ volume\ (mL) \div Residence\ time\ (min)$$

Equation 2-4

Whereby the reactor volume was determined when the reactor was fully assembled: i.e. rods, coupons and baffle.

$$330\ mL\ \div 30\ mins = \mathbf{11\ mL\ min^{-1}}$$

Immediately before beginning the continuous phase, all tubing was aseptically connected where necessary, input media was purged through the tubing and the effluent spout was opened. Media flow rate was then set at 11 mL min^{-1} and the CDC biofilm reactor was operated

with continuous flow of media for 24 hours. The reactor stir rate and temperature was kept constant at 125 rpm, and 22.5°C, respectively.

2.6.3 Biofilm treatment and enumeration

After a continuous phase of 24 hours, the media flow was stopped, as well as the stirring of the baffle within the CDC biofilm reactor. Each rod was aseptically removed from the reactor, rinsed with quarter strength ringer solution, then each coupon was then aseptically placed into a 50 mL falcon tube (Sarstedt, Germany), containing 3 mL of disinfectant (5, 25, 50, 75, 100 or 150 mg L^{-1} free chlorine) or sterile DI (control, 0 mg L^{-1} free chlorine).

After a 5 minute reaction time, 27 mL of neutralising solution (as per Section 2.5.2) was added to the tubes and incubated for 10 minutes. To remove biofilm from the surface of the coupon, each falcon tube was vortexed for 30 seconds, and then sonicated at 35 kHz in a sonicating water bath (FB11078 FisherBrand, Fisher Scientific, UK) for 1 minute. This was repeated three times. Finally, the disaggregated cell suspension was serially diluted in quarter strength ringers, before being plated onto R2A agar (CM0906; Oxoid, Thermo Scientific, UK) using a Spiral Plater (50 µL volume (Whitley Automated Spiral Plater, Don Whitely Scientific, Bingley, UK)). Plates were incubated at 37°C for 24 hours, and colonies counted to provide numbers of surviving colony forming units(Log_{10} CFU $coupon^{-1}$).

2.6.4 Statistical analysis

A two-way analysis of variance (ANOVA) and Bonferroni post-test was carried out to determine significant differences in antimicrobial efficacy between disinfectant type and free chlorine concentration (GraphPad Prism version 8.0 for Windows, San Diego, CA). A *P* value of <0.05 was regarded as significant.

2.7 *In-situ* Dosing CDC Biofilm Reactor Assays

The amended CDC biofilm reactor model was used to determine the effect of *in-situ* disinfectant dosing at MIC concentration (Section 2.5.1) to investigate the effect of managing biofilm formation on polycarbonate [PC] and polyethersulphone [PES] surfaces (Figure 2.6 [C]). The development of an *in-situ* dosing model is explained further in Chapter 6.

2.7.1 *In-situ* ECAS dosing CDC biofilm– single species

An amended model set up for the CDC biofilm reactor (Biosurface Technologies Corporation, Montana, USA) was designed to allow for *in-situ* disinfectant dosing (Figure 2.8). PC coupons were used as the substrate for single-species (*P. aeruginosa* ATCC 15422) biofilm formation.

2.7.1.1 CDC biofilm reactor model with *in-situ* dosing set up

To determine the effect of *in-situ* dosing on biofilm formation on PES surface material, with a dosing regimen commensurate with Chapter 3 (0.5% [v]), an *in-situ* dosing biofilm model was developed (Figure 6.1). The model was adapted from a standard CDC biofilm method (Figure 2.7), whereby, an inoculated 20 L carboy containing 100 mg L^{-1} TSB and *P. aeruginosa* (ATCC 15442) was the input medium. This *in-situ* model did not include a batch phase, which allows for initial bacterial attachment and biofilm formation, to determine the effect of *in-situ* dosing at inhibiting biofilm formation. This model investigated whether constant low ECAS dosing (0.5% [v/v]) would inhibit biofilm formation, with a continuous flow rate (11 mL min^{-1}) over 8 days.

The model consisted of a sterile 20 L carboy containing the inoculated input media (Figure 6.1 [1]), which was then dosed *in-situ* by either ECAS [2A], or sterile DI [2B]. Immediately after the point of dosing a sample tap allowed for planktonic samples to be taken immediately

before [3A/B] and after [5A/B] the CDC biofilm reactor [4]. An outlet port on the CDC biofilm reactors allowed for excess media to empty into a 20 L sterile carboy [6].

2.7.1.2 Operational procedure

Samples of cultured *P. aeruginosa,* as well as the inoculated input media, were taken after a 24-hour incubation period, or at point of input media renewal, which occurred every 12 hours from the start of the model beginning. Overnight cultures were standardised to an optical density of OD_{600} 0.1, equivalent to 8.11 ± 0.275 x 10^8 CFU mL^{-1}, and diluted in 1:500 to a final density of 5.461 ± 0.293 log_{10} CFU mL^{-1}. The first planktonic and biofilm samples were taken 6 hours after the initial start of the CDC biofilm model, and then every 24 hours thereafter (i.e. 30 hours, 54 hours).

2.7.1.3 Enumeration of planktonic samples taken pre-CDC biofilm reactor

One coupon rod (three PES coupons per rod), per reactor were aseptically removed at the determined sampling times (i.e. 6 hours, and then every 24 hours thereafter; 30 hours, 54 hours) from the CDC biofilm reactor and replaced with sterile blank rods. Coupons were then removed from the rods and washed with sterile ringers to remove any planktonic bacteria before being placed into 10 mL sterile quarter strength ringers in a falcon tube. Biofilm was disaggregated as per Section 2.6.3. R2A plates were incubated at 37°C for 24 hours, and colonies counted to provide numbers of surviving colonies (log_{10} CFU $coupon^{-1}$).

2.7.2 *In-situ* dosing CDC biofilm for managing multi-species environmental biofilms

Polyethersulfone (PES) coupons (Ryan Plastics Ltd, Earls Barton, UK), 24 coupons per reactor, were used for surfaces for biofilm formation (Figure 2.6 [C]).

Environmental water from the artificial water body (Figure 2.3) was stored in a sterile 20L Nalgene carboy. The carboy was placed on a magnetic stir plate set at approximately 1200 rpm to minimise particulate matter settling. A sample tap [A2] was installed to allow for environmental water samples to be taken without disruption to the model. A sterile 10 L Nalgene carboy held a disinfectant (Figure 2.8 [A1]), either NaOCl, HOCl or ECAS. A single channel peristaltic pump draws environmental water from the media carboy, towards the CDC reactor, whilst a multi-channel peristaltic pump [M] (IP24 Ismatec, Wertheim, Germany) draws from the disinfectant carboy. The multi-channel peristaltic pump rotation speed was adjusted to achieve a final free chlorine concentration of 50 mg L^{-1}, whilst the single channel peristaltic pump (feed water) was adjusted to produce a total flow rate of 12 mL min^{-1}. After purging the tubing from the environmental water carboy and disinfectant carboy, a continuous flow of environmental water was drawn into the CDC biofilm reactor. The amended CDC biofilm reactor model ran for a total of 48 hours.

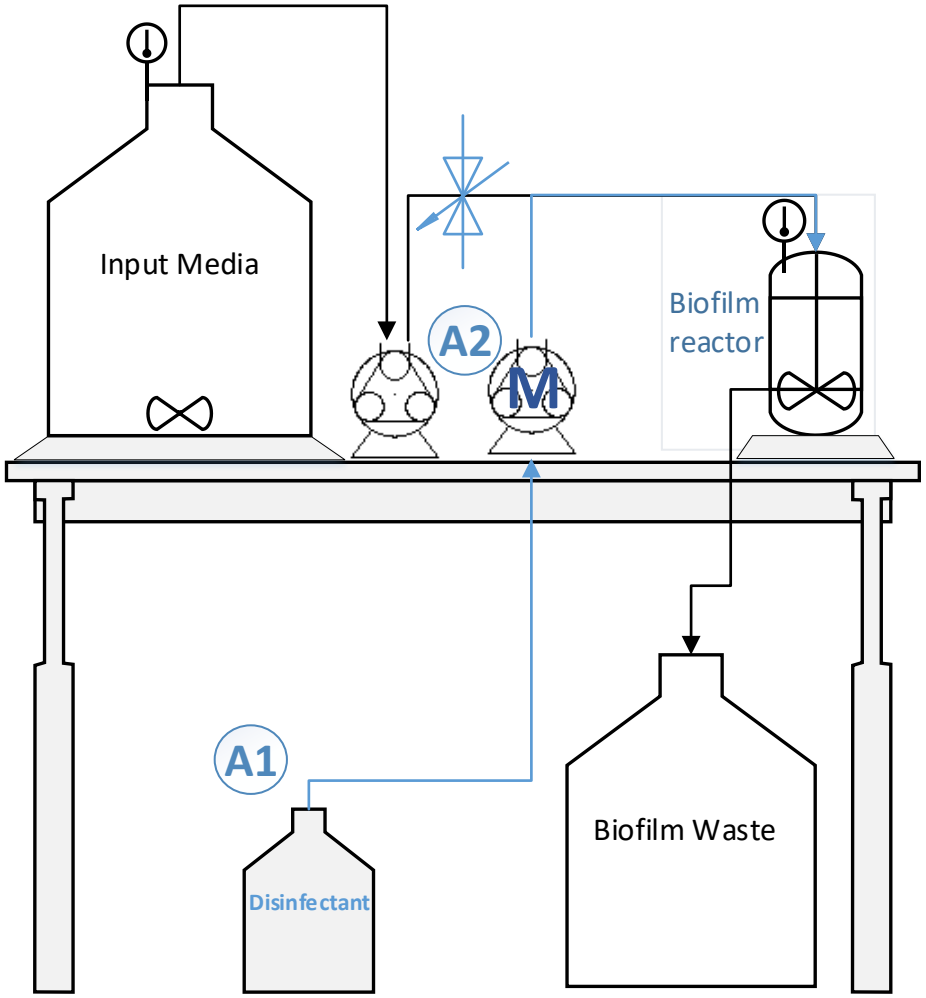

Figure 2.8: Experimental set up of *in-situ* dosing CDC biofilm reactor set up to allow for *in-situ* dosing of disinfectants. [A1] Disinfectants were stored in sterile 10L Nalgene carboys, and drawn into silicone tubing through a multi-channel peristaltic pump [M]. A sample tap was installed immediately after the single channel peristaltic pump [A2].

2.7.3 *In-situ* dosing CDC planktonic and biofilm enumeration

Coliform counts were carried on Membrane Lactose Glucuronide Agar (MLGA) using the membrane filtration method (The Environment Agency, 2009). Heterotrophic plate counts (HPC) were carried out on R2A agar using a Spiral Plater (50 µL volume (Whitley Automated Spiral Plater, Don Whitley Scientific, Bingley, UK)) and incubated at 30°C for 3 days and 22°C for 5 - 7 days. Environmental water samples were diluted in sterile quarter strength ringers.

Two coupon rods (three PES coupons per rod) were aseptically removed at 24 and 48 hours from the CDC biofilm reactor and replaced with sterile blank rods. Coupons were then removed from the rods and washed with sterile ringers to remove any planktonic bacteria before being placed into 10 mL sterile quarter strength ringers. Biofilm was disaggregated as per Section 2.6.3. Plates were incubated at 30°C for 3 days and 22°C for 5 - 7 days.

2.7.4 Data analysis

Comparative statistical analysis of biofilm formation (between time and disinfectant type) was performed using a two-way ANOVA with Sidak multiple comparison post-hoc test (GraphPad Prism version 8.0 for Windows, San Diego, CA). A *P* value of <0.05 was regarded significant.

Chapter 3. Proof-of-concept; the development of a decentralised drinking water treatment system

Data presented in this chapter is adapted from Clayton, Thorn and Reynolds, (2019) Journal of Water Process Engineering [DOI: 10.1016/j.jwpe.2017.08.018] (Appendix III: Publications)

Data presented in this chapter was collected by Dr Robin Thorn as part of a consultancy project with Portsmouth Aviation Ltd. Data analysis was carried out by Gillian Clayton.

3.1 Introduction

Decentralised drinking water treatment systems (DWTS) designed for communities often use a combination of filtration (Zhu *et al.*, 2014; Peter-Varbanets *et al.*, 2009; Ali Baig *et al.*, 2011; Mahmood *et al.*, 2011; Chaidez *et al.*, 2016; Huang, Jacangelo and Schwab, 2011) and disinfection (Martínez-Huitle *et al.*, 2008; Carratalà *et al.*, 2016; Attisani, 2016). A key feature needed for decentralised systems which are designed at a community level, is the potential for scalable, modular capability, whereby output capacity can be varied to cope with demand. Point-of-use DWTS should ensure biologically and chemically safe water is produced without adversely impacting the environment.

The European Council set guidelines for water quality which is safe for human consumption (The Council of the European Union, 1998), which is interpreted by each European Union member state. In the United Kingdom the Drinking Water Inspectorate (DWI) interprets and regulates drinking water quality (Drinking Water Inspectorate, 2012).

The aim of this chapter is to demonstrate the capability of a novel DWTS for the production of biologically safe drinking water from a raw water source (artificial water body) to DWI

standards. This novel DWTS uses *in-situ* dosing of electrochemically activated solutions, as described in Section 2.1.1.

Two field trials were performed over 18 operational days, see Section 2.2.1, Field trial 1 dosed 0.5% (v/v) ECAS pre- and post- UF membranes, resulting in a total of 1% (v/v) ECAS directly dosed into the DWTS pipework, equivalent to a final free chlorine concentration of 1.5 mg L^{-1}. Field trial 2, the control, had no dosing of ECAS pre- and post- UF membranes, resulting in 0% (v/v) ECAS dosage into the DWTS pipework. There were 18 consecutive days between Field trial 1 and 2; throughout this time UF membranes were cleaned using alkaline and acid washes using NaOCl, HCl and NaOH (Appendix I: UF membrane cleaning protocols). Methods used to determine water quality and permeability of UF membranes are detailed in Section 2.2.2 and Section 2.2.3, respectively.

3.2 Results

3.2.1 Field trial 1: 1% (v/v) total ECAS dosing

Field trial 1 ran for 18 days whereby 0.5% (v/v) ECAS was dosed pre- and post-UF membrane directly into the DWTS pipework, providing a total of 1% (v/v) ECAS throughout the system (see Figure 2.4 for a schematic of this system). Treated and raw water samples were tested for various water quality parameters by an independent ISO 17025 accredited laboratory (see Table 2-1). These tests include the basic, biological, chemical and metal parameters of the raw and treated water samples. All raw water samples taken throughout the 18 operational days failed to meet the Drinking Water Inspectorate (DWI) standards for safe drinking water in the UK (See Table 3-1 and Table 3-8). In comparison, the water quality parameters for all the treated water samples were determined to have met the DWI standard requirements, and so deemed fit for human consumption. A key basic parameter that consistently failed in the raw water was turbidity (Table 3-1). The mean turbidity of the raw water was 15.6 ± 3.1 FTU, which

was significantly reduced within treated water samples by 97.8% to 0.34 ± 0.27 FTU ($p < 0.0001$), well below the maximum DWI threshold of 4 FTU for drinking water. This demonstrates that particulate matter is effectively removed by the novel DWTS.

All test and raw water samples were analysed for their microbiological properties. Raw water samples failed with the presence of coliforms (12 ± 8.29 CFU 100 mL^{-1}), *Clostridium perfringens* (95.83 ± 11.7 CFU 100 mL^{-1}), and Enterococci (52.67 ± 42.04 CFU 100 mL^{-1}), where 0 CFU 100 mL^{-1} is permissible in drinking water. A 1% total dosing of ECAS resulted in zero coliforms (0 CFU 100 mL^{-1}), *Escherichia coli (E. coli)*, *Enterococci* and *Clostridium perfringens* within the treated water samples (Table 3-2). No heterotrophic bacteria (incubated at 37°C for 48 hours) were detected in treated water samples, except for operational day 5 ([2 CFU mL^{-1}] Figure 3.1). It should be noted that there is no threshold for heterotrophic bacteria within DWI regulations (Drinking Water Inspectorate, 2010).

Table 3-1: Analytical results of basic water parameters of the raw water samples and treated water samples. Field trial 1: 1% total ECAS dosing UF membrane. Results shown are the calculated mean from the independent ISO 17025 accredited laboratory reports (n = 6 ± SD). Significant difference (Sig. diff) calculated through an unpaired, two tailed t-test, with a confidence interval of 95% (**** = p<0.0001; *** = p<0.001; ** = p<0.01; * = p<0.05; ns = not significant). Red values represent results that fail to meet the DWI standard.

		FIELD TRIAL 1 (1% total ECAS dosing)					
Water type		**Raw water**		**Treated water**			
	UNIT	Mean	SD	Mean	SD	Sig. diff	*DWI Limit*
BASIC WATER PARAMETERS							
Alkalinity		139.00	2.65	131.50	7.78	ns	
Colour (spectrophotometer)	mg L^{-1} Pt/Co	5.60	0.55	4.00	1.79	ns	
Colour estimated	Deg Hazen	5.00	0.00	5.00	0.00	ns	
Conductivity	µS cm^{-1} @ 20°C	708.00	69.80	764.17	151.18	ns	*2500*
pH		8.88	0.18	8.70	0.37	ns	*6.5 - 10*
Total hardness	Mg Ca L^{-1}	118.67	13.31	114.93	16.75	ns	
Turbidity	FTU	15.60		0.34	0.27	****	*4*

Table 3-2: Analytical results of biological parameters of the raw water samples and treated water samples. Field trial 1: 1% total ECAS dosing UF membrane. Results shown are the calculated mean from the independent ISO 17025 accredited laboratory reports (n = 6 ± SD). Significant difference (Sig. diff) calculated through an unpaired, two tailed t-test, with a confidence interval of 95% (*** = p<0.001; ** = p<0.01; * = p<0.05; ns = not significant). Red values represent results that fail to meet the DWI standard.

		FIELD TRIAL 1 (1% total ECAS dosing)					
Water type		**Raw water**		**Treated water**			
	UNIT	Mean	SD	Mean	SD	Sig. diff	*DWI Limit*
BIOLOGICAL							
Plate count (2 day @ 37°c)	mL^{-1}	538.83	753.19	0.33	0.82	***	
Plate count (3 day @ 22°c)	mL^{-1}	2685.33	770.77	2690.67	757.71	ns	
Non-lactose fermenters	100 mL^{-1}	33.33	100.00	1.17	2.86	ns	
Presumptive coliform bacteria	100 mL^{-1}	49.17	43.19	0.00	0.00	***	
Coliform bacteria	100 mL^{-1}	**12.00**	8.29	0.00	0.00	***	*0*
Presumptive *E.coli*	100 mL^{-1}	1.50	0.71	0.00	0.00	***	
Escherichia coli	100 mL^{-1}	1.50	0.71	0.00	0.00	***	
Clostridium perfringens	100 mL^{-1}	**95.83**	11.70	0.00	0.00	***	*0*
Enterococci	100 mL^{-1}	**52.67**	42.04	0.00	0.00	***	*0*

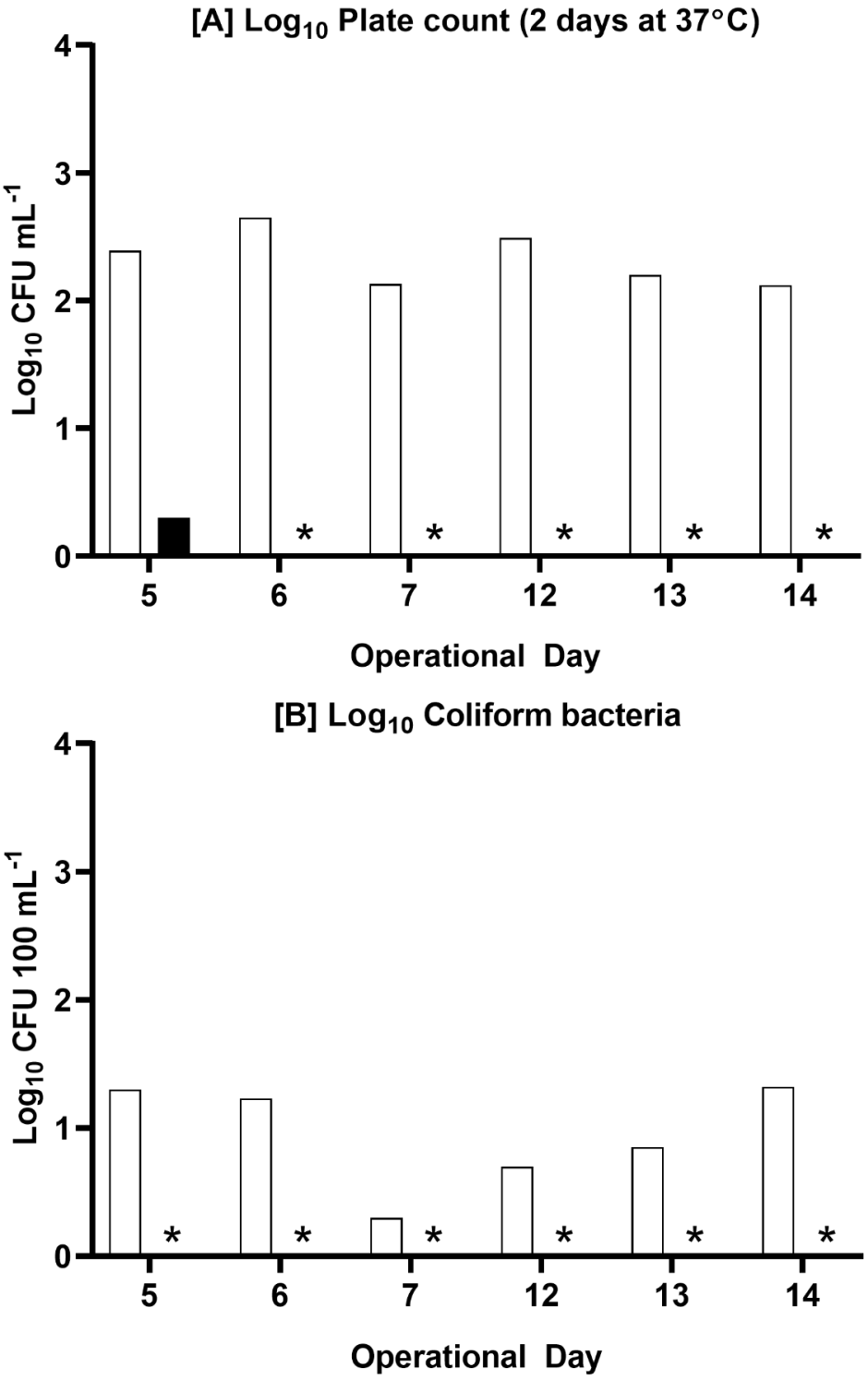

Figure 3.1: [A] Heterotrophic plate count and [B] coliform bacteriological results for water samples taken during Field Trial 1 (1% total ECAS dosing). White bars represent raw water samples. Black bars represent treated water samples. Data taken from independent ISO 17025 accredited laboratory reports (n = 1 per sampling day). * indicates a value of 0.

No significant differences in chemical parameters were observed between the raw and treated water samples, except for a 30% increase in chloride concentration from the raw to the treated water samples (Table 3-3). An increase in chloride is frequently observed in treated waters, compared to raw waters due to the addition of chlorine or chloride-based disinfectants (World Health Organization, 2003a). The dosing of ECAS which is an electrolysed NaCl solution, results in the increase of chloride in treated water samples, it yet remained below the DWI limit of 250 mg L^{-1}. Chloride is indicative of chlorine disinfection, but does not infer the residual disinfection present within treated water.

Monitoring chlorine concentration ensures that a sufficient disinfection is present throughout distribution networks. A residual chlorine concentration of 0.5 and 5 mg L^{-1} should be maintained throughout a distribution network, and at point of consumption, a chlorine concentration of between 0.2 – 0.5 mg L^{-1} (World Health Organization, 2011). Treated water quality was monitored in real-time through an in-line chlorine probe (Figure 2.4) and remained significantly below the DWI limit (Figure 3.2). The frequent spikes observed in the dataset are the result of when the UF membranes go through a backwash cycle, occurring for 30 seconds every 30 minutes.

Table 3-3: Analytical results of chemical parameters of the raw water samples and treated water samples. Field trial 1: 1% total ECAS dosing UF membrane. Results shown are the calculated mean from the independent ISO 17025 accredited laboratory reports (n = 6 ± SD). Significant difference (Sig. diff) calculated through an unpaired, two tailed t-test, with a confidence interval of 95% (*** = p<0.001; ** = p<0.01; * = p<0.05; ns = not significant).

		FIELD TRIAL 1 (1% total ECAS dosing)					
Water type		**Raw water**		**Treated water**			
	UNIT	Mean	SD	Mean	SD	Sig. diff	*DWI Limit*
CHEMICAL ANALYSIS							
Ammonium	mg L^{-1}	0.05	0.04	0.03	0.01	ns	*0.5*
Chloride	mg L^{-1}	96.50	7.66	137.50	5.54	***	*250*
Nitrate	mg L^{-1}	3.50	0.46	3.77	0.42	ns	*50*
Nitrite	mg L^{-1}	0.05	0.01	0.04	0.01	ns	*0.5*
Orthophosphate	mg L^{-1}	0.10	0.02	0.12	0.02	ns	
Silica	mg L^{-1}	0.40	0.20	0.45	0.35	ns	
Sulphate	mg L^{-1}	158.00	40.31	156.83	36.86	ns	*250*

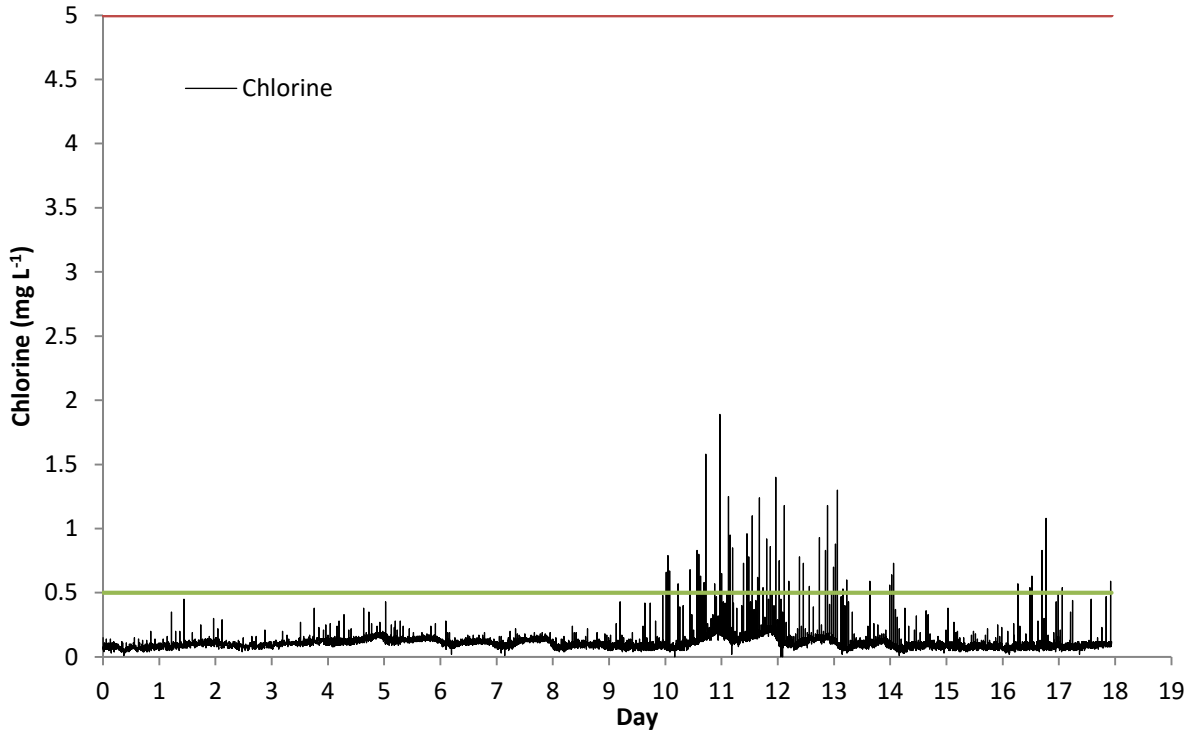

Figure 3.2: Residual chlorine concentration (mg L^{-1} [–]) of treated water samples (as recorded by the in-line DWTS probe) for Field Trial 1 (1% total ECAS dosing). WHO maximum residual chlorine limit for distribution networks (–), and POU systems (–).

Metal water quality parameters, the aluminium (256.67 ± 183.16 µg L^{-1}) and iron (316.67 ± 180.85 µg L^{-1}) concentrations within the raw water samples exceeded what is acceptable for drinking water, 200 µg L^{-1} (World Health Organization, 2011). All treated water samples were within the threshold limits, due to significant reductions in aluminium, iron, lead, manganese and zinc within treated water samples compared to raw water samples (Table 3-4). This is believed to be a result of the multi-step filtration within the DWTS, as significant reductions were observed between both field trials (Table 3-4 and Table 3-8). It should be noted that there was a significant increase in sodium within treated water samples ($p<0.0001$), which is likely a result of chemical relaxation of ECAS. An increase sodium was not seen in metallic analysis in Field Trial 2. This is expected since ECAS (containing NaCl) was directly dosed into the DWTS pipework (World Health Organization, 2003c).

Table 3-4: Analytical results of metallic parameters of the raw water samples and treated water samples. Field trial 1: 1% total ECAS dosing UF membrane. Results shown are the calculated mean from the independent ISO 17025 accredited laboratory reports (n = 6 ± SD). Significant difference (Sig. diff) calculated through an unpaired, two tailed t-test, with a confidence interval of 95% (**** = <0.0001; *** = p<0.001; ** = p<0.01; * = p<0.05; ns = not significant). Red values represent results that fail to meet the DWI standard.

		FIELD TRIAL 1 (1% total ECAS dosing)					
Water type		**Raw water**		**Treated water**			
	UNIT	Mean	SD	Mean	SD	Sig. diff	*DWI Limit*
METAL ANALYSIS							
Aluminium	µg L^{-1}	**256.67**	183.16	16.67	5.16	**	*200*
Cadmium	µg L^{-1}	0.12	0.04	0.10	0.00	ns	*5*
Calcium	mg L^{-1}	103.45	11.07	100.28	14.07	ns	
Copper	mg L^{-1}	0.01	0.00	0.01	0.00	ns	
Iron	µg L^{-1}	**316.67**	180.85	10.00	0.00	**	*200*
Lead	µg L^{-1}	5.38	2.43	0.62	0.26	***	*25*
Magnesium	mg L^{-1}	9.28	1.36	8.95	1.67	ns	
Manganese	µg L^{-1}	21.00	3.85	4.33	1.97	****	*50*
Nickel	µg L^{-1}	2.00	0.00	2.00	0.00	ns	
Potassium	mg L^{-1}	3.90	0.49	3.65	0.57	ns	
Sodium	mg L^{-1}	53.17	4.02	75.83	3.66	****	
Zinc	µg L^{-1}	35.00	13.78	11.67	4.08	**	

During field trials, the health of the UF membranes was determined through continuously monitoring the pre- and post-UF membrane pressure. Membrane health infers good operation functionality, whereby no significant blocking or biofouling had occurred. Recording of pressure enabled the calculation of the filtration flux (Section 2.2.2, Equation 2-1), transmembrane pressure filtration (Section 2.2.2, Equation 2-2), and ultimately the permeability of the membrane (Section 2.2.2, Equation 2-3). When the DWTS was dosed with 0.5% ECAS pre-UF, the UF permeability reduced gradually between operational days 0 to 7 from 94.33 ± 9.03 L m^2h^{-1} bar at 20°C to 52.27 ± 2.24 L m^2h^{-1} bar at 20°C, and then stabilised at 51.81 ± 5.50 L m^2h^{-1} bar at 20°C from operational day 7 onwards (Figure 3.3). This stability indicates that the membranes were still in a healthy condition, whereby no significant blocking or biofouling of the UF membranes had occurred. Biofouling of UF membranes is caused by either particulate matter, or the formation of biofilm blocking the UF pores (Flemming, 2002), consequently increasing the pre-UF membrane pressure. Managing biofilm formation can result in maintained permeability, and ECAS has been demonstrated in managing biofilm formation, through reducing microbial loads (Thorn *et al.*, 2012; Liao, Chen and Xiao, 2007; Thantsha and Cloete, 2006). Similarly to the results obtained for the in-line chlorine probe, the frequent spikes observed in the dataset are the result of when the UF membranes go through a backwash cycle (30 seconds every 30 minutes), which affects the measured pressure differential.

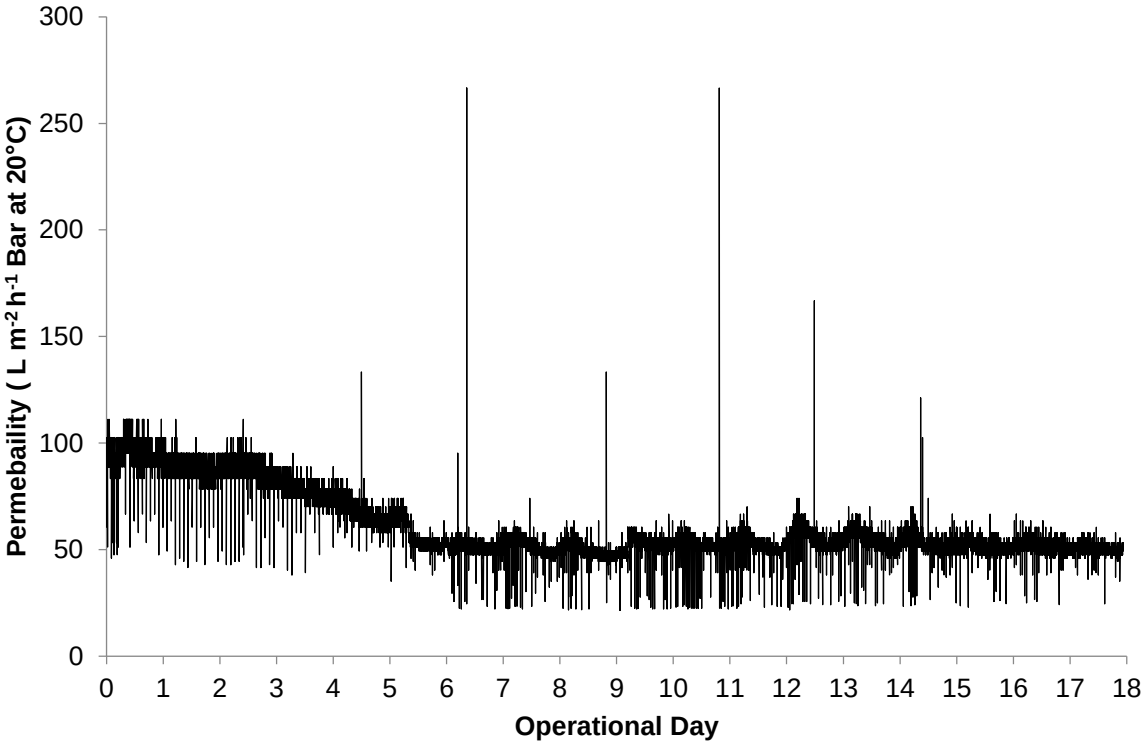

Figure 3.3: UF membrane column permeability within the drinking water production system during Field trial 1 (1% total ECAS dosing).

3.2.2 Field trial 2: 0% ECAS dosing (Control period)

Field trial 2 was run for 18 operational days, whereby ECAS was not dosed pre- and post- UF membranes. Treated and raw water samples were tested for various water quality parameters by an independent ISO 17025 accredited laboratory (Table 3-5 to Table 3-8). All raw water samples taken from the environmental water source during the trial failed to meet DWI standards, in accordance with the results from Field trial 1.

The basic water quality parameters of the treated water samples taken from the DWTS all met the DWI standards, as shown in Table 3-5. Similarly to Field trial 1, the turbidity of treated water samples significantly decreased from 27.33 ± 6.86 FTU to 0.19 ± 0.13 FTU), ($p<0.0001$), a decrease of over 99%. This reduction of turbidity demonstrates that the DWTS was capable of removing particulate matter even in the absence of ECAS.

Table 3-5: Analytical results of basic water parameters of the raw water samples and treated water samples. Field trial 2: Control, 0% ECAS UF membrane. Results shown are the calculated mean from the independent ISO 17025 accredited laboratory reports (n = 6 ± SD). Significant difference (Sig. diff) calculated through an unpaired, two tailed t-test, with a confidence interval of 95% (*** = p<0.001; ** = p<0.01; * = p<0.05; ns = not significant). Red values represent results that fail to meet the DWI standard.

		FIELD TRIAL 2 (Control; 0% ECAS dosing)					
Water type		**Raw Water**		**Treated water**			
	UNIT	Mean	SD	Mean	SD	Sig diff.	*DWI Limit*
BASIC WATER PARAMETERS							
Alkalinity		155.00	31.11	154.00	31.11	ns	
Colour (spectrophotometer)	mg L^{-1} Pt/Co	8.33	1.37	7.67	1.53	ns	
Colour estimated	Deg Hazen	5.00	0.00	5.00	0.00	ns	
Conductivity	µS cm^{-1} @ 20°C	610.33	53.71	613.17	52.53	ns	*2500*
pH		8.33	0.66	8.22	0.69	ns	*6.5 - 10*
Total hardness	Mg Ca L^{-1}	109.00	11.33	102.93	8.23	ns	
Turbidity	FTU	**27.33**	6.86	0.19	0.13	***	*4*

In terms of the microbiological water quality, for Field trial 2 the DWTS treated samples (in the absence of ECAS dosing) failed to meet the DWI standards. The microbial quality of treated water was not achieved in field trial 2 due to the presence of coliforms in the treated water (Table 3-6 and Figure 3.4). A mean of 76.67 (± 73.55) CFU 100 mL^{-1} was observed in treated water samples, which is above the required 0 coliforms result to adhere to DWI standards (Figure 3.4). High numbers of heterotrophic bacteria and non-lactose fermenters (Figure 3.4) were consistently detected within the treated water samples, compared to the raw water (Table 3-6). Conversely, no presumptive *E. coli*, *E. coli*, *Clostridium perfringens* or Enterococci were recovered from the treated water samples.

When analysing the chemical parameters of treated water samples, no significant difference was observed from that determined within the raw water samples (Table 3-7), although all chemical parameters were within the DWI standard limits. Free chlorine concentration of the treated water was below the reliable limit of detection for the probe, (< 0.15 mg L^{-1}), which was expected for Field trial 2 as no ECAS (containing NaCl) was dosed into the DWTS pipework (Figure 3.5).

Table 3-6: Analytical results of biological parameters of the raw water samples and treated water samples. Field trial 2: Control, 0% ECAS UF membrane. Results shown are the calculated mean from the independent ISO 17025 accredited laboratory reports (n = 6 ± SD). Significant difference (Sig. diff) calculated through an unpaired, two tailed t-test, with a confidence interval of 95% (*** = p<0.001; ** = p<0.01; * = p<0.05; ns = not significant). Red values represent results that fail to meet the DWI standard.

		FIELD TRIAL 2 (Control; 0% ECAS dosing)					
Water type		**Raw Water**		**Treated water**			
	UNIT	Mean	SD	Mean	SD	Sig diff.	*DWI Limit*
BIOLOGICAL							
Plate count (2 day @ 37°c)	mL^{-1}	672.60	778.93	457.33	518.80	ns	
Plate count (3 day @ 22°c)	mL^{-1}	12769.40	11209.42	2330.00	596.80	ns	
Non-lactose fermenters	100 mL^{-1}	0.00	0.00	13.67	33.48	***	
Presumptive coliform bacteria	100 mL^{-1}	1913.33	3977.37	86.50	66.51	ns	
Coliform bacteria	100 mL^{-1}	**1913.33**	3977.37	**76.67**	73.55	ns	*0*
Presumptive *E.coli*	100 mL^{-1}	573.33	832.99	0.00	0.00	***	
Escherichia coli	100 mL^{-1}	573.33	832.99	0.00	0.00	***	
Clostridium perfringens	100 mL^{-1}	**115.33**	82.65	0.00	0.00	***	*0*
Enterococci	100 mL^{-1}	**88.67**	88.59	0.00	0.00	***	*0*

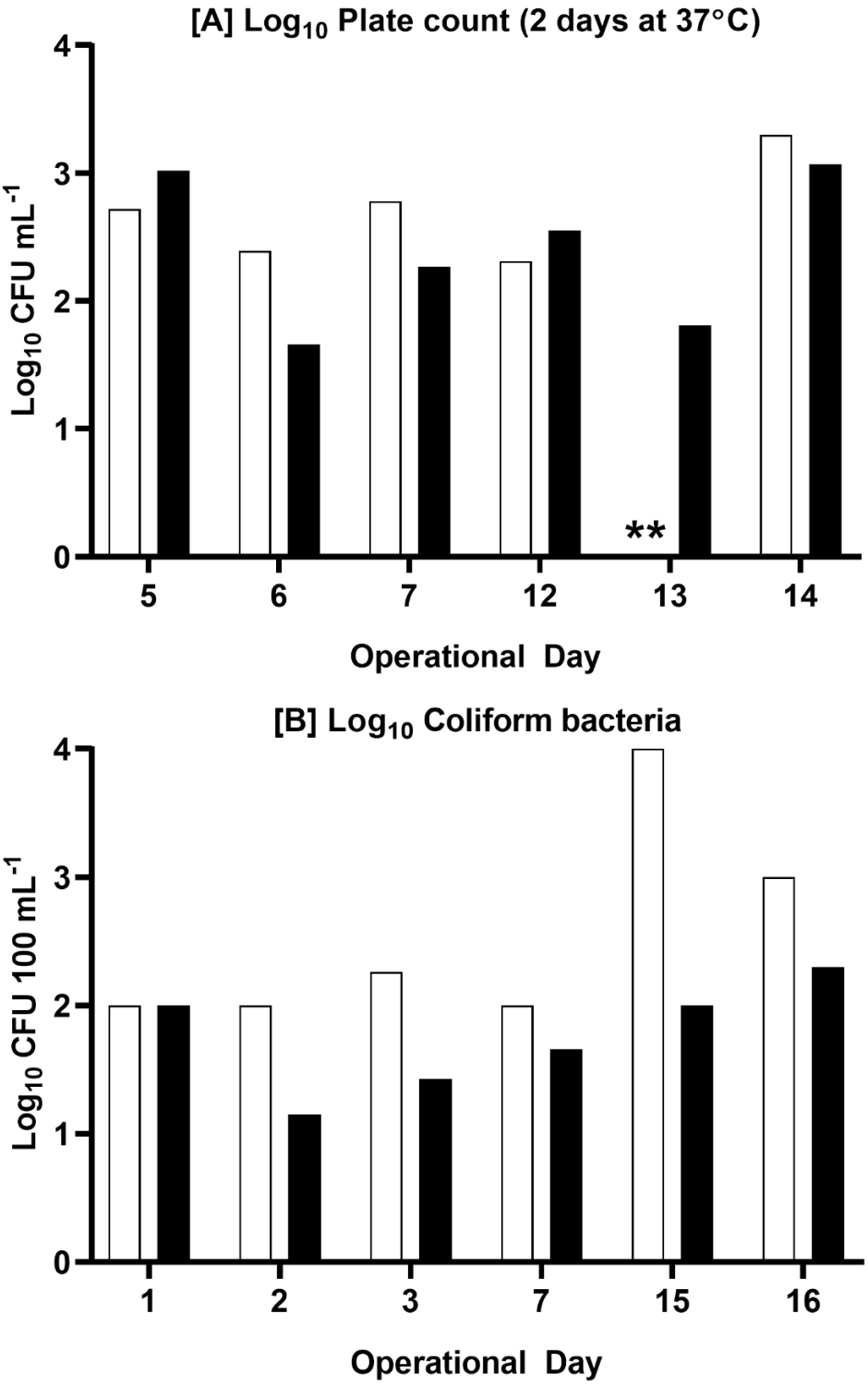

Figure 3.4: Heterotrophic plate count [A] and coliform [B] bacteriological results for water samples taken during Field Trial 2 (0% total ECAS dosing). White bars represent raw water samples. Black bars represent treated water samples. Data taken from independent ISO 17025 accredited laboratory reports (n = 1 per sampling day). ** represents no sample taken.

Table 3-7: Analytical results of chemical parameters of the raw water samples and treated water samples. Field trial 2: Control, 0% ECAS UF membrane. Results shown are the calculated mean from the independent ISO 17025 accredited laboratory reports (n = 6 ± SD). Significant difference (Sig. diff) calculated through an unpaired, two tailed t-test, with a confidence interval of 95% (*** = p<0.001; ** = p<0.01; * = p<0.05; ns = not significant). Red values represent results that fail to meet the DWI standard.

		FIELD TRIAL 2 (Control; 0% ECAS dosing)					
Water type		**Raw Water**		**Treated water**			
	UNIT	Mean	SD	Mean	SD	Sig diff.	*DWI Limit*
CHEMICAL ANALYSIS							
Ammonium	mg L^{-1}	0.27	0.29	0.21	0.16	ns	*0.5*
Chloride	mg L^{-1}	63.17	3.54	62.17	3.54	ns	*250*
Nitrate	mg L^{-1}	1.02	0.55	1.08	0.71	ns	*50*
Nitrite	mg L^{-1}	0.06	33.68	0.38	33.21	ns	*0.5*
Orthophosphate	mg L^{-1}	0.09	0.08	0.12	0.08	ns	
Silica	mg L^{-1}	0.50	0.74	0.41	0.58	ns	
Sulphate	mg L^{-1}	126.00	13.53	129.00	13.00	ns	*250*

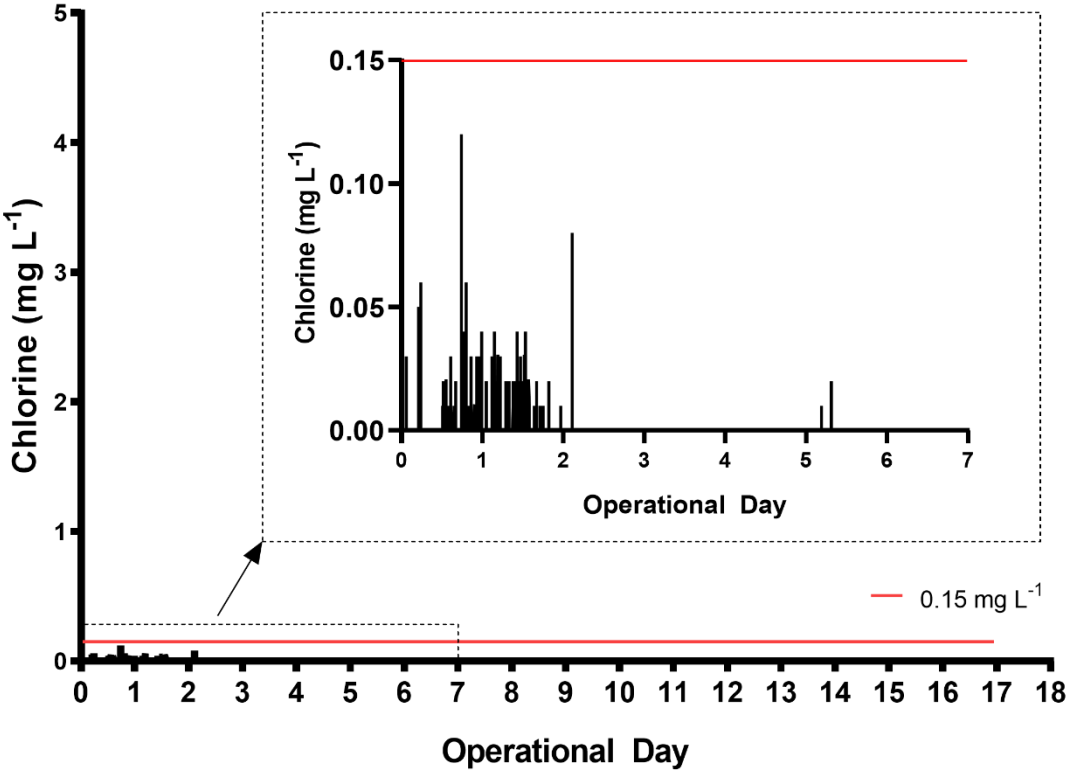

Figure 3.5: Free chlorine concentration (mg L^{-1}) of treated water samples (as recorded by the in-line DWTS probe) for Field Trial 2 (0% total ECAS dosing). Inset figure represents the free chlorine concentrations recorded throughout Field Trial 2 (< 0.15 mg L^{-1}).

The metal water quality parameters of the treated water samples were all within the DWI standard limits, whereby a significant reduction of aluminium ($p < 0.0001$), cadmium ($p = 0.0101$), iron ($p < 0.0001$), lead ($p < 0.0001$) and zinc ($p = 0.0412$) compared to the raw water samples was observed (Table 3-8). This significant reduction was observed within both Field trials (except for cadmium), i.e. in the presence and absence of ECAS dosing. It is likely that this reduction is due to the multi-step filtration process within the DWTS. Average percentage reductions were comparable for aluminium (93.51% and 94.96%), iron (96.84% and 98.18%) and lead (88.54% and 95.72%) between Field Trial 1 and Field Trial 2, respectively The percentage reduction of zinc was greater in Field Trial 1 (65.67%) compared to Field trial 2 (35.19%), despite comparable starting concentrations.

Table 3-8: Analytical results of metallic parameters of the raw water samples and treated water samples. Field Trial 2: Control, 0% ECAS UF membrane. Results shown are the calculated mean from the independent ISO 17025 accredited laboratory reports (n = 6 ± SD). Significant difference (Sig. diff) calculated through an unpaired, two tailed t-test, with a confidence interval of 95% (**** = p<0.0001; *** = p<0.001; ** = p<0.01; * = p<0.05; ns = not significant). Red values represent results that fail to meet the DWI standard.

		FIELD TRIAL 2 (Control; 0% ECAS dosing)					
Water type		**Raw Water**		**Treated water**			
	UNIT	Mean	SD	Mean	SD	Sig diff.	*DWI Limit*
METAL ANALYSIS							
Aluminium	µg L^{-1}	**463.33**	124.85	23.33	5.77	****	*200*
Cadmium	µg L^{-1}	0.10	0.00	0.10	0.00	*	*5*
Calcium	mg L^{-1}	95.50	9.78	92.40	6.22	ns	
Copper	mg L^{-1}	0.01	0.00	0.01	0.01	ns	
Iron	µg L^{-1}	**548.33**	160.18	10.00	0.00	****	*200*
Lead	µg L^{-1}	8.57	2.38	0.37	0.20	****	*25*
Magnesium	mg L^{-1}	8.22	0.91	7.50	0.66	ns	
Manganese	µg L^{-1}	62.67	32.96	25.40	23.89	ns	*50*
Nickel	µg L^{-1}	1.68	0.78	1.70	0.73	ns	
Potassium	mg L^{-1}	3.87	0.38	3.58	0.34	ns	
Sodium	mg L^{-1}	37.50	1.87	36.83	1.33	ns	
Zinc	µg L^{-1}	36.00	15.17	18.00	8.37	*	

The health of the UF membranes was continuously monitored throughout Field Trial 2, through calculating the permeability across the UF membranes (Figure 3.6). The permeability between operational day 0 and 7 did seemingly not stabilise, and fluctuated a lot throughout each day. Between operational day 8 and 15, the data logging system failed to record the pressure values, therefore no data has been plotted. On day 15 when the data logger began recording again, the permeability dropped from an average of 124.95 L m^2h^{-1} bar at 20°C on day 7, to 20.06, 17.59 and 12.17 L m^2h^{-1} bar at 20°C, on days 14, 15 and 16 respectively. This indicates that the UF membranes are not in a healthy state and had begun to block, indicating potential biofouling.

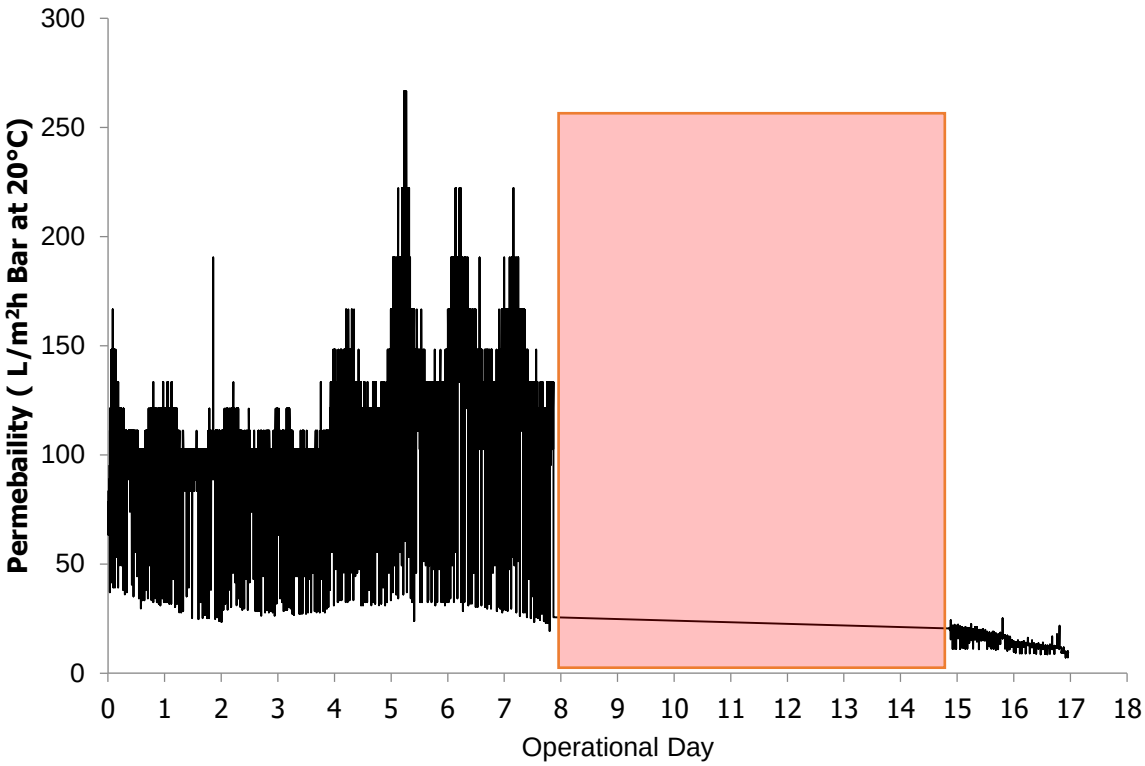

Figure 3.6: UF membrane column permeability within the drinking water production system during Field Trial 2 (0% ECAS dosing). The red rectangle represents data logging failure.

3.3 Discussion

Two DWTS field trials were conducted in either the presence (1% total [v/v]; Field Trial 1) or absence (0% [v/v]; field trial 2) of ECAS dosed directly into the system pipework pre- and post-UF membranes (Figure 2.4). Accredited drinking water analysis was undertaken for all water samples: both raw and treated water. Throughout both field trials, regular samples were taken from the DWTS raw water source, an urban drainage holding pond (on UWE, Bristol's Frenchay Campus). None of these raw water samples met DWI drinking water standards, and as such this water source would not be deemed fit for human consumption. Within both field trials the DWTS produced water that adhered to the DWI limits for basic, chemical and metal water quality parameters. However, only when ECAS dosing was used within Field Trial 1 (1% [v/v] ECAS), did the DWTS produce treated water which met the DWI biological safety standards for potable water. Collectively, this data demonstrates that in the presence of active ECAS dosing, the decentralised DWTS was capable of consistently producing DWI standard drinking water with all basic, biological, chemical and metal parameters within stated threshold limits (this includes: coliform bacteria, *Clostridium perfingens*, enterococci, conductivity, pH, turbidity, ammonium, chloride, nitrate, nitrite, sulphate, aluminium, cadmium, iron, lead and manganese).

The DWTS multi-step filtration process, culminating with ultrafiltration membranes, proved capable of efficiently reducing turbidity between the raw water source and the treated water (even in the absence of ECAS dosing). High turbidity can be associated with organic and bacterial contamination (Lechevallier, Evans and Seidler, 1981), and in remote locations, filtration of raw waters is frequently the sole stage in providing improved water (Mahmood *et al.*, 2011; Ali Baig *et al.*, 2011). It has been

demonstrated that reducing turbidity in waters can be effective in removing organic matter and some bacterial contamination (Lechevallier, Evans and Seidler, 1981; Chowdhury *et al.*, 1997), but the field trial data presented here demonstrates that turbidity alone cannot be used as an indicator of biologically safe drinking water.

The comparative biological (microbiological) results from Field Trial 1 and 2 demonstrate the need for ECAS dosing within the DWTS to produce biologically safe water to DWI standards. The consumption of biologically contaminated drinking water (i.e. coliforms) is the cause of many gastrointestinal illnesses or deaths (Corcoran *et al.*, 2010; World Health Organization, 2016b; Prüss-Üstün *et al.*, 2008). Treated water samples from Field Trial 1 had zero coliforms present (0 CFU 100 mL^{-1}), see Table 3-2 and Figure 3.1. Decentralised DWTSs need to sufficiently reduce microbial loads from input waters (i.e. ground or surface waters) which can potentially be contaminated from faecal matter from humans or animals. The consumption of contaminated waters which have not been efficiently treated can result in dysentery, diarrhoea or typhoid (World Health Organization, 2011; Cabral, 2010). Direct dosing of ECAS into the DWTS pipework (total 1% [v/v]) biologically safe water was produced, therefore the potential hazards associated with key groups of pathogenic bacteria are reduced, when compared to the control field trial (Field trial 2).

Field Trial 1 demonstrated that with the presence of ECAS dosing (0.5% [v/v]) pre-UF membranes resulted in a stable permeability of the UF membranes (Figure 3.3), in comparison to Field Trial 2 (Figure 3.6). The permeability of the UF membranes remained stable throughout Field Trial 1, with 0.5% (v/v) ECAS dosed directly into the DWTS pipework prior to the UF membranes (Figure 2.4). This pre-UF dose

indicates that ECAS could be managing biofilm formation within the UF membranes, or interacting with organic matter (particulate matter), to reduce biofouling (Huang, Schwab and Jacangelo, 2009). Throughout Field Trial 2, where no ECAS dosing occurred (control), greater fluctuations in permeability across the UF membranes was observed, indicating less stability, and potential biofilm formation, speculatively resulting in reduced operational time due to biofouling (Figure 3.6). Manufacturers of UF membranes do not recommend constant dosing of oxidising disinfectants as a pre-treatment due to unwanted changes to surface properties (Inge GmbH, 2015). Dosing NaOCl to a free chlorine concentration of 1 mg L^{-1} has shown shown to effectively reduce the microbial load in UF feed water, as well as maintain UF performance (Yu *et al.*, 2014). However, studies have also demonstrated that membrane permeate flux can be stabilised through natural biological predation within biofilms (Derlon *et al.*, 2012; Peter-Varbanets *et al.*, 2010), inferring a reduction in biofilm formation, and reducing the potential for biofouling using a low ECAS dose (0.5% [v/v]) pre-UF membranes, which requires further investigation. Chapter 6 discusses initial investigations into the comparison of chlorine-based disinfectants at managing biofilm formation within a model biofilm system with *in-situ* dosing.

To ensure water quality within the decentralised DWTS is maintained, a real-time telemetry network (WebMaster data logger) monitors key parameters, including free chlorine and ORP (Figure 2.4). Continuous monitoring ensures that risks associated with biological and physicochemical contamination within the DWTS are minimised. Free chlorine is monitored as the maximum European guideline chlorine concentration at point-of-use, or consumption is between 0.2 and 0.5 mg L^{-1}. Continuously monitoring the ORP of the treated water is to ensure that ECAS generated, and stored, maintains its antimicrobial activity. ECAS characteristics

which contribute to its efficacious nature are a high ORP of ECAS (> + 1130 mV), low pH (< 3.5), low free chlorine concentration (compared to conventional drinking water disinfectants), and rapid kill kinetics (< 10 seconds). Thus, within the DWTS developed, monitoring ORP is vital in ensuring biologically safe drinking water is produced. Along with the real-time telemetry network, regular spot samples should be taken for accredited laboratory analysis for the basic, biological, chemical and metal parameters to confirm compliance to DWI standards.

Field trial 1 demonstrated that the treated water meets DWI drinking water standards. Interest has grown regarding ECAS, or similar solutions, as part of drinking water treatment due to increasing evidence relating to its antimicrobial activity and reduced environmental impact (Petrushanko and Lobyshev, 2001; Huang *et al.*, 2008; Thorn, Robinson and Reynolds, 2013). A potential benefit of using ECAS as part of decentralised DWTS also include the reduced formation of DBPs, such as THMs, due to reduced free chlorine concentration at point of generation, this is investigated and discussed in the next chapter (Chapter 4). Combined with the on-site generation of ECAS generators and its broad spectrum antimicrobial properties, allowing for it be used in a wide variety of applications, the need to transport and store hazardous chemicals is reduced. These properties provide a safe and effective alternative to conventional disinfectants in remote locations. There are also alternative ECAS generation systems which are capable of being powered by photovoltaics (Centrego, 2015).

ECAS is produced from electrochemical generator cells through combined use of NaCl, treated water and electricity (Section1.4.2.2; Figure 1.12), therefore minimal resources are required, which is favourable in remote locations where transporting

goods can be expensive, and potentially hazardous. Moreover, the ECAS generated within the DWTS could be used directly as a general disinfectant, and has been shown to be effective in clinical and healthcare environments (Thorn *et al.*, 2012; Robinson *et al.*, 2010; Selkon, Babbt and Morris, 1999; Kirkpatrick, 2009; Inoue *et al.*, 1997; Tanaka *et al.*, 1996; Tagawa *et al.*, 2000), as well part of food production (Thorn, Pendred and Reynolds, 2017; López-Gálvez *et al.*, 2010; Rahman, Khan and Oh, 2016; Park, Hung and Chung, 2004; Huang *et al.*, 2008; Tomás-Callejas *et al.*, 2011; Gómez-López *et al.*, 2015; Gil *et al.*, 2015; Ovissipour *et al.*, 2015; Han *et al.*, 2017; Veasey and Muriana, 2016; Ayebah *et al.*, 2006; Kim, Hung and Brackett, 2000). A self-contained system, which can produce safe drinking water from contaminated water sources, as well as have a safe and efficacious general disinfectant could be invaluable maintaining sanitation and hygiene conditions, or as part of disaster relief efforts, within refugee camps, or remote communities.

An overview of current decentralised drinking water treatment technologies was provided in Chapter 1 (Table 1-2). Many of the technologies described combine filtration and disinfection stages, utilising conventional chlorination (Chaidez *et al.*, 2016; Derlon *et al.*, 2014, 2013; Wendt *et al.*, 2015; Sartor *et al.*, 2008; Loizidou *et al.*, 2015; Zhu *et al.*, 2014). Many of the decentralised technologies are built within the immediate environment around a community or within a household, i.e. sand filter or solar still. The benefit of the decentralised DWTS described in this chapter is that by being self-contained, where all filters, except the intake filter pump (Figure 2.4) and disinfectant dosing are contained within a unit, allows for the potential to be modular and varied output volume. Such self-contained and modular units are beneficial as contamination or wear on equipment is reduced from external factors, such as weather events. A self-contained DWTS can provide an alternative solution in

providing potable water in disaster relief efforts (Clayton, Thorn and Reynolds, 2019b; Garsadi *et al.*, 2009).

The field trials undertaken as part of the proof-of-concept study highlighted additional research questions in the production of chemically and biologically safe drinking water as part of POU systems. Disinfection by-products (DBPs), specifically chlorinated-DBPs can form as part of chlorination processes when organic matter is present (Rook, 1976), posing hazards to humans as they exhibit mutagenic and carcinogenic properties (Llopis-González *et al.*, 2010; Chowdhury, Rodriguez and Sadiq, 2011). Trihalomethanes (THMs) are an important group of regulated DBPs and their formation throughout distribution networks is well understood (Brown *et al.*, 2010; Toroz and Uyak, 2005; Shehawy and Awad, 2012). THMs are known to be harmful to human health, but little is known about their production within POU systems. Chapter 4 investigates the comparative formation of total THMs in water when treated with three disinfectants (ECAS, NaOCl, and HOCl) for applications in POU decentralised drinking water treatment systems.

This chapter demonstrated the need for ECAS dosing to ensure the production of biologically safe drinking water (Clayton, Thorn and Reynolds, 2019b). The efficacy of ECAS, or similar solutions, has been proven in the context of food production (Thorn, Pendred and Reynolds, 2017; Huang *et al.*, 2008; Rahman, Khan and Oh, 2016; Han *et al.*, 2017) and healthcare settings (Thorn *et al.*, 2012; Kirkpatrick, 2009; Selkon, Babbt and Morris, 1999). However, to the authors' knowledge, no comparative studies between ECAS and conventional chlorination solutions used in drinking water treatment have been published. Therefore, Chapter 5 compares the antimicrobial efficacy of ECAS against NaOCl and HOCl through equivalent free

chlorine concentrations, against model organisms (*Escherichia coli* ATCC 10536 and *Pseudomonas aeruginosa* ATCC 15442).

Further understanding of the antimicrobial effect of disinfectants in representative systems requires the development of dynamic models, which incorporate *in-situ* disinfectant dosing. This chapter demonstrated the need for ECAS to produce biologically safe water, but to also maintain stable permeability across the UF membranes. It was postulated that permeability was maintained through inhibiting, or managing, biofilm formation. Chapter 6 compares the effect of *in-situ* disinfectant dosing in a model biofilm system in managing biofilm formation.

3.4 Conclusions

- Field trial 1 (total 1% [v] ECAS dosing) demonstrated;
    - A decentralised DWTS produced DWI standard drinking water from a heavily biologically contaminated artificial water body meeting all basic, biological, chemical and metallic parameters.
    - UF membrane permeability remained stable throughout Field trial 1, whereas membrane permeability appeared to deteriorate throughout Field trial 2 (control).
- Field trial 2 resulted in;
    - All treated water samples were within DWI limits for basic, chemical and metal water quality parameters. Treated water produced was not biologically safe due to the presence of coliform bacteria.
- The potential modular and scalable capability of the decentralised drinking water treatment system could be beneficial in remote, rural or temporary communities, where population numbers can fluctuate.

Chapter 4. The comparative formation of trihalomethanes using chlorine-based disinfectants within a model system

Data presented in this chapter is adapted from Clayton, Thorn and Reynolds (2019) Frontiers in Environmental Science: Water and Wastewater Management [DOI: 10.3389/fenvs.2019.00035]. (Appendix III: Publications)

4.1 Introduction

Chlorination has been a fundamental treatment stage in the production of biologically safe drinking water for the past 100 years. Disinfection by-products, which include trihalomethanes (THMs), form through the reaction of chlorine with natural organic matter (NOM) over time, and have shown to be hazardous to human health. However, little is known about THM formation in POU systems, and alternative disinfection approaches (e.g. electrochemically activated solutions) require further investigation and development, to minimise trihalomethane formation within such systems. Electrochemically activated solutions (ECAS) are to known to exhibit; fast-acting antimicrobial properties (>5 log reduction within 10 seconds), reduced free chlorine concentration at point of generation, compared to conventional chlorine-based disinfectants (Section 2.1), therefore potentially reducing THM formation within water treatment processes.

This chapter investigates the comparative formation of total THMs in water when treated with three disinfectants (ECAS, NaOCl and HOCl) as a function of contact time and free chlorine, with respect to their potential to produce THMs within POU

drinking water systems. The methods used to generate the results presented throughout this chapter are detailed in Section 2.3.

4.2 Results

4.2.1 HS-SPME calibration curves

Calibration curves for the peak area of individual THM species (Figure 4.1 [A]) and total THMs are shown in Figure 4.1 [B]. Total THMs refer to the sum of the individual THM species ($CHCl_3$, $CHCl_2Br$, $CHClBr_2$ and $CHBr_3$). A summary of regression analysis (r^2 values) and mean retention times for each individual THM species, and total THMs (sum of individual THM species) extracted from the standard mixed solution are shown in Figure 4.1. Due to the volatility of THMs, the extraction efficiency and quantitative analysis of THMs in water via HS-SPME vary with temperature and molecular weight. The regression values (r^2 values) for the standard THM solutions increase in the order: chloroform < BDCM < DBCM < Bromoform (Table 2-3). THM compounds become more stable in the headspace with increasing molecular weight i.e. chloroform = 119.38 g mol^{-1}; bromoform = 252.73 g mol^{-1}, and therefore boiling point (chloroform = 62°C; bromoform = 149°C). This is supported by the retention times of the individual THM species and regression values Table 2-3. The greatest deviations along the regression are associated with the determination of higher concentrations of individual THMs (>60 µg L–1). However, increased linearity for individual and total THM species were observed at lower concentrations between 0, 10, and 60 µg L–1 (regression value of 0.9674 for tTHMs). Only three instances occurred in this study where tTHM concentration values exceeded 60 µg L–1, in the presence of NaOCl at 5 and 10min reaction times (Figure 4.2).

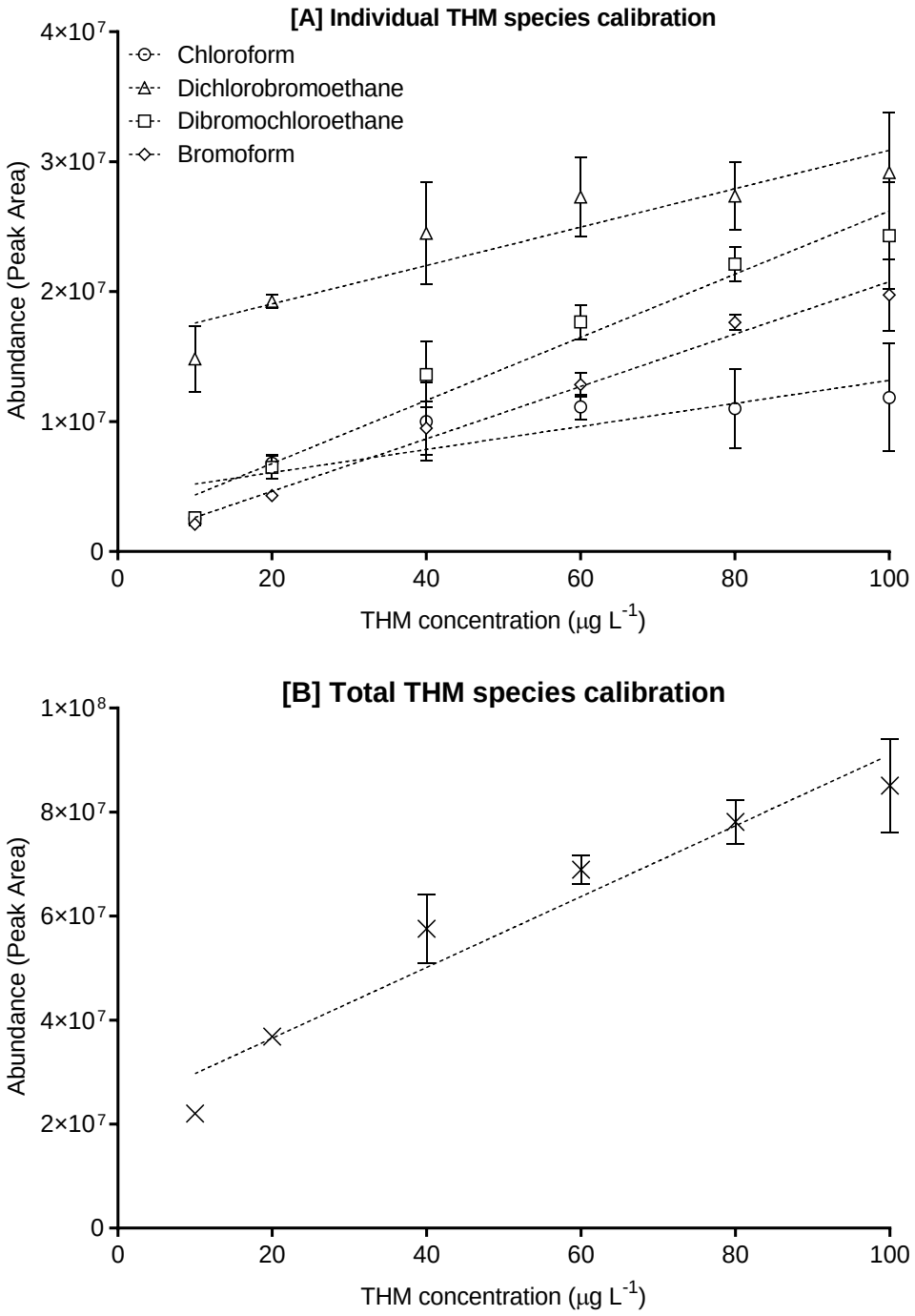

Figure 4.1: Peak area calibration curves for [A] individual THM species (chloroform [○], bromodichloromethane [△], dibroochloromethane [□] and bromoform [◇]), and [B] total THMs (X), whereby total THMs refer to the sum of individual THM species. n = 3 (± SD).

4.2.2 tTHM formation as a function of free chlorine concentration and reaction time

The reaction of the three disinfectants (NaOCl, HOCl and ECAS) with NOM generated tTHMs at a free chlorine concentration of 3 and 5 mg L^{-1} at reaction times of 5 and 10 minutes in all instances (Figure 4.2). At a free chlorine concentration of 3 mg L^{-1} all disinfectants generated tTHMs after a 10 minute reaction time, whilst only NaOCl generated tTHMs at shorter reaction times (1 and 5 minutes). The most abundant THM species was chloroform (> 75% of the total), followed by the brominated THM species [bromodichloromethane, dibromochloromethane, and bromoform] (Figure 4.3). The formation of low concentrations of brominated THM species are a consequence of bromide present with Suwannee River humic acid [NOM] solution, as reported previously (Chowdhury and Champagne, 2008). The high relative abundance of chloroform is in accordance with literature regarding the formation of THMs within drinking water treatment (Ikem, 2010; Cho, Kong and Oh, 2003; Zhang *et al.*, 2015).

At a free chlorine concentration of 1 mg L^{-1} the observed formation of all tTHMs for all disinfectants was low or below the limit of detection (0.86 µg L^{-1}; Figure 4.2). Nevertheless, although starting from a low baseline, the formation of tTHMs reached a maximum concentration (according to the experimental design), at a reaction time of 10 minutes for all disinfectants at 1 mg L^{-1} (Figure 4.2). Whereby tTHM concentrations for NaOCl and HOCl were, 14.23 ± 3.75 µg L^{-1} and 3.88 ± 2.44 µg L^{-1}, respectively (Figure 4.2 [A] and [B]). The tTHM concentrations for ECAS was below the LoD of 0.86 µg L^{-1} after a 10-minute reaction time with a free chlorine concentration of 1 mg L^{-1} (Figure 4.2 [C]).

Increasing the free chlorine concentration (3 and 5 mg L^{-1}) significant differences were observed between reaction times (Table 4-1). Increasing the free chlorine concentration to 3 mg L^{-1} resulted in NaOCl forming tTHMs at all reaction times: 1, 5 and 10 minutes (Figure 4.2 [A]). The maximum tTHM concentration detected was 63.79 ± 24.27 µg L^{-1} after a 5-minute reaction time, and surprisingly, reduced after a 10-minute reaction time to 56.21 ± 17.65 µg L^{-1}. This is contradictory to other published studies which demonstrate an increase in tTHM formation in reaction times in excess of 10 minutes (i.e. hours, days) (Brown *et al.*, 2010; Ghebremichael *et al.*, 2011; Saidan, Rawajfeh and Fayyad, 2013; Werner *et al.*, 2016). For HOCl and ECAS, tTHMs were below the LoD at 1 and 5 minutes whereas tTHMs were formed after a 10-minute reaction time, resulting in concentrations of 16.94 ± 4.1 µg L^{-1} and 5.28 ± 3.82 µg L^{-1}, respectively.

Total THM formation at 5 mg L^{-1} free chlorine resulted in all disinfectants forming tTHMs from 5 minutes onwards (Figure 4.2). In line with tTHM formation at 1 mg L^{-1} and 3 mg L^{-1} free chlorine concentrations, NaOCl produced the highest concentration of tTHMs, where a maximum concentration was again reached at 5 minutes (135.12 ± 34.188 µg L^{-1}), exceeding the permissible level for UK drinking water of 100 µg L^{-1} (Drinking Water Inspectorate, 2012). Between 5 and 10 minutes the tTHM concentration significantly reduced to 71.24 ± 12.69 µg L^{-1} [$p = < 0.0001$]. Maximum tTHM formation for HOCl and ECAS was observed after a 10-minute reaction time: 27.41 ± 11.46 µg L^{-1} and 26.19 ± 23.65 µg L^{-1}, respectively.

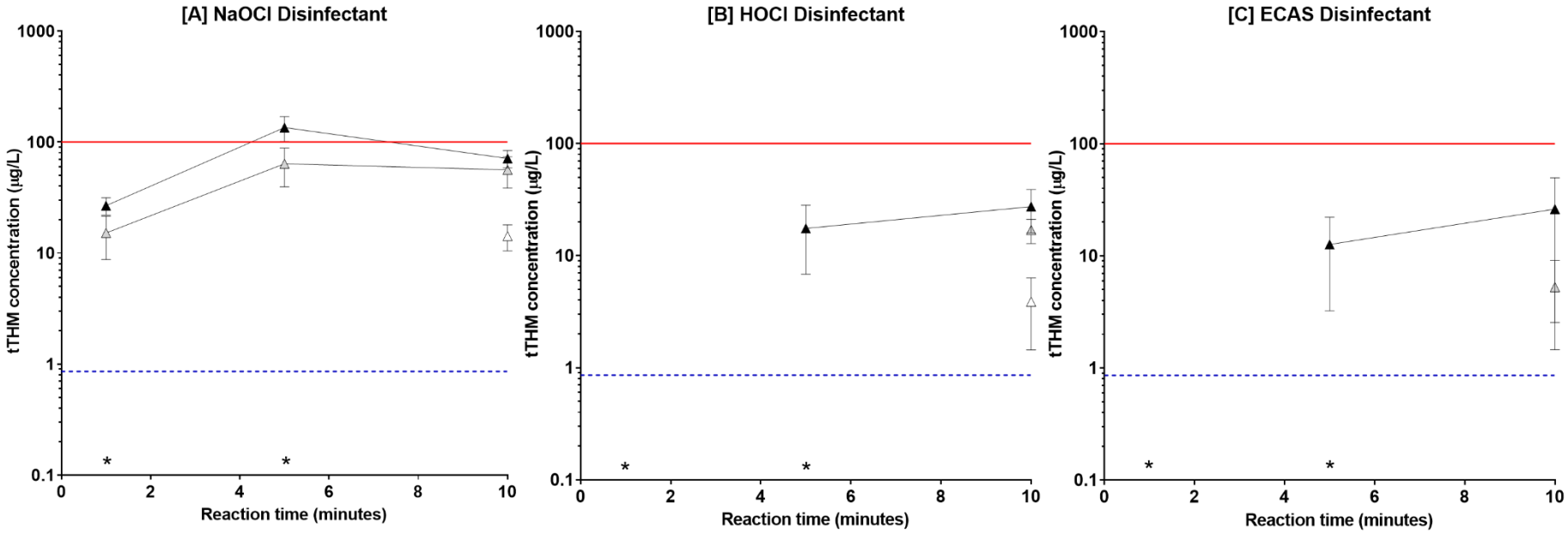

Figure 4.2: Total THM (tTHM) formation when a standard organic load was reacted with disinfectants NaOCl (△) [A], HOCl (☐) [B] or ECAS (○) [C] at free chlorine concentrations between 1 (clear), 3 (grey) and 5 mg L-1 (black). Contact times were 1 minute, 5 minutes and 10 minutes (n = 6 ± SD). tTHM Maximum (red line) refers to the maximum guideline value permissible in drinking water (within the UK), the blue botted line refers to the limit of detection (0.86 µg L^{-1}), tTHMs concentrations below the limit of detection are reported by an asterisk (*)

Chloroform was the most abundant THM species across all free chlorine concentrations and reaction times (Figure 4.3). Increased chloroform composition at 5 mg L^{-1} free chlorine, compared to 1 mg L^{-1} after a 1-minute reaction time for NaOCl (98.604 ± 0.592 % - 98.903 ± 1.009%) and HOCl (86.994 ± 8.532% - 97.595 ± 1.914%). This trend continued for NaOCl and HOCl with increasing reaction time. Chloroform was also the most abundant THM species in the presence of ECAS; yet, lower percentage compositions were observed across all reaction times and free chlorine concentrations, compared to NaOCl and HOCl. However, chloroform composition increased as a result of reaction time (i.e. 1 mg L^{-1} free chlorine at 1-minute: 83.46 ± 3.59%; 5 minutes 88.301 ± 2.208%; and 10 minutes: 89.969 ± 1.402%). Interestingly, in the presence of ECAS chloroform composition decreased between 1 and 3 mg L^{-1}, before increasing at 5 mg L^{-1} (Figure 4.3 [C]). This trend was observed throughout all tested experiments, and could be a result of the overall low concentrations of THMs formed in the presence of ECAS (Figure 4.2).

Reduced formation of tTHMs were observed with HOCl and ECAS disinfectants across all free chlorine concentrations and reaction times (Figure 4.1 [B] and [C]), compared to NaOCl (Figure 4.1 [A]). Statistical analysis reveals no significant differences between tTHMs formed by HOCl and ECAS disinfectants at equivalent free chlorine concentrations and reaction times (Table 4-1). However, NaOCl consistently resulted in significantly higher tTHM formation at all reaction times and equivalent free chlorine concentrations, when compared to HOCl and ECAS ($P < 0.01$).

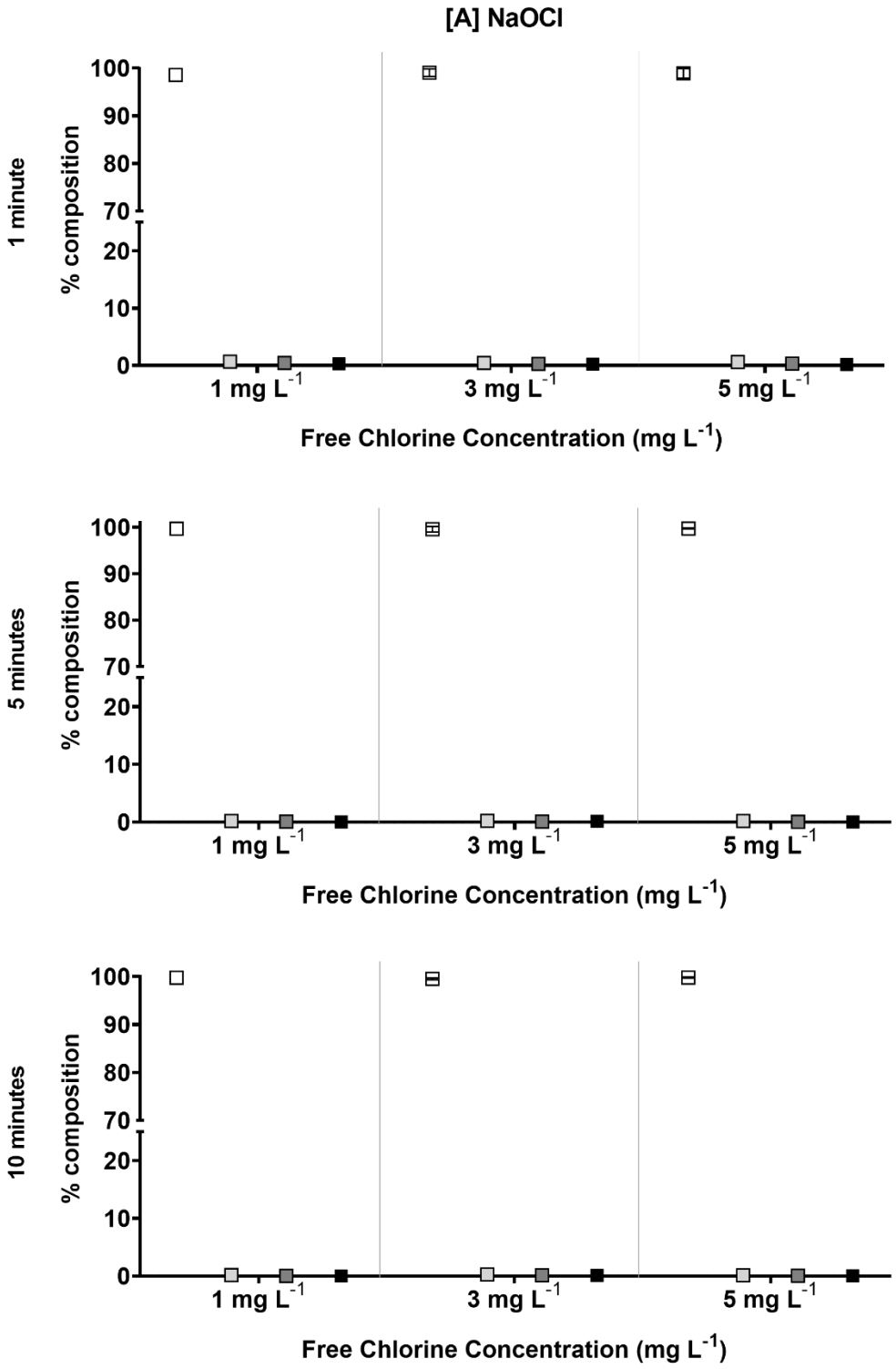

Figure 4.3 [A]: Mean percentage composition of THM species for disinfectants: [A] NaOCl, [B] HOCl or [C] ECAS; as a function of reaction time (rows) and free chlorine; Chloroform (white), bromodichloromethane (light grey), dibromochloromethane (dark grey) and bromoform (black). n = 6 ±SD.

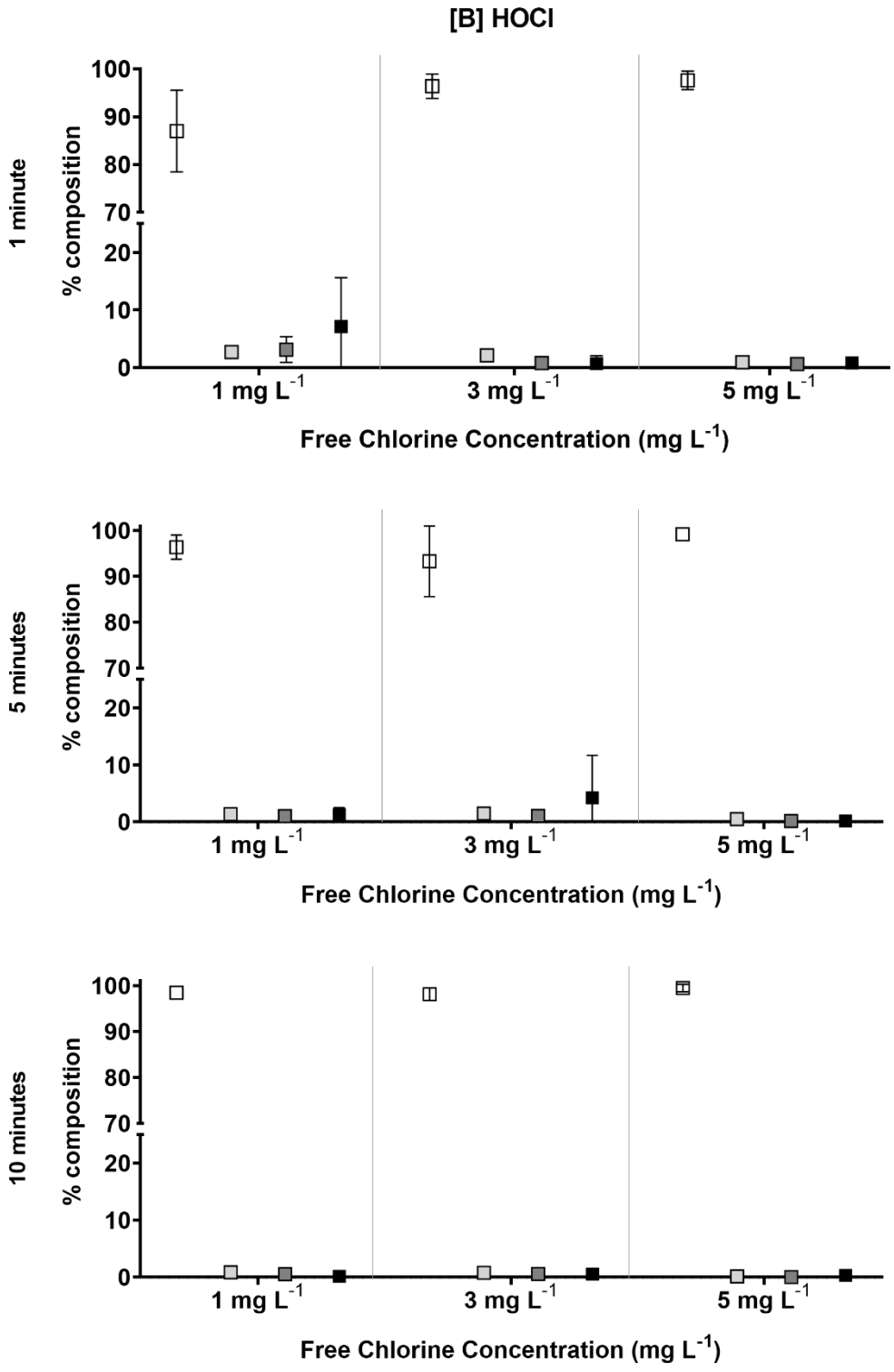

Figure 4.3 [B]: Mean percentage composition of THM species for disinfectants: [A] NaOCl, [B] HOCl or [C] ECAS; as a function of reaction time (rows) and free chlorine; Chloroform (white), bromodichloromethane (light grey), dibromochloromethane (dark grey) and bromoform (black). n = 6 ±SD.

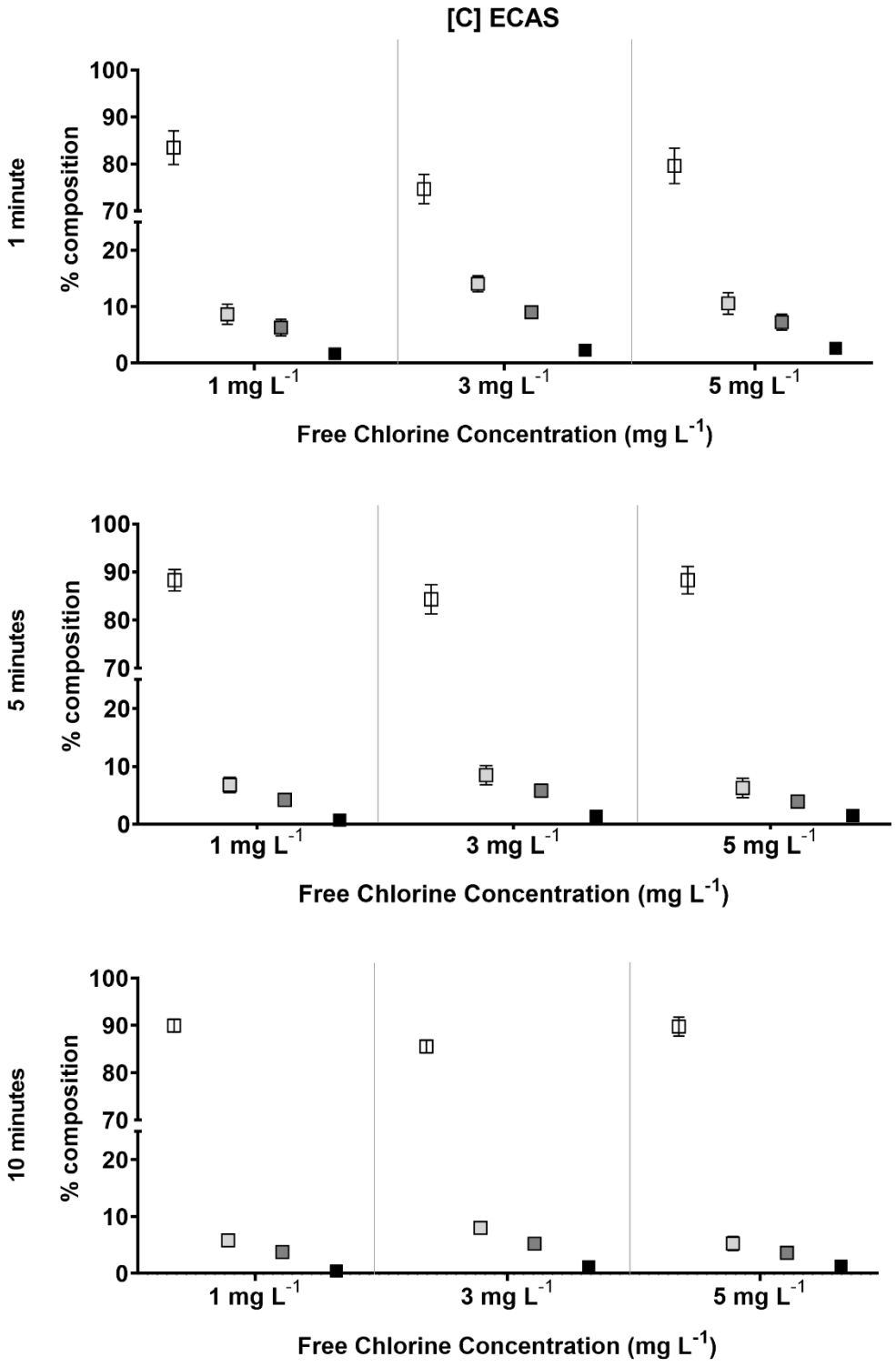

Figure 4.3 [C]: Mean percentage composition of THM species for disinfectants: [A] NaOCl, [B] HOCl or [C] ECAS; as a function of reaction time (rows) and free chlorine; Chloroform (white), bromodichloromethane (light grey), dibromochloromethane (dark grey) and bromoform (black). n = 6 ±SD.

Table 4-1: Analysis of total THM formation between reaction time (minutes) and disinfectant type (NaOCl, HOCl and ECAS), for each free chlorine concentration (mg L^{-1}) Significant difference calculated through a two-way ANOVA, with a confidence interval of 95% (*** = p < 0.001; ** = p < 0.01; * = p < 0.05; ns = not significant).

Free chorine concentration	**1 mg L^{-1}**			**3 mg L^{-1}**			**5 mg L^{-1}**		
Reaction time (minutes) / **Disinfectants**	1	5	10	1	5	10	1	5	10
ECAS vs NaOCl	**	***	***	***	***	***	**	***	***
ECAS vs HOCl	ns	ns	ns	ns	ns	ns	ns	ns	ns
HOCl vs NaOCl	***	***	***	***	***	***	**	***	***

4.2.3 Changes in pH over a 10 minute reaction time

Free chlorine species present in chlorine solutions are pH dependant (Sivey, McCullough and Roberts, 2010; Stoner *et al.*, 1982):

$$HOCl \leftrightarrow OCl^{-} + H + pKa = 7.5$$

Equation 4-1 (Heeb *et al.*, 2014; Liu and Margerum, 2001)

THMs have shown to have a greater affinity to form at higher pH, with increased free chlorine concentrations (Brown *et al.*, 2010; Peters, Young and Perry, 1980; Rasheed *et al.*, 2017; Saidan, Rawajfeh and Fayyad, 2013; Urano, Wada and Takemasa, 1983). HOCl is the dominant compound which results in tTHM formation, although, since tTHM formation is base-catalysed (Yee *et al.*, 2009), there is a trade-off in terms of pH effect. Therefore, to further understand variation in tTHM formation between each of the disinfectants tested, changes in physicochemical parameters (pH, ORP,

free and total chlorine) within the reaction vessel were measured over the full 10 minute reaction time, for each of the disinfectants (Section 2.3.2).

The pH of NaOCl (11.4 ± 0.1) remained greater than HOCl (5.6 ± 0.25) and ECAS (3.3 ±0.16) over the 10-minute reaction time (Figure 4.4). All disinfectant reaction vials showed a decrease in pH over the 10-minute contact time, when reacting with 4 mg L^{-1} humic acid (Figure 4.4). However, no significant reduction was observed at a starting free chlorine concentration of 1 mg L^{-1}. At a starting free chlorine concentration of 3 mg L^{-1}, the pH of NaOCl was significantly greater than HOCl ($p = 0.0132$) and ECAS ($p = 0.013$). Whereby the pH of HOCl was also significantly greater than ECAS ($p = 0.0236$). This trend continues when the starting free chlorine concentration was increased to 5 mg L^{-1}, whereby the pH of NaOCl is significantly greater than HOCl ($p = 0.0002$) and ECAS ($p < 0.0001$), and the pH of HOCl was significantly greater than ECAS ($p = 0.0005$).

It is interesting to note, that for ECAS at the highest starting free chlorine (5 mg L^{-1}) concentration the starting pH was lower, than at the lowest free chlorine concentration tested (1 mg L^{-1}). The opposite trend was observed in NaOCl and HOCl, whereby a higher starting free chlorine concentration resulted in an increased in pH. This phenomenon, was also observed when calibrating the ECAS generator (Chapter 2, Figure 2.2 [B]).

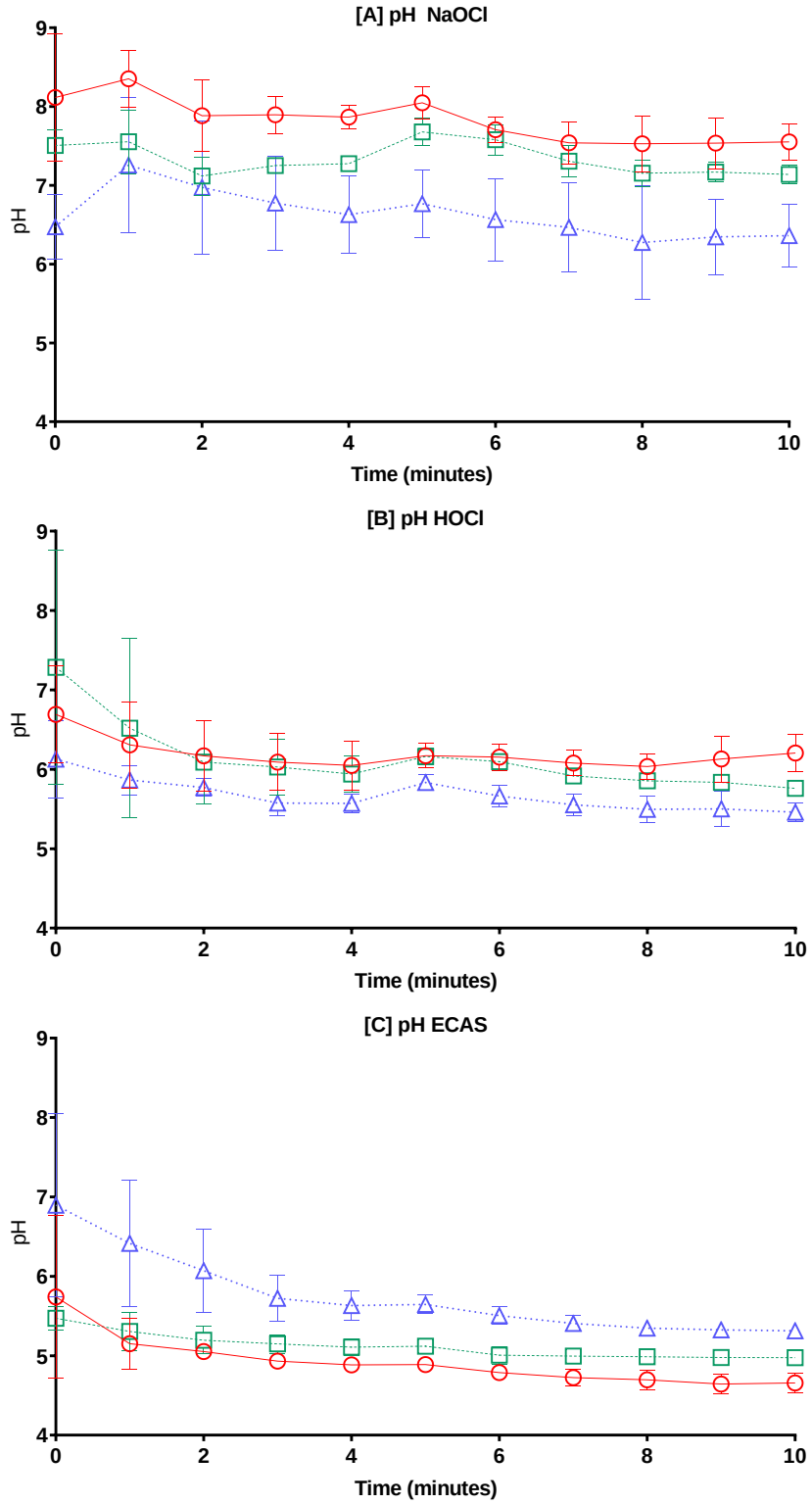

Figure 4.4: The change in pH over a 10-minute reaction time for three disinfectants when reacted with an aqueous standard organic load (4 mg L^{-1} SRHA). 1 mg L^{-1} (△); 3 mg L^{-1} (□) [B] and 5 mg L^{-1} (○). n = 3 (± SD).

4.2.4 Changes in free and total chlorine over a 10 minute reaction time

All reactions showed a reduction in total and free chlorine concentrations over the 10-minute reaction time (Figure 4.5). A starting free chlorine concentration of 5 mg L^{-1} resulted in the greatest comparative reduction throughout the 10 minute reaction, compared to the lower starting free chlorine starting concentrations (1 and 3 mg L^{-1}). ECAS treatment resulted in the greatest free chlorine decrease after 10 minutes of 34.68%, whereas NaOCl had the smallest decrease in free chlorine by 19.34%. The total chlorine concentration of the disinfectants were also measured, and demonstrated the same reducing trend as free chlorine, across all reaction times and starting free chlorine concentrations.

Free and total chlorine remained in excess after 10 minutes for all experiments conducted, which is consistent with previous studies (Brown *et al.*, 2010). Therefore, further investigations should investigate whether the remaining excess free chlorine present after ECAS or HOCl reacting with NOM results in increased THMs at significantly extended contact times (i.e. > 10 minutes).

Collectively, all physicochemical data is in-line with previous research studies, whereby a higher affinity has been observed between higher free chlorine concentrations, higher pH and increased THM formation (Stevens *et al.*, 1976; Kim *et al.*, 2002; Liang and Singer, 2003; Hua and Reckhow, 2007; Chowdhury and Champagne, 2008; David, 2014).

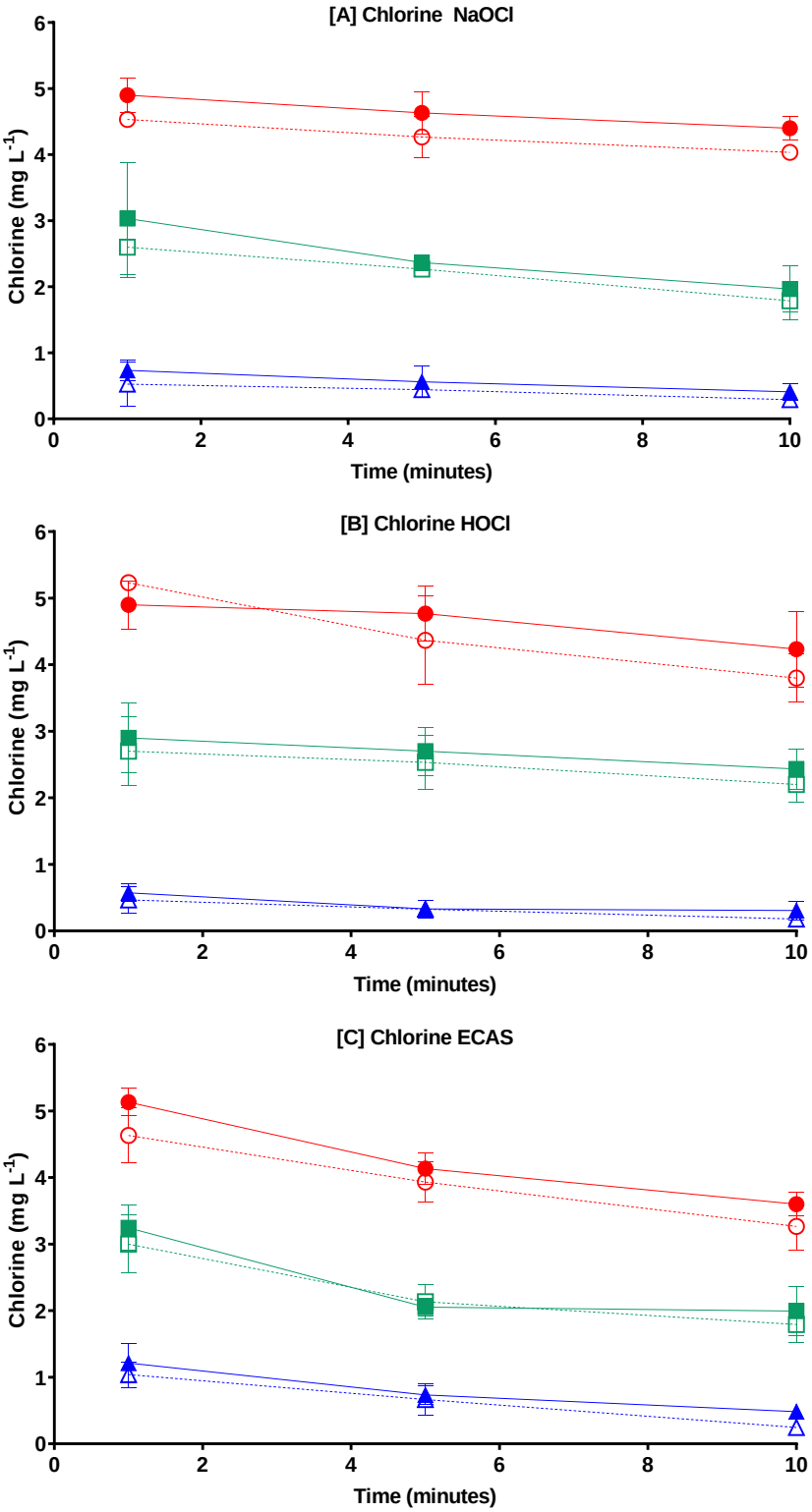

Figure 4.5:The change in free (open shapes/dashed line) and total (closed shapes/ solid line) chlorine concentrations over a 10 minute reaction time for three disinfectants when reacted with an aqueous standard organic load (4 mg L^{-1} SRHA). 1 mg L^{-1} (△); 3 mg L^{-1} (□) [B] and 5 mg L^{-1} (○). n = 3 (± SD).

4.2.5 Changes in ORP over a 10 minute reaction time

The oxidation reduction potential [ORP] of the three disinfectants tested was measured over the 10 minute reaction time (Figure 4.6). This parameter was measured as a high ORP is a key antimicrobial characteristic of ECAS (Liao, Chen and Xiao, 2007). The ORP of all disinfectants increased gradually over the 10-minute reaction time (i.e. NaOCl [5 mg L^{-1}] 25.5%; HOCl [5 mg L^{-1}] 5.7%; and ECAS [5 mg L^{-1}] 11.3%), or had no significant change (1 mg L^{-1}; $p = 0.5367$).

The ORP of NaOCl consistently remained below +800 mV for all free chlorine concentrations (Figure 4.6 [A]), and studies have shown that electrolysed solutions with an ORP > +800 mV can effectively inactivate bacteria (Liao, Chen and Xiao, 2007; Kimbrough *et al.*, 2006). By contrast, the ORP for ECAS was above the functional bacterial ORP threshold at a starting free chlorine concentration of 5 mg L^{-1} free chlorine, across the 10-minute reaction time. At the highest free chlorine concentration tested (5 mg L^{-1}) the ORP of ECAS was significantly greater than HOCl ($p = 0.0006$) and NaOCl ($p = 0.0001$), whilst the ORP of HOCl was significantly greater than NaOCl ($p = 0.0105$).

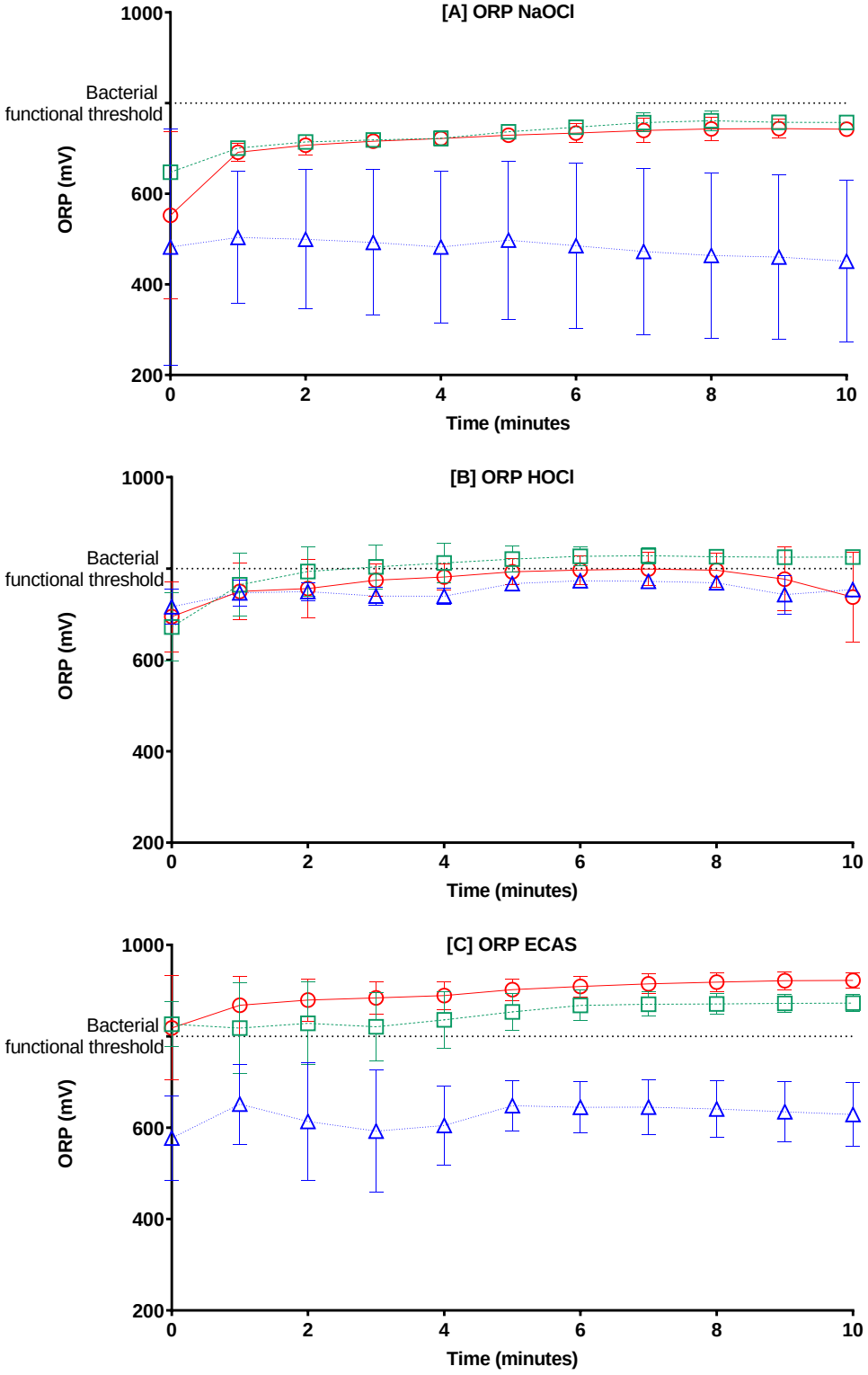

Figure 4.6: The change in ORP over a 10-minute reaction time for three disinfectants when reacted with an aqueous standard organic load (4 mg L^{-1} SRHA). 1 mg L^{-1} (blue dotted line △); 3 mg L^{-1} (green dashed line □) [B] and 5 mg L^{-1} (red solid line ○). Black dotted line (+800 mV) refers to the minimum ORP to inhibit bacterial functionality. n = 3 (± SD).

4.3 Discussion

This study demonstrated that all disinfectants resulted in the formation of THMs under the experimental conditions. Reactions between NaOCl and NOM resulted in significantly increased tTHM formation compared to HOCl and ECAS. The increased formation of tTHMs in the presence of NaOCl is likely a result of the higher pH of the disinfectant (11.4 ± 0.1) compared to HOCl (5.6 ± 0.25) or ECAS (3.3 ± 0.16). THMs have shown to have a greater affinity at alkaline conditions (Brown *et al.*, 2010; Peters, Young and Perry, 1980; Rasheed *et al.*, 2017; Saidan, Rawajfeh and Fayyad, 2013; Urano, Wada and Takemasa, 1983).

The maximum observed tTHM concentrations occurred in the presence of NaOCl; however, a decline in tTHMs between 5 and 10-minute reaction times was observed at 3 and 5 mg L^{-1}. This is contradictory to other published studies which demonstrate an increase in tTHM formation in reaction times in excess of 10 minutes (i.e. hours, days) (Werner *et al.*, 2016; Saidan, Rawajfeh and Fayyad, 2013; Ghebremichael *et al.*, 2011; Brown *et al.*, 2010; Ramavandi *et al.*, 2015). This decline is potentially due to hydrolysis (Mabey and Mill, 1978; Rahman, 2015), or dehalogenation (Abusallout, Rahman and Hua, 2017; Hua and Reckhow, 2012; Rahman, 2015) of already formed tTHMs present in solution. The extent of which the hydrolysis reaction can occur over such a short contact time (i.e. 10 minutes) is unknown, however, the percentage composition of chloroform increased with reaction time, whilst brominated species decline (Figure 4.3). Bromine-carbon bonds are more tolerant to dissociation, compared to chlorine, as a result of lower dissociation energies (Abusallout, Rahman and Hua, 2017). Dehalogenation is affected by pH, whereby, more alkaline condition increase the rate of dehalogenation (Rahman, 2015). The tTHM decline was not observed with either HOCl or ECAS, potentially as a result of the neutral/acidic

disinfectant properties, reducing the overall pH of the reaction (Figure 4.4). Combined with the low concentrations of tTHMs formed and the rapid reaction kinetics of such disinfectants (Robinson *et al.*, 2011; Liao, Chen and Xiao, 2007). This study investigated THM formation over a short reaction time (<10 minutes), therefore investigating tTHM formation over longer reaction times (i.e. tens of minutes, hours, days), requires further investigations. This would provide answers as to how the chemical quality of drinking water changes if dosed with ECAS or HOCl, and subsequently stored as part of a decentralised POU DWTS. This study was primarily concerned with quantifying the formation of tTHMs, therefore any THM derivatives or other DBPs formed as part of the experimental design were not identified. Further studies that can identify other DBPs formed as part of this reaction are required to be carried out.

4.3.1 The effect of disinfectant physicochemical properties on tTHM formation

The physiochemical parameters influence the formation potential of the three disinfectants used in this study. A positive correlation was observed between higher free chlorine concentrations, higher pH and increased THM formation, in accordance with previous studies (Chowdhury and Champagne, 2008; David, 2014; Stevens *et al.*, 1976; Kim *et al.*, 2002; Liang and Singer, 2003; Hua and Reckhow, 2007). Whereby NaOCl which had the highest pH leading to the highest formation of tTHMs, as has been observed previously (Brown *et al.*, 2010; Rasheed *et al.*, 2017; Saidan, Rawajfeh and Fayyad, 2013). An interesting note is that the concentration of free and total chlorine remained in excess after 10 minutes for all experiments conducted. Therefore, further studies should investigate whether the excess of free and total chlorine in HOCl and ECAS disinfection results in further formation of THMs at extended reaction times (i.e. > 10 minutes).

This study demonstrated a reduced formation of THMs by HOCl and ECAS over a 10-minute reaction time, compared to NaOCl. These results correspond with the limited number of other studies which have investigated THM formation in the presence of a neutral ECAS, when reacted with NOM (Ghebremichael *et al.*, 2011), or within food processing applications (Gómez-López *et al.*, 2013). An important difference between HOCl and ECAS is the biochemistry dependent antimicrobial mode of action, whereby HOCl, and NaOCl, rely upon free chlorine for effective disinfection (Fair *et al.*, 1948; National Academy of Sciences, 1980; Clasen and Edmondson, 2006). Conversely, ECAS relies upon numerous transient reactive oxidative chemical species, including OH^-, O_3, H_2O_2 and O_2^- (Jeong, Kim and Yoon, 2006; Martínez-Huitle *et al.*, 2008), resulting in a high ORP (> +1130 mV (Suslow, 2004)), in addition to free chlorine. Disinfecting solutions which have a high ORP have been shown to cause rupture of microbial inner and outer membranes, impacting on microbial functionality, such as energy generation (Liao, Chen and Xiao, 2007). To achieve comparable disinfection, lower free chlorine concentrations of ECAS are needed, in contrast to conventional chlorine solutions, such as NaOCl and HOCl, reducing the formation potential of THMs (Di Cristo, Esposito and Leopardi, 2013).

4.3.2 Alternative analytical methods to measure tTHM formation

In recent years, several methods of gas chromatography (GC) have been trialled for reliable and robust quantification of THMs. Such methods include GC mass spectrometry [GC/MS] (Xue *et al.*, 2014; Rasheed *et al.*, 2017) and GC with electron capture detector [GC-ECD] (Werner *et al.*, 2016; Kim *et al.*, 2002; Chowdhury and Champagne, 2008; Saidan, Rawajfeh and Fayyad, 2013). These techniques can have various sample preparation techniques, such as: liquid-liquid extraction [LLE] (Rasheed *et al.*, 2017), purge and trap [PAT] (Kim *et al.*, 2002; Xue *et al.*, 2014), direct aqueous injection [DAI], head space [HS] (Saidan, Rawajfeh and Fayyad, 2013) or

headspace solid-phase micro extraction [HS SPME] (Guilherme and Rodriguez, 2015). However, these techniques do not allow for continuous analysis of samples. Formation and decay dynamics of DBPs, including THMs, is consequently based on discrete sampling at specified time points.

It would be beneficial to dynamically investigate the formation and decay of DBPs and THMs, allowing peak formation to be determined, as well as identify potential decay. Dynamically monitoring formation and decay could also provide an insight to derivative formation, such as identifying compounds forming as a result of hydrolysis or dehalogenation. Selected ion flow tube mass spectrometry (SIFT-MS) can detect and analyse volatile organic compounds (VOCs) in real-time (Smith and Španěl, 2005). This method of analysis removes the need for sample preparation, whereby headspace is sampled directly from laboratory or environmental samples or environments. Direct sampling can potentially provide a quantitative real-time analysis of VOCs throughout the disinfection process of a water treatment system. This would be interesting to investigate in future studies.

4.3.3 The effectiveness of ECAS compared to conventional chlorine disinfectants for use in point-of-use treatment systems

Many studies focus on tTHM formation dynamics as part of centralised water treatment systems, which feed into extensive distribution networks (Saidan, Rawajfeh and Fayyad, 2015; Rodriguez and Sérodes, 2001; Toroz and Uyak, 2005; Brown *et al.*, 2010; Shehawy and Awad, 2012). Decentralised POU drinking water treatment systems require rapid, broad spectrum antimicrobials to provide biologically and chemically safe drinking water. Previous studies have demonstrated

that ECAS can disinfect in less than 10 seconds (Liao, Chen and Xiao, 2007; Robinson *et al.*, 2011).

To ensure sufficient disinfection in centralised drinking water treatment, and distribution systems (i.e. sodium hypochlorite, calcium hypochlorite and chlorine gas), as well as maintain water quality throughout the distribution network, residual chlorine concentration of 0.5 mg L^{-1} is required after a contact time of 30 minutes (World Health Organization, 2011). Residual free chlorine within distribution networks can react with NOM, or other organic material (i.e. biofilms), resulting in the formation of THMs, or other DBPs. Limiting the contact time, reducing the pH of the disinfectants used (HOCl or ECAS), and reducing the organic load (i.e. humic acid or biofilm) within bulk water can help reduce the formation of (Amy, Chadik and Chowdhury, 1987; Brown, Bridgeman and West, 2011b; Di Cristo, Esposito and Leopardi, 2013). However, this study has demonstrated that ECAS treatment of water could lead to reduced formation of THMs, and other DBPs, compared to conventional chlorination (NaOCl). Further investigations are required to determine whether this observation occurs in scaled-up POU water treatment systems (i.e. Figure 2.4), as well as the role of pH, specifically within the treated water holding tank, in comparison to traditional clearwells.

Conventional drinking water disinfectants, i.e. NaOCl, are required to be transported and safely stored to minimise potential hazards to people and the environment. If stored incorrectly (i.e. direct sunlight or inadequate seal closure) disinfectants can have short 'shelf-lives', causing the antimicrobial activity to deteriorate over time (Clarkson, Moule and Podlich, 2001). ECAS, however, can be generated on-site and *in-situ*, requiring only salt, water and energy to produce the disinfectant (Clayton,

Thorn and Reynolds, 2019b; Thorn *et al.*, 2012; Kim, Hung and Brackett, 2000). The bespoke ECAS generator (60 L hr^{-1}) used in this study has an operating current range of between 4 and 30 amps, therefore the power requirement ranges between 0.345 kW (4 amps) and 0.69 kW (30 amps). Alternative ECAS generators are available which have lower power requirements, and can generate ECAS utilizing solar power (Centrego, 2015; Witt and Reiff, 1993). Such alternatives provide practical solutions for remote locations, or as part of disaster relief efforts.

On-site disinfectant generation is advantageous in remote and rural locations where purchasing, transporting and storing disinfectants is expensive and unrealistic. Such locations can have limited, or no, access to improved drinking water, therefore, safe disinfectants with a short environmental legacy could be beneficial in small-scale point-of-use decentralised drinking water treatment systems. The system discussed in Chapter 3 established that DWI standard drinking water can be produced with a low dose of ECAS pre- and post-ultrafiltration membrane (Clayton, Thorn and Reynolds, 2019b). If ECAS generators are able to be efficiently installed within a self-contained decentralised drinking water treatment system, then these systems could be deployed in remote areas or communities, or as part of disaster relief efforts. In such locations or applications, access to centralised drinking water distribution networks can be difficult, or non-existent. Such locations and applications require reliable, robust and straightforward treatment systems that are capable of producing chemically and biologically safe drinking water.

Source waters that feed into decentralised treatment systems in remote locations can be heavily contaminated with faeces. Bacteria associated with faecal matter, i.e. coliforms, can result in diarrhoeal diseases result in dehydration, malnourishment

and can be fatal if untreated, especially vulnerable groups such as the elderly and young children (Cabral, 2010). In the UK, the DWI states that zero coliform are permissible in drinking water, due to associated health implications. ECAS exhibits significant antimicrobial activity against a range of pathogenic microorganisms including *E. coli* (Robinson *et al.*, 2011), with proven efficacy against *E. coli* O157:H7 [95% reduction <10 seconds] (Liao, Chen and Xiao, 2007), which can cause haemolytic uraemic syndrome (HUS). Young children and the elderly are most at risk of HUS, which can be fatal (The Environment Agency, 2002). Therefore, the combination of ECAS properties; low pH, high ORP, low comparative free chlorine concentrations and broad spectrum antimicrobial activity, demonstrate that ECAS could be a viable alternative for use within decentralised drinking water treatment systems. Both in producing chemically and biologically safe drinking water, including the potential for reduced THM formation.

4.4 Conclusions

- NaOCl disinfectant produced significantly greater THMs ($p < 0.01$) compared to HOCl and ECAS at all reaction times and free chlorine concentrations, this is due to the higher pH.
- Comparable THM concentrations were formed by HOCl and ECAS ($p > 0.05$) disinfectants, under experimental conditions tested.
- HOCl and ECAS should be considered for point-of-use drinking water treatment systems.

Chapter 5. The comparative antimicrobial efficacy of chlorine-based disinfectants for use in point-of-use drinking water applications

5.1 Introduction

Chlorine disinfection has been integral to the production of biologically safe drinking water since the beginning of the 20th Century (McGuire, 2016). Chlorine has been used due to its effective biocidal properties, low cost and wide availability (Farghaly *et al.*, 2013; Kumari and Gupta, 2015; Rodriguez and Sérodes, 2001), see Section 1.4.2. Electrochemically activated solutions (ECAS) have potential advantages over conventional chlorine-based disinfectants in decentralised POU drinking water treatment systems, as they can be produced on-site only requiring salt, water and energy (Robinson, Thorn and Reynolds, 2013; Robinson *et al.*, 2010; Thorn, Robinson and Reynolds, 2013).

The aim of this chapter is to compare the antimicrobial activity of ECAS against the commonly used chlorine-based drinking water disinfectants sodium hypochlorite (NaOCl) and hypochlorous acid (HOCl). The antimicrobial activity of NaOCl, HOCl and ECAS was assessed against standard microbiological challenges in planktonic phase (*Escherichia coli* ATCC 10536), and as biofilms (*Pseudomonas aeruginosa* ATCC 15422). The methods used to generate data presented in this chapter are detailed in Section 2.1, Section 2.4, Section 2.5 and Section 2.6.

5.2 Results

5.2.1 Minimum inhibitory and minimum bactericidal concentration assays

The minimum inhibitory concentration [MIC] and minimum bactericidal concentration [MBC] for three chlorine-based disinfectants was determined for *E. coli* ATCC 10536 and *P. aeruginosa* ATCC 15422. The lowest MIC was for ECAS (23.5 mg L^{-1} free chlorine), followed by HOCl (37.0 mg L^{-1}) and NaOCl (55.0 mg L^{-1}), see Table 5-1. The MBC for NaOCl or HOCl were equal to the MIC; 55.0 and 37.0 mg L^{-1}, respectively. However, the MBC for ECAS was higher than the MIC, at 47.0 mg L^{-1} free chlorine. It is interesting to note that ECAS was most efficacious in inhibiting *E. coli* and *P. aeruginosa*, whilst HOCl demonstrated the greatest bactericidal activity, followed by ECAS then NaOCl.

The MIC/MBC results (shown in Table 5-1) provide inhibitory and bactericidal concentrations for the three disinfectants tested. However, a quantified log reduction to assess comparative disinfectant efficacy is not determined for this test assay. Standard assays that are more representative of real-world conditions (see Section 5.2.2), can assess the bactericidal activity of chemical disinfectants under different organic load conditions (i.e. interfering solutions of bovine serum albumen).

Table 5-1: Minimum inhibitory concentrations [MIC] and minimum bactericidal concentrations [MBC] as a function of free chlorine (mg L^{-1}) for *Escherichia coli* ATCC 10536 and *Pseudomonas aeruginosa* ATCC 15422 against NaOCl, HOCl and ECAS as determined by optical density (n = 3 ± SD).

	NaOCl		**HOCl**		**ECAS**	
	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	55.0	55.0	37.0	37.0	23.5	47.0
Pseudomonas aeruginosa	55.0	55.0	37.0	37.0	23.5	47.0

5.2.2 The comparative antimicrobial activity of ECAS, compared to NaOCl and HOCl, against planktonic *E. coli*

The antimicrobial activity of three chlorine-based disinfectants against *E.coli* was assessed using a standard bactericidal method (Section 2.5). The standard method tests whether a bactericidal product can demonstrate ≥5 log reduction, with a contact time of 5 minutes at 20°C (British Standards Institution, 2005b).

Figure 5.1 shows the antimicrobial efficacy of the test disinfectants against *E. coli* in sterile DI water (i.e. with no interfering solution). At free chlorine concentrations ≥ 50 mg L^{-1}, a log reduction of > 7.366 $\log_{10}$ CFU mL^{-1} of *E. coli* was achieved for all disinfectants tested (NaOCl, HOCl and ECAS), consequently no significant differences were observed between the disinfectants ($p > 0.05$) as shown in Table 5-2. At a free chlorine concentration of 25 mg L^{-1}, HOCl resulted in a complete log reduction (7.366 ± 0.048 $\log_{10}$ CFU mL^{-1}), whereas ECAS resulted in a log reduction of 5.676 $\log_{10}$ CFU mL^{-1}, whilst NaOCl resulted in a log reduction of 3.8 $\log_{10}$ CFU mL^{-1}. At 25 mg L^{-1}, NaOCl did not exhibit bactericidal activity with a 5 minute contact time at 20°C, as defined by the standard test assay undertaken (British Standards

Institution, 2005a). At this concentration, NaOCl was significantly less efficacious than both ECAS ($p = 0.0067$) and HOCl ($p < 0.0001$) as shown in Table 5-2, whilst HOCl was significantly more efficacious than ECAS ($p = 0.0194$).

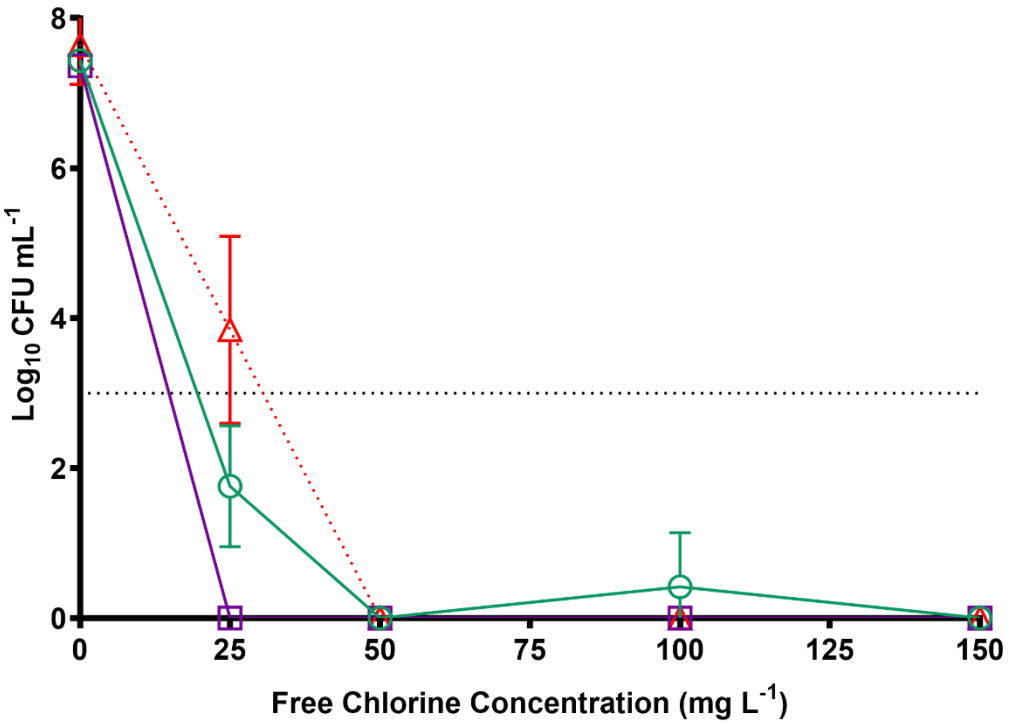

Figure 5.1: Antimicrobial efficacy of ECAS [○], HOCl [□] and NaOCl [△] using standardised free chlorine concentrations against *E. coli* ATCC 10536, and assessed using the standard method BS EN 1040 (British Standards Institution, 2005a). Dotted line represents the minimum log reduction (5 log CFU mL^{-1}) required to demonstrate basic bactericidal activity under the experimental conditions of the assay ($n = 3 \pm SD$).

Table 5-2: Significant differences between disinfectant efficacy (ECAS, HOCl and NaOCl) using standardised free chlorine concentrations against *E. coli* ATCC 10536. Analysis undertaken by a two-way ANOVA with Tukey's multiple comparison test, with a confidence interval of 95% (**** = < 0.0001, *** = p < 0.001; ** = p < 0.01; * = p < 0.05; ns = not significant [> 0.05])

Disinfectant	25 mg L^{-1}	50 mg L^{-1}	100 mg L^{-1}	150 mg L^{-1}
ECAS vs. HOCl	*	ns	ns	ns
ECAS vs. NaOCl	**	ns	ns	ns
HOCl vs. NaOCl	****	ns	ns	ns

Figure 5.2 and Figure 5.3 show the antimicrobial activity of the test disinfectants using the standard BS EN 1276 method (British Standards Institution, 2009). These assays utilise bovine serum albumen (BSA) solutions that simulate 'clean' (0.3 g L^{-1} BSA; Figure 5.2), and 'dirty' conditions (3.0 g L^{-1} BSA; Figure 5.3).

Under 'clean' BSA conditions, a complete log reduction occurred for NaOCl (8.29 $\log_{10}$ CFU ml^{-1}) and HOCl (7.30 $\log_{10}$ CFU mL^{-1}) at the highest standardised free chlorine concentration tested of 150 mg L^{-1} (Figure 5.2). A log reduction of 6.96 $\log_{10}$ CFU mL^{-1} reduction was achieved in the presence of ECAS (150 mg L^{-1} free chlorine). HOCl demonstrated complete log reductions at 50 and 100 mg L^{-1} free chlorine concentrations. At 100 mg L^{-1} free chlorine NaOCl treatment resulted in a 7.871 $\log_{10}$ CFU mL^{-1} reduction, whilst ECAS resulted in a 6.806 $\log_{10}$ CFU ml^{-1} reduction. At 50 mg L^{-1} NaOCl did not achieve the minimum 5-log reduction, resulting in a reduction of 4.531 $\log_{10}$ CFU mL^{-1}. HOCl and ECAS achieved the minimum log reduction required, reducing *E. coli* by 7.3 $\log_{10}$ CFU mL^{-1}, and a 6.549 $\log_{10}$ CFU mL^{-1}, respectively. Interestingly, at the lowest free chlorine concentration tested (25 mg L^{-1}) ECAS was the only disinfectant to reduce the bacterial load ≥ 5 $\log_{10}$ CFU mL^{-1}

(Figure 5.2), resulting in a 6.077 log_{10} CFU mL^{-1} log reduction. The log reductions for NaOCl and HOCl were 1.945 log_{10} CFU $mL^{-1}$and 3.207 log_{10} CFU mL^{-1}, respectively.

No significant differences were observed between all disinfectants at the two highest standardised free chlorine concentrations tested; 100 and 150 mg L^{-1} (Table 5-3:). However, at free chlorine concentrations of 25 and 50 mg L^{-1}, NaOCl was significantly less efficacious than HOCl ($p < 0.0001$ [25 mg L^{-1}] and $p = 0.0102$ [50 mg L^{-1}]) and ECAS ($p = 0.0003$ [25 mg L^{-1}] and $p < 0.0001$ [50 mg L^{-1}]).

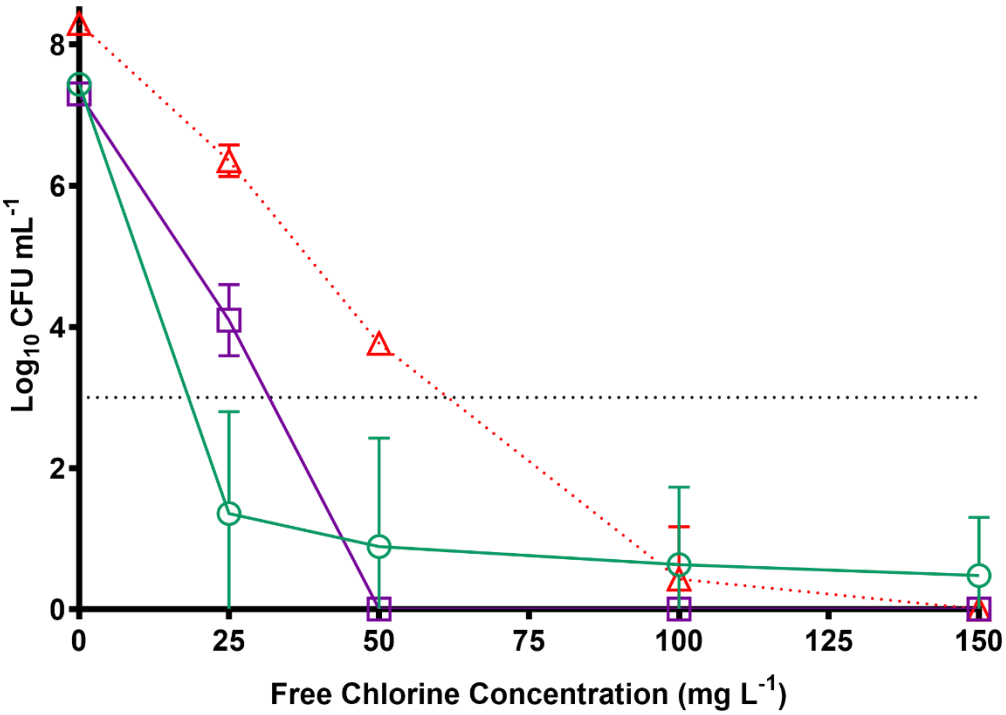

Figure 5.2: Antimicrobial efficacy of ECAS [○], HOCl [□] and NaOCl [△] when free chlorine matched at a range of standardised concentrations against *E. coli* ATCC 10536 with an interfering solution of 0.3 g L^{-1} BSA (British Standards Institution, 2009). Dotted line represents the minimum log reduction (5 log CFU mL^{-1}) required to demonstrate basic bactericidal activity under the experimental conditions of the assay (n = 3 ± SD).

Table 5-3: Significant differences between disinfectant efficacy (ECAS, HOCl and NaOCl) when standardised free chlorine concentrations against *E. coli* ATCC 10536, with an interfering solution of 0.3 g L^{-1} BSA (with reference to figure 5.3). Analysis undertaken by a two-way ANOVA with Tukey's multiple comparison test, with a confidence interval of 95% (**** = < 0.0001, *** = p < 0.001; ** = p < 0.01; * = p < 0.05; ns = not significant [> 0.05]).

Disinfectant	**25 mg L^{-1}**	**50 mg L^{-1}**	**100 mg L^{-1}**	**150 mg L^{-1}**
ECAS vs. HOCl	ns	****	ns	ns
ECAS vs. NaOCl	***	****	ns	ns
HOCl vs. NaOCl	****	*	ns	ns

When the concentration of BSA within the interfering solution was increased from 0.3 g L^{-1} (clean conditions) to 3.0 g L^{-1} (dirty conditions), the antimicrobial activity of the three disinfectants tested was reduced (Figure 5.3). Complete log reductions of *E. coli* was achieved by HOCl at 100 and 150 mg L^{-1} of standardised free chlorine concentrations (7.282 log_{10} CFU mL^{-1}). A 5-log reduction of *E. coli* was not achieved by NaOCl or ECAS at any standardised free chlorine concentrations tested. At 150 mg L^{-1}, NaOCl reduced the bacterial load by 1.365 log_{10} CFU mL^{-1}, whilst ECAS reduced *E. coli* by 3.131 log_{10} CFU mL^{-1}. At 100 mg L^{-1} standardised free chlorine, NaOCl reduced the bacterial load by 0.982 log_{10} CFU mL^{-1} and ECAS reduced the bacterial load by 3.125 log_{10} CFU mL^{-1}. At 50 mg L^{-1} HOCl reduced *E. coli* by 5.964 log_{10} CFU mL^{-1} followed by ECAS 1.771 log_{10} CFU mL^{-1} and NaOCl 0.345 log_{10} CFU mL^{-1}. However, at the lowest standardised free chlorine concentration tested (25 mg L^{-1}), ECAS resulted achieved a log reduction of 1.606 log_{10} CFU mL^{-1}, followed by HOCl (0.978 log_{10} CFU mL^{-1}) and NaOCl (0.025 log_{10} CFU mL^{-1}).

At all standardised free chlorine concentrations tested (25, 50, 100 and 150 mg L^{-1}), HOCl had significantly higher antimicrobial activity in comparison to NaOCl (p < 0.0001; Table 5-4:). Whilst HOCl had significantly higher antimicrobial activity

compared to ECAS at 100 mg L^{-1} ($p < 0.0001$) and 150 mg L^{-1} ($p = 0.0079$). However, ECAS was significantly more efficacious at 25 mg L^{-1} compared to HOCl ($p < 0.0001$), and more efficacious than NaOCl at 50 and 150 mg L^{-1} ($p < 0.0001$).

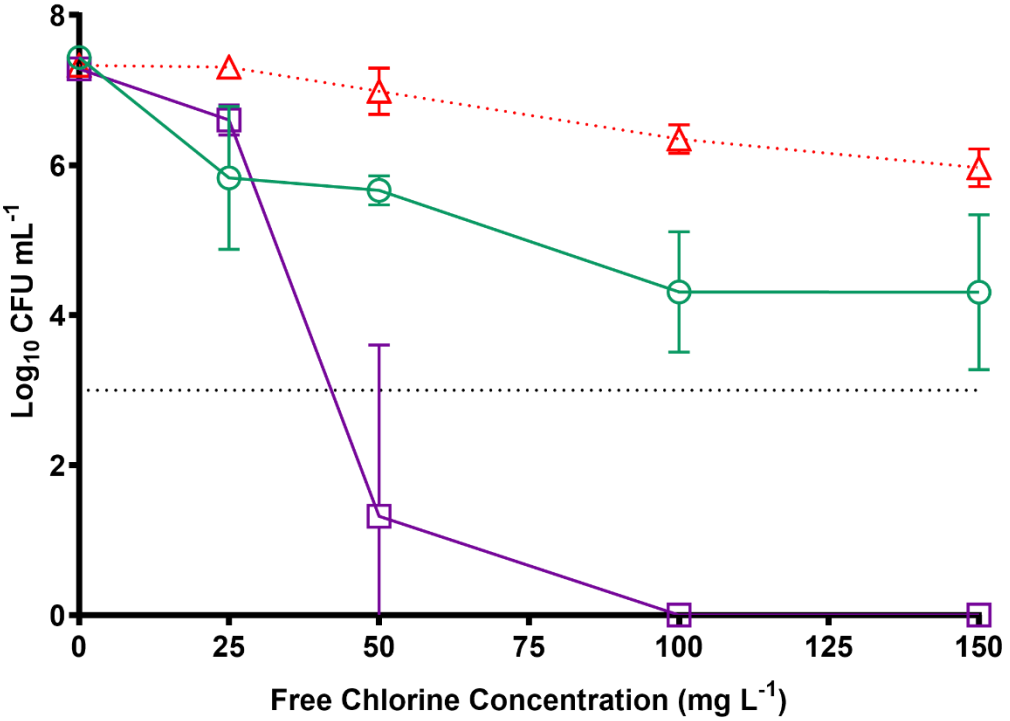

Figure 5.3: Antimicrobial efficacy (expressed as log reduction) of ECAS [○], HOCl [□] and NaOCl [△] when standardised free chlorine concentrations against *E. coli* ATCC 10536 with an interfering solutions of 3.0 g L^{-1} BSA (British Standards Institution, 2009). Dotted line represents the minimum log reduction (5 log CFU mL^{-1}) required to demonstrate basic bactericidal activity under the experimental conditions of the assay (n = 3 ± SD).

Table 5-4: Significant differences between disinfectant efficacy (ECAS, HOCl and NaOCl) when standardised free chlorine concentrations against *E. coli* ATCC 10536, with an interfering solution of 3.0 g L^{-1} BSA (with reference to figure 5.3). Analysis undertaken by two-way ANOVA with Tukey's multiple comparison test, with a confidence interval of 95% (**** = < 0.0001, *** = p < 0.001; ** = p < 0.01; * = p < 0.05; ns = not significant [> 0.05]).

Disinfectant	**25 mg L^{-1}**	**50 mg L^{-1}**	**100 mg L^{-1}**	**150 mg L^{-1}**
ECAS vs. HOCl	****	ns	****	**
ECAS vs. NaOCl	ns	****	ns	****
HOCl vs. NaOCl	****	****	****	****

Collectively the results of these experiments (Figure 5.1, 5.2 and 5.3) demonstrate that for all disinfectants tested, except for HOCl, at standardised free chlorine concentrations of ≥100 mg L^{-1}, as the organic load increases, the antimicrobial activity decreases [1] (Table 5-5).

Table 5-5: Significant differences between disinfectant efficacy (NaOCl, HOCl, and ECAS) as a function of organic load of interfering solutions (sterile water, clean BSA and dirty BSA). Analysis undertaken by two-way ANOVA with Tukey's multiple comparison test, with a confidence interval of 95% (**** = < 0.0001, *** = p < 0.001; ** = p < 0.01; * = p < 0.05; ns = not significant [> 0.05]).

Disinfectant	**Free Chlorine concentration**	**Sterile Water vs. Clean BSA**	**Sterile Water vs. Dirty BSA**	**Clean BSA vs. Dirty BSA**
NaOCl	25 mg L^{-1}	****	****	*
	50 mg L^{-1}	ns	****	****
	100 mg L^{-1}	ns	****	****
	150 mg L^{-1}	*	****	****
HOCl	25 mg L^{-1}	ns	****	**
	50 mg L^{-1}	**	**	ns
	100 mg L^{-1}	ns	ns	ns
	150 mg L^{-1}	ns	ns	ns
ECAS	25 mg L^{-1}	ns	*	*
	50 mg L^{-1}	*	***	ns
	100 mg L^{-1}	*	****	****
	150 mg L^{-1}	**	****	****

5.2.3 The comparative antimicrobial activity of ECAS, NaOCl and HOCl, against microbial biofilms

The antimicrobial activity of NaOCl, HOCl and ECAS was determined against mature *Pseudomonas aeruginosa* biofilms grown within a Centre for Disease Control (CDC) Biofilm Reactor (Figure 5.4) as a function of free chlorine. *P. aeruginosa* has been widely used as a model organism in biofilm studies, and is a bacterial strain used in standard quantifiable methods using the CDC biofilm reactor (US Enviromental Protection Agency Office of Pesticide Programs, 2013).

The mean biofilm density that formed on untreated control polycarbonate coupons (sterile DI; 0 mg L^{-1} [control]), was 9.927 ± 0.172 $\log_{10}$ CFU $coupon^{-1}$ (n = 18). The highest reduction in biofilm density occurred in the presence of ECAS (150 mg L^{-1} free chlorine), resulting in a 3.852 $\log_{10}$ CFU $coupon^{-1}$ reduction. NaOCl and HOCl resulted in reductions of 2.018 $\log_{10}$ CFU $coupon^{-1}$ and 2.005 $\log_{10}$ CFU $coupon^{-1}$, respectively, at the highest free chlorine concentration tested (150 mg L^{-1}). A complete reduction in *P. aeruginosa* ATCC 15442 biofilm density was not achieved for any of the disinfectants, at any of the free chlorine concentrations tested (Figure 5.4).

There was no significant difference in the antimicrobial efficacies exhibited between NaOCl or HOCl at any of the standardised free chlorine concentrations tested ($p > 0.05$) against *P. aeruginosa*. However, ECAS exhibited a significantly greater antimicrobial effect than NaOCl and HOCl, at free chlorine concentrations ≥ 50 mg L^{-1} ($p < 0.0001$). Therefore, ECAS was more efficacious at reducing *P. aeruginosa* biofilm density compared to NaOCl and HOCl at equivalent standardised free chlorine concentrations. Increasing free chlorine concentration had little effect on reducing biofilm density with NaOCl or HOCl treatments, except between 50 - 75 mg

L^{-1} for NaOCl ($p = 0.0334$) and 5 – 25 mg L^{-1} for HOCl ($p = 0.0124$), see Figure 5.5. However, for ECAS significant reductions in biofilm density were exhibited as standardised free chlorine concentrations increased, between 25 and 150 mg L^{-1} ($p < 0.0001$ [Figure 5.4]). Antimicrobial activity was significantly reduced at standardised free chlorine concentrations 50, 75, 100 and 150 mg L^{-1}, in the presence of NaOCl and HOCl, compared to ECAS ($p < 0.0001$). However, no significant differences in biofilm log reduction was observed between all disinfectants at 5 and 25 mg L^{-1} free chlorine ($p > 0.05$).

Reduced antimicrobial activity of disinfectants against mature biofilms, compared to planktonic bacteria, has been observed (Bridier *et al.*, 2011; Mah and O'Toole, 2001; Théraud *et al.*, 2004). Furthermore, at free chlorine concentrations commonly present throughout drinking water distribution networks (i.e. < 5 mg L^{-1}), log reductions of < 0.202 $\log_{10}$ CFU $coupon^{-1}$ (NaOCl) were observed. This indicated that all disinfectants tested have low antimicrobial activity against pre-formed microbial biofilms, after a 5-minute disinfection contact time (see Section 2.6.3).

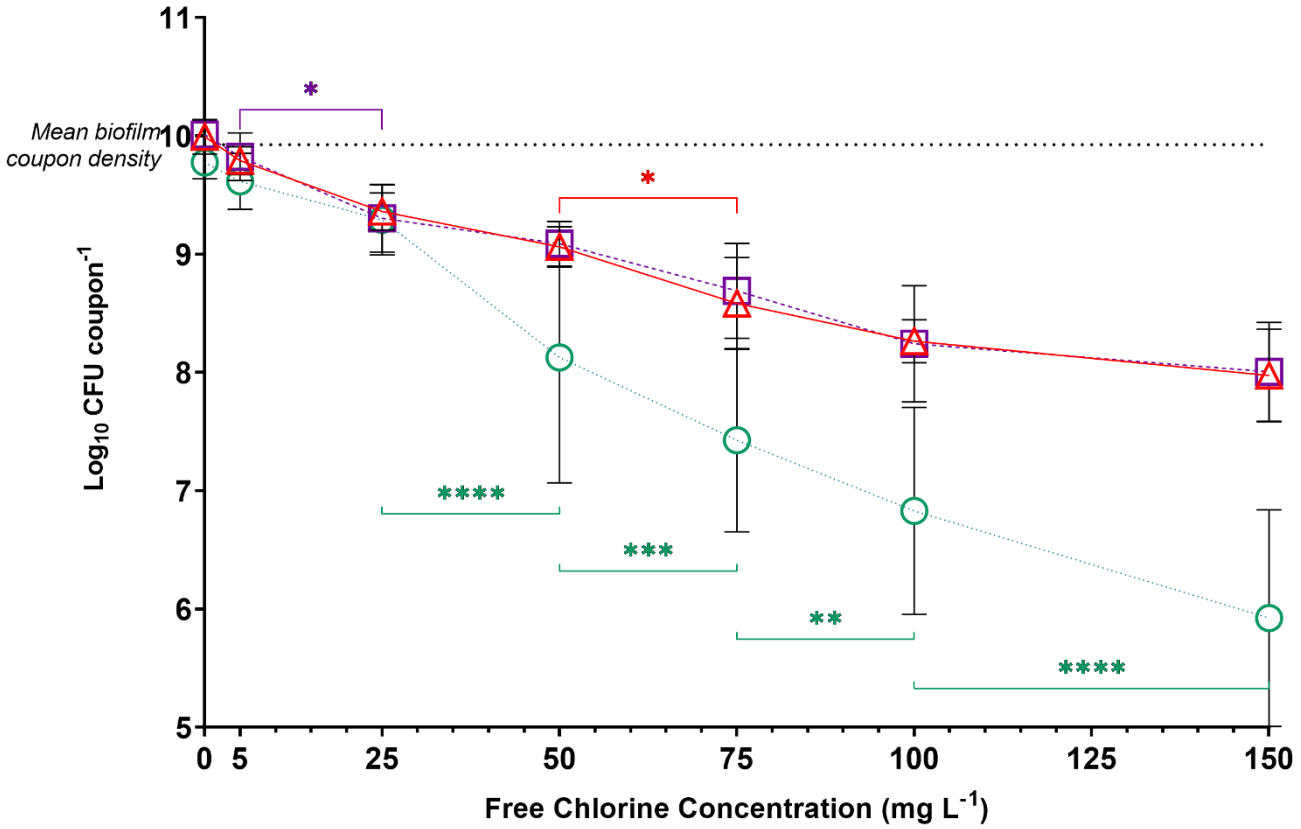

Figure 5.4: Antimicrobial activity of ECAS [○], HOCl [□] and NaOCl [△] when standardised free chlorine concentrations against *Pseudomonas aeruginosa* ATCC 15442 biofilm (n = 9 ± SD). Untreated biofilm density (-) refers to the mean CFU coupon^{-1} recovered from control treatment (0 mg L^{-1}); n = 18. Significant difference calculated through a two-way ANOVA with Tukey's multiple comparison post-test, with a confidence interval of 95% (**** = < 0.0001, *** = p < 0.001; ** = p < 0.01; * = p < 0.05).

5.3 Discussion

5.3.1 The antimicrobial activity of disinfectants against planktonic microorganisms

The primary role of disinfection processes throughout drinking water treatment is to control pathogenic microorganisms, ensuring the production of biologically safe water. The presence of indicator organisms such as *E. coli*, coliforms and *Clostridium perfringens* (Drinking Water Inspectorate, 2012; World Health Organization, 2011) can infer whether faecal contamination has occurred, as well as monitor the effectiveness of disinfection treatment processes. However, conventional disinfectants have shown to be less effective against some opportunistic pathogenic microorganisms (including; *Mycobacterium* species, viruses and eukaryotes [Section 1.2.2]). For example, *Mycobacterium avium* strains been shown to be over 500 times more resistant to chlorine compared to *E. coli* (Taylor *et al.*, 2000). In addition, evidence has shown that chlorine is unable to penetrate biofilm EPS effectively (Chen and Stewart, 1996; De Beer, Srinivasan and Stewart, 1994; Stewart *et al.*, 2001), and this is discussed further in Section 5.3.2. Investigating alternative disinfectant processes in comparison to conventional chlorination (i.e. NaOCl) help to determine the impact that such approaches may have on minimising DBPs, including THM formation, which are undesirable due to their mutagenic and carcinogenic properties (Bellar, Lichtenberg and Kroner, 1974; Rook, 1976; World Health Organization, 2005), as previously discussed in Chapter 4.

The efficacy of ECAS, compared to commonly used surface disinfectants (i.e. Virkon, ethanol, NaOCl) has been assessed through a variety of different methods such as bioluminescence (Robinson *et al.*, 2011) and bacterial recovery through viable counting (Ding *et al.*, 2016; Thorn, Robinson and Reynolds, 2013). Robinson e*t al.*

(2011) determined that with no interfering solution an 80% concentration of ECAS resulted in a 2.336 $\log_{10}$ RLU [relative light units] s^{-1} reduction of *E. coli* Nissle 1917/pGLITE over a two second contact time. However, the addition of a 10% foetal bovine serum [FBS] solution reduced the antimicrobial activity of ECAS to 0.165 $\log_{10}$ RLU s^{-1}. The results from the assays carried out in this study also exhibited reduced antimicrobial activity of ECAS in the presence of increased organic (BSA) loading (see Figure 5.1, 5.2 and 5.3). The antimicrobial efficacy of aerosolized ECAS has been compared to NaOCl (both 100 mg L^{-1} free chlorine) against methicillin resistant *Staphylococcus aureus* [MRSA], methicillin susceptible *Staphylococcus aureus* [MSSA] and *P. aeruginosa* on a variety of different substrates (Thorn, Robinson and Reynolds, 2013). ECAS was consistently more efficacious after a 20-minute treatment regimen on plastic substrates, compared to NaOCl, against MRSA, MSSA and *P. aeruginosa*. Future studies should test how continuous ECAS exposure over increased contact times affects the efficacy in the context of drinking water treatment. Ding *et al.* (2016) assessed inactivation of *S. aureus* between NaOCl and ECAS, with available chlorine concentrations of 30 and 33 mg L^{-1}, respectively, after a 1-minute treatment time. ECAS resulted in a significantly greater log reduction of *S. aureus* (5.8 log CFU mL^{-1}) compared to NaOCl (3.26 CFU mL^{-1}). Collectively, the results from this study have further developed the evidence that ECAS can be more efficacious in comparison to other chlorine-based disinfectants over shorter contact times. Whilst there have been investigations into the antimicrobial activity of differing ECAS solutions (i.e. acidic, slightly acidic, neutral, or alkaline) against pathogenic organisms (Venczel *et al.*, 1997; Bari *et al.*, 2003; Ovissipour *et al.*, 2015; Tomás-Callejas *et al.*, 2011; Ding *et al.*, 2016), no direct comparison of the antimicrobial activity of ECAS, NaOCl and HOCl solutions standardised to equivalent free chlorine concentrations has been conducted in published literature. Studies have compared antimicrobial activity of frequently used drinking water disinfectants, including; free

chlorine (typically NaOCl), chlorine dioxide, ozone and chloramines (Hoff and Geldreich, 1981; Loret *et al.*, 2005; Diao *et al.*, 2004), but the focus of this research has not been for POU systems.

Organic matter reduces the antimicrobial efficacy of disinfectants (Harrison and Hand, 1981; Oomori *et al.*, 2005), which is in line with the results of this study (Figure 5.1 to 5.3). Solutions with a high organic load can reduce the antimicrobial activity of disinfectants (Figure 5.3). This has been shown in solutions which have a higher turbidity as a result of particulate organic matter (Lechevallier, Evans and Seidler, 1981; Obi *et al.*, 2008) or as a result of organic loading (Robinson *et al.*, 2011; Oomori *et al.*, 2005). The reduced antimicrobial activity of disinfectants is likely a result of indiscriminate reactions between antimicrobial species (i.e. chlorine species, or transient oxidative functional groups) and organic matter, such as particulate matter (Huang *et al.*, 2008).

The efficacy of the test disinfectants in the presence of organic matter appears pH or ORP dependant. NaOCl the most alkaline (p11.4 ± 0.1) and with the lowest ORP value (ORP 588 ± 0.95 mV), seemingly had the lowest antimicrobial activity compared to HOCl (pH 5.6 ± 0.25, ORP 958 ± 18.98 mV) and ECAS (pH 3.3 ± 0.16, ORP 1134 ± 3.26 mV). At lower free chlorine concentrations tested (25 mg L^{-1}) in the presence of an organic load (i.e. BSA), disinfectants with a higher ORP (i.e. ECAS and HOCl) were more efficacious compared to NaOCl, which have a lower ORP. This infers that free chlorine is not the only antimicrobial species present, but that other factors, such as transient oxidative functional groups (e.g. OH-, O_3, H_2O_2 and O_2-(Jeong, Kim and Yoon, 2006; Martínez-Huitle *et al.*, 2008; Liao, Chen and Xiao, 2007)), contribute to the overall antimicrobial efficacy of these disinfectants (Diao *et al.*, 2004).

Therefore, within water treatment it has been shown that reducing the turbidity or organic load within input water, through filtration, coagulation and flocculation, can increase antimicrobial efficacy against microorganisms (Lechevallier, Evans and Seidler, 1981). Studies have also shown that reactions between free chlorine and proteins result in organochloramine formation; and that these have reduced antimicrobial activity (Black and Veatch Corporation, 2010; Hricova, Stephan and Zweifel, 2008), but a greater potential for penetrating biofilms (Lee *et al.*, 2011).

5.3.2 The comparative antimicrobial activity of ECAS, NaOCl and HOCl, against microbial biofilms

Free chlorine concentrations of 5 and 25 mg L^{-1} resulted in comparable log reductions of *P. aeruginosa* for all disinfectants tested; HOCl, NaOCl or ECAS (Figure 5.4). However, ECAS was significantly more efficacious than NaOCl and HOCl at free chlorine concentrations ≥ 50 mg L^{-1} ($p < 0.0001$). At the highest free chlorine concentration tested a 3.853 $\log_{10}$ CFU $coupon^{-1}$ reduction was achieved through ECAS treatment; almost double that of NaOCl (2.018 $\log_{10}$ CFU $coupon^{-1}$) and HOCl (2.005 $\log_{10}$ CFU $coupon^{-1}$). Despite ECAS displaying the greatest antimicrobial activity against mature *P. aeruginosa* biofilms, it also exhibited the greatest variability in antimicrobial activity (SD 0.775 [75 mg L^{-1}] – 1.061 [50 mg L^{-1}]), compared to HOCl (SD ≤ 0.492) and NaOCl (SD ≤ 0.393). This could be a result of the transient antimicrobial species formed as part of ECAS generation (see Section 1.4.2.2). Disinfectants were free chlorine matched, but this does not take into account transient antimicrobial species present in solutions, which were not investigated as part of this study. Transient antimicrobial species are very reactive, with much of the antimicrobial activity occurring within the first 10 seconds (Robinson *et al.*, 2011; Liao, Chen and Xiao, 2007), leaving chlorine species (HOCl or OCl^{-1}) to provide further disinfection.

Antimicrobial disinfectants containing halogen species (i.e. chlorine) have shown to have a reduced efficacy on reducing biofilm density due to halogen species reacting with unspecified biofilm matter, becoming neutralised, and being unable to penetrate and diffuse into the biofilm (Stewart, 2015; Stewart *et al.*, 2001). However, a combination of factors increase a biofilms tolerance to antimicrobials. In addition to ineffective penetration of antimicrobials into the biofilm (Chen and Stewart, 1996; De Beer, Srinivasan and Stewart, 1994; Stewart *et al.*, 2001), other factors include; adaptations in stress responses (Davies, 2003), heterogeneous nature of biofilms (i.e. multispecies (Schwering *et al.*, 2013)) and persister cells (Wood, 2016; Lewis, 2010). Furthermore, the reaction between chlorine and proteins can result in organochloramine formation, which have lower antimicrobial activity in comparison to chlorine or transient antimicrobial species (Hricova, Stephan and Zweifel, 2008; Black and Veatch Corporation, 2010) yet chloramine species have demonstrated greater penetration into biofilms (Lee *et al.*, 2011).

This study investigated the direct antimicrobial activity of chlorine-based disinfectants against mature mono-species biofilms. However, biofilms rarely exist as mono-species (Hall-Stoodley, Costerton and Stoodley, 2004). To develop a greater understanding of the antimicrobial activity of these disinfectants, multispecies biofilms would provide more representative models of real-world applications. Developing a biofilm model representative of POU DWTSs, such as the one discussed in Chapter 3, would provide greater insight into the effectiveness of the disinfectants within such applications. Incorporating *in-situ* disinfectant dosing as part of a biofilm model can determine how disinfectants manage initial bacterial attachment and potentially inhibit biofilm formation. To investigate the effect of *in-situ* disinfectant dosing on managing biofilm formation a model was developed, and preliminary results are presented in Chapter 6.

5.3.3 Comparing ECAS to conventional chlorine based disinfectants

The antimicrobial activity determined for ECAS consistently exhibited greater variation (standard deviation) across all free chlorine concentrations, compared to NaOCl and HOCl, in the presence of an organic load (Figure 5.2 and Figure 5.3), or against a mature biofilm (Figure 5.4). This could be a result of transient antimicrobial species present in ECAS, which were not quantified and standardised within this study since the disinfectants were compared by matched free chlorine concentrations. Many studies have investigated the antimicrobial activity of acidic ECAS, or more frequently neutral ECAS, on a variety of indicator bacterial and fungal species. Such applications include: food processing or production (Cui *et al.*, 2009; Gil *et al.*, 2015; Huang *et al.*, 2008; Kim, Hung and Brackett, 2000; Oomori *et al.*, 2005; Park *et al.*, 2008; Park, Hung and Chung, 2004; Thorn, Pendred and Reynolds, 2017; Rahman *et al.*, 2012; Rahman, Khan and Oh, 2016; Veasey and Muriana, 2016; Hricova, Stephan and Zweifel, 2008), healthcare settings (Kirkpatrick, 2009; Thorn *et al.*, 2012; Selkon, Babbt and Morris, 1999; Tagawa *et al.*, 2000; Tanaka *et al.*, 1996), general disinfection (Helme *et al.*, 2010; Cloete *et al.*, 2009; Liao, Chen and Xiao, 2007; Robinson *et al.*, 2010, 2011; Robinson, Thorn and Reynolds, 2013; Thorn, Robinson and Reynolds, 2013) and water treatment (Gonzalez, 2002; Clayton, Thorn and Reynolds, 2019b; Venczel *et al.*, 1997). However, few studies have studied the effect of ECAS (neutral or acidic) on reducing biofilm density through direct disinfection (Ayebah *et al.*, 2006; Cloete, 2002; Sandvik *et al.*, 2013). With even fewer directly comparing the efficacy of ECAS (neutral or acidic) against chlorine solutions frequently used in POU drinking water treatment (i.e. NaOCl and HOCl), as a function of equivalent free chlorine (Venczel *et al.*, 2004; Ghebremichael *et al.*, 2011).

Conventional chlorine is well established as an effective disinfection process (World Health Organization, 2004a); however, with the formation of disinfection by-products (i.e. THMs) (Rook, 1976), as discussed previously in chapter 4, investigating alternative disinfection processes to produce both biologically and chemically safe drinking water as part of POU treatment systems is required. DBPs form as a result of organic matter reacting with chlorine, see Figure 1.5 (Rook, 1976). In fact, it is now known that the formation of DBPs can result from chlorine reacting with organic matter present in biofilms (Abokifa *et al.*, 2016; Wang *et al.*, 2013). Several studies have demonstrated that free chlorine used to target biofilms is diffusion limited (Flemming, 2002; Chen and Stewart, 1996; De Beer, Srinivasan and Stewart, 1994; Buckingham-Meyer, Goeres and Hamilton, 2007), thus reducing its antimicrobial activity. This was reflected in the relatively low reduction in biofilm density shown in Figure 5.4. At the highest free chlorine concentration tested (150 mg L^{-1}), ECAS treatment resulted in the greatest reduction in biofilm density (3.85 ± 0.914 $\log_{10}$ CFU $coupon^{-1}$). The increased reduction in biofilm density, compared to NaOCl and HOCl, could be a result of transient antimicrobial species present at the point of ECAS generation (Diao *et al.*, 2004), which rupture cell membranes inhibiting crucial microbial functions (Liao, Chen and Xiao, 2007). Transient antimicrobial species increase the oxidation reduction potential and lower the pH (Suslow, 2004), at point of generation (i.e. 150 mg L^{-1} free chlorine), and have been thought to provide greater disinfection capabilities, in comparison to conventional chlorine solutions (Diao *et al.*, 2004).

5.4 Conclusions

- ECAS exhibited the lowest MIC against *E. coli* and *P. aeruginosa*, whilst HOCl exhibited the lowest MBC against the organisms followed by ECAS, and NaOCl.
- The antimicrobial efficacy of ECAS against *E. coli* was comparable to NaOCl and HOCl at free chlorine concentrations ≥ 50 mg L^{-1}, with no interfering solution (sterile DI), or in the presence of clean BSA (0.3 g L^{-1}).
- In the presence of a high organic load (3.0 g L^{-1} BSA), HOCl demonstrated the highest antimicrobial activity against *E. coli* at free chlorine concentrations ≥ 50 mg L^{-1}.;NaOCl or ECAS did not achieve a ≥ 5 log reduction of E. coli after a 5-minute contact time.
- ECAS exhibited significantly higher antimicrobial activity against *P. aeruginosa* biofilms at free chlorine concentrations ≥ 50 mg L^{-1}, compared to NaOCl and HOCl.

Chapter 6. Biofilm management; investigating the inhibitory effects of chlorine-based disinfectants on biofilms

6.1 Introduction

As part of the biofilm formation process (Figure 1.6), cells can irreversibly attach to a surface through secretion of EPS (Flemming and Wingender, 2010). Through the inhibition or disruption of microbial attachment, biofilm formation can be managed (Yang *et al.*, 2012). Managing biofilm formation in water treatment systems is beneficial as blockages and corrosion, as a result of biofouling, can result in non-operational phases (Simoes and Simoes, 2013; Vrouwenvelder *et al.*, 1998). Biofilms which form in drinking water systems can pose hazards to end users, as potential pathogenic microorganisms can exist in the biofilm matrix (Sanchez-Vizuete *et al.*, 2015; Skraber *et al.*, 2005). Therefore, developing benchtop biofilm models representative of real-world conditions within water treatment systems are required to reflect the complexity of naturally occurring biofilms.

This chapter investigates the effect of ECAS *in-situ* dosing on managing the formation of a single species (*P. aeruginosa* ATCC 15422) biofilm. Subsequently, the effect of NaOCl, HOCl and ECAS *in-situ* dosing on a multi-species (environmental) biofilm was studied. The first objective was to investigate the formation of *P. aeruginosa* ATCC 15442 on polycarbonate [PC] surface material, with an *in-situ* disinfectant dosing regimen commensurate with the levels used in the decentralised drinking water treatment system (0.5% [v/v]). Amended bactericidal assays determined the

antimicrobial activity of ECAS, NaOCl and HOCl, against planktonic bacteria (*Escherichia coli* ATCC 10536) in which environmental water was used to simulate a realistic organic load as an interfering solution. Investigating the inhibitory effect of *in-situ* disinfectant dosing on multi-species biofilms required the development of an amended *in-situ* disinfectant dosing CDC reactor model. This model enabled an investigation of the efficacy of NaOCl, HOCl and ECAS at managing environmental biofilm formation over a 48-hour period on polyethersulfone [PES] coupons. The methods used to generate data presented throughout this chapter are described in Section 2.7.

6.2 Results

6.2.1 Managing *P. aeruginosa* biofilms in a long-term *in-situ* ECAS dosing CDC reactor model

6.2.1.1 Development of a long-term *in -situ* ECAS dosing CDC reactor model

To determine the effect of *in-situ* dosing on biofilm formation on PC surface material, with a dosing regimen commensurate with the decentralised drinking water treatment system described in Chapter 3 (0.5% [v/v]), a long-term *in-situ* ECAS dosing CDC reactor model was developed (Figure 6.1). The model was adapted from a standard CDC biofilm method (Figure 2.7), whereby, a 20L carboy containing 100 mg L^{-1} tryptone soy broth (TSB) was inoculated to a density of 5.461 ± 0.293 $\log_{10}$ CFU mL^{-1} with *Pseudomonas aeruginosa* (ATCC 15442) and used as the input medium. To determine the effect of *in-situ* dosing at inhibiting biofilm formation, this *in-situ* model, unlike the standard CDC reactor model (Figure 2.7), does not incorporate a batch phase. A key research aim was to investigate the effect of constant low dosing (0.5% [v/v]) of ECAS on biofilm formation, with a continuous flow rate (12 mL min^{-1}; 30-minute residence time) over 8 days.

The model used throughout these studies is represented in Figure 6.1. A sterile 20 L carboy containing the inoculated input media [1], was dosed (*in-situ*) prior to the biofilm reactor, by either ECAS [2A], or sterile DI [2B]. Immediately after the point of dosing, a sample tap was included to allow planktonic samples to be taken before [3A/B] the CDC biofilm reactor [4]. An effluent spout on the CDC biofilm reactors allowed for excess media to empty into a 20 L sterile carboy [5]. The first planktonic and biofilm samples were taken 6 hours after the start of the CDC biofilm model, and then every 24 hours thereafter (i.e. 30 hours, 54 hours), for a total of 174 hours (8 days).

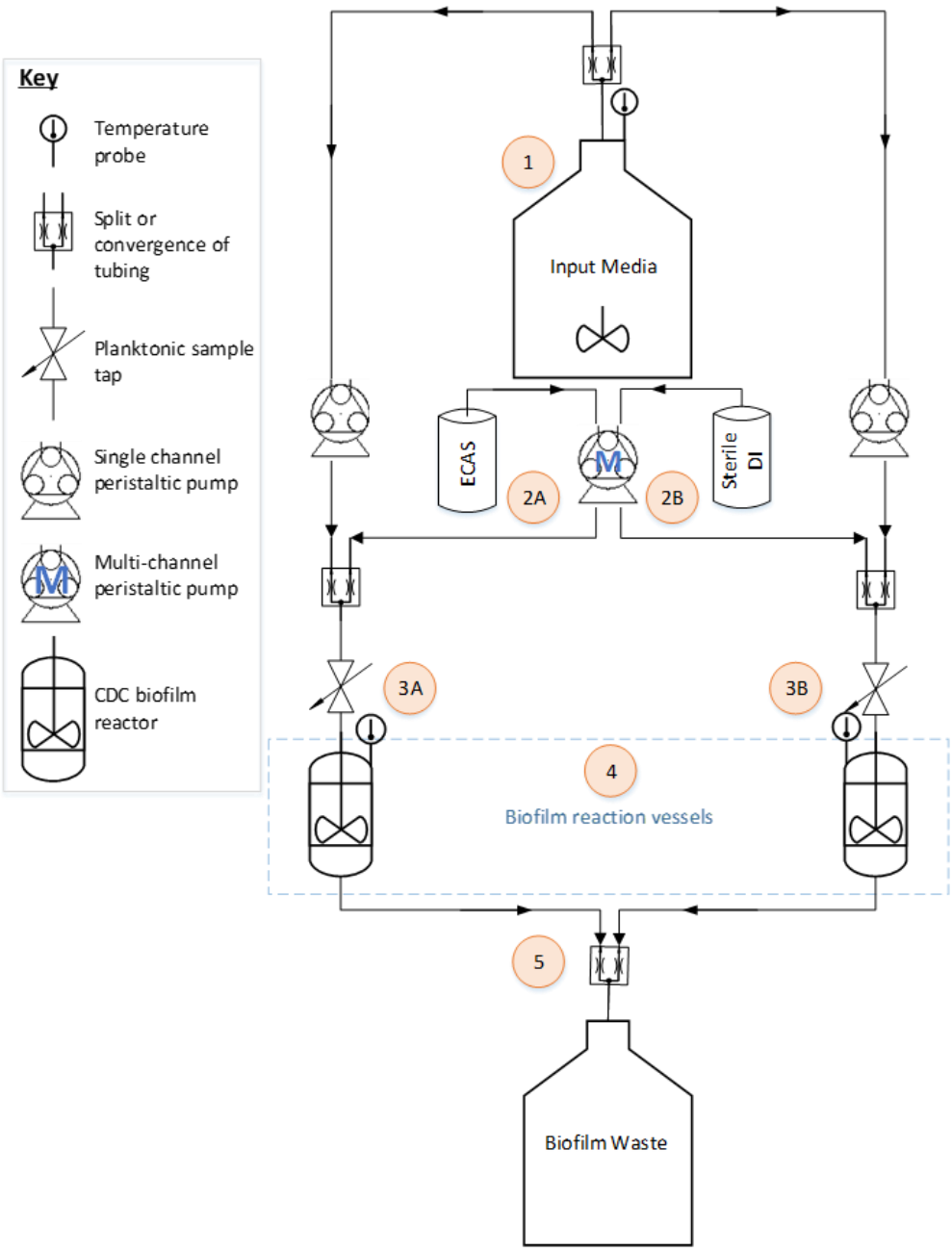

Figure 6.1: Schematic of long-term *in-situ* dosing modified CDC reactor model. [1] Input media (100 mg L^{-1} TSB innoculated with *P. aeruginosa*) is drawn by a single pertistalic pump towards the point of dosing; with either ECAS [2A] or sterile DI [2B]. Planktonic sample taps [3A/B] were located immediately before the CDC biofilm reactors [4]. Waste media was collected in a 20 L Nalgene carboy [5].

6.2.1.2 Enumeration of planktonic samples taken pre-CDC reactor

The long-term *in-situ* ECAS dosing CDC reactor model (Figure 6.1) ran continuously for 174 hours (8 days). The effect of this treatment regimen on planktonic *P. aeruginosa* is shown in Figure 6.2. Samples taken pre-CDC (in planktonic phase) throughout the control (sterile DI dosing) experiment demonstrated increased microbial numbers between 6 and 78 hours, increasing from 5.70 $\log_{10}$ CFU mL^{-1} to 7.13 $\log_{10}$ CFU mL^{-1}. Planktonic plate counts were then relatively stable between 78 and 174 hours (7.075 ± 0.049 $\log_{10}$ CFU mL^{-1} - 7.29 ± 0.014 $\log_{10}$ CFU mL^{-1}).

For planktonic samples taken pre-CDC reactor, the ECAS dosing regimen resulted in no significant increase in microbial numbers between 6 and 30 hours. Thereafter, from 30 to 78 hours, an increase from 5.59 ± 0.035 $\log_{10}$ CFU mL^{-1} to 7.35 ± 0.071 $\log_{10}$ CFU mL^{-1}, was observed, before plateauing between 78 and 126 hours (7.35 ± 0.071 $\log_{10}$ CFU mL^{-1} – 7.33 ± 0.071 $\log_{10}$ CFU mL^{-1}). Between 126 and 174 hours, microbial numbers increased to 7.63 ± 0.085 $\log_{10}$ CFU mL^{-1}.

The antimicrobial efficacy of the two treatment regimens were compared for all time points across the continuous running of the long-term *in-situ* ECAS dosing CDC reactor model. After 6-hours, there was no significant difference between planktonic samples treated with ECAS or sterile DI water. For control planktonic samples (pre-CDC) higher microbial loads were observed in comparison to ECAS dosed samples, up to 54 hours. Thereafter, for ECAS dosed samples, the microbial load was higher than the control (sterile DI water) dosing regimen. However, at 126 hours, an increase of 0.19 $\log_{10}$ CFU mL^{-1} in the control treatment regimen was observed.

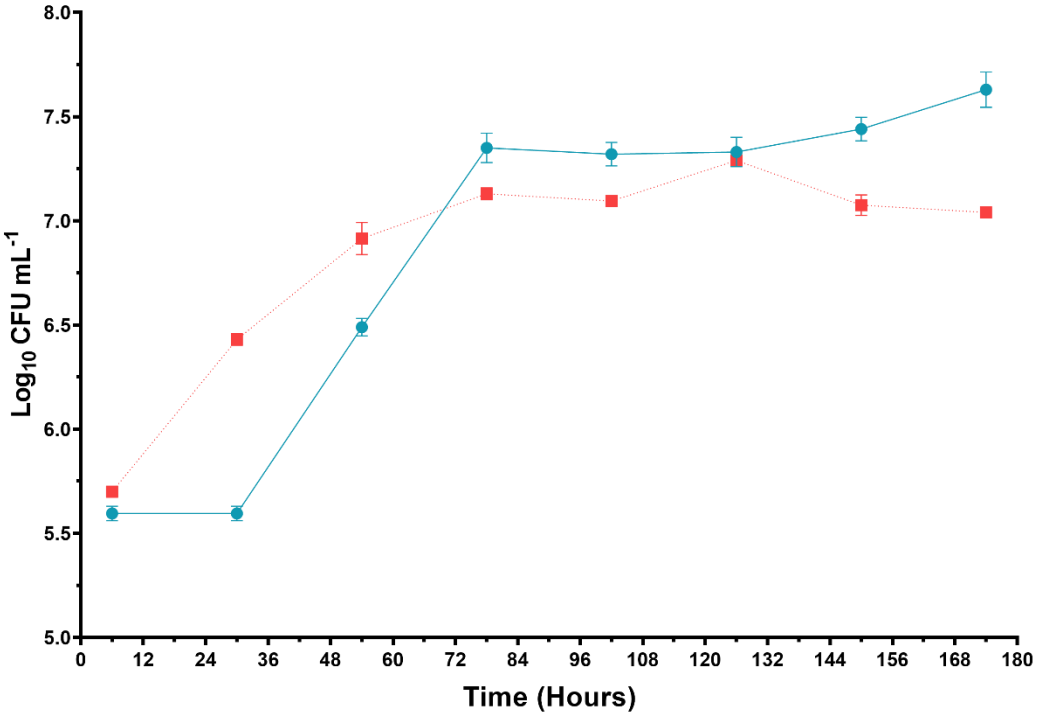

Figure 6.2: Antimicrobial efficacy of *in-situ* ECAS dosing [●] and control (sterile DI) dosing [■] against planktonic *Pseudomonas aeruginosa* (ATCC 15442) compared to sample taps before the biofilm reactor (Figure 6.1). n = 2 (± SD).

6.2.1.3 Enumeration of *P. aeruginosa* biofilm

Biofilm densities of 4.157 ± 0.297 log_{10} CFU coupon^{-1} and 4.194 ± 0.182 log_{10} CFU coupon^{-1}, were recovered for ECAS and control dosing regimens after 6 hours, respectively (Figure 6.3). Over the next 24 hours, biofilm densities increased to 8.123 ± 0.057 log_{10} CFU coupon^{-1} (ECAS) and 7.950 ± 0.0.024 log_{10} CFU coupon^{-1} (control), respectively.

Both dosing regimens resulted in a gradual increase in biofilm density, before peaking at 102 hours for both ECAS (9.043 ± 0.625 log_{10} CFU coupon^{-1}) and control dosing regimens (9.007 ± 0.476 log_{10} CFU coupon^{-1}). Biofilm density then decreased for both dosing regimens between 102 and 174 hours. A reduction in biofilm density of 0.807

log_{10} CFU coupon^{-1} and 1.065 log_{10} CFU coupon^{-1} was exhibited throughout the control and ECAS treatment regimen, respectively. After 30 hours, the ECAS dosing regimen resulted in a significantly higher biofilm density, compared to the control ($p = <0.0001$). However, at 150 hours, the biofilm density for the control dosing regimen was significantly greater than ECAS dosing ($p = 0.0073$). No significant differences were observed between the dosing regimens at the any other time points.

After operation of the model for 174 hours, cleaning and decontamination of the system was undertaken. It was noted that there was a marked visual difference between the biofouling present on the CDC biofilm reactor flow breaks (see Figure 6.4). In the presence of ECAS dosing, virtually no biofilm was observed on the flow break [A] that was located post-dosing, but prior to entering the CDC biofilm reactor (Figure 2.10). Conversely, for the control dosing (sterile DI water), biofilm was clearly present on the flow breaks [B].

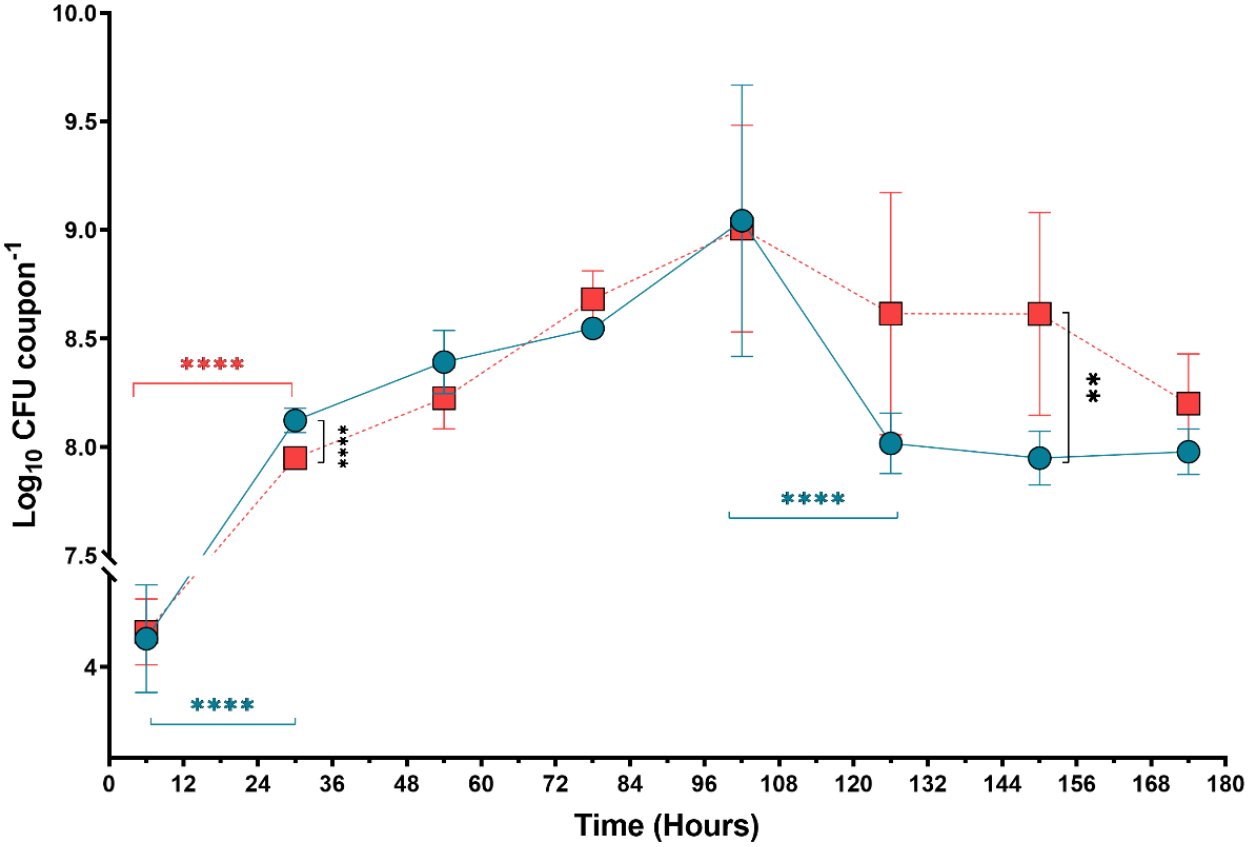

Figure 6.3: Efficacy of ECAS dosing [●] or control dosing of sterile DI [■] in inhibiting the formation of *Pseudomonas aeruginosa* ATCC 15442 biofilms on polycarbonate coupons (n = 3 ± SD). Significant differences between dosing regimen (ECAS and sterile DI) were calculated through t-tests with multiple comparisons using Holm-Sidak. Significant differences within each dosing regimen (green [ECAS] or red [control]) were calculated using a two-way ANOVA with Tukey's multiple comparison test, with a confidence interval of 95% (**** = < 0.0001, ** = p < 0.01).

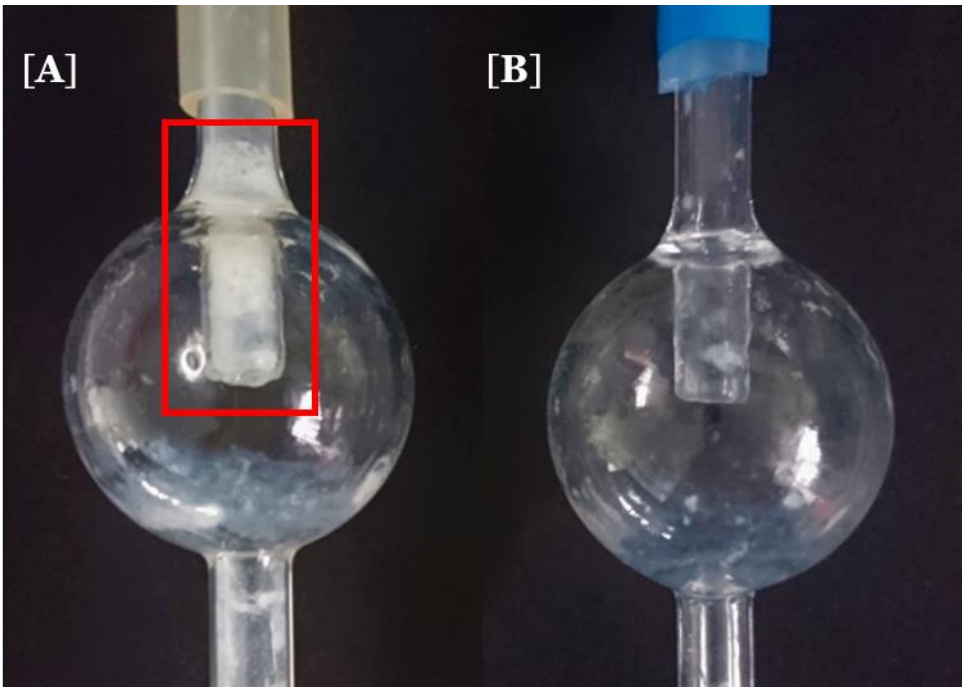

Figure 6.4: Long-term *in-situ* dosing modified CDC biofilm model flow breaks after 174 hours with [A] control and [B] ECAS dosing regimens. Biofilm shown by □.

6.2.2 Managing environmental biofilm formation in an amended *in-situ* disinfectant dosing CDC reactor model

6.2.2.1 Determining stability of bacterial numbers within environmental water

The maximum time between changing the input environmental water media during operation of the in-situ dosing model was 24 hours. Therefore, understanding the bacterial variations in the input environmental water, in terms of coliform and heterotrophic bacterial counts, over this period was necessary. The bacterial stability of input environmental over a 24 hour period, at ambient laboratory temperature (approximately 25°C), was established by taking samples (in duplicate) at 0, 6 and 24 hours.

The mean presumptive *E. coli* counts at 0 and 24 hours were 2.977 ± 0.032 $\log_{10}$ CFU 100 mL^{-1} and 3.190 ± 0.020 $\log_{10}$ CFU 100 mL^{-1}, whilst presumptive non-*E. coli* counts

were 3.123 ± 0.115 log_{10} CFU 100 mL^{-1} and 3.034 ± 0.113 log_{10} CFU 100 mL^{-1}, at 0 and 24 hours. Total coliform counts, the sum of presumptive and non-presumptive *E. coli*, remained somewhat constant throughput the 24 hours; 3.36 ± 0.054 log_{10} CFU 100 mL^{-1} (0 hours) and 4.045 ± 0.001 log_{10} CFU 100 mL^{-1}.

Heterotrophic bacterial plate counts incubated at 30°C for 3 days increased between 0 and 24 hours from 4.674 ± 0.167 log_{10} CFU mL^{-1} to 5.534 ± 0.081 log_{10} CFU mL^{-1}. However, the heterotrophic bacterial plate counts incubated at 22°C for 5 days remained relatively stable between 0 (5.339 ± 0.153 log_{10} CFU mL^{-1}) and 24 hours (5.638 ± 0.021 log_{10} CFU mL^{-1}).

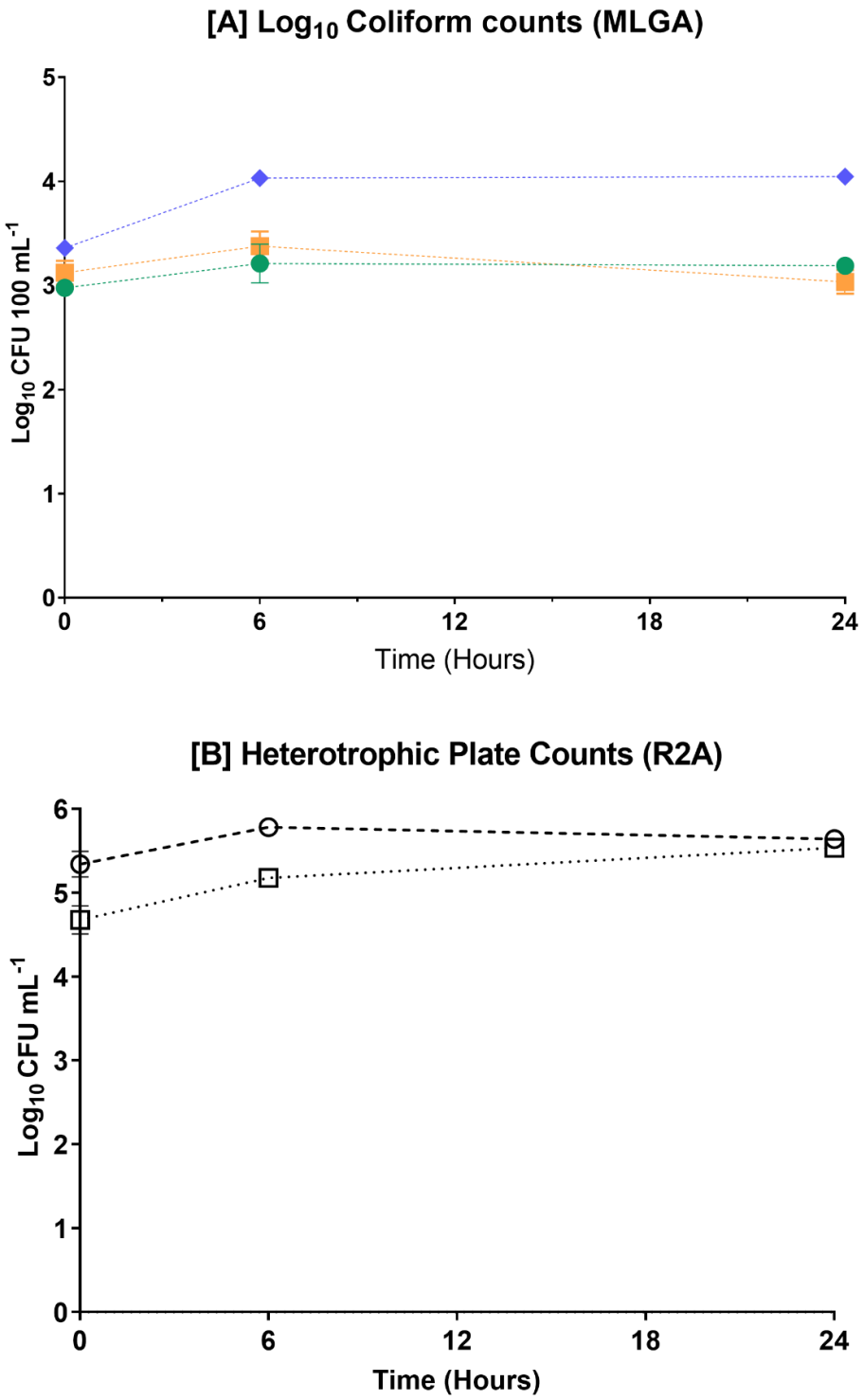

Figure 6.5: Coliforms [A] and heterotrophic bacteria [B] recovered from input environmental water over a 24-hour period, when kept at ambient laboratory temperature (approximately 25°C). Coliforms isolated through the filtration method and plated on selective MLGA. Presumptive *E.coli* (●) presumptive non-*E. coli* (■), and total coliform (◆). Heterotrophic bacteria were cultured on R2A agar and incubated at 22°C for 5 days (○) or 30°C for 3 days (□). (n = 2 ± SD).

6.2.2.2 Environmental water as bacterial source for (input) environmental biofilm formation

The Centre for Disease Control (CDC) biofilm reactor method was amended to develop a representative *in-situ* dosing environmental biofilm model. A schematic of this model is shown in Figure 6.6, and the method is described in Section 2.7.2.

This model was developed to determine the efficacy of NaOCl, HOCl and ECAS at managing environmental biofilm formation over a 48-hour period. The model allowed for *in-situ* dosing of disinfectants directly into tubing prior to the CDC biofilm reactor to a final free chlorine concentration of 50 mg L^{-1} (Figure 6.6). Polyethersulfone (PES) coupons (Figure 2.6[C]) were used as the substrate for biofilm formation. This material is commonly used within ultrafiltration membrane columns, due to its increased hydrophilicity, reducing the adsorption of organics, therefore improving operating performance (Inge GmbH, 2015).

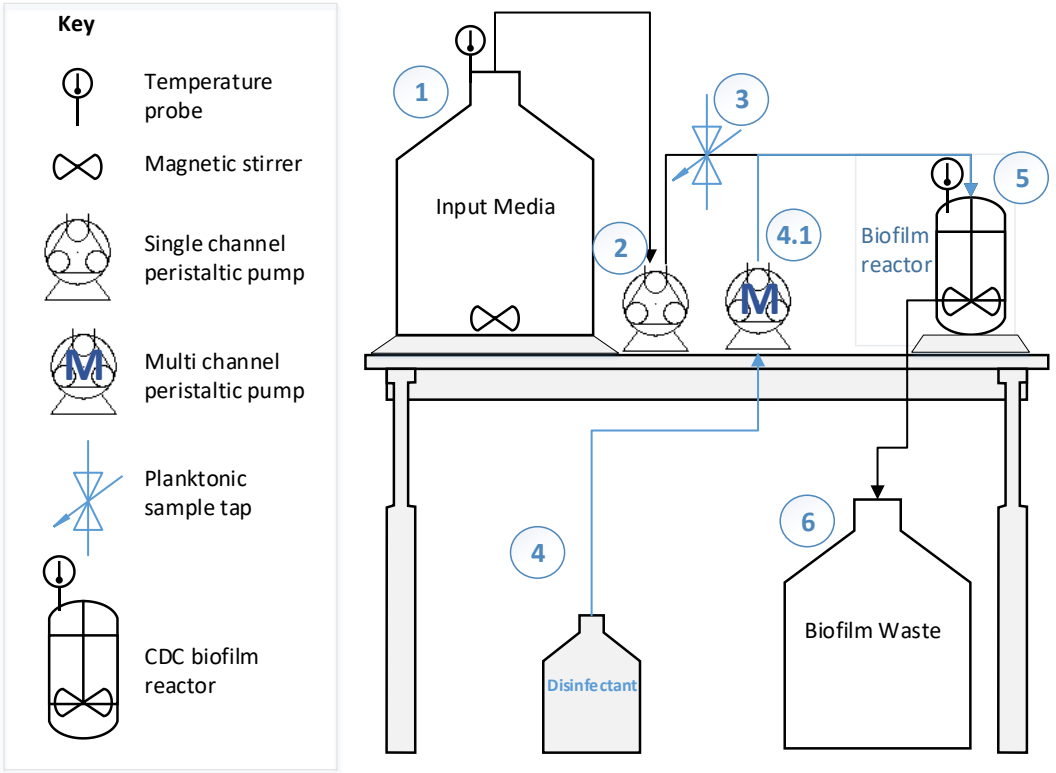

Figure 6.6: Schematic of the amended *in-situ* disinfectant dosing CDC reactor for managing environmental biofilm formation. [1] Input environmental water was stored in a sterile 20 L Nalgene carboy, [2] a single channel peristaltic pump, draws feed water towards the [3] sample tap. [4] Disinfectant solutions were stored in a sterile 10 L Nalgene carboy, and dosed into the CDC biofilm reactor through a [4.1] multichannel peristaltic pump to a final free chlorine concentration of 50 mg L^{-1} before entering the [5] CDC biofilm reactor. [6] Waste media from the CDC biofilm reactor was collected in a 20 L Nalgene carboy.

To demonstrate that environmental biofilms form on PES coupons (Figure 2.7 [C]) when using environmental water as the bacterial source (Figure 2.3) within the amended *in-situ* disinfectant dosing CDC reactor model, a dosing control (no disinfectant) was tested (see Figure 6.6).

Input environmental water required replacing after 24 hours as part of the amended *in-situ* disinfectant dosing CDC reactor model. Therefore, understanding the starting bacterial density within each media batch (environmental water) was needed. Environmental water heterotrophic plate counts (HPCs) were greater than total coliforms, for both incubation temperatures of 22°C [$HPC^{22°C/5days}$] and 30°C [$HPC^{30°C/3\ days}$] (Figure 6.7). Environmental water $HPC^{22°C/5days}$ were 4.686 ± 0.163 log_{10} CFU mL^{-1} and 3.802 ± 0.062 log_{10} CFU mL^{-1} for media batch 1 and 2, respectively, a significant difference ($p < 0.0001$). There were significant differences between media batch 1 and 2 for $HPC^{30°C/3\ days}$ ($p = 0.0036$), with bacterial counts of 4.952 ± 0.119 log_{10} CFU mL^{-1} to 4.440 ± 0.165 log_{10} CFU mL^{-1}, respectively. Total coliform counts in environmental water were 3.256 ± 0.334 log_{10} CFU 100 mL^{-1} for media batch 1, and 3.195 ± 0.524 log_{10} CFU 100 mL^{-1} for media batch 2 (Figure 6.7), with no significant difference ($p > 0.05$).

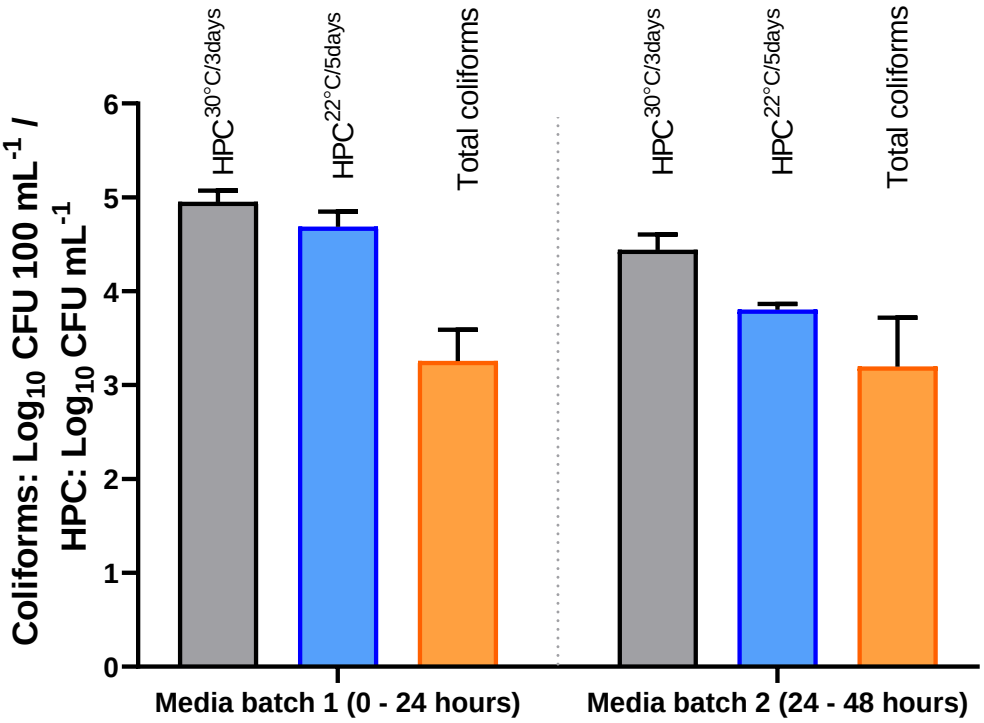

Figure 6.7: Heterotrophic plate counts recovered from environmental feed water. $HPC^{30°C/3\ days}$ [grey bar] refer to plates incubated at 30°C for 3 days, and $HPC^{22°C/5days}$ [blue bar] refer to plates incubated at 22°C for 5 days. Total coliforms [orange bar]. n = 6 ± SD.

The biofilm density recovered from PES coupons after 24 hours with continuous input environmental water flow, was 5.019 ± 0.440 log_{10} CFU $coupon^{-1}$ ($HPC^{22°C/5days}$) and 4.183 ±0.408 log_{10} CFU $coupon^{-1}$ ($HPC^{30°C/3\ days}$). After 48 hours the $HPC^{22°C/5days}$ biofilm density did not increase (4.761 ±0.281 log_{10} CFU $coupon^{-1}$), whereas the $HPC^{30°C/3\ days}$ biofilm density significantly increased to 5.230 ±0.064 log_{10} CFU $coupon^{-1}$ ($p < 0.0001$). The $HPC^{22°C/5days}$ biofilm density was less at 48 hours, than at 24 hours, this is likely a result of the significantly reduced bacterial load between media batch 1 and 2 (Figure 6.7). After 24 or 48 hours, biofilms from control experiments were disaggregated, from which no coliforms (sum of presumptive *E. coli* and presumptive non-*E. coli*) were recovered (Figure 6.8). Previous studies have shown that initial biofilm colonisation within water treatment systems is dominated

by heterotrophic bacteria (Leclerc, 2003; Wingender and Flemming, 2004), and this is supported by Figure 6.8.

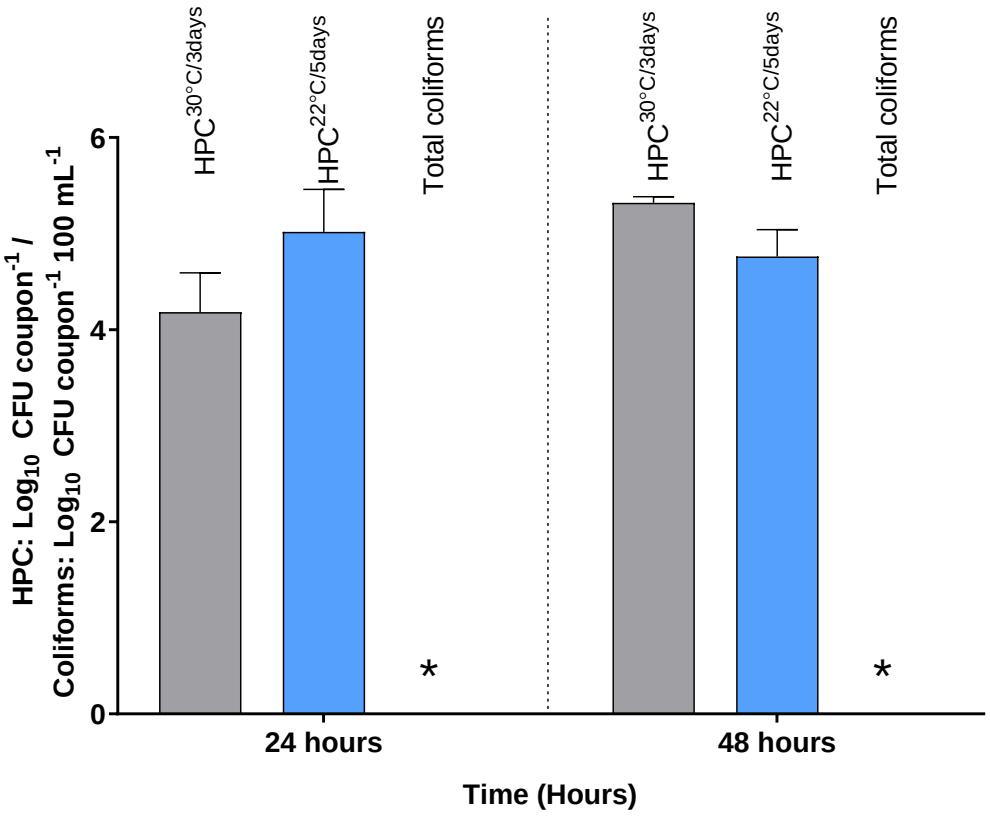

Figure 6.8: Heterotrophic plate counts recovered from environmental biofilms formed on PES coupons; $HPC^{30°C/3\ days}$ [grey bar] refer to plates incubated at 30°C for 3 days, and $HPC^{22°C/5days}$ [blue bar] refer to plates incubated at 22°C for 5 days. [*] Refer to no recoverable coliforms in disaggregated environmental biofilms. n = 6 ± SD.

6.2.2.3 *In-situ* dosing of disinfectants to manage environmental biofilm formation on PES coupons

The formation of environmental biofilms on PES coupons within the amended *in-situ* disinfectant dosing CDC reactor model over a 48-hour period (Figure 6.8), with continuous flow of environmental water is shown in Figure 6.8. The determination of the effectiveness of *in*-situ dosing of NaOCl, HOCl or ECAS on managing

environmental biofilm formation over a 48-hour period was undertaken. A free chlorine concentration of 50 mg L^{-1} was chosen, since this was the lowest free chlorine concentration whereby all disinfectants reduced *E. coli* ATCC 10536 by at least 5 log when tested using amended experimental conditions of BS EN 1276 (British Standards Institution, 2009), see Figure 6.9. At a standardised free chlorine concentration of 25 mg L^{-1}, NaOCl was the only disinfectant not to achieve a > 5 log reduction. This amended assay used input environmental water, taken from the modified artificial water body (Figure 2.3), as an interfering solution.

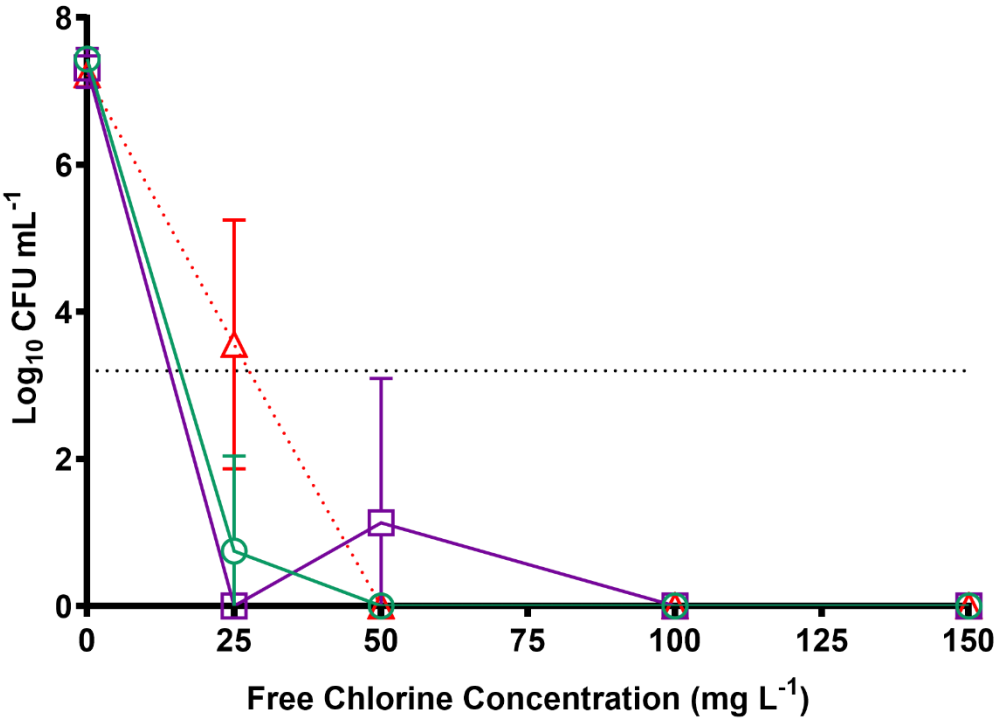

Figure 6.9: Antimicrobial efficacy of ECAS [○], HOCl [□] and NaOCl [△] using standardised free chlorine concentrations against *E. coli* ATCC 10536, with an interfering solution of environmental water. An amended BS EN 1276 assay was used (British Standards Institution, 2009). Dotted line represents the minimum log reduction (5 log CFU mL^{-1}) required to demonstrate basic bactericidal activity under experimental conditions of the assay n = 3 ± SD.

Figure 6.10 shows the comparative biofilm recovered from the PES coupons with no dosing (Figure 6.8 [control]), or dosed with either ECAS, HOCl or NaOCl, at a

standardised free chlorine concentration of 50 mg L^{-1}. Due to no coliforms being recovered in the control biofilm (no dosing [Figure 6.8]), they were not tested for as part of the *in-situ* disinfectant dosing experiments. All disinfectants resulted in significantly reduced biofilm density in comparison to the control ($p < 0.0001$). No significance differences were observed between any of the dosing regimens after 24 hours at either of the incubation temperatures. After 24 hours, no viable cells from disaggregated biofilms were recovered from PES coupons when dosed with either NaOCl or HOCl (Figure 6.10). Conversely, ECAS did not completely inhibit biofilm formation on PES coupons (Figure 6.10), resulting in a low density biofilm forming; 0.933 ± 2.158 log_{10} CFU $coupon^{-1}$ ($HPC^{30°C/3\ days}$) and 0.507 ± 1.716 log_{10} CFU $coupon^{-1}$ ($HPC^{22°C/5\ days}$).

After 48 hours the biofilm density when in the presence of ECAS dosing had increased to 1.156 ± 2.064 log_{10} CFU $coupon^{-1}$ ($HPC^{30°C/3\ days}$) and 1.160 ± 2.076 log_{10} CFU $coupon^{-1}$ ($HPC^{22°C/5\ days}$), although this was not significant ($p > 0.05$). Interestingly, after 48 hours a biofilm had formed in the presence of NaOCl dosing: 1.39 ± 2.071 log_{10} CFU $coupon^{-1}$ ($HPC^{30°C/3\ days}$) and 0.902 ± 1.817 log_{10} CFU $coupon^{-1}$ ($HPC^{22°C/5\ days}$). After 48 hours, biofilm density in the presence of NaOCl was significantly greater than HOCl ($p = 0.0075$ [$HPC^{30°C/3\ days}$]). The biofilm density in the presence of ECAS was also significantly greater than HOCl ($p = 0.0172$ [$HPC^{22°C/5\ days}$]). The only dosing regimen to have a significant increase in biofilm density between 24 and 48 hours was NaOCl ($p = 0.0025$ [$HPC^{30°C/3\ days}$] and $p = 0.0496$ [$HPC^{22°C/5\ days}$]). It is worth noting that HOCl was the only disinfectant tested not to have any recoverable biofilm after 48 hours, suggesting it exhibited the greatest antimicrobial activity under the experimental conditions.

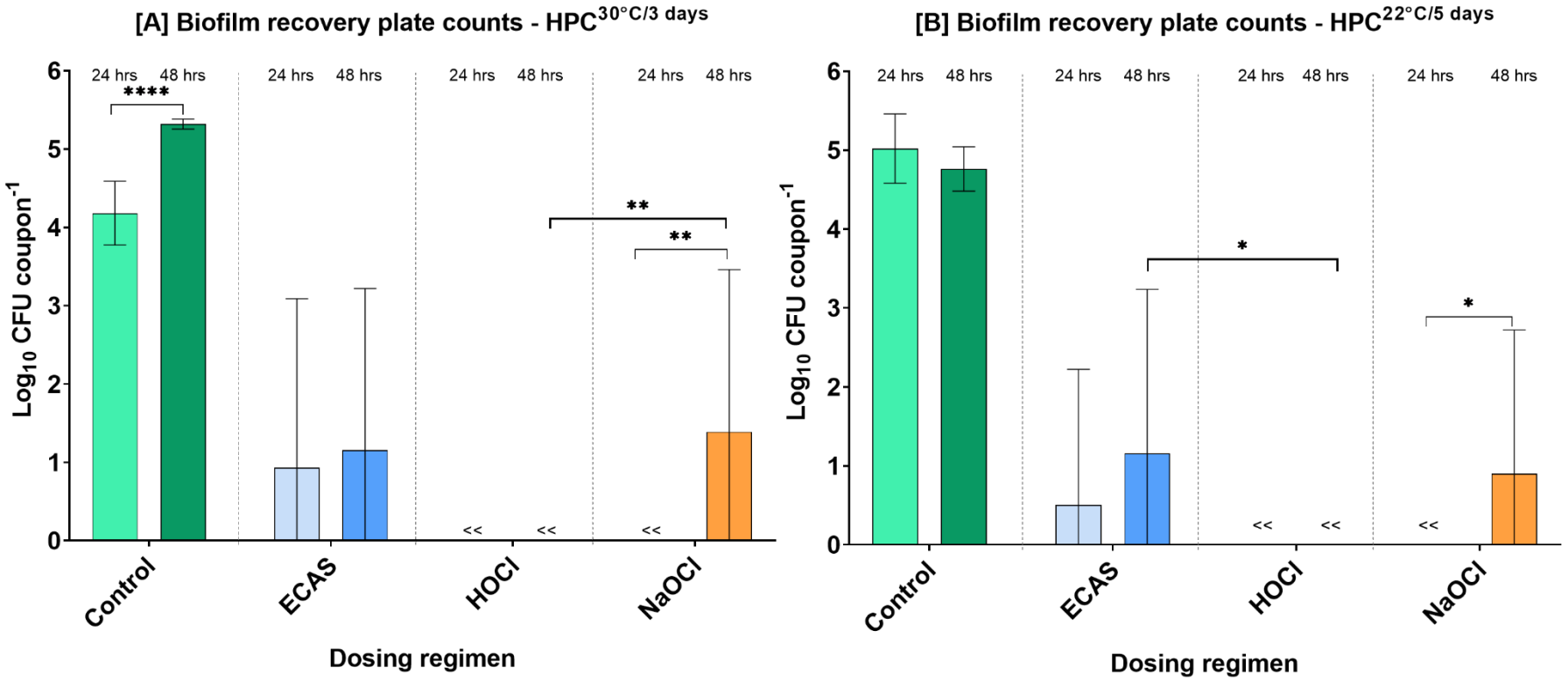

Figure 6.10: Viable biofilm density recovered from PES coupons (HPC) with no *in-situ* disinfectant dosing (control [green]), or in the presence of ECAS [blue], HOCl [white] or NaOCl [orange], at a matched final free chlorine concentration of 50 mg L^{-1}. [A] HPC incubated at 30°C for 3 days, and [B] HPC incubated at 22°C for 5 days. [<<] Plate counts below limit of detection n = 12 ± SD. Significant difference calculated through a two-way ANOVA with Sidak multiple comparison test, with a confidence interval of 95% (** = $p < 0.01$; * = $p < 0.05$).

6.3 Discussion

In summary, the results in this chapter demonstrate that ECAS dosing (0.5% [v/v]) does not significantly reduce *P. aeruginosa* biofilm, when compared to control (sterile DI) dosing. This is relevant as the ECAS dosing regimen used was commensurate with the decentralised drinking water treatment system discussed in Chapter 3 (Clayton, Thorn and Reynolds, 2019). Final biofilm densities of 7.978 ± 0.105 $\log_{10}$ CFU coupon^{-1} (ECAS) and 8.20 ± 0.230 $\log_{10}$ CFU coupon^{-1} (control [sterile DI]) were recovered from PC coupons after 174 hours.

However, single species biofilms rarely exist in natural environments (Hall-Stoodley, Costerton and Stoodley, 2004), thus, the experimental design was developed to represent an amended *in-situ* disinfectant dosing CDC rector model against environmental multi-species biofilms. Input environmental water, from the same artificial water body used to test the decentralised DWTS (Figure 2.3), was used as the bacterial source. The stability of the bacterial load within environmental water was determined over a 24-hour period (Figure 6.5). Coliform and heterotrophic bacteria monitored over 24 hours remained relatively constant, and so was used as the environmental water feed water source. PES coupons were used as the substrate for the environmental biofilm to form. To ensure that environmental bacteria would attach, resulting in the formation of biofilms that were recoverable and culturable, environmental water with no disinfectant dosing was used as a control (Figure 6.6). After 48 hours biofilm densities of 4.761 ±0.281 $\log_{10}$ CFU coupon^{-1} ($HPC^{22°C/5days}$) and 5.230 ±0.064 $\log_{10}$ CFU coupon^{-1} ($HPC^{30°C/3\ days}$) were recovered (Figure 6.8). The presence of coliforms within the biofilm were also tested for; however, none were recovered which is inline with previous literature (Leclerc, 2003; Wingender and Flemming, 2004). The introduction of *in-situ* disinfectant dosing regimens (final free

chlorine concentration of 50 mg L^{-1}) resulted in significantly reduced biofilm densities, compared to no dosing ($p < 0.0001$). However, after 24 hours, ECAS was the only disinfectant to result in recoverable bacterial cells from disaggregated biofilm, 0.933 ± 2.158 log_{10} CFU $coupon^{-1}$ ($HPC^{30°C/3\ days}$) and 0.507 ± 1.716 log_{10} CFU $coupon^{-1}$ ($HPC^{22°C/5\ days}$), see Figure 6.10. Conversely, no bacterial cells from disaggregated biofilms were recovered from NaOCl or HOCl dosing regimens, after 24 hours. However, biofilm was recovered from PES coupons in the presence of NaOCl after 48 hours, with 1.39 ± 2.071 log_{10} CFU $coupon^{-1}$ ($HPC^{30°C/3\ days}$) and 0.902 ± 1.817 log_{10} CFU $coupon^{-1}$ ($HPC^{22°C/5\ days}$) recovered. Throughout the ECAS dosing regimen there was no significant increase in biofilm density between 24 and 48 hours, whilst no bacterial cells were recovered throughout the HOCl dosing regimen. The experiments carried out within this and the preceding chapters (4 and 5) standardised the disinfectants by free chlorine concentration. However, one of the benefits of NaOCl, and its wide use as part of drinking water distribution networks, is the residual chlorine concentration (free available chlorine). This is also a trait of HOCl, but there is no difference between free and total available chlorine in ECAS, see Figure 2.2 [C]. Future work should compare the antimicrobial activity of the disinfectants when standardised to total available chlorine concentrations; this is discussed further in Section 7.2. Reducing the microbial load within bulk water through *in-situ* disinfectant dosing is one method to limit the potential of bacteria to attach to surfaces and form biofilms (Figure 1.6). However, managing biofilm formation within drinking water treatment infrastructure is far more complex as many factors need to be considered including nutrient availability (Percival and Walker, 1999; Chandy and Angles, 2001) and hydraulic conditions (Mathieu *et al.*, 2014; Fish, Osborn and Boxall, 2017).

6.3.1 Managing biofilm formation through *in-situ* disinfectant dosing within bench-scale models

One of the conclusions from chapter 3 was that UF membrane permeability throughout Field Trial 1 (0.5% [v/v]) was more stable (Figure 3.3), in comparison to Field trial 2, where no ECAS dosing regimen was employed (Figure 3.6). It was postulated that the low ECAS dosing regimen was sufficient to manage biofilm within the UF membranes. However, Figure 6.3 demonstrated that ECAS dosing (0.5% [v/v]) alone did not significantly reduce *P. aeruginosa* ATCC 15442 biofilm forming on PC coupons, in comparison to the control (sterile DI). Nonetheless, there was a marked visual difference between the biofouling present on the CDC biofilm reactor flow breaks between ECAS and control dosing regimens (Figure 6.4). This difference infers that ECAS may have been having an inhibitory effect in biofilm formation within the model tubing prior to the CDC biofilm reactor, but due to the low dosing regimen, did not maintain activity throughout the model. This could be due to the rapid antimicrobial properties associated with ECAS (Robinson *et al.*, 2011; Liao, Chen and Xiao, 2007), whereby, maintaining residual chlorine concentrations is unlikely. Single species biofilms are not representative of biofilms that may form as part of POU decentralised drinking water treatment systems. However, using environmental water as the bacterial source for multispecies biofilms provides more representative biofilms, which form within POU decentralised drinking water treatment systems, such as that outlined in Chapter 3 (Clayton, Thorn and Reynolds, 2019b).

The microbial load of the artificial water body used as the input environmental water for the biofilm models was determined, whereby both heterotrophic and coliform bacteria were present in the feed water (Figure 6.5). The densities obtained were in-line with the results obtained from water samples taken as part of the proof-of-

concept study (Clayton, Thorn and Reynolds, 2019b), where, as expected, heterotrophic bacteria and coliform were present in all untreated water samples, see Chapter 3. Control experiments demonstrated that environmental biofilms formed on PES coupons. This resulted in recoverable heterotrophic bacteria, although no coliforms were recovered and so were not tested for throughout the *in-situ* dosing experiments (Figure 6.7). However, it would be beneficial to monitor for coliforms in future experiments, to understand whether coliforms ever form part of the biofilm community. The experiments described in this chapter focussed on bacterial biofilms, and not on viruses, fungi and archaea present within environmental biofilms. Future experimental studies should consider including biofilm community analysis and this is discussed further in Section 6.3.2. It is worth noting that despite culturing viable bacteria from the environmental water source, it is likely that the actual bacterial load was greater due to viable but nonculturable (VBNC) bacteria present (Li *et al.*, 2014; Ramamurthy *et al.*, 2014). Such instances occur when bacteria become stressed after being taken from favourable environmental conditions, i.e. taken from environmental water source and plated onto foreign media, such as R2A, a low nutrient agar (Uhl and Schaule, 2004). Consequently, some bacterial species are unable to be cultured on laboratory media. However, alternative analytical methods, such as microscopy, co-culturing or 16S rRNA sequencing, their presence in environmental samples can be confirmed (Stewart, 2012).

The BS EN 1276 bactericidal assay (British Standards Institution, 2009) was amended to include environmental water as an interfering solution, to assess antimicrobial activity against *E. coli* ATCC 10536, under more representative conditions. HOCl and ECAS resulted in a significant reduction in *E. coli* at all free chlorine concentrations tested; 25, 50, 75, 100 and 150 mg L^{-1} (Figure 6.9), whereas NaOCl did not result in a 5-log reduction at the lowest free chorine concentration

tested, 25 mg L^{-1}. The physicochemical properties (i.e. ORP, pH and free chlorine availability) of the three disinfectants vary at point of generation. NaOCl has the lowest ORP (588 ± 0.95 mV) and highest pH (11.4 ± 0.1) of the three disinfectants tested. ECAS, however, has the highest ORP (1134 ± 3.26 mV) and most acidic pH (3.3 ± 0.16). A high ORP is representative of higher concentrations of rapid antimicrobial transient reactive species (Liao, Chen and Xiao, 2007), indicating that free chlorine is not the only antimicrobial property of the disinfectant. However, NaOCl which has the highest pH and ORP, is widely used throughout drinking water distribution networks due to residual chlorine concentrations which can maintain water quality.

Chapter 3 demonstrated that ECAS can produce Drinking Water Inspectorate standard drinking water (Clayton, Thorn and Reynolds, 2019b) from a highly biologically contaminated water source. It also showed that through continuous low dosing of ECAS, ultrafiltration membrane health can be maintained, and permeability can remain stable, over 18 operational days. One hypothesis for this stability, was that ECAS reduced the microbial load in the bulk water, decreasing the potential for biofilms for form. The total reduction of *E. coli* in the presence of ECAS with environmental water as an interfering solution, indicated that a reduction of microbial load in bulk water could manage biofilm formation (Figure 6.9). However, further amended BS EN assays, and up-scaled experiments with a wide variety of bacterial species (i.e. environmental isolates), would be required to validate this hypothesis.

Environmental biofilms were recovered after 48 hours in the presence of NaOCl and ECAS (50 mg L^{-1} free chlorine) dosing regimens (Figure 6.10). This demonstrates the

ability of environmental bacteria, whilst under unfavourable conditions, to continue to attach to surfaces and form biofilms (Campanac *et al.*, 2002; Schwering *et al.*, 2013; Mulamattathil, Bezuidenhout and Mbewe, 2014; Bernstein *et al.*, 2014). HOCl was the only disinfectant to result in no recoverable biofilm as part of this dosing regimen (Figure 6.10). This variability in environmental biofilm formation in the presence of chlorine-based disinfectants could potentially be due to their physicochemical properties. ECAS was the least effective at inhibiting bacterial attachment and managing biofilm formation. This could be due to the antimicrobial mode of action. ECAS contains high concentrations of reactive oxygen species (i.e. OCl^-, HOCl, OH) which when in contact with organic matter, react and then chemically relax, causing the solution to revert back to a weak saline (Petrushanko and Lobyshev, 2001; Huang *et al.*, 2008; Thorn, Robinson and Reynolds, 2013). The neutralisation of ECAS will result in decreased ORP, potentially reducing the antimicrobial efficacy. This hypothesis could be determined through monitoring the ORP throughout the CDC biofilm reactor model system, and should be considered in future work.

The antimicrobial mode of action for NaOCl relies on available chlorine to modify sulfhydryl groups in enzymes, inhibiting vital metabolic functions (Venkobachar, Iyengar and Prabhakara Rao, 1977; Virto *et al.*, 2005; Collivignarelli *et al.*, 2018; Fair *et al.*, 1948). NaOCl has been shown to disinfect rapidly (5.25%, < 30 seconds); however, antimicrobial activity reduces through dilution, requiring a longer reaction time to achieve equivalent disinfection (0.5%; 2 minutes) (Harrison and Hand, 1981). The observed increased antimicrobial activity of HOCl, in comparison to NaOCl, could be a combination a high ORP (958 ± 18.98 mV), which is indicative of the presence of reactive oxygen species (Liao, Chen and Xiao, 2007), whilst also relying on free chlorine. To further understand the effect of disinfectant pH on the

management of biofilm formation, measuring pH, free and total available chlorine throughout the CDC biofilm reactor model system should be considered for future work.

6.3.2 Quantitative and qualitative methods to measure biofilm formation

Biofilm reactor models are used to form biofilms under defined experimental conditions (Horn and Lackner, 2014; Tan *et al.*, 2017; Ashbolt and Storey, 2001). Traditionally, biofilm density is determined through plate count methods, using selective and non-selective agar. However, alternative analytical methods can provide additional information regarding quantification, cell viability, topography, density and community composition. Flow cytometry is a quantitative method which can rapidly quantify heterogenic populations of planktonic and biofilm populations (Kerstens *et al.*, 2015; Van Nevel *et al.*, 2017). Flow cytometry can mitigate issues with VBNC (Khan, Pyle and Camper, 2010), as cells are not cultured and quantified on laboratory agar media. Fluorescent staining kits (e.g. Live/Dead BacLight kit) can allow for the quantification of viable and total cells in samples (Berney *et al.*, 2007).

Confocal laser scanning microscopy (CLSM) can complement flow cytometry quantification methods by producing multidimensional images of biofilms (Schlafer and Meyer, 2017; Neu and Lawrence, 2015; Waller, Packman and Hausner, 2018a). CLSM can observe *in-situ* disinfectant activity on biofilms with additional fluorescent stains, such as Live/Dead (Sanchez-Vizuete *et al.*, 2015). Quantitative biofilm analysis can occur through inputting multidimensional CLSM images into modelling software (Schlafer and Meyer, 2017) which can determine co-localisation of colonies or the diffusion, or penetration, of disinfectants. CLSM imaging can also draw attention to

any significant or interesting features in biofilm architecture. A widely use alternative is scanning electron microscopy (SEM) which visualises biofilms at high resolution. Preparation of biofilms for SEM varies, but typically involve fixation onto a substrate, dehydrating before sputter coating with an electrically conducting metal, such as gold (Diao *et al.*, 2004; Priester *et al.*, 2007; Nguyen *et al.*, 2012). Control environmental biofilms (no treatment) formed over 48 hours, see Section 6.2.2.2, were prepared for SEM (Appendix II: SEM preparation protocol) to further understand the community architecture (Figure 6.11). However, these micrographs did not provide useful information with regards to the environmental biofilms formed, such as topography, density or composition. The difficulty in imaging the environmental biofilms could be a result of the fixation and dehydration method used (Appendix II: SEM preparation protocol), or that the biofilms were relatively low density (4.761 ± 0.281 $\log_{10}$ CFU coupon^{-1} - 5.230 ± 0.064 $\log_{10}$ CFU coupon^{-1}). Future experiments should explore alternative fixation/dehydration methods, or negating the need for such methods through environmental SEM (eSEM).

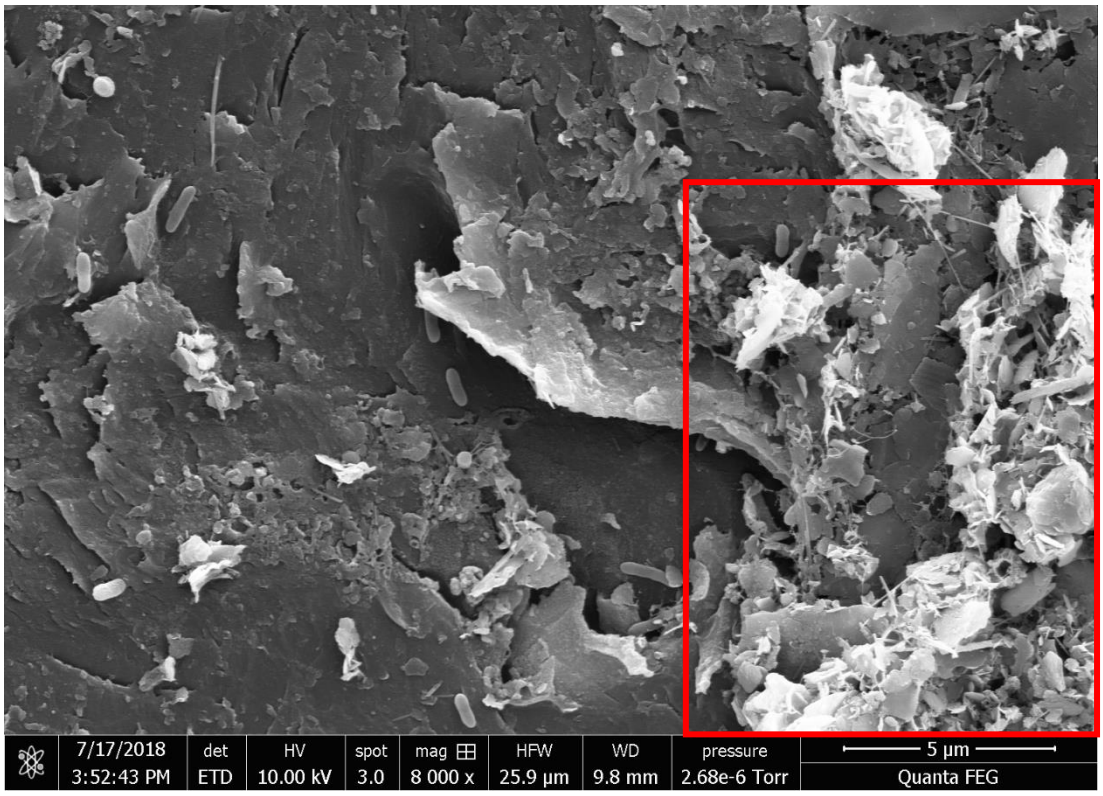

Figure 6.11: Scanning electron micrograph of an environmental biofilm formed after 48 hours. Red box (□) highlights potential disrupted environmental biofilm.

Visualising biofilm diversity through CLSM or SEM does not provide detail regarding organisms present. However, multispecies biofilm composition and diversity can be determined through molecular methods such as quantitative real-time polymerase chain reaction (qPCR) 16S rRNA gene sequencing. Understanding biofilm communities enables for VBNC organisms to be accounted for (Ramamurthy *et al.*, 2014), as well as provide an overview regarding community composition and diversity (Douterelo *et al.*, 2014; Montoya-Pachongo *et al.*, 2018). Such analytical techniques allow for an understanding of how microbial biofilm communities vary with differing input waters. This is important to ensure efficient treatment regimens are appropriately implemented to manage biofilm formation, and maintain biological water quality.

6.3.3 Managing biofilm formation in POU decentralised drinking water treatment systems

Developing methods that represent biofilm formation in POU decentralised drinking water treatment systems [DWTSs] are challenging. By way of comparison, an extensive number of studies have investigated managing the formation of biofilms throughout drinking water distribution networks (Fish, Osborn and Boxall, 2017; Reilly and Kippin, 1983; Ashbolt and Storey, 2001; Waller, Packman and Hausner, 2018b; Wingender and Flemming, 2004; Deines *et al.*, 2010; Juhna *et al.*, 2007). Potential control measures to prevent biofouling on membranes have been reviewed for water and wastewater treatment processes (Nguyen *et al.*, 2012). Biofouling is just as prevalent in POU decentralised systems (Pooi and Ng, 2018), but with resources (i.e. cleaning chemicals) potentially difficult to access, reliable technologies should be incorporated. Control measures include surface modification (Pasmore *et al.*, 2001; Yadav, Morison and Staiger, 2009), amended operating conditions (Crozes *et al.*, 1997; Yadav, Morison and Staiger, 2009; Derlon *et al.*, 2016), and the use of biocides (Shi *et al.*, 2014; Huang, Schwab and Jacangelo, 2009).

Biocides as a pre-treatment to membrane filtration reduces the number of viable bacteria within the bulk water, thus manages the potential for biofilm formation, providing a suitable dosing regimen be in operation. However, oxidising disinfectant solutions can damage membrane surfaces (Inge GmbH, 2015; Huang, Schwab and Jacangelo, 2009), although installing UV as a pre-treatment can minimise membrane damage. Biofouling reduces operational time through corrosion (Lehtola *et al.*, 2004; Beech and Sunner, 2004) or blockages (Renner and Weibel, 2011; Shi *et al.*, 2014; Bachmann and Edyvean, 2005; Flemming, 2002; Vargas *et al.*, 2014). There is a difficulty in developing representative models that determine and/or quantify biofilm density on ultrafiltration membranes without being destructive, or interrupting

operation. Conversely, there is evidence that, in UF membranes, biofilm structure is self-regulated to allow for nutrient channels to form which help maintain stable permeability (Peter-Varbanets *et al.*, 2010; Derlon *et al.*, 2014).

In-situ approaches to monitoring biofouling on membranes is through monitoring permeability (Equation 2-3), which is calculated using the transmembrane pressure (Equation 2-2) and filtration flux (Equation 2-1). A decrease in UF membrane permeability is indicative of biofouling (Nguyen *et al.*, 2012). Figure 6.3 indicates that ECAS dosing, in the absence of hydraulic conditions present in the UF membrane (i.e. cross-flow), was not the sole reason for stable UF membrane permeability observed within the decentralised DWTS (Figure 3.3 and Figure 3.6). Therefore, it is postulated that the combination of cross-flow hydraulics (Pentair, no date) and biocide (ECAS) dosing (1% total [v/v]) contributed towards the management of biofilm formation within this system, and ultimately reducing biofouling potential. However, further investigations are required to better understand these interactions. It would also be worthwhile investigating the use of HOCl within a decentralised POU DWTS, as it exhibited the greatest antimicrobial activity throughout bench-scale biofilm models experiments, as well as in standard chemical bactericidal assays (see Chapter 5).

6.4 Conclusions

- When dosing a single species biofilm model with ECAS or sterile DI (control) for 174 hours (8 days), with a 0.5% [v/v] dosing regimen, there was no significant difference in the density of *P. aeruginosa* biofilms that formed on PC coupons.
- When using a multi-species environmental water source, biofilms formed on PES coupons after 48 hours (no disinfectant dosing);
    - All disinfectants (NaOCl, HOCl and ECAS) resulted in significantly reduced biofilm density in comparison to the control;
    - Residual environmental biofilms were recovered from PES coupons in the presence of ECAS and NaOCl after 48 hours, whilst no environmental biofilm was recovered in the presence of HOCl.
- Future work should determine the effect of *in-situ* disinfectant dosing at managing biofilm formation on UF membranes within a POU decentralised DWTS.

Chapter 7. Final discussion

7.1 Summary

The overall aim of the research outlined in the preceding chapters was to develop point-of-use [POU] water treatment technologies for the production of drinking water. This was demonstrated by showing the capability of a proof-of-concept decentralised drinking water treatment system (Chapter 3), as well as the production of chemically (Chapter 4) and biologically (Chapters 5 and 6) safe drinking water by comparing three chlorine-based disinfectants, ECAS, sodium hypochlorite (NaOCl) and hypochlorous acid (HOCl).

Chapter 3 detailed the proof-of-concept decentralised drinking water treatment system [DWTS] that was used to demonstrate that drinking water could be produced from a heavily biologically contaminated artificial water body (chapter 3) to EU drinking water standards, specifically UK Drinking Water Inspectorate [DWI]. A field trial that incorporated a 1% total [v/v] ECAS dosing regimen resulted in the production of DWI standard water and stable UF membrane permeability over the 18-day field trial period. In contrast, the control field trial (i.e. no ECAS dosing) resulted in biologically unsafe drinking water being produced alongside a deterioration in UF membrane permeability. Numerous decentralised POU drinking water treatment technologies have been developed (Table 1-2) which vary in complexity, scale (e.g. personal, household or community), reliability and resource requirements (Pooi and Ng, 2018; Loo *et al.*, 2012; Peter-Varbanets *et al.*, 2009). These decentralised treatment technologies improve treated water quality, but not to internationally recognised drinking water regulation standards, such as those stated by the Drinking Water Inspectorate [DWI] (Drinking Water Inspectorate, 2012) or the World Health Organization (World Health Organization, 2011). However, the

quality of the treated water from the field trails described in chapter 3 were compared to such standards (see Table 2-1). The research undertaken as part of the proof-of-concept study raised additional research questions in the production of chemically and biologically safe drinking water as part of POU systems. Firstly, to ascertain the comparative formation of total trihalomethanes (tTHMs) in water when treated with NaOCl, HOCl or ECAS, as a function of free chlorine and reaction time and secondly ensuring that alternative disinfectants (e.g. ECAS) in comparison to commonly used disinfectants (e.g. NaOCl and HOCl), do not produce substantially higher tTHM concentrations that represent a health concern due to their carcinogenic and mutagenic properties (Liang and Singer, 2003; Dodds and King, 2001; King, Marrett and Woolcott, 2000; Grazuleviciene *et al.*, 2013; Siddique *et al.*, 2015).

The research described in Chapter 4 demonstrates that NaOCl disinfectant produced significantly greater ($p < 0.01$) amounts of tTHMs, compared to HOCl and ECAS, at all free chlorine concentrations and reaction times. The comparable tTHM concentrations between natural organic matter [NOM], and HOCl or ECAS is potentially a result of the neutral/acidic disinfectant properties, i.e. lower reaction pH (Stevens *et al.*, 1976; David, 2014; Rasheed *et al.*, 2017). Chloroform was the dominant THM species present for all disinfectants for all reaction times and all free chlorine concentrations (> 75%), which is in accordance with literature regarding the formation of THMs within drinking water treatment (Ikem, 2010; Cho, Kong and Oh, 2003; Zhang *et al.*, 2015). This study investigated the comparative formation of tTHMs between three chlorine-base disinfectants, but in future work where a more quantitative approach is required, alternative analytical techniques, such as GC-ECD (see Section 4.3.2), should be employed.

Developing a greater understanding of the antimicrobial activity of ECAS, in the context of POU drinking water, for the production of biologically safe drinking water was investigated. Ensuring the production of biologically safe water can help manage the spread of waterborne diseases (e.g. diarrhoeal) which are known to cause approximately 800,000 deaths each year (Corcoran *et al.*, 2010; World Health Organization, 2016b; Prüss-Ustün *et al.*, 2014). Therefore, managing the spread of waterborne diseases through POU DWTS is working to achieve Sustainable Development Goal 6.1: "*achieving universal and equitable access to safe and affordable drinking water for all".*

Chapter 5 compared the antimicrobial activity of ECAS against NaOCl and HOCl against standard microbial challenges in planktonic phase, and as biofilms. Within planktonic assays against *Escherichia coli* ATCC 10536, neutral (HOCl) and acidic (ECAS) disinfectants exhibited greater antimicrobial activity in comparison to NaOCl, an alkaline disinfectant (see Figure 5.1, Figure 5.2 and Figure 5.3). Against a mature *Pseudomonas aeruginosa* ATCC 15442 biofilm, ECAS exhibited significantly higher antimicrobial activity at free chlorine concentration ≥ 50 mg L^{-1}, compared to NaOCl and HOCl ($p < 0.0001$). The increased antimicrobial activity of ECAS is thought to be due to transient antimicrobial species (e.g. OH^-, O_3, H_2O_2 and O_2^-), which are very reactive, providing much of the antimicrobial activity within the first 10 seconds (Robinson *et al.*, 2011; Liao, Chen and Xiao, 2007), leaving chlorine species (HOCl or OCl^{-1}) to provide further disinfection. The lowest free chlorine concentration tested against *P. aeruginosa* biofilms (5 mg L^{-1}), resulted in a significant log reduction for all disinfectants tested. However, at this concentration NaOCl disinfectant produced significantly higher tTHMs in comparison to HOCl and ECAS after a 5-minute reaction time. Thus, HOCl and ECAS would be the suggested disinfectant in providing biologically safe water, without producing high concentrations of unwanted tTHMs.

The decentralised DWTS discussed in chapter 3 utilises *in-situ* ECAS disinfectant dosing to produce biologically safe water, and maintain stable ultrafiltration [UF] membrane permeability. Consequently, representative biofilm models were developed to compare chlorine-based disinfectants to manage single (*P. aeruginosa* ATCC 15442) and multi-species (environmental) biofilms (chapter 6). The long-term *in-situ* ECAS dosing CDC reactor model employed a dosing regime commensurate with the decentralised DWTS (0.5% [v/v]) resulted in no significant difference in biofilm formation after 8 days with an ECAS or sterile DI (control) dosing regime ($p = 0.0572$). This is likely due to ECAS not maintaining antimicrobial activity throughout the model. Development of the *in-situ* ECAS dosing CDC reactor model included the use of a representative substrate (polyethersulfone [PES]) and environmental water from the artificial water body (Figure 2.3), as the bacterial source. This resulted in the amended *in-situ* disinfectant dosing CDC reactor model, which compared the efficacy of NaOCl, HOCl and ECAS in managing environmental biofilm formation when dosed at 50 mg L^{-1} free chlorine. This was the lowest free chlorine concentration where all disinfectants reduced *E. coli* ATCC 10536 by ≥ 5 log, under amended BS EN 1276 experimental conditions (British Standards Institution, 2009). All disinfectants resulted in significantly reduced biofilm density in comparison to the control ($p < 0.0001$). However, after 48 hours, residual environmental biofilms were recovered from PES coupons under ECAS (1.156 ± 2.064 log_{10} CFU $coupon^{-1}$ [$HPC^{30°C/3\ days}$] and 1.16 ± 2.076 log_{10} CFU $coupon^{-1}$ [$HPC^{22°C/5\ days}$]) and NaOCl (1.392 ± 2.071 log_{10} CFU $coupon^{-1}$ [$HPC^{30°C/3\ days}$] and 0.902 ± 1.817 log_{10} CFU $coupon^{-1}$ [$HPC^{22°C/5\ days}$]) dosing regimes (Figure 6.10), whilst no environmental biofilm was recovered in the presence of HOCl. The density of cells from environmental biofilms recovered from the amended *in-situ* disinfectant dosing CDC reactor model is likely to be lower than the actual microbial numbers present (Li *et al.*, 2014; Ramamurthy *et al.*, 2014). This is mostly due to the difficulty in culturing and enumerating environmental microbial species on laboratory media. However,

alternative quantitative or qualitative analysis can be employed in future experiments (see Section 6.3.2) to provide more accurate and representative environmental biofilm densities. Such analysis can shed light on community composition, diversity and architecture, which would be important through developing and improving the decentralised DWTS.

The experiments outlined within chapters 3 – 6 have subsequently informed the development of a POU minimum viable product [MVP] by Portsmouth Aviation Ltd (PAqua 1000D-2 Fresh Water Purification System). This system specifications can produce 1000 L of drinking water per hour to WHO drinking water standards (World Health Organization, 2011). This MVP unit is due to be trialled in India as part of a Natural Environment Research Council [NERC] India-UK Water quality project (NE/R003106/1). This project combines fresh water quality sensing technologies with the deployment of a decentralised DWTS to remove pollutants, to "enhance water protection and security."

The key findings from this research are:

- ECAS or HOCl resulted in the formation of significantly lower tTHM concentrations, compared to NaOCl, across all reaction times and free chlorine concentrations, with standardised NOM source water;
- The antimicrobial efficacy of ECAS was comparable to HOCl and NaOCl, when assessed against standard microbial challenges;
- HOCl exhibited the greatest antimicrobial activity throughout *in-situ* disinfectant dosing experiments, whilst ECAS was comparable to NaOCl.

7.2 Recommendations for Future Work

The decentralised POU DWTS outlined in Chapter 3, is capable of producing DWI standard drinking water from biologically contaminated water sources (Clayton, Thorn and Reynolds, 2019b). To understand the effectiveness of each process stage (i.e. settle tank, UF membranes), future trials should sample at each process stage within the DWTS. It would also be interesting to investigate the treated water quality if no pre-UF dosing occurred, and only an ECAS post-UF dose occurred, potentially reducing the volume of ECAS required within the system.

To assess the capability of the DWTS, long-term trials to understand the lifetime of the UF membranes before permeability drops and water quality falls would be beneficial. The DWTS has been assessed with water taken directly from a modified artificial water body with a high microbial load, however, trialling the system on a wide variety of source waters would be useful to determine how effective the system is with varying water qualities and contaminants (i.e. fertilisers, heavy metals, saline concentration). However, in order for the decentralised POU DWTS to be suitable for use in a wide number of applications and locations, further developments would be required. It is currently unknown whether the decentralised POU DWTS can treat waters with high concentrations of agricultural fertilisers (e.g. phosphates [PO_4^-] or nitrates [NO_3^-]), or waters with high salt (NaCl) content (e.g. brackish or seawater). Installing nanofiltration [NF] membranes could provide solutions to reduce fertilisers and salts in treated waters (Hilal *et al.*, 2005; Al-Zoubi and Omar, 2009). NF membranes are lower energy alternatives to reverse osmosis membranes (Mohammad *et al.*, 2015). The installation of NF membranes into the decentralised POU DWTS would provide the ability to treat a wide variety of fresh water sources contaminated with agricultural fertilisers, reducing eutrophication events. They

would also allow the decentralised POU DWTS to be deployed for communities living in coastal areas, or as an alternative solution for disaster relief where freshwater sources have been contaminated with seawater (e.g. hurricanes).

The decentralised POU DWTS currently utilises low ECAS dosing and UF membranes to control bacteria throughout the system. However, UF membrane permeability reduces due to biofilm formation (e.g. biofouling), causing reduced operational periods, and necessitating subsequent chemical cleaning. Biofilters have been utilised in decentralised water treatment systems (Wendt *et al.*, 2015), due to low energy requirements, in comparison to commonly used UF membranes. The concept of biofilters are to utilise a biofilm community's need for nutrients (e.g. natural organic matter, phosphates, nitrates, ammonia), which reduces nutrient concentrations in bulk waters (Proctor and Hammes, 2015; Lautenschlager *et al.*, 2013). Nutrient reductions are most effective after several treatment cycles, creating an unfavourable environment for planktonic bacteria to survive. Incorporating biofilters into a decentralised POU DWTS can also reduce DBP precursors (e.g. NOM) within input waters (McKie *et al.*, 2019; Fu *et al.*, 2017), as well as remove pharmaceuticals and personal care products (Fu *et al.*, 2019).

The results within chapters 4 – 6 demonstrate that HOCl and ECAS were comparable in tTHM formation and antimicrobial efficacy. Future work should investigate the formation of other DBPs, through ECAS disinfection; this would be beneficial to ensure that treated water is chemically safe. It would also be interesting to determine tTHM formation in the presence of varying organic concentrations and types (i.e. fulvic acid, microbial biofilms), as well as determine tTHMs formation at extended contact times which are representative of treated water stored in drinking water

tanks. It would also be beneficial to determine the tTHMs concentration from treated waters sampled from the DWTS, with various input waters.

Chapter 5 compared the antimicrobial activity of ECAS, HOCl and NaOCl against *E. coli* and *P. aeruginosa*, future experiments should include a wide variety of microbial organisms that are frequently associated with poor water quality (i.e. *Enterococci spp., Campylobacter spp.,* and *Aeromonas spp.*). It would also be beneficial to determine the effect of the disinfectants at various contact times and at lower free chlorine concentrations, commensurate with *in-situ* dosing in POU systems.

However, one of the benefits of ECAS is *in-situ* generation (see Section 1.4.2.2) requiring only salt, water and energy. This reduces the need for hazardous chemicals to be stored. Additionally, ECAS has been proven to be effective as a surface disinfectant in a wide number of settings, allowing for it to be used as a general disinfectant improving hygiene and sanitation. There are many variations in ECAS generators, and some low-energy ECAS generators are able to produce solutions with solar power (Witt and Reiff, 1993; Centrego, 2015). ECAS generators that produce a neutral solution (e.g. pH 5 - 6.5), by combining the anodic and cathodic solutions, have greater total available chlorine concentrations, in comparison to acidic ECAS where free and total chlorine are equivalent (Figure 2.2 [C]). Total chlorine provides residual disinfection, which is beneficial if treated water is stored or distributed.

Based on the findings within this thesis, investigating the use of HOCl within decentralised POU DWTS systems should be considered due to its high antimicrobial properties and the reduced tTHM formation, in comparison to NaOCl. However, the environmental legacy of HOCl (formed from NaDCC) is far greater than ECAS, which

reverts back to a weak saline during chemically relaxation (Petrushanko and Lobyshev, 2001; Huang *et al.*, 2008; Thorn, Robinson and Reynolds, 2013). HOCl is very toxic to aquatic life, and can cause eye and respiratory irritation (Guest Medical, 2018). This can pose hazards to humans and the environment if incorrectly stored or managed.

Managing decentralised POU DWTS infrastructure is important in ensuring the reliable production of high quality drinking water. Biofilms, if ineffectively managed, can result in biofouling and non-operational periods. Investigating sufficient management strategies, such as disinfection and backflush procedures, to minimise their effect is necessary. The interaction between natural organic matter [NOM] and disinfectants which result in disinfection by-products has been widely investigated (Rook, 1976; Brown, Bridgeman and West, 2011b; Liang and Singer, 2003). However, an emerging area of research is investigating biofilms as a source of organic material which, when react with disinfectants, result in the formation of disinfection by-products [DBPs] (Wang *et al.*, 2013; Abokifa *et al.*, 2016). Understanding the interactions of disinfectants as part of biofilm management within decentralised POU DWTS is beneficial in ensuring that biologically and chemically safe drinking water is produced, whilst managing infrastructures. Future experiments should include the development of analytical models representative of dosing regimes within POU DWTS which can monitor, in real-time, the effect of *in-situ* disinfectant dosing of biofilms, and resultant DBP formation, to ensure that high quality drinking water is produced.

With developments in water quality testing and increased awareness of pollutants entering water sources, there are a vast number of emerging contaminants, such as microplastics [MPs], endocrine disrupting chemicals [EDCs] and antibiotics, which

require additional remediation strategies. MPs, EDCs, chemicals present in pesticides, metals, food preservatives, pharmaceuticals and personal care products World Health Organization, 2016a), and antibiotics have become ubiquitous in nature, as a result of increased population growth, industrialisation and mismanagement. These contaminants not only pose potential operational issues for drinking water treatment (i.e. blockages of membranes and pipework), but could lead to adverse, or unknown, effects on human health after water consumption. EDCs have been associated with negative impacts on reproduction, increased incidences of breast and testicular cancer (Bergman *et al.*, 2012), neurodevelopmental delays in children and changes in immune function (World Health Organization, 2016a). Whilst antibiotic resistance in low/middle income countries has increased dramatically due to the misuse of antibiotics, insufficient water management, as well as poor sanitation and hygiene services (Reardon, 2014; Okeke, Lamikanra and Edelman, 1999). Developing analytical methods, as well as treatment processes, that can rapidly and reliably quantify MPs, EDCs and antibiotics present in treated water is necessary to ensure safe drinking water is produced.

Research into alternative drinking water treatment technologies, such as ECAS or biofiltration, which can be installed within decentralised POU DWTS should be assessed to determine their ability to manage emerging contaminants. For example, monitoring the presence of antibiotic resistant bacteria before and after treatment can be achieved through susceptibility testing (Mulamattathil, Bezuidenhout and Mbewe, 2014; Bernstein *et al.*, 2014) or through metagenomic studies profiling antibiotic resistance genes (Li et al., 2015).

Further developing the decentralised POU DWTS to mitigate for emerging contaminants, whilst producing high quality drinking water requires long-term trials.

Such trials should ensure that the decentralised POU DWTS is capable of treating water from a variety of input waters, with various contamination concerns (e.g. EDCs and antibiotic resistant bacteria). This will occur as part of the NERC India-UK Water quality project (NE/R003106/1).

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Appendix I: UF membrane cleaning protocols

Pentair Protocol for column cleaning

Stock solutions:

- Sodium hypochlorite (5% bleach) at 2000 mgL^{-1}
- Sodium hydroxide(NaOH) at5250 mgL^{-1}
- Hydrochloric acid (HCl) 4500 mgL^{-1}

Note: ALL stock solutions shall be diluted 1:10 before use

Protocol

1. Ensure cleaning takes place OUTSIDE 0J17 (Environmental Field Centre)
2. Remove Modules retaining screws and remove modules
3. Drain modules by holding upside down over drainage channel
4. Attach inlet and outlet connectors. Remove O-rings to reduce damage
5. Place of level surface and SLOWLY add 5 litres of tap water to INLET port to flush out
6. Make note of flushed water (colour, particles, contaminants etc.)
7. Make up 8 litres of CEB 1 (200 mgL^{-1} of sodium hypochlorite and 525 mgL^{-1} of sodium hydroxide)
8. Slowly pour solution into INLET port of modules and allow to overflow out of the PERMEATE and CONCENTRATE ports.
9. Leave CEB 1 solution in modules for 10 minutes
10. After 10 minutes flush out CEB 1 solution using tap water
11. Make up 8 litres of CEB 2 (450 mgL^{-1} of hydrochloric acid)
12. Slowly pour CEB 2 solution into INLET port of modules and allow to overflow out of the PERMEATE and CONCENTRATE ports.
13. Leave CEB 2 solution in modules for 10 minutes
14. After 10 minutes, flush out CEB 2 solution using tap water until running clear with no visible particles.
15. Drain modules fully
16. Re-attach modules into LineGuard fixture.

Appendix II: SEM preparation protocol

Protocol provided by Patton (2018).

Fixation

Fix in 4% glutaraldehyde in buffer (usually 0.1M)*

Leave for 1 hr at room temperature or 24hrs in the fridge. Remove glutaraldehyde and store for official disposal.

Rinse in buffer x3 (total storage time should exceed fixation time by a factor of 3).

SEM Dehydration

- 5m in 20% ethanol
- 5m in 30% ethanol
- 5m in 50% ethanol
- 5m in 70% ethanol
- 5m in 80% ethanol
- 5m in 90% ethanol
- 5m in 100% ethanol
- 5m in 100% ethanol
- 5m in 100% ethanol

- 5m in 100% ethanol / HMDS (2:1)
- 5m in 100% ethanol / HMDS (2:2)
- 5m in 100% ethanol / HMDS (1:2)
- 5m in 100% HMDS
- 5m in 100% HMDS
- 5m in 100% HMDS

Remove specimen from HMDS and place on filter paper in a petri dish and leave in fume hood until dry.

***For 4% glutaraldehyde in 0.1M buffer or PBS**

Take, say, 50mls of 0.2M buffer, 34ml d water and 16ml of 25% glutaraldehyde.

Appendix III: Publications

[1] Clayton, G.E., Thorn, R.M.S. and Reynolds, D.M. (2019) Development of a novel off-grid drinking water production system integrating electrochemically activated solutions and ultrafiltration membranes. *Journal of Water Process Engineering* DOI: 10.1016/j.jwpe.2017.08.018.

[2] Clayton, G E; Thorn, R.M.S and Reynolds, D.M (2019) Comparison of trihalomethane formation using chlorine-based disinfectants within a model system; applications within drinking water treatment *Frontiers in Environmental Science: Water and Wastewater Management*. DOI: 10.3389/fenvs.2019.00035

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Development of a novel off-grid drinking water production system integrating electrochemically activated solutions and ultrafiltration membranes

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ABSTRACT

Approximately 800 million people live without clean drinking water. Diarrhoea is responsible for between 1.7 and 2 million deaths each year (primarily children) which are the result of poor drinking water quality and sanitation. The main aim of this study was to demonstrate the production of drinking water from a raw water source using an off-grid drinking water production system. The off-grid drinking water production system (DWPS) developed at UWE Bristol, combines an ultra-filtration (UF) system with in situ generation of electrochemically activated solutions (ECAS). ECAS has two functional roles within the system; to manage biofilms within the UF system and as a disinfectant. Integrated in-situ probes (pH, oxidation reduction potential, chlorine, conductivity and dissolved oxygen) coupled with a water quality sensing network (pH, water temperature, conductivity and dissolved oxygen) enabled real time monitoring of; the operational efficiency of the DWPS, and the physicochemical parameters of both the raw water source and the produced drinking water. Spot samples of both raw and treated water were sent for independent chemical and microbial analysis at an accredited laboratory which demonstrated that the DWPS produced biologically safe potable drinking water according to the Drinking Water Inspectorate (DWI) standards. Samples from the raw water source were shown to be consistently unsuitable for human consumption, failing several of the DWI standards for potable water supply, including coliform bacteria. This study demonstrated that the novel off-grid DWPS was capable of producing DWI standard drinking water from a heavily biologically contaminated water source.

1. Introduction

An estimated 800 million people worldwide do not have access to improved drinking water sources [1–3], with 1.2 billion people unable to access reliable electricity sources [4,5]. Therefore, there is a need for low energy technological solutions for the provisioning of safe drinking water. By the end of the 21st century the global population is expected to increase to 9–10 billion [6], this is likely to generate increased stress on water and power (gas and electricity) resources worldwide. Sufficient safe drinking water provisioning for an increasing population will require the development of sustainable, reliable and robust water treatment systems. The consumption of contaminated water, or poor water quality, is the cause of between 1.7 and 2 million deaths each year from diarrhoeal diseases [7–10]. The majority of these deaths are in developing or transitional countries which have inadequate sanitation conditions [11], and do not have established water distribution systems. Developing countries have economies with little industrial development, whilst aiming to improve quality of life through increasing food and water security [12]. Transitional countries often have emerging economies with a prominent secondary manufacturing industry; however, there is still considerable rural and peri-urban poverty [12]. Developed countries have established centralised water, gas and electricity (power) networks, which supply the majority of a country's population with sufficient water, gas and electricity [4]. Developing and transitional countries do not have the same established water and power networks, resulting in many remote, rural or temporary communities unable to access reliable and safe power and drinking water [4]. In developing or transitional countries, communities which are unable to access improved water sources often live in remote or transitory locations, therefore 'centralised' drinking water treatment facilities and distribution systems are not sustainable options from a financial or resource efficient perspective.

Research into decentralised, or off-grid, drinking water treatment systems for developing countries has gained momentum due to unfeasible practicalities with centralised provision [10,13–15], and are an important element in the process of reaching the Millennium

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Development Goals. Some decentralised systems focus on rainwater harvesting [10,16,17], solar based disinfection [18,19], or the physical removal of contaminants within treated water through sand bed filters [20,21] or ultrafiltration (UF) [15,22]. Since the main drinking water risks in developing countries are still associated with microbial contamination, many decentralised systems continue to use established disinfection techniques such as UV [18], chlorination [23,24], or ozonation [14]. Even when disinfection agents (e.g. chlorine) are used, the presence of suspended material and colloids in the water can reduce their efficacy, ultimately enabling bacterial growth after treatment [10]. In addition, these disinfection techniques require the regular purchase, transportation and storage of hazardous chemicals and for developing or transitioning countries, this can prove expensive and logistically challenging. A key advantage of off-grid systems is the modular capability, whereby, the production of drinking water output can be increased to cope with increasing populations/demand.

Electrochemically activated solutions (ECAS), are known by several terms, the most common being electrochemically activated water (ECAW), electrolyzed water (EW), electrolyzed oxidising water (EOW) and mixed oxidant (MIOX) solutions. These solutions are generated by passing a weak salt solution (e.g. NaCl), through an electrochemical cell, whereupon a direct current is applied. Electrochemically activated solution generated at the anode, referred to in this paper as ECAS, is acidic in nature and possesses antimicrobial chemical species including hypochlorous acid (HClO) and other transient oxidative functional groups [25–27]. ECAS have a short environmental legacy, reverting back to a saline solution during chemical relaxation [25], and are often referred to as 'green biocides' [28,29]. These solutions have been shown to have a beneficial application within; the fresh produce industry [27,30–34], healthcare settings [25,26,35] and drinking water treatment [24,36,37], due to extremely fast acting kill kinetics e.g. < 10 s [38,39].

The main aim of this study was to demonstrate the production of drinking water from a raw water source (artificial water body) to Drinking Water Inspectorate (DWI) standards, using a decentralised, off-grid, *drinking water production system* (DWPS). The European Council set guidelines for water quality which is safe for human consumption [40], which is interpreted by each European Union member state. In the United Kingdom the Drinking Water Inspectorate (DWI) interprets and regulates drinking water quality.

2. Materials and methods

2.1. Off-grid drinking water production system

A technical schematic of the off-grid drinking water production system (DWPS) is shown in Fig. 1. Raw water, from an artificial water body (an urban drainage holding pond, UWE Bristol, UK [N51°29′56″, W2°32′39″]), is pumped to a settle tank within the drinking water production system through an intake submersible filter pump (115 µm) and a reverse flushing filter (100 µm). A peristaltic pump draws water from the settle tank into the UF membrane columns ([0.02 µm] Line-Guard UF-100, Pentair). ECAS is generated as per details in Section 2.2, and subsequently stored in the ECAS reservoir tank (100L). ECAS is dosed directly into the DWPS pipework, immediately before (A) and after (B) the UF membrane columns via automated peristaltic dosing pumps. Treated water is then stored in the 400 L treated water tank. To monitor the health of the UF membranes, pressure gauges are installed before and after the UF membrane columns.

2.2. Electrochemically activated solution (ECAS) generation

ECAS was generated using a 60 L ESOL™ generator (Bridge Biotechnology, Fife, Scotland) through the electrolysis of a 1% (w/v) NaCl solution under a direct current (Fig. 2). Solutions were generated (anodic solution) to an oxidation reduction potential (ORP) of 1130 mV, and subsequently held and stored within a 100 L reservoir tank until required. Peristaltic dosing pumps enabled precise dosing of ECAS directly into the DWPS pipework pre- and post- ultrafiltration (UF) column membranes.

2.3. DWPS field trials

Two field trials were performed. Field trial 1 consisted of dosing 0.5% (v/v) ECAS pre- and post- UF membranes. Resulting in a total of 1% (v/v) ECAS dosed directly into the DWPS pipework. Field trial 2, the control period, had no ECAS dosed pre- and post- UF membranes, resulting in 0% (v/v) ECAS dosage into the DWPS pipework. Both field trials were conducted over 16 operational days. The total time between the end of one field trial and the start of next was 18 days. This downtime between trials allowed for UF membranes to be thoroughly cleaned using alkaline and acid washes using sodium hypochlorite, hydrochloric acid and sodium hypochlorite.

2.4. Water sampling and analysis

Six water samples (both raw water and treated water) were collected and sent for analysis during each field trial. Water samples were collected from the raw water source, and the treated water outlet within the DWPS, before being immediately transported to an independent ISO 17025 accredited laboratory for standard suite analysis. See Table 1 for a full list of parameters tested within the standard suite analysis.

To determine the significant difference between raw water and treated water samples throughout field trial 1 and field trial 2, a *t-test* was performed for each parameter listed in Table 1. A P value of < 0.05 was considered significant. Graph construction and statistical analysis was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA), and Microsoft Excel 2013 for Windows (Microsoft Corporation, Redmond, WA).

Real-time monitoring of treated water quality (conductivity, oxidation reduction potential [ORP], pH, dissolved oxygen and chlorine), as well as pre- and post- membrane pressures, was performed using a WebMaster data logging system (Walchem, Holliston, MA, USA).

UF membrane health was determined by calculating the pressure differential across the UF membrane column module (Eq. (1) and Eq. (2)), and converting this to membrane permeability, the industry standard for membrane health (Eq. (3)).

Eq. (1): Filtration flux.

$$\text{Filtration flux}(L^{-1}\,m^{-1}\,h^{-1}) = \frac{\text{Feed flow}[m^3\,h^{-1}] \times 1000}{A \times B[m^2]} \quad (1)$$

Whereby; UF feedflow is measured on the module, A = Number of membrane housings, and B = Membrane area per membrane housing [m^2]

Eq. (2): Transmembrane pressure filtration.

$$\text{Transmembrane Pressure (TMP) filtration}[\text{bar}] = PT_{feed} - PT_{permeate} \quad (2)$$

Eq. (3): Permeability UF module.

$$\text{Permeability UF module}[L^{-1}\,m^{-1}\,h^{-1}] = \frac{\text{Filtration flux}(L^{-1}\,m^{-1}\,h^{-1})}{\text{Transmembrane Pressure (TMP) filtration}[\text{bar}]} \quad (3)$$

3. Results and discussion

3.1. Field trial 1: 1% (v/v) total ECAS dosing

Field trial 1, dosing 0.5% ECAS pre- and post- UF membranes, ran for 16 operational days. Table 1 shows the biological, basic water

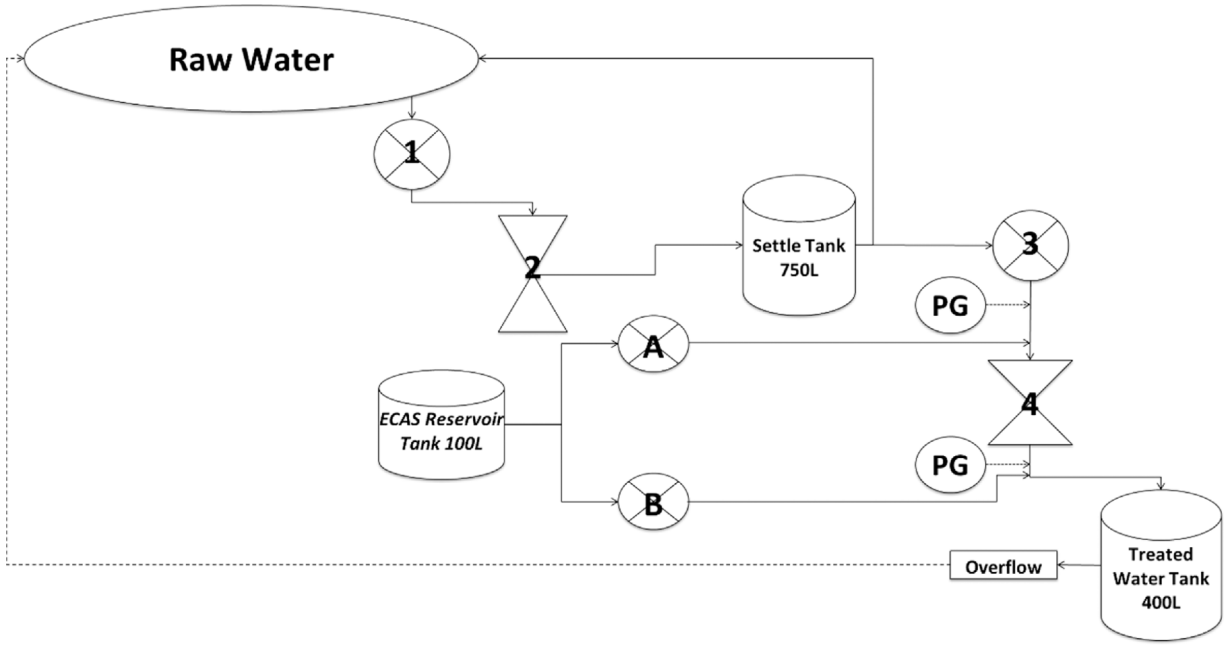

Fig. 1. Technical schematic of the off-grid drinking water production system. Direction of arrows refer to water flow direction. (1) Submersible filter pump (115 µm); (2) Reverse flushing filter (100 µm); (3) Peristaltic pump; (4) UF membrane columns (0.02 µm); ECAS reservoir tank 100L for ECAS generated outside of the DWPS; (A) & (B) ECAS peristaltic dosing pumps for delivering ECAS into the bulk treated water stream; (PG) Pressure gauges.

parameters, chemical and metal analysis results for the 6 water samples taken during DWPS operation. Over the entire sampling period all raw water samples analysed prior to treatment failed to meet DWI standards, and were deemed unsafe for human consumption (Table 1). All tested parameters for the DWPS treated water samples were within the specified DWI limits. For example, Table 1 and Fig. 3 demonstrate the achieved biological quality of drinking water produced using 1% (v/v) total ECAS as a disinfectant.

Water that was treated by the DWPS was shown to contain zero (0 cfu 100 ml^{-1}) levels of coliforms, *Escherichia coli*, Enterococci and *Clostridium perfringens*. The complete log reduction of bacteria within heterotrophic plates counts at 37 °C for 48 h was achieved for every treated water sample, except operational day 5 ([2 cfu ml^{-1}] Fig. 3). However, all treated water samples contained significantly lower

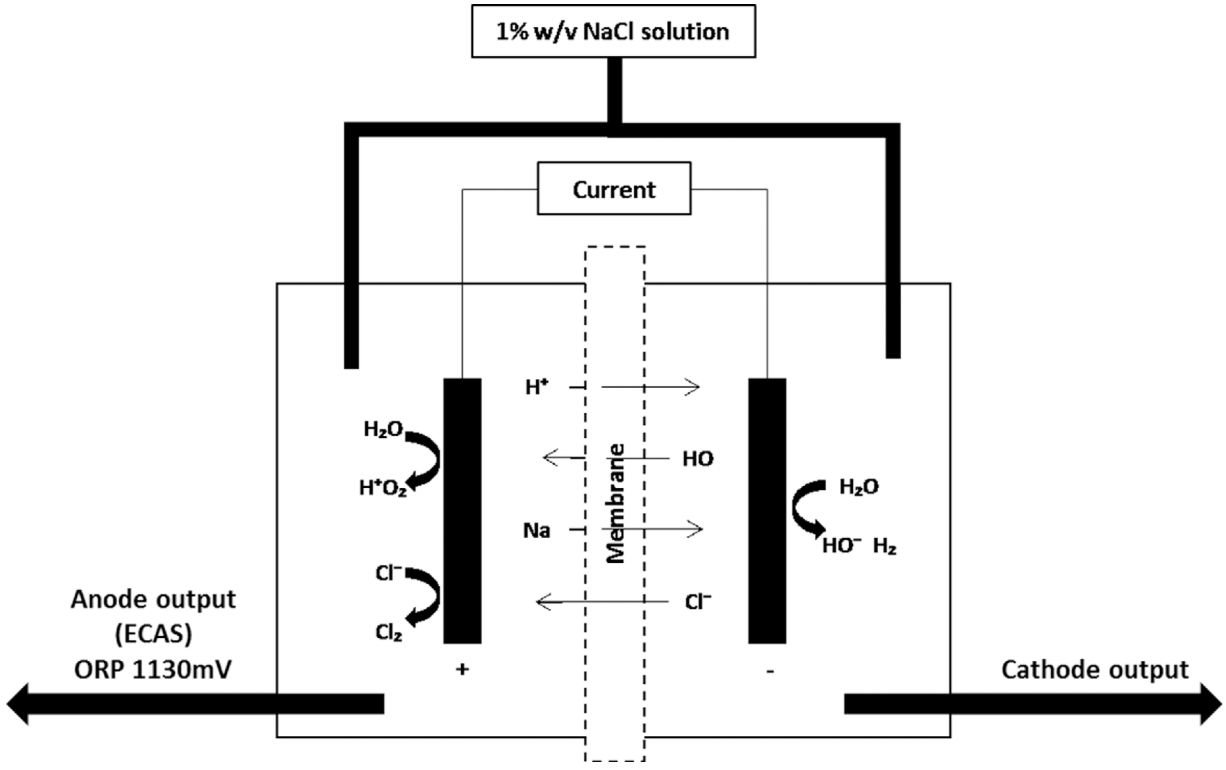

Fig. 2. Schematic of ECAS generation. A direct current is applied across two electrodes, an anode (+) and cathode (−) separated by a permeable ion exchange membrane, allowing constant perfusion of an electrolyte solution (1% w/v NaCl). The anolyte solution generated (ECAS) has a high oxidising potential, whilst the catholyte solution has a high reducing potential.

Table 1
Analytical results of the raw water samples and treated water samples. Field trial 1: 1% total ECAS dosing pre- and post- UF membrane. Field trial 2: Control, 0% ECAS pre- and post- UF membrane. Results shown are the calculated mean from the independent ISO 17025 accredited laboratory reports (n = 6 ± SD). Significant difference (Sig. diff) calculated through an unpaired, two tailed *t*-test, with a confidence interval of 95% (*** = p < 0.001; ** = p < 0.01; * = p < 0.05; ns = not significant). Bold figures = Above DWI limit value.

Water type		FIELD TRIAL 1 (1% total ECAS dosing)					FIELD TRIAL 2 (Control; 0% ECAS dosing)					
		Raw water		Treated water			Raw Water		Treated water			
	UNIT	Mean	SD	Mean	SD	Sig. diff	Mean	SD	Mean	SD	Sig diff.	*DWI Limit*
Biological analysis												
Plate count (2 day @ 37 °c)	/ml	538.83	753.19	0.33	0.82	***	672.60	778.93	457.33	518.80	ns	
Plate count (3 day @ 22 °c)	/ml	2685.33	770.77	2690.67	757.71	ns	12769.40	11209.42	2330.00	596.80	ns	
Non-lactose fermenters	/100 ml	33.33	51.64	1.17	2.86	***	0.00	0.00	13.67	33.48	***	
Presumptive coliform bacteria	/100 ml	49.17	43.19	0.00	0.00	***	1913.33	3977.37	86.50	66.51	ns	
Coliform bacteria	/100 ml	**12.00**	8.29	0.00	0.00	***	**1913.33**	3977.37	**76.67**	73.55	ns	*0*
Presumptive *E.coli*	/100 ml	1.50	0.71	0.00	0.00	***	573.33	832.99	0.00	0.00	***	
Escherichia coli	/100 ml	1.50	0.71	0.00	0.00	***	573.33	832.99	0.00	0.00	***	
Clostridium perfringens	/100 ml	**95.83**	11.70	0.00	0.00	***	**115.33**	82.65	0.00	0.00	***	*0*
Enterococci	/100 ml	**52.67**	42.04	0.00	0.00	***	**88.67**	88.59	0.00	0.00	***	*0*
Basic water parameters												
Alkalinity		139.00	2.65	131.50	7.78	ns	155.00	31.11	154.00	31.11	ns	
Colour (spectrophotometer)	mg L^{-1} Pt/Co	5.60	0.55	4.00	1.79	ns	8.33	1.37	7.67	1.53	ns	
Colour estimated	Deg Hazen	5.00	0.00	5.00	0.00	ns	5.00	0.00	5.00	0.00	ns	
Conductivity	µS cm^{-1} @ 20 °C	708.00	69.80	764.17	151.18	ns	610.33	53.71	613.17	52.53	ns	*2500*
pH		8.88	0.18	8.70	0.37	ns	8.33	0.66	8.22	0.69	ns	*6.5 – 10*
Total hardness	Mg Ca L^{-1}	118.67	13.31	114.93	16.75	ns	109.00	11.33	102.93	8.23	ns	
Turbidity	FTU	15.60		0.34	0.27	***	27.33	6.86	0.19	0.13	***	*4*
Chemical analysis												
Ammonium	mg L^{-1}	0.05	0.04	0.03	0.01	ns	0.27	0.29	0.21	0.16	ns	*0.5*
Chloride	mg L^{-1}	96.50	7.66	137.50	5.54	***	63.17	3.54	62.17	3.54	ns	*250*
Nitrate	mg L^{-1}	3.50	0.46	3.77	0.42	ns	1.02	0.55	1.08	0.71	ns	*50*
Nitrite	mg L^{-1}	0.05	0.01	0.04	0.01	ns	0.06	33.68	0.38	33.21	ns	*0.5*
Orthophosphate	mg L^{-1}	0.10	0.02	0.12	0.02	ns	0.09	0.08	0.12	0.08	ns	
Silica	mg L^{-1}	0.40	0.20	0.45	0.35	ns	0.50	0.74	0.41	0.58	ns	
Sulphate	mg L^{-1}	158.00	40.31	156.83	36.86	ns	126.00	13.53	129.00	13.00	ns	*250*
Metal analysis												
Aluminium	µg L^{-1}	**256.67**	183.16	16.67	5.16	**	**463.33**	124.85	23.33	5.77	***	*200*
Cadmium	µg L^{-1}	0.12	0.04	0.10	0.00	ns	0.10	0.00	0.10	0.00	ns	*5*
Calcium	mg L^{-1}	103.45	11.07	100.28	14.07	ns	95.50	9.78	92.40	6.22	ns	
Copper	mg L^{-1}	0.01	0.00	0.01	0.00	ns	0.01	0.00	0.01	0.01	ns	
Iron	µg L^{-1}	**316.67**	180.85	10.00	0.00	***	**548.33**	160.18	10.00	0.00	***	*200*
Lead	µg L^{-1}	5.38	2.43	0.62	0.26	***	8.57	2.38	0.37	0.20	***	*25*
Magnesium	mg L^{-1}	9.28	1.36	8.95	1.67	ns	8.22	0.91	7.50	0.66	ns	
Manganese	µg L^{-1}	21.00	3.85	4.33	1.97	***	62.67	32.96	25.40	23.89	ns	*50*
Nickel	µg L^{-1}	2.00	0.00	2.00	0.00	ns	1.68	0.78	1.70	0.73	ns	
Potassium	mg L^{-1}	3.90	0.49	3.65	0.57	ns	3.87	0.38	3.58	0.34	ns	
Sodium	mg L^{-1}	53.17	4.02	75.83	3.66	***	37.50	1.87	36.83	1.33	ns	
Zinc	µg L^{-1}	35.00	13.78	11.67	4.08	**	36.00	15.17	18.00	8.37	*	

heterotrophic bacteria at 37 °C than in raw water samples (Table 1), and there is no DWI maximum limit for heterotrophic plate counts (37 °C) [41]. Therefore treated water within field trial 1 was deemed fit for human consumption.

The multi-step filtration within the DWPS (Fig. 1) resulted in a significant reduction in turbidity between raw water samples and treated water samples, whereby treated water turbidity was within the DWI maximum limit of 4 FTU (Table 1).

The observed increase in the chloride concentration of treated water samples is due to dosing an electrolysed saline solution (ECAS) directly into the water treatment system. However, chloride concentrations for raw and treated water samples were consistently below the DWI limit of 250 mg L^{-1} (Table 1). Fig. 4 shows the real-time free chlorine concentration data of the treated water (using the in-line probes), whereby a reading was automatically taken every minute. The frequent chlorine spikes are a result of UF membranes back-flushing, which occur every 30 min, resulting in ECAS being dosed into the DWPS pipework in the absence of bulk water flow. Despite this, free chlorine concentrations within the treated water were significantly below the WHO recommended concentration of 5 mg L^{-1} free chlorine in drinking water.

It is evident from Table 1, that there were significant reductions in aluminium, iron, lead, manganese and zinc concentrations in the treated water compared to the raw water source. The reduction in these metals is due to the multi-step filtration process within the DWPS. A significant increase of sodium concentration in treated water samples is due to dosing an electrolysed saline solution (ECAS) directly into the water treatment system. All metals levels measured in the treated water were below the DWI limit for safe drinking water.

Permeability of the UF membranes initially decreased prior to stabilising, indicating no significant blocking or biofouling of the UF columns during the course of this field trial (Fig. 5). Biofouling can be a result of biofilm formation [42,43]; however, ECAS has been shown to be effective in inhibiting biofilm formation [25,37,44] The regular spikes in permeability are a result of UF membrane back-flushing every 30 min, which artificially impacts on the measured pressure differential across the columns.

3.2. Field trial 2: 0% ECAS dosing (Control period)

Table 1 shows the biological, basic water parameters, chemical and metal analysis results for the 6 water samples taken during DWPS operation in the absence of ECAS dosing. All water samples taken and analysed from the raw water source failed to meet DWI specifications and were deemed unsafe for human consumption (Table 1).

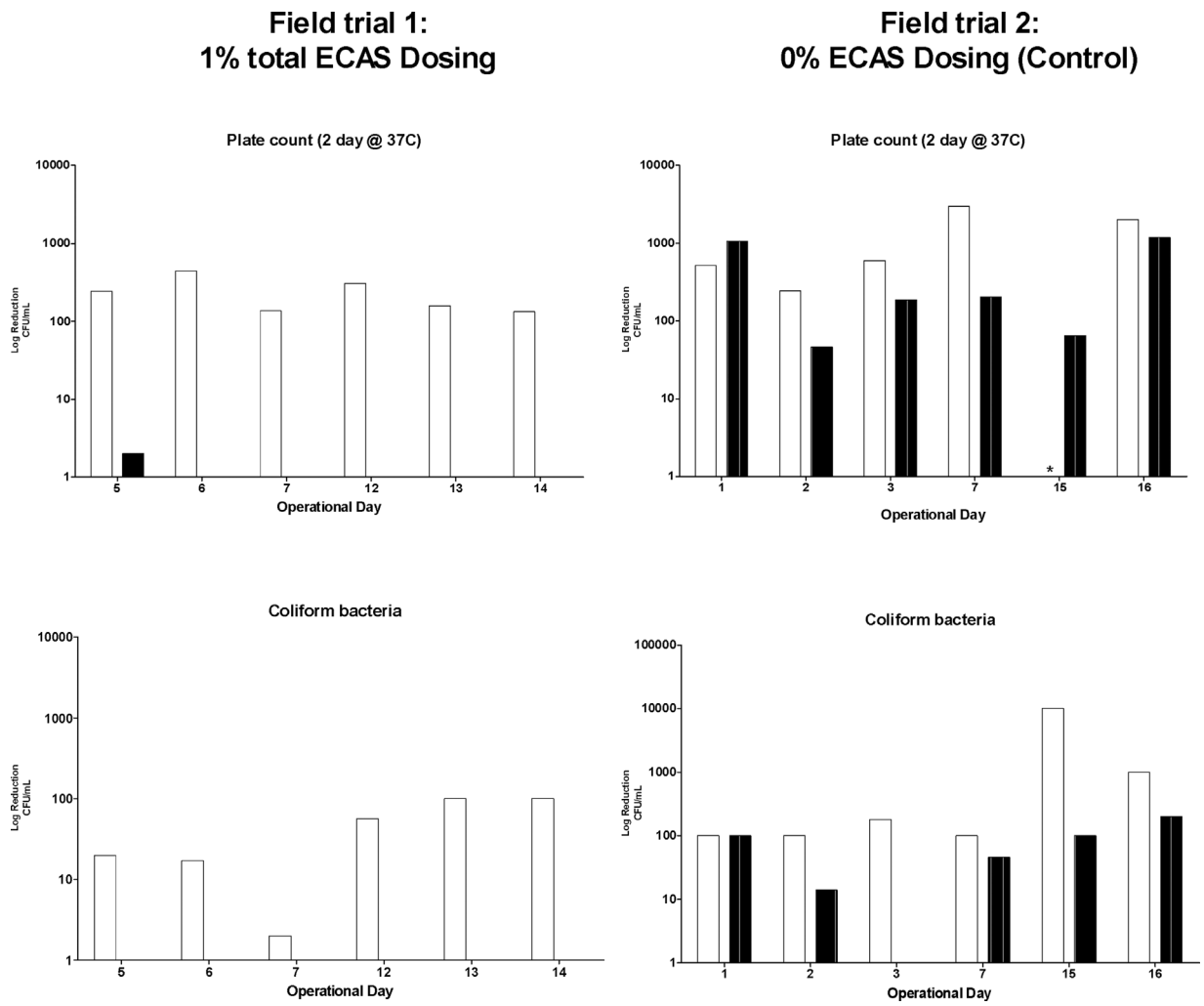

Fig. 3. Heterotrophic plate count and coliform bacteriological results for water samples taken during Field Trial 1 (1% total ECAS dosing) and Field Trial 2 (control; no dosing). White bars represent raw water samples. Black bars represent treated water samples. Data taken from independent ISO 17025 accredited laboratory reports (n = 1 per sampling day).

During Field Trial 2 (in the absence of ECAS dosing), the DWPS did not produce drinking water to DWI standards. Coliform bacterial counts exceeded the maximum allowance of 0 cfu 100 ml^{-1}, producing a mean result of 76.67 cfu ml^{-1}. Non-lactose fermenters within treated water samples were significantly higher compared to raw water samples. However, there were no recovered presumptive *E. coli*, *E. coli*, *Clostridium perfringens* and enterococci from treated water samples (Table 1).

Table 1 demonstrates that multi step filtration within the DWPS resulted in a significant reduction in turbidity between the raw water

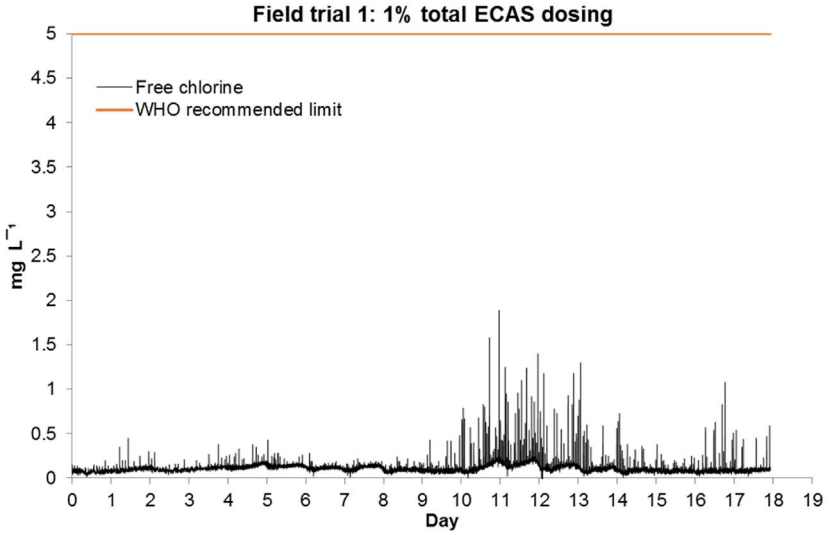

Fig. 4. Free chlorine concentration (mg L^{-1}) of treated water samples (as recorded by the in-line DWPS probe) for Field Trial 1 (1% total ECAS dosing).

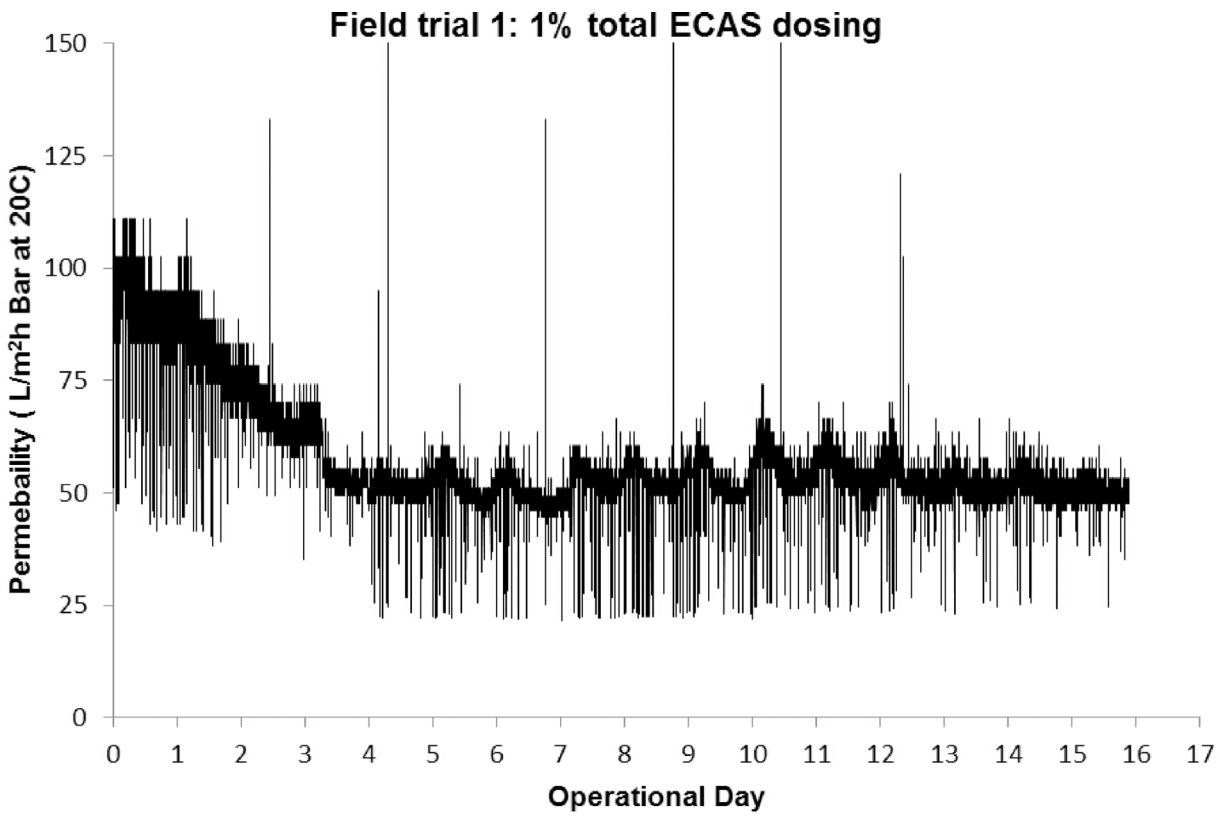

Fig. 5. UF membrane column permeability within the drinking water production system during Field trial 1 (1% total ECAS dosing).

and the treated water, bringing the treated water sample to within DWI limits. No significant difference was observed between the raw water and treated for any of the measured chemical parameters (ammonium, chloride, nitrate, nitrite, orthophosphate, silica and sulphate), and were within the DWI limits. The free chlorine concentration of treated water over the 16 operational days was below the limit of reliable detection for the in-line sensor (< 0.12 mg L^{-1}), which is expected since no ECAS was dosed into the DWPS.

Metal analysis of the raw and treated water samples resulted in significant reduction of aluminium, iron, lead and zinc (Table 1). Since this was observed in both field trials (presence and absence of ECAS dosing), it can be concluded that reduction is due to the multi-step filtration process alone within the DWPS.

4. Conclusions

Two field trials were conducted over two 16 day periods to evaluate the off-grid drinking water production system shown in Fig. 1. Field trial 1 performed direct dosing of 1% (v/v) total ECAS into the DWPS pipework, pre- and post- UF membranes. Field trial 2 was a control period, whereby the DWPS was operated in the absence of ECAS (0% ECAS dosing) pre- and post- UF membranes.

All raw water source samples taken throughout the two field trials failed to meet DWI standards and were deemed unsafe for human consumption.

During the control period (Field trial 2) all treated water samples were within DWI limits for basic, chemical and metal parameters. However, the treated water produced was not biologically safe due to the presence of coliform bacteria (Table 1 and Fig. 3).

The analysis of all treated water samples resulting from field trial 1, demonstrated that the off-grid DWPS was consistently capable of producing DWI standard drinking water, with all basic, biological, chemical and metal parameters falling within the DWI threshold limits (coliform bacteria, *Clostridium perfingens,* enterococci, conductivity, pH, turbidity, ammonium, chloride, nitrate, nitrite, sulphate, aluminium, cadmium, iron, lead and manganese).

In particular, the microbiological results from field trial 1 treated water samples demonstrated the importance of ECAS dosing in the production of biologically safe drinking water to DWI standards.

The stable permeability of the UF membranes during field trial 1 (whereby 0.5% (v/v) ECAS was dosed pre-UF membranes), indicates that ECAS may help manage biofilm formation on the UF membranes. During Field trial 2 (control; no dosing) greater fluctuations in permeability within the UF membranes was observed, indicative of less stability, and possible biofilm formation (data not shown). This inference of reduced biofilm formation, reducing the possibility of biofouling using 0.5% (v/v) ECAS dosing pre-UF membranes, requires further investigation.

Through a systems based hazard analysis the critical control points of the DWPS focus on the in-line monitoring parameters, specifically ORP and free chlorine. Two key critical variables are continuously measured in the WebMaster data logger, chlorine and ORP, ensuring the risks of biological and physicochemical contamination in the DWPS final treated water are minimised. The guideline value for chlorine is 5 mg L^{-1}, a European requirement for ensuring adequate residual disinfection within distribution systems. In addition, due to the nature of ECAS as a disinfectant; high ORP ($+1130$ mV), low free chlorine concentration, compared to conventional chlorination, and very fast acting kill kinetics (< 10 s), ORP is a key parameter to ensure production of biologically safe drinking water. Regular spot sampling of the treated water for biological, basic, chemical and metal analysis is required to ensure DWI compliance. A complete assessment regarding the hazards and critical control points of the DWPS shall be carried out as part of any future work.

This study has shown that a novel off-grid drinking water production system can produce DWI standard drinking water from a heavily biologically contaminated water source, when a 1% (v/v) total ECAS dosing regimen is implemented. The DWPS was developed with the

intention of use in a wide variety of applications and locations, such as developing and transitional countries, many of which lack established centralised water treatment networks. The potential modular and scalable capability of the DWPS could be beneficial in remote, rural or temporary communities, which can have fluctuating populations. The self-contained nature of the DWPS, all filtration and disinfection processes are within the DWPS (except for intake filter pump), could be beneficial for temporary communities such as long-term research expeditions, or during disaster relief efforts. Long-term field trials are now required to obtain data for more representative applications, such as raw water sources from surface and ground waters, which have differing 'contaminants' (e.g. fertilisers, heavy metals, faecal contamination), ensuring the DWPS is capable and versatile in a wide variety of applications. Investigations into the energy requirements for the DWPS are currently being conducted to ensure that the DWPS is robust and reliable for long-term operation.

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Comparison of Trihalomethane Formation Using Chlorine-Based Disinfectants Within a Model System; Applications Within Point-of-Use Drinking Water Treatment

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Point-of-use (POU) drinking water treatment systems provide solutions for communities where centralized facilities are unavailable. Effective POU systems treat and reduce the number of pathogens in POU water supplies often employing disinfection. Chlorine disinfection results in the formation of disinfection by-products (DBPs), such as trihalomethanes (THMs), through the reaction of chlorine with natural organic matter (NOM) over time. Although THMs are known to be harmful to human health, little is known about their production within POU systems. This study compares the disinfectants; Electrochemically Activated Solutions (ECAS), hypochlorous acid (HOCl), and sodium hypochlorite (NaOCl), with respect to their potential to produce THMs within POU drinking water systems. Headspace solid-phase microextraction (HS-SPME) gas chromatography mass spectrometry (GC-MS) was utilized to quantify THMs in treated water samples containing NOM (Suwannee River humic acid, 4 mg L^{-1}). All disinfection treatments were matched to free chlorine concentrations of 1, 3, and 5 mg L^{-1}, using reaction times of 1, 5, and 10 min. THMs were produced at free chlorine concentrations of 5 mg L^{-1} and at reaction times of 5 and 10 min for all disinfectants. ECAS or HOCl, resulted in the formation of significantly lower total THM concentrations across all reaction times and free chlorine concentrations, compared to NaOCl. ECAS can be generated at the POU requiring only water, salt and energy for production, and this study demonstrates that its use results in reduced formation of THMs, compared with NaOCl. Further work is required to replicate these findings within scaled-up POU water treatment systems.

Keywords: electrochemically activated solutions, hypochlorous acid, point-of-use decentralized drinking water treatment, sodium hypochlorite, trihalomethane formation

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INTRODUCTION

Approximately 1 billion people worldwide are living without access to improved drinking water sources (Corcoran et al., 2010; Unicef and World Health Organization, 2014; Ardakanian et al., 2015; World Health Organization, 2015). For the past 100 years chlorine has been crucial in the production of biologically safe drinking water. Chlorine is the most widely used disinfectant in drinking water treatment due to its availability, low cost, and broad spectrum antimicrobial efficacy

(Rodriguez and Serodes, 2001; Farghaly et al., 2013; Kumari and Gupta, 2015). Decentralized point-of-use (POU) drinking water treatment systems typically utilize alternative disinfectant solutions (Mbilinyi et al., 2005; Peter-Varbanets et al., 2009; Domènech, 2011; Attisani, 2016; Carratalà et al., 2016; Pooi and Ng, 2018) or chlorine release tablets (Jain et al., 2010; Werner et al., 2016), rather than conventional chlorination solutions (i.e., NaOCl) for the production of biologically safe water. Alternatives to conventional chlorination are adopted due to quicker disinfection times, ease of transport and storage (Clasen and Edmondson, 2006; Jain et al., 2010). Our recent study has shown that Electrochemically Activated Solutions (ECAS) can be utilized within a POU drinking water system to produce biologically safe drinking water (Clayton et al., in press), and this disinfection system is now a Drinking Water Inspectorate (DWI) approved product for use in public water supply in the UK (Drinking Water Inspectorate, 2018). The production of trihalomethanes within drinking water systems is known over the medium (hours) to long-term (days/weeks) (Rodriguez and Serodes, 2001; Rossman et al., 2001; Emmert et al., 2009; Brown et al., 2010; Guilherme and Rodriguez, 2015). However, within POU drinking water treatment systems where alternative disinfectants are applied over a short contact time ($\leq$10 min), THM formation is largely unknown.

It is well-understood that the chlorination of drinking water containing natural organic matter (NOM) can result in the formation of disinfection by-products [DBPs] (World Health Organization, 2000; Grunwald et al., 2002; Di Cristo et al., 2013). Trihalomethanes (THMs) are an important group of DBPs that form during the chlorination of drinking water, and are known to be hazardous to human health (Liang and Singer, 2003). THMs form when free chlorine reacts with natural organic material (NOM) over an extended contact time throughout water treatment processes, as occurs during conventional chlorination (Amy et al., 1987; Brown et al., 2011a; Di Cristo et al., 2013). The short-term (i.e., <10 min) formation of disinfection by-products (THMs) is relatively unknown for decentralized POU drinking water treatment applications, where treated water is often utilized immediately. THMs are a health concern since they have been shown to possess carcinogenic and mutagenic properties, and can be attributed to health concerns such as cancer, liver and kidney damage, miscarriages and birth defects in new born babies (King et al., 2000; Dodds and King, 2001; Wright et al., 2004; Bove et al., 2007; Wang et al., 2007; Rahman et al., 2010; Chowdhury et al., 2011; Grazuleviciene et al., 2013; Siddique et al., 2015). Drinking water quality guidelines state maximum individual concentrations for all THMs; chloroform ($CHCl_3$) [300 $\mu g\ L^{-1}$], bromoform ($CHBr_3$) [100 $\mu g\ L^{-1}$], dibromochloromethane (DBCM; $CHBr_2Cl$) [100 $\mu g\ L^{-1}$], and bromodichloromethane (BDCM; $CHBrCl_2$) [60 $\mu g\ L^{-1}$] (World Health Organization, 2011). This has resulted in maximum guideline values for total THMs (tTHMs) in drinking water worldwide to vary between 80 (USEPA, 2010) and 100 $\mu g\ L^{-1}$ (The Council of the European Union, 1998; Health Canada, 2017). In the UK, the drinking water inspectorate enforces a tTHM limit of 100 $\mu g\ L^{-1}$ (Drinking Water Inspectorate, 2012).

Typically, drinking water treatment systems incorporate disinfection stage(s) that utilize chlorine gas (Cl_2), sodium hypochlorite (NaOCl), or calcium hypochlorite [$Ca(OCl)_2$] (World Health Organization, 2000; Drinking Water Inspectorate, 2012) to achieve effective chlorination. These chlorination techniques result in high concentrations of free and residual chlorine that are distributed throughout the network achieving long residual reaction times (World Health Organization, 2011). A minimum free chlorine residual concentration of 0.5 mg L^{-1} is recommended throughout distribution systems to ensure biologically safe water (World Health Organization, 2011).

The formation of THMs in drinking water supplies occurs when free chlorine reacts with NOM over time (World Health Organization, 2004). Minimizing NOM [encompassing dissolved, suspended, particulate organic carbon, or matter which occur in aquatic systems (Demiral et al., 2006)] concentrations in raw water supplies in decentralized point-of-use (POU) drinking water treatment systems, via filtration prior to chlorination, limits THM formation (World Health Organization, 2000). Other considerations such as temperature, pH, and contact time within the distribution network (World Health Organization, 2000) are also known to drive THM formation (Brown et al., 2010, 2011b; Rasheed et al., 2017). As the knowledge of the toxicological effects of THMs on human health has increased, so has the need to investigate alternative disinfection techniques such as ozonation (Schlichter et al., 2004; Zhu et al., 2014) and UV sterilization (Carratalà et al., 2016). Electrochemical disinfection, either direct or via the application of electrochemically activated solutions (ECAS) has also gained interest (Kerwick et al., 2005; Ghebremichael et al., 2011). Electrochemically activated solutions are referred to as electrolyzed water (EW), electrolyzed oxidizing water (EOW), oxidized water (OW), and mixed oxidants (MIOX) solutions (Venczel et al., 1997). A schematic of ECAS generation is shown in **Figure 1**, whereby a weak saline solution (typically 1% [w/v]) is passed through an electrochemical cell containing separate anodic and cathodic chambers (Robinson et al., 2010, 2013; Thorn et al., 2013). Solutions produced at the cathode (catholyte) are highly reductive [−800 mV], compared to anodic solutions (anolyte) which are highly oxidative [+1,000 mV] in nature (Inoue et al., 1997; Morita et al., 2000; Liao et al., 2007; Robinson et al., 2010). The reactions which occur at the anodic surface result in the production of chlorine (Cl_2) and oxygen, as well as a hydroxyl radicals and transient oxidative functional groups [e.g., OH^-, O_3, H_2O_2, and O_2^-; (Jeong et al., 2006; Martínez-Huitle and Brillas, 2008)]. The myriad of transient reactive species increase the ORP of the solution, resulting in a pH shift toward the acidic range. This is dependent on redox reactions of strongly adsorbed electro-active water derived intermediate molecular species (Burke and O'Neill, 1979; Erenburg et al., 1984; Boggio et al., 1985; Trasatti, 1991). Initially, water is decomposed at the anode surface

$$H_2O_{ad} \rightarrow OH_{ad} + H^+ + e^- \quad (1)$$

$$OH_{ad} \rightarrow O_{ad} + H^+ + e^- \quad (2)$$

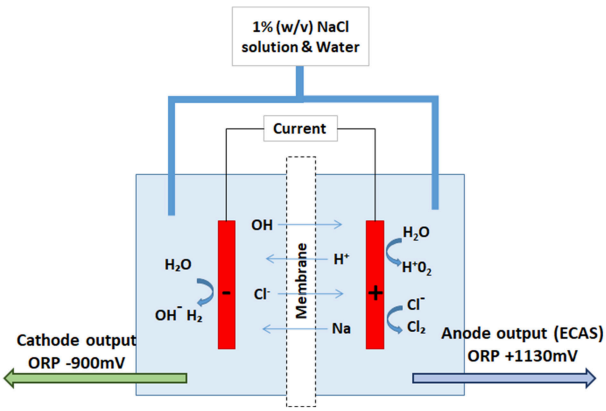

FIGURE 1 | Schematic of ECAS generation. A direct current is applied across two electrodes, an anode (+) and cathode (–) separated by a permeable ion exchange membrane, allowing constant perfusion of an electrolyte solution (1% w/v NaCl). Anolyte solution generated (ECAS) has a high oxidizing potential, the catholyte solution has a high reducing potential.

Dissociated chloride ions from NaCl through direct current polarization are then adsorbed;

$$O_{ad} + Cl^{-} \rightarrow OCl_{ad} + e^{-} \quad (3)$$

Chlorine gas and oxygen are both then produced from these intermediates;

$$OCl_{ad} + Cl^{-} + H^{+} \rightarrow OH_{ad} + Cl_{2(g)} \quad (4)$$

$$O_{ad} + O_{ad} \rightarrow O_{2(g)} \quad (5)$$

A large scientific body of evidence now exists for the two 1-electron processes shown above (Equations 1 and 2) (Stoner et al., 1982; Trasatti, 1987; Cai, 2005), and the electrochemically generated chlorine then reacts with water producing hypochlorous acid;

$$Cl_2 + H_2O \rightarrow HOCl + HCl \quad (6)$$

This reaction is pH dependent, and (according to the Nernst equation) dictates which free form of chlorine is most prevalent within generated solution; Cl2, HClO, or ClO (Stoner et al., 1982; Sivey et al., 2010). The anodic solutions exhibit antimicrobial properties as a result of chlorine and their high oxidation reduction potential (ORP), disrupting cell membrane function (Inoue et al., 1997; Kumon, 1997; Thorn et al., 2012). This high ORP environment has been shown to damage and rupture both inner and outer microbial membranes, prohibiting microbial functionality, including energy generating mechanisms (Liao et al., 2007). ECAS solutions have been shown to exhibit rapid antimicrobial kinetics, exerting a significant effect after short contact times between 2 (Robinson et al., 2011) and 10 s (Liao et al., 2007), and are still efficacious with suitable storage after 12 months (Robinson et al., 2013). ECAS have shown comparable efficacy to other chlorine based disinfectants at lower free chlorine concentrations (Thorn et al., 2013). The low free chlorine concentrations of ECAS coupled with its high ORP and broad spectrum antimicrobial activity could potentially reduce THM formation within water treatment systems.

ECAS has the potential to be used within decentralized POU drinking water systems, in remote areas or communities, or as part of disaster relief efforts which do not have access to centralized drinking water distribution networks. Decentralized point-of-use applications require reliable and robust treatment systems capable of producing chemically and biologically safe drinking water. Source waters used to feed decentralized POU water treatment systems can often be contaminated with feces, therefore if treatment is not effective then consumed water can result in diarrheal diseases. These diarrheal diseases can result in dehydration, malnutrition and can be fatal, especially to vulnerable groups within the populations such as young children (Cabral, 2010). ECAS has been shown to exhibit significant antimicrobial activity against a range of microorganisms, such as *Escherichia coli* [*E. coli*] (Robinson et al., 2011), including a pathogenic enterohemorrhagic *E. coli* strain: O157:H7 [95% reduction <10 s] (Liao et al., 2007), which can cause hemolytic uremic syndrome (HUS). Young children and the elderly are most at risk of HUS, which can be fatal (The Environment Agency, 2002). Under such challenging situations the formation of THMs resulting from POU disinfection within such systems is often overlooked. The combination of ECAS properties; low pH, high ORP, low comparative free chlorine concentrations and broad spectrum antimicrobial activity, demonstrate that ECAS could be a viable alternative for use within decentralized drinking water treatment systems. Both in producing chemically and biologically safe drinking water, including the potential for reduced THM formation.

This study investigates the comparative formation of total THMs in water when treated with three disinfectants (ECAS, NaOCl, and HOCl) as a function of contact time and free chlorine, for application in point-of-use decentralized drinking water treatment systems.

MATERIALS AND METHODS

Reagents

Ultrapure water with a resistivity output of 18.2 MΩ, was used for preparation of humic acid solutions (Purite Water Purification Solutions, United Kingdom). Calibration solutions, the internal standard Fluorobenzene and THM standard solution (comprising of chloroform, DBCM, BDCM and bromoform) (Sigma Aldrich, Dorset, United Kingdom), were prepared using high grade (HPLC) methanol (Fisher Scientific, United Kingdom).

Disinfectant Solutions

Hypochlorous acid stock solution was produced through the dissolution of sodium dichloroisocyanurate (NaDCC) within 1 L of deionized water producing a free chlorine concentration of 201 ± 13.55 mg L^{-1}, pH of 5.6 ± 0.25, and an ORP of 958 ±

18.98 mV. Stock solution of sodium hypochlorite was prepared by diluting a commercial bleach (Pattersons bleach; Pattersons Ltd., Bristol, United Kingdom) in deionized water to a final free chlorine concentration of 508 ± 18.19 mg L^{-1}, pH of 11.4 ± 0.1, and an ORP of 588 ± 0.95 mV. **Figure 1** shows how electrochemically activated solutions (ECAS) were generated using an electrochemical cell supplied by Bridge Biotechnology Ltd (Fife, Scotland, United Kingdom). Generated ECAS solutions had free chlorine concentrations of 158.63 ± 18.66 mg L^{-1}, pH of 3.3 ± 0.16, and an ORP of 1,134 ± 3.26 mV. ECAS solutions were stored at 4°C in the dark, and used within 5 days of production (Robinson et al., 2011). Disinfectant solutions were diluted in deionized water to produce equal concentrations of free chlorine (1–5 mg L^{-1}) as determined using the N, N-diethyl-p-phenylenediamine sulfate (DPD) no. 1 Palintest test (Palintest Ltd., Gateshead, United Kingdom). The pH and ORP of disinfectant and test solutions were measured using an Orion Dual Star (Fisher Scientific, United Kingdom).

Preparation of THM and NOM Standard Solutions

THM standard solutions containing chloroform ($CHCl_3$), bromodichloromethane ($CHCl_2Br$), dibromochloromethane ($CHClBr_2$), and bromoform ($CHBr_3$) each at 20 μg mL^{-1} were prepared from a standard THM stock solution (200 μg mL^{-1}) and HPLC grade methanol, both supplied by Sigma Aldrich (Dorset, United Kingdom).

A NOM stock solution was prepared by dissolving 4 mg of Suwannee River humic acid (SRHA; International Humic Substances Society, St Paul, MN, United States of America), in 100 mL of ultrapure water (overnight) to achieve a concentration of 40 mg L^{-1} (Gadad et al., 2007). From this, standard NOM solutions of 4 mg L^{-1} were prepared (Boggs et al., 1985; Venczel et al., 2004).

Gas Chromatography and Mass Spectrometry

The quantification of THMs in pre and post disinfected water samples were determined using the standard method [BS EN ISO 17943, (British Standards Institution, 2016)], which incorporates headspace solid-phase microextraction (HS-SPME) gas chromatography and mass spectrometry (GC/MS). GC/MS was carried out using an Agilent 7820A GC System with an Agilent 5977B high efficiency source with Mass Selective Detection, and a phenyl methyl silox capillary column (Agilent Technologies, Santa Clara, California, United States), see **Table 1** (British Standards Institution, 2016). An internal standard (IS) of fluorobenzene (British Standards Institution, 2016), was diluted to a working concentration of 20 μg mL^{-1} in HPLC grade methanol from a solution of 2,000 μg mL^{-1} (Sigma Aldrich, Dorset, United Kingdom).

HS-SPME Experimental Procedure

A Supelco solid-phase microextraction (SPME) fiber holder for manual sampling was fitted with an 85 μm carboxen/polydimethylsiloxane (CAR/PDMS) fiber (Sigma Aldrich, Dorset, United Kingdom). The fiber was conditioned at 280°C for 2 h.

A working calibration curve for HS-SPME extracted THMs from water samples was constructed by dissolving mixed THM standard solutions (each at 20 μg mL^{-1}) in ultrapure water containing 6 g NaCl, to produce solutions at 10, 20, 40, 60, 80, and 100 μg L^{-1}, respectively. Regression analysis (r^2-values) and mean retention times for each individual THM(s) extracted from the standard mixed solution are shown in **Table 2**. The total THMs (tTHMs) extracted from standard mixed solutions (i.e., the sum of $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$) is also shown in **Table 2**.

TABLE 1 | Operational conditions for GC/MS analysis.

Parameter	Conditions
Capillary column, dimensions	Phenyl methyl silox; 60–325°C 30 m × 250 μm × 0.25 μm
Carrier gas	Helium 1.2 mL min^{-1}
GC equipment	Agilent 7820a GC system
MS detector	Agilent 5977b MSD
Selected ion monitoring (SIM) ions used (dwell time 100 ms)	82.9, 84.9, 96, 128.8, 207.8, 251.7
Temperature programme	35°C, 5 min; 20°C/min to 250°C; 5 min hold at 250°C

TABLE 2 | THM calibration regression values for THM calibration mix with dilutions of 10, 20, 40, 60, 80, and 100 μg L^{-1} ($n = 3$).

Compound	r^2 (linear)	Mean retention time (minutes) [± SD]
	10–100 μg L^{-1}	
Chloroform	0.7453	2.52 ± 0.0075
Bromodichloromethane	0.8555	3.87 ± 0.0068
Dibromochloromethane	0.9657	6.48 ± 0.0054
Bromoform	0.9883	8.95 ± 0.0059
tTHM	*0.9404*	

Mean retention time across all THM calibration mix dilutions (n = 18 ± SD).

Preparation of Test Samples

For the reaction vials (sterile glass universals with solid high-density polyethylene screw caps), disinfectants were added to standard NOM solution (4 mg L^{-1} IHSS Humic acid), maintaining a total reaction volume of 30 mL, to achieve free chlorine concentrations of 0, 1, 3, and 5 mg L^{-1}. Reaction times (1, 5, or 10 min) were controlled by taking a 20 mL sample from the test reaction vial, and injected into a test extraction vial. Test extraction vials (30 mL sterile extraction glass universals with high density polyethylene screw cap with silicone septum) contained 6 g laboratory grade NaCl, 5 g L^{-1} sodium thiosulfate (British Standards Institution, 2005; Antoniou et al., 2006; Environmental Protection Agency, 2011) and the internal standard, fluorobenzene, at a final concentration of 100 μg L^{-1}. Prior to headspace extraction all samples were incubated

at 40°C for 20 min, inclusive of 10 min headspace extraction (fiber exposed). During SPME fiber exposure the manual SPME holder was injected through the septum into the headspace of the sample vial, exposing the CAR/PDMS fiber. After the 10-min fiber exposure period, care was taken to ensure the CAR/PDMS fiber was retracted into the manual SPME holder and inserted into the GC/MS inlet (<30 s), minimizing extraneous exposure of the fiber. All sample fibers had a desorption period of 2-min prior to analysis.

Data Analysis

Individual THM concentrations were calculated using Agilent Mass Hunter Enhanced Data Analysis Software (Santa Clara, California, United States). tTHMs were calculated from the sum of $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$. Values reported were blank corrected, and a limit of detection (LoD) of 0.86 μg L^{-1} for all samples was determined experimentally. Where analysis is below the LoD, then data values are represented by an asterisk (*).

Comparative statistical analysis of THM concentrations (between experimental variables) was performed using a two-way ANOVA with Bonferroni *post-hoc* test (GraphPad Prism version 5.00 for Windows, San Diego, CA). A $P < 0.05$ was regarded as significant.

RESULTS

Due to the volatility of THMs, the extraction efficiency and quantitative analysis of THMs in water via HS-SPME vary with temperature and molecular weight. The regression values (r^2-values) for the standard THM solutions increase in the order: chloroform < BDCM < DBCM < Bromoform (**Table 2**). THM compounds become more stable in the headspace with increasing molecular weight, and therefore boiling point, which supports the observed regression values shown in **Table 2**. The greatest deviations along the regression are associated with the determination of higher concentrations of individual THMs (>60 μg L^{-1}). However, increased linearity for individual and total THM species were observed at lower concentrations between 0, 10, and 60 μg L^{-1} (regression value of 0.9674 for tTHMs). Only three instances occurred in this study where tTHM concentration values exceeded 60 μg L^{-1}, in the presence of NaOCl at 5 and 10 min reaction times (**Figure 2**).

The reaction of the three disinfectants with NOM produced tTHMs at free chlorine concentrations of 3 and 5 mg L^{-1} and at reactions times of 5 and 10 min on all occasions (**Figure 2**). The most abundant individual THM species in all reactions was chloroform (>75% of the total), followed by the three brominated species [bromodichloromethane, dibromochloromethane, and bromoform] (**Figure 3**). The formation of low concentrations of brominated THM species are a consequence of bromide present within Suwannee River humic acid [NOM] solution, as reported previously (Chowdhury, 2013). The high relative formation of chloroform is in accordance with previous findings regarding THM formation within drinking water (Cho et al., 2003; Ikem, 2010; Yang et al., 2015).

At a concentration of 1 mg L^{-1} of free chlorine the observed production of tTHMs for all disinfectants was low or below the level of detection. Regardless, the formation of tTHMs, although low, reached a maximum concentration, for this experimental design, at a reaction time of 10 min for all disinfectants at 1 mg L^{-1} (**Figure 2**). Upon increasing the free chlorine concentration (3 and 5 mg L^{-1}) significant differences were then observed between the reaction times (**Table 3**). NaOCl resulted in peak

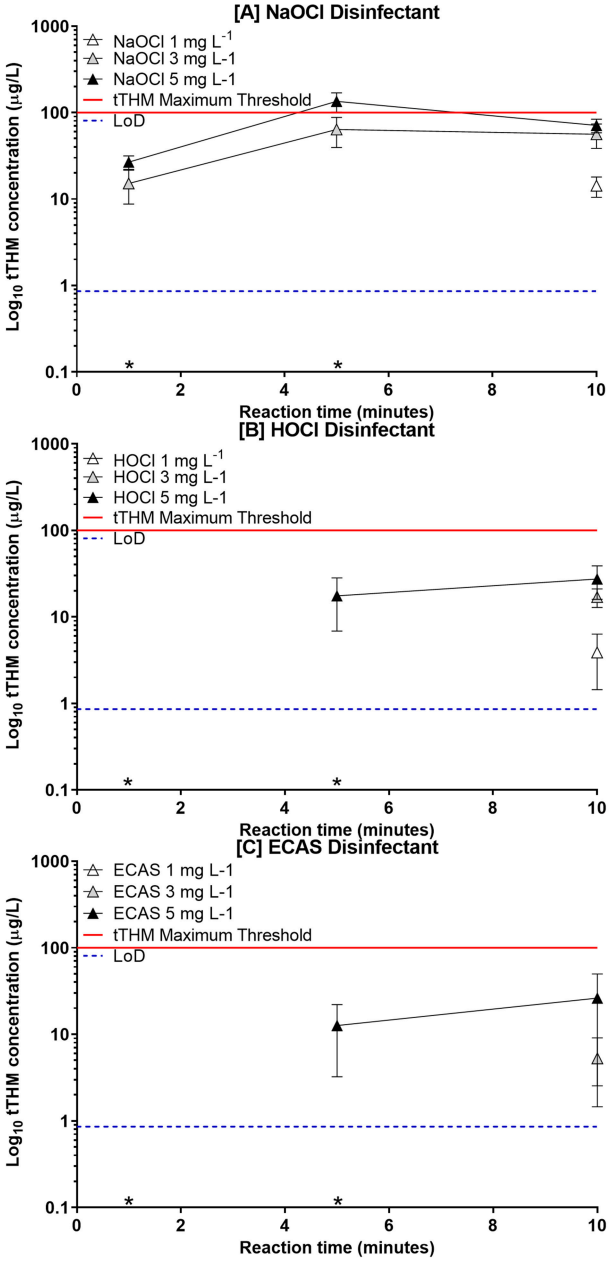

FIGURE 2 | Total THM formation with disinfectants **(A)** NaOCl (△), **(B)** HOCl (□), or **(C)** ECAS (○) at free chlorine concentrations between 1 (white), 3 (gray), and 5 mg L^{-1} (black). Contact times were 1, 5, and 10 min ($n = 6 \pm$ SD). tTHM Maximum (horizontal dashed line) refers to the maximum guideline value permissible in drinking water (within the UK). tTHMs concentrations below the limit of detection (*).

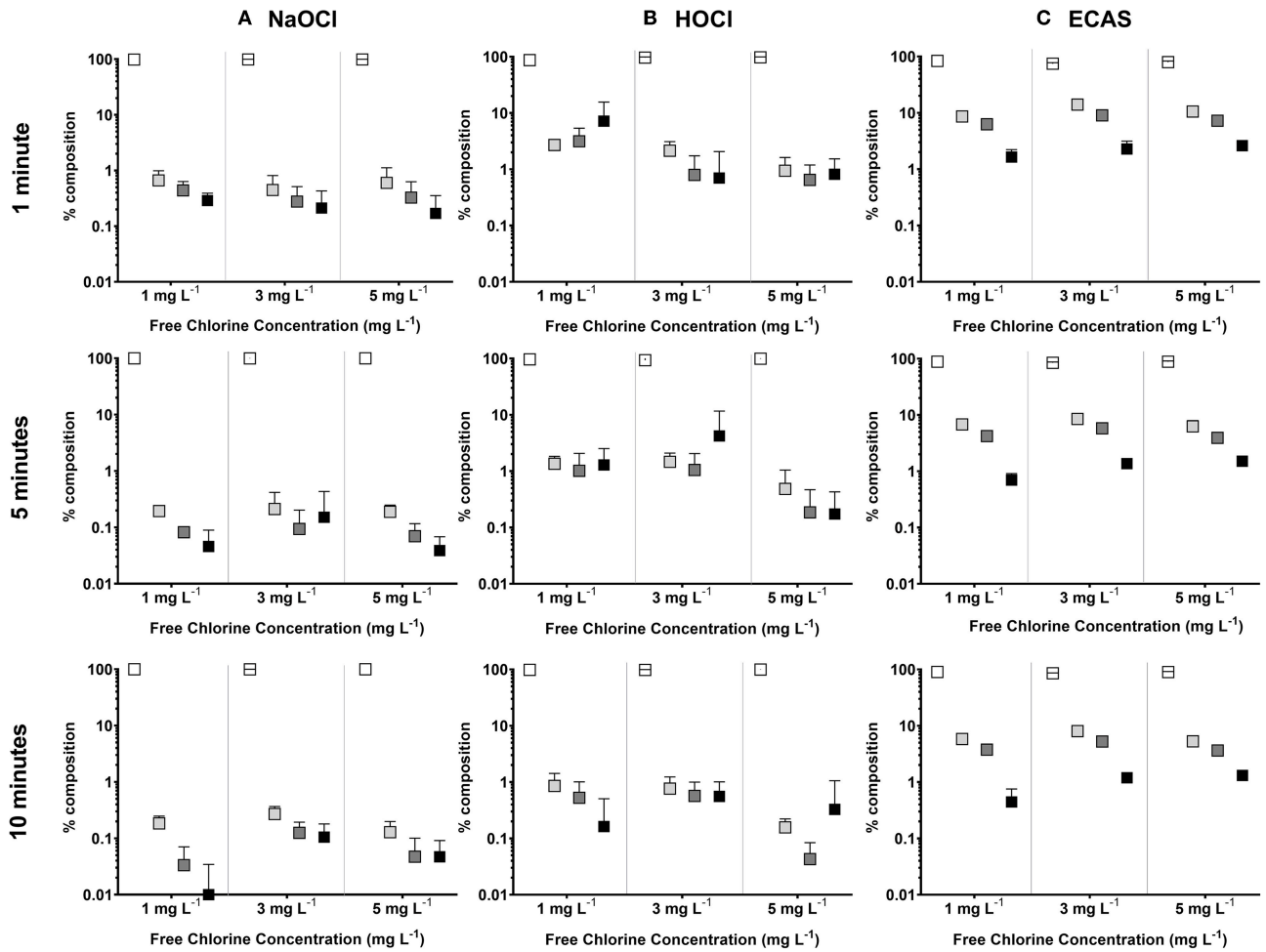

FIGURE 3 | Mean percentage composition of THM species for disinfectants: **(A)** NaOCl (△), **(B)** HOCl (□), or **(C)** ECAS (○); as a function of reaction time (rows) and free chlorine; Chloroform (white), bromomodichloromethane (light gray), dibromochloromethane (dark gray), and bromoform (black). $N = 6$ (±SD).

tTHM concentrations after a 5 min reaction time, except for 1 mg L^{-1} free chlorine concentration which resulted in peak concentration after 10 min (**Figure 2A**). Disinfectants HOCl and ECAS both resulted in peak tTHM concentration at 10 min reaction times at all free chlorine concentrations (**Figures 2B, C**).

When comparing the three disinfectants, the maximum observed concentrations of tTHMs were formed by NaOCl across all free chlorine concentrations for all reaction times (**Figure 2A**). At a free chlorine concentration of 5 mg L^{-1} and a reaction time of 5 min the mean tTHM formation exceeded the maximum regulatory threshold of 100 μg L^{-1}, the permissible level for UK drinking water (Drinking Water Inspectorate, 2012). However, surprisingly, the mean level of tTHMs decreased between 5 and 10 min reaction time, at free chlorine concentrations 3 and 5 mg L^{-1}. This is contradictory to other published studies which demonstrate an increase in tTHM formation in reaction times in excess of 10 min (i.e., hours, days) (Brown et al., 2010; Ghebremichael et al., 2011; Saidan et al., 2013; Werner et al., 2016). It is postulated that this observed decline could be due to the hydrolysis, or dehalogenation of already formed tTHMs present in solution (Mabey and Mill, 1978; Rahman, 2015; Abusallout et al., 2017), although, it is unknown the extent to which these reactions can occur over such a short contact time (i.e., 10 min).The decline in tTHMs was not observed with either HOCl or ECAS, probably due to a combination of the low tTHM concentrations formed, the rapid reaction kinetics of these agents, and the lower pH of the disinfectant solutions (Robinson et al., 2011; Liao et al., 2017). Further investigations into how tTHM concentrations change over longer reaction times (i.e., tens of minutes, hours, and days) for all of the disinfectants tested would be required. This would answer how the chemical quality of drinking water changes over time if dosed with either HOCl or ECAS and subsequently stored as part of a decentralized point-of-use drinking water treatment system. This study was only concerned with the formation of tTHMs, therefore THM derivatives or other DBPs formed as part of the reactions were not identified. Further studies would be required to investigate other DBPs formed as part of this reaction.

TABLE 3 | Analysis of total THM formation between reaction time (minutes) and free chlorine concentration (mg L-1), for each disinfectant type (ECAS, HOCl, and NaOCl).

Disinfectants	ECAS			HOCl			NaOCl		
Free chlorine concentration (mg L^{-1}) / Reaction time (minutes)	1	3	5	1	3	5	1	3	5
1 vs. 5 min	ns	ns	***	ns	ns	***	ns	***	***
5 vs. 10 min	ns	ns	ns	ns	***	*	ns	*	***
1 vs. 10 min	ns	ns	***	ns	***	***	ns	*	*

*Significant difference calculated through a two-way ANOVA, with a confidence interval of 95% (***$p < 0.001$; *$p < 0.05$; ns, not significant).*

Many studies into THM formation focus on formation dynamics as part of centralized water treatment systems which feed into extensive distribution networks. Decentralized systems require rapid, broad spectrum antimicrobials, and ECAS has been shown to disinfect in <10 s (Liao et al., 2007; Robinson et al., 2011), therefore is a potential alternative within decentralized POU drinking water treatment systems, where there are no extensive distribution networks (Clayton et al., in press).

Lower tTHM concentrations were observed from HOCl and ECAS disinfectants, at all free chlorine concentrations and reaction times (see **Figures 2B, C**), compared to the NaOCl disinfectant (**Figure 2A**). Statistical analysis reveals no significant difference between tTHMs formed by HOCl and ECAS disinfectants at matched free chlorine concentrations and contact times (**Table 4**). However, significant differences were observed between NaOCl, and both HOCl and ECAS ($P < 0.01$) at all matched reaction times and free chlorine concentrations (**Table 4**).

Free chlorine species present in chlorine solutions are pH dependent (Stoner et al., 1982; Sivey et al., 2010):

$$\mathrm{HOCl} \rightleftharpoons \mathrm{OCl-} + \mathrm{H+} \quad \mathrm{pKa} = 7.5 \tag{7}$$

(Liu and Margerum, 2001; Heeb et al., 2014)

THMs have been shown to have a greater affinity to form at a higher pH and with increased free chlorine concentrations (Peters et al., 1980; Urano et al., 1983; Brown et al., 2010; Saidan et al., 2013; Rasheed et al., 2017). It is known that HOCl is the dominant compound that results in tTHM formation, although, since tTHM formation is base-catalyzed (Yee et al., 2009), there is a trade-off in terms of the effect of pH. To further understand this phenomenon, changes in these physicochemical parameters within the reaction vessel were measured over the full 10 min reaction time, for each of the disinfectants. The pH and free chlorine concentration of NaOCl remained greater than both HOCl and ECAS over the 10 min reaction time (**Figure 4**). All disinfectant reaction vials showed a decrease in pH over the 10 min contact time, when reacting with 4 mg L^{-1} humic acid (**Figure 4**). Significant reductions in pH were observed for all disinfectants at 5 mg L^{-1} free chlorine ($p = \leq 0.01$). In terms of pH, no significant differences were observed between disinfectants at 1 mg L^{-1}; however, significant differences were observed at free chlorine concentrations of 3 and 5 mg L^{-1}, whereby the pH of NaOCl was greater than HOCl, and HOCl greater than ECAS (**Table 5**). All disinfectant reaction vials showed a reduction in free chlorine over the 10 min contact time from a starting free chlorine concentration of 5 mg L^{-1} (**Figure 4**). ECAS treatment resulted in the greatest comparative decrease after 10 min (34.68%), whilst NaOCl had the smallest reduction (19.34%). Collectively, this physicochemical data is in-line with previous research studies, whereby a positive correlation has been observed between higher free chlorine concentrations, higher pH and increased THM formation (Stevens et al., 1976; Kim et al., 2002; Liang and Singer, 2003; Hua and Reckhow, 2007; Chowdhury and Champagne, 2008; David, 2014). Free chlorine remains in excess after 10 min for all experiments conducted, which is consistent with previous studies (Brown et al., 2010). Therefore, further investigations should investigate whether the remaining excess free chlorine present after ECAS or HOCl reacting with NOM results in significantly increased THMs at extended contact times (i.e., >10 min).

DISCUSSION

This study has demonstrated that both HOCl and ECAS result in reduced THM formation under the experimental conditions. Reactions between NaOCl and NOM resulted in significantly increased tTHM formation compared to HOCl and ECAS under the experimental conditions. The increased formation of tTHMs in the presence of NaOCl is likely a result of the higher pH of the disinfectant (11.4 ± 0.1) compared to HOCl (5.6 ± 0.25) or ECAS (3.3 ± 0.16). THMs have shown to have a greater formation affinity in more alkaline conditions (Peters et al., 1980; Urano et al., 1983; Brown et al., 2010; Saidan et al., 2013; Rasheed et al., 2017).

The maximum observed tTHM concentrations occurred in the presence of NaOCl; however, a decline in tTHMs between 5 and 10 min was observed at 3 and 5 mg L^{-1}. This decline is potentially a result of hydrolysis (Mabey and Mill, 1978; Rahman, 2015), or dehalogenation (Hua and Reckhow, 2012; Rahman, 2015; Abusallout et al., 2017) of already formed tTHMs present in solution. The extent of tTHM degradation as a result of hydrolysis or dehalogenation over such a short contact time (i.e., 10 min) is unknown, however, the percentage composition of chloroform increases with reaction time, whilst brominated species decline (**Figure 3**). Bromine-carbon bonds are more tolerant to dissociation, compared to chlorine, as a result of lower dissociation energies (Abusallout et al., 2017). Dehalogenation is

TABLE 4 | Analysis of total THM formation between reaction time (minutes) and disinfectant type (NaOCl, HOCl, and ECAS), for each free chlorine concentration (mg L^{-1}).

Free chorine concentration	1 mg L^{-1}			3 mg L^{-1}			5 mg L^{-1}		
Reaction time (minutes) / Disinfectants	1	5	10	1	5	10	1	5	10
ECAS vs. NaOCl	**	***	***	***	***	***	**	***	***
ECAS vs. HOCl	ns	ns	ns	ns	ns	ns	ns	ns	ns
HOCl vs. NaOCl	***	***	***	***	***	***	**	***	***

*Significant difference calculated through a two-way ANOVA, with a confidence interval of 95% (***$p < 0.001$; **$p < 0.01$; ns, not significant).*

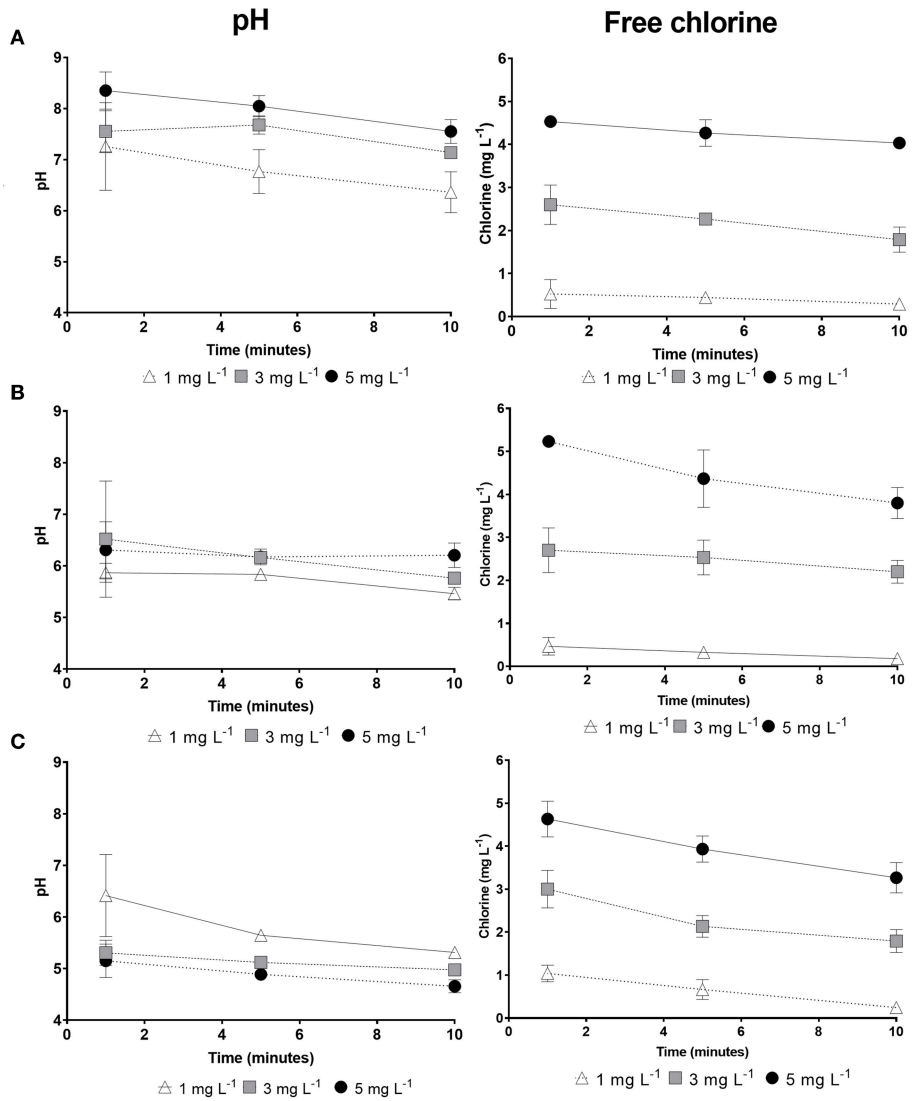

FIGURE 4 | Physicochemical parameters of disinfectant solutions reacting with 4 mg L^{-1} SRHA, over a 10 min reaction time. **(A)** NaOCl, **(B)** HOCl, and **(C)** ECAS. Left column: pH; Right column: free chlorine concentrations: 1 mg L^{-1} (Δ); 3 mg L^{-1} (■), and 5 mg L^{-1} (●). $N = 3$ (± SD).

TABLE 5 | Significant difference of physicochemical characteristics between NaOCl, HOCl, and ECAS at 1, 3, and 5 mg L^{-1} free chlorine dosing concentrations.

	pH			Free chlorine		
Dosing concentration (mg L^{-1})	**NaOCl vs HOCl**	**NaOCl vs ECAS**	**HOCl vs ECAS**	**NaOCl vs HOCl**	**NaOCl vs ECAS**	**HOCl vs ECAS**
1	ns	ns	ns	ns	*	*
3	*	**	*	ns	ns	ns
5	***	****	***	ns	ns	ns

*Significant difference calculated through a two-way ANOVA, with a confidence interval of 95% (***p < 0.001; **p < 0.01; *p < 0.05; ****P < 0.0001; ns, not significant) (N = 3).*

affected by pH, whereby, more alkaline conditions increase the rate of dehalogenation (Rahman, 2015). The decline in tTHMs was not observed with either HOCl or ECAS, potentially as a result of the neutral/acidic disinfectant properties, reducing the overall pH of the reaction (**Figure 4**). To better understand the formation of tTHMs over these short reaction times (<10 min) future studies could utilize real-time monitoring of formation and decay using selected ion flow tube mass spectrometry (SIFT-MS) (Smith and Španěl, 2005, 2011; Ioannidis et al., 2018).

The results of this study are in line with the limited number of other studies which have investigated THM formation in the presence of a neutral ECAS, when reacted with NOM (Ghebremichael et al., 2011), or within food processing applications (Gómez-López et al., 2013). A key difference between HOCl and ECAS is the antimicrobial mode of action. HOCl relies upon free chlorine for effective disinfection (Fair et al., 1948; National Academy of Sciences, 1980; Clasen and Edmondson, 2006), whereas ECAS relies upon a myriad of reactive oxidative chemical species such as OH^-, O_3, H_2O_2, and O_2^- (Jeong et al., 2006; Martínez-Huitle and Brillas, 2008), in addition to free chlorine, resulting in a high ORP (> +1,130 mV; Suslow, 2004). This high ORP is known to result in the rupture of inner and outer microbial membranes, prohibiting microbial functionality, such as the energy generating mechanism (Liao et al., 2007). Therefore, to achieve the same levels of disinfection, lower free chlorine concentrations of ECAS are required in comparison to HOCl, or indeed NaOCl, therefore reducing the potential for THM formation (Di Cristo et al., 2013). In addition, ECAS can be generated on site and *in-situ*, requiring only water, salt and energy to produce the disinfectant (Kim et al., 2000; Huang et al., 2008; Thorn et al., 2012; Clayton et al., in press). Whereas, conventional treatment systems require the transport, storage and use of, potentially hazardous chemicals such as NaOCl or chlorine gas, which can have short "shelf-lives" if stored incorrectly (direct sunlight or inadequate closure) whereby the antimicrobial efficacy can decay over time (Clarkson et al., 2001). The bespoke ECAS generator (60 L h^{-1}) used in this study has an operating current range of between 4 and 30 amps, therefore the power requirement ranges between 0.345 kW (4 amps) −0.69 kW (30 amps). Alternative ECAS generators are available which have lower power requirements, and can generate ECAS utilizing solar power (Witt and Reiff, 1993; Centrego, 2015). Such alternatives provide more practical solutions for remote locations, or as part of disaster relief efforts.

Ensuring sufficient disinfection in conventional centralized drinking water treatment and distribution systems (i.e., sodium hypochlorite or calcium hypochlorite) a residual free chlorine concentration of 0.5 mg L^{-1} is required after a contact time of 30 min (World Health Organization, 2011). Disinfectant contact in excess of minutes can cause residual free chlorine to react with NOM present in treated water, or within organic material (e.g., biofilms), in distribution network pipeworks, leading to the formation of THMs, or other DBPs. Limiting the contact time, reducing the pH of the disinfectant used (HOCl or ECAS), and reducing the organic load (i.e., humic acid or biofilm) within bulk water can help to reduce the formation of DBPs/THMs through drinking water disinfection processes (Amy et al., 1987; Brown et al., 2011a; Di Cristo et al., 2013). The results of this study have shown that ECAS treatment of water could lead to reduced formation of THMs, or other DBPs, compared to traditional chlorination (NaOCl). Further investigations are required to determine whether this observation occurs in scaled-up POU water treatment systems, as well as the role of pH, specifically within the treated water holding tank, in comparison to traditional clearwells. In addition, the free chlorine concentration of ECAS used within water treatment systems can be lower compared to conventional chlorination solutions, due to comparatively higher efficacy as a result of a high ORP and the presence of other transient oxidative functional groups. As such, this integral THM precursor will be reduced at the point of disinfection, diminishing the potential for THM formation.

CONCLUSIONS

Formation of tTHMs were quantified for three disinfectants, NaOCl, HOCl, and ECAS, when reacted with NOM for 1, 5, and 10 min. NaOCl produced the highest concentration of THMs across all reaction times and free chlorine concentrations (1, 3, and 5 mg L^{-1}). The reaction between NOM, and ECAS or HOCl, formed comparable tTHM concentrations, significantly lower than NaOCl at all free chlorine concentrations and contact times. Chloroform was the dominant THM species formed for all three disinfectants (NaOCl, HOCl, and ECAS) at all contact times and free chlorine concentrations tested. The analytical technique employed in this study (HS SPME GC/MS) appropriately allowed for the comparison of tTHMs formed in the presence of three disinfectants in model water. As part of any future work, where a more quantitative approach is required (i.e., focusing on

individual THM species formation), then an alternative analytical technique, such as GC-ECD, could be employed.

The comparative disinfection properties of ECAS, at point of generation, including reduced free chlorine compared to conventional disinfectants, high ORP (+1,130 mV), and rapid reaction time (<10 s), considerably reduces key precursors required for THM formation i.e., contact time and free chlorine availability. It has been shown that generating and storing ECAS in a reservoir tank, and dosing a low concentration (v/v) within a decentralized POU drinking water production system, is effective at producing Drinking Water Inspectorate drinking water (Clayton et al., in press). ECAS can be generated as a concentrated stock, and continuously dosed in-line to achieve the desired final concentration within the bulk water. No tTHMs were detected after a 1 min contact time, and only low concentrations of tTHMs were detected after 5 and 10 min, which were considerably lower than the maximum guideline value permissible in drinking water. Therefore, ECAS could be considered a safe alternative to conventional chorine disinfection for decentralized point-of-use water treatment systems, as free chlorine concentrations can be lower compared to conventional chlorination solutions due to comparative higher efficacy. This reduces an integral THM precursor, decreasing the formation potential of THMs.

This study focussed on the quantification of four specific THMs (chloroform, BDCM, DBCM, and bromoform). As such, other associated THM derivatives or DBPs were not identified, and identification of these possible derivatives would be of interest. Further work is required to determine any DBP derivatives that may form in place of, or in addition to, THMs. Finally, scaled-up testing is required to determine whether the results obtained in this study are representative within decentralized POU water treatment systems.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the supplementary files.

AUTHOR CONTRIBUTIONS

GC: method development, data collection and analysis, writing—original draft manuscript preparation, editing and review; DR: conceptualization, methodology; RT and DR: supervision, funding acquisition, writing—review and editing.

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