A fundamental design study of electrochemical processes for the control of pathogenic bacteria

A thesis submitted for the degree of Doctor of Philosophy

By

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Abstract

Water systems in buildings have been reported to contribute to pseudomonal infection transmission and have been associated with Legionnaires' disease (LD) outbreaks, for they provide the perfect conditions for bacteria proliferation and biofilms formation. An overview of the problem has highlighted that the economic burden, the healthcare and mortality costs of both LD and pseudomonal infections are significant. Although critical to the safe delivery of water, pathogen control continues to remain a challenge as current hot water treatments are not always effective, are often energy intensive and require expensive maintenance.

This thesis was set out to evaluate the potential use of electrochemical disinfection (ED) in controlling pathogens in hot water systems of buildings. In this project, we performed a fundamental systematic study on the effect of geometrical and operational parameters in a flask, to gather an understanding of the effect of each parameter on the rate of bacteria elimination, crucial for the design and optimization of electrolytic cells. ED prototypes were then installed in in the hot water systems of two different buildings operating at 60°C, the temperature recommended for *Legionella* control (HSE, 2013), and their efficacy was monitored long term. In one of the buildings, 2 to 4– log reductions in total bacteria counts was observed, while *Pseudomonas* species counts were reduced by 3 log. The apparent failure in the other building was due to the inadequate operation of the water system.

In order to achieve the 2019 zero carbon targets for new non-domestic buildings set by the UK government, the energy demand associated with heating water needs to be addressed, but maintaining systems at such high temperatures renders difficult the use of greener technologies that could further reduce the CO_2 impact of heating water. Given that ED generates disinfectants and that the Health and Safety Executive advises that if hot water is treated with biocides, water temperatures can be reduced, the efficacy of the prototype device was evaluated under laboratory conditions at

temperatures between 30 and 45˚C. The prototype was found to be effective both on laboratory-grown biofilm and on planktonic *Legionella pneumophila* serogroup 1, with 5-log reduction on bacteria counts.

Declaration

I declare that this thesis is my original work except where otherwise stated.

Signature: _____ Date: _____

Statement of Confidentiality

The information enclosed in this report is strictly confidential and as such it shall not be used, sold, assigned or divulged to any other person, organisation or corporation without the permission of Environmental Scientific Group Ltd.

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Symbols

A	Area (cm^2)
Ag^+	Silver + ion
c	Specific heat capacity (J/K)
$CaCO_3$	Calcium carbonate
Cl_2	Chlorine
ClO_2	Chlorine Dioxide
C^n	Concentration of disinfectants
CO_2	Carbon Dioxide
CT	CT value (mg/L min)
Cu^+	Copper + ion
Cu^{2+}	Copper 2+ ion
E	Energy (J)
Fe^{2+}	Iron
G	Surface factor
H_2	Hydrogen
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen Sulfide
HCl	Hydrochloric acid
HClO	Hypochlorous acid
I	Current (A or mA)
Ir	Iridium

J	Current density (A/cm^2 or mA/cm^2)
-k	Empirical organism, temperature and pH dependent constant
m	Mass (kg)
$Mg(OH)_2$	Magnesium Hydroxide
Mn^{2+}	Manganese
N	Bacteria concentration at any given time
N_0	Initial bacteria concentration
$NaClO_2$	Sodium Chlorite
NCl_3	Thrichloramine
NH_3	Ammonia
NHCl	Monochloramine
$NHCl_2$	Dichloramine
O	Oxygen atom
O_2	Oxygen molecule
O_3	Ozone
OCl^-	Hypochlorite ion
OH^-	Hydroxyl radicals
Pt	Platinum
Ru	Ruthenium
t	Time (min)
T_f	Final temperature (°C)
T_i	Initial temperature (°C)

Ti	Titanium
u_c	Combined Uncertainty (%)
ω_f^2	Uncertainty of the dilution factor (%)
ω_v^2	Uncertainty of the total volume (%)
ω_z^2	Poisson scatter (%)

Abbreviations used in the text

ACoP	Approved Code of Practice
BCYE	Buffered Charcoal Yeast Extract
CFU	Colony Forming Units
CIBSE	Chartered Institution of Building Services Engineers
CT value	Concentration and Time Value
DBPs	Disinfection by-products
DECC	Department of Energy and Climate Change
DEFRA	Department for Environment, Food and Rural Affairs
DFA	Direct Fluorescent antibody
DHW	Domestic Hot Water
E.coli	*Escherichia coli*
EAA	European Economic Area
ECDC	European Centre for Disease Prevention and Control
ED	Electrochemical Disinfection
ELDsNet	European Legionnaires ' Disease Surveillance Network

EPS	Extracellular Polymeric Substances
ESG	Environmental Scientific Group
EU	European Union
HAA	Haloacetic acids
HAI	Healthcare-acquired Infections
HPS	Health Protection Scotland
HSCIC	Health and Social Care Information Centre
HSE	Health and Safety Executive
HTM	Health Technical Memorandum
ICU	Intensive Care Units
L. pneumophila	*Legionella pneumophila*
LD	Legionnaires' Disease
NHS	National Health Service
P. *aeruginosa*	*Pseudomonas aeruginosa*
PCR	Polymerase Chain Reaction
PHE	Public Health England
RSD	Repeatability Standard Deviation
SEM	Scanning Electron Microscopy
Sg.	serogroup
Spp.	Species
THM	Trihalomethane
TMV	Thermostatic Mixing Valves

toe	Tonnes of equivalent oil
TVC	Total Viable Count
UK	United Kingdom
UNICEF	United Nations International Children's Emergency Fund
USA	United States of America
UV	Ultraviolet
WHO	World Health Organisation
WSG	Water Safety Group

Chapter 1 : Introduction

‘In an age when man has forgotten his origins and is blind even to his most essential needs for survival, water along with other resources has become the victim of his indifference.’

Rachel Carson

1.1 Background

The control of pathogens is critical to the safe delivery of water as its contamination can lead to disease outbreaks in both developed and developing countries. It is estimated that 780 million people still have no access to clean water at a global scale, making unsafe water the leading cause of sickness and death (WHO and UNICEF, 2012). In developing countries, the lack of clean water and sanitation causes 3.41 million deaths a year, 1.5 million of which are children. Meanwhile, in developed countries the ever increasing number of systems storing water that by design, harbour bacteria such as cooling towers, hot water systems, spas and air-conditioning has resulted in disease outbreaks caused by opportunistic bacteria such as *Legionella* and *Pseudomonas* (BBC News, 2012, The Regulation and Quality Improvement Authority, 2012, Health and Safety Executive, 2013, Queensland Health, 2013).

Whilst larger outbreaks of Legionnaires' disease are generally associated with cooling towers, the more frequent smaller outbreaks and individual cases are attributed to hot water systems. In recent years, there has been evidence that *Legionella* is not the only danger from contaminated hot water systems, but that *Pseudomonas aeruginosa* can also pose a risk, if it is not kept within control limits (Department of Health, 2013). Large buildings often comprise central hot water storage with extensive pipework, providing ideal breeding grounds for bacteria; thus, non-domestic buildings such as universities, hospitals, schools, health clubs, leisure centres and hotels require measures to minimise the risk.

Standard methods of controlling pathogens in the hot water systems of non-domestic buildings include pasteurisation and chlorination. Despite their effectiveness, biofilm is not often eliminated and there are some drawbacks. The generation of toxic by-products is the main disadvantage of chlorination, whilst temperature control is associated with high running costs, considerable CO_2 emissions and increased risk of scalding. Of the alternative water treatments, many researchers have identified electrochemical

disinfection (ED) as having the potential to overcome those limitations (Patermarakis and Fountoukidis, 1990, Kerwick et al., 2005, Kraft, 2008, Martinez-Huitle and Brillas, 2008). No transport, storage and dosing of hazardous chemicals are required. It might also allow hot water to be distributed at lower temperatures, leading to a decrease in energy consumption and resultant CO_2 emissions.

Three years ago, Brunel University was approached by Waterwise Technology Ltd (now part of the Environmental Scientific Group), a company specialised in the development, design and manufacture of innovative systems for the treatment of water. They asked the team at Brunel University to evaluate the performance of an ED device in controlling the growth of pathogens in hot water systems. The device comprised a set of low voltage electrodes which, when placed in water services pipework, apparently produced chlorine, chlorine dioxide and other disinfectant products without the need to dose any chemicals to the water.

It was clear from the outset that if the claims were accurate, then there could be considerable potential for the use the ED device in hot water systems in buildings. There are some 2 million hot water installations in non-domestic buildings in the UK alone where Protex might offer effective water hygiene protection at reduced temperatures with resulting reduction in energy consumption and CO_2 emissions together with simpler and cheaper maintenance. Furthermore, it was apparent that there were other applications where disinfection of water is required that could benefit from Protex.

1.2 Objectives

The main objectives of this investigation were:

1. to contribute to the understanding of how geometrical and operational parameters influence the performance of the electrolytic process with the scope of optimizing the process at a later stage. This was achieved by performing experiments under controlled laboratory conditions, both in a flask experiment and in a purposely built rig, designed to simulate a scaled-down hot water system.
2. to evaluate the effectiveness of the electrolytic process in controlling *Legionella* and other pathogens in hot water systems operating at 55-60°C, the temperatures recommended for *Legionella* control (HSE, 2013), by installing and monitoring ED devices in the hot water recirculation systems of two different buildings. Even though many studies have confirmed the ability of several ED devices in eliminating various pathogens under controlled laboratory conditions, (Patermarkis and Fountoukidis, 1990, Furuta et al., 2004, Kerwick et al., 2005, Fang et al., 2006, Jeong et al., 2007, Polcaro et al., 2007, Delaedt et al., 2008, Sarkka et al., 2008), no data are available on the long-term monitoring of electrolytic devices in the field.
3. to evaluate the efficacy of the electrolytic process at lower water temperatures (40-45°C) under laboratory conditions. The Health and Safety Executive (2013) advises that if hot water is treated with biocides, water temperatures can be reduced, providing Legionella levels are monitored. Given that ED generates disinfectants, water temperatures can be lowered, reducing the energy associated with heating water in buildings. Temperature reduction is not currently permitted in hospitals and healthcare premises (Department of Health, 2006).
4. to determine if the residual disinfectants generated in water at lower temperatures could decrease or eliminate the formation of biofilms in pipes. No data are available in the literature on the effect of

electrochemical disinfection on biofilm.

The results are aimed at the control of pathogens in hot water systems but can be used by engineers, researchers and scientists developing electrochemical cells for any application aimed at bacterial disinfection.

1.3 Thesis outline

The overall structure of the study takes the form of seven chapters including this chapter with an introduction to the topic, the thesis objectives and the thesis outline.

Chapter 2 begins by highlighting the importance of pathogen control in hot water systems of commercial buildings, and comprises an overview of the most problematic pathogens. Bacteria facts, detection methods, incidence, infections contracted and treatment methods are discussed for both *Legionella* and *Pseudomonas* bacteria. Furthermore, the economic burden of diseases related to *Legionella* and *Pseudomonas* is also quantified by estimating the costs arising from healthcare and lost working days through illness and by evaluating the likely cost of monitoring *Legionella* in those buildings. This is followed by a critical overview of typical water treatments, including filtration, heat treatment, UV lights and chemical methods such as chlorination, chloramination, chlorine dioxide, copper-silver and ozone. ED is then introduced as an alternative method of hot water disinfection, and a comprehensive review of the literature published on the use of ED for bacteria inactivation is presented.

The results of small scale experiments undertaken in a flask to determine the effect of geometrical and operational parameters on the inactivation rate of bacteria are presented in Chapter 3.

Chapter 4 comprises the results of the long-term monitoring of two ED prototypes installed in a large university building and in a small-size health-care centre. Furthermore, an estimate of the energy and CO_2 savings that might arise from operating hot water systems in non-domestic buildings at

lower temperatures is provided.

The results of the experiments at lower water temperatures undertaken in the scaled-down hot water purposely designed are shown in Chapter 5.

The final chapter includes an overall critique of the findings and identifies areas for future research.

Chapter 2 : The fight against water pathogens

'When antibiotics became industrially produced following World War II, our quality of life and our longevity improved enormously. No one thought bacteria were going to become resistant.'

Bonnie Bassler (2009)

2.1 Water treatment

Attempts at treating water have been recorded as early as 2000 B.C. The Sanskrit writings suggest methods of treating water such as boiling, exposure to the sun and filtration through gravel and sand. Images of water purification systems as the one in Figure 2.1 have been found in Egyptian tombs dating back to the 13th and 15th century B.C.

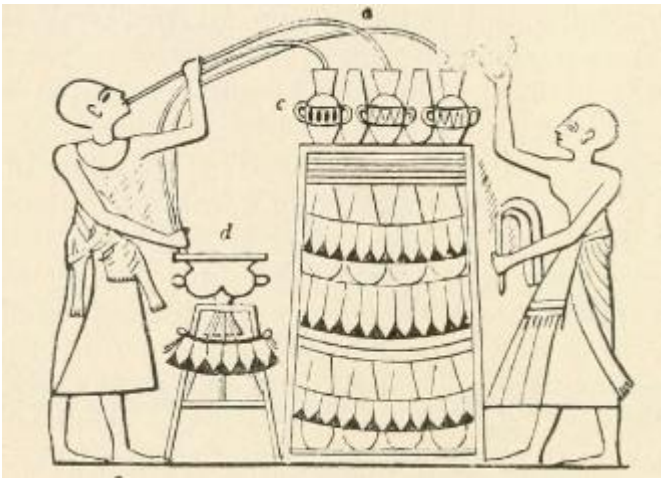

Figure 2.1: Image of a water purification system found in Egyptian tombs (Wilkinson and Birch, 1879).

Although water is essential to life, it is taken for granted. Often, we forget that the tap water we use could have killed us only a century ago. Indeed, one of the major tributes to public health was the adoption of water chlorination that resulted in the elimination of common waterborne diseases such as typhoid, cholera and dysentery. This was followed by the construction of additional water supply lines to meet the ever increasing water demand of fast-growing cities.

A schematic of the water distribution process from source to consumer can be seen in Figure 2.2. As shown in the diagram, water starts its long journey to our taps after being disinfected with chlorine or filtered at the source, during the process defined as primary disinfection. While the water leaving the plant should meet thorough quality standards, it is not practicable or economical to sterilize it, so it is likely that a small amount of bacteria might

enter the water distribution system there. Once in the distribution system, bacteria can multiply, persist in biofilms and harbour in amoebae where they can survive until the conditions are favourable for dispersal. Bacteria can also be introduced to potable water supplies when leaks occur or when maintenance work is carried out. Hence, water utilities are in charge of maintaining residual disinfection along the distribution system to minimise the growth of microorganisms; this process is defined as secondary disinfection. Both primary and secondary disinfection ensures water in our taps is safe to drink.

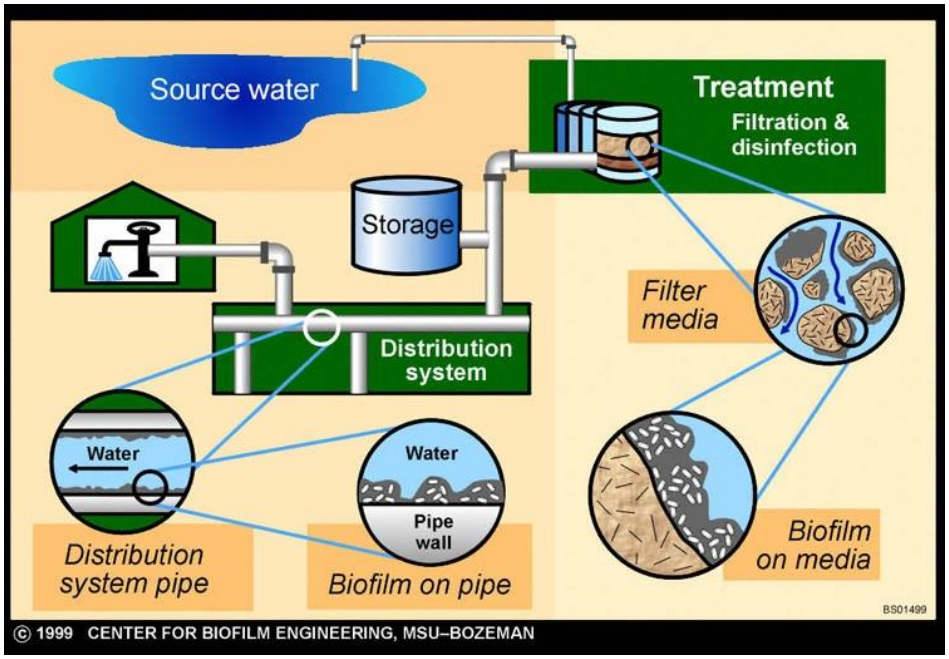

Figure 2.2: Schematic diagram of a water distribution system, showing locations for biofilm growth. Reproduced with permission (Camper and Dirckx, 1999).

Once a luxury for the privileged, hot water and air conditioning are nowadays the norm in hotels, offices, hospitals and houses. Unfortunately, the inadequate disinfection and maintenance of the growing number of systems storing water have resulted in outbreaks of illnesses associated with pathogenic bacteria such as *Legionella* and *Pseudomonas* species.

2.2 Hot water systems in commercial buildings

2.2.1 The problem with commercial buildings

The UK Water supply Regulations requires that in non-domestic buildings, the property owners and/or facilities managers maintain the water quality (The Water Supply Regulations, 1999). Under Health and Safety Law, they have the legal duty to safeguard the water users from contracting Legionnaire's disease by identifying sources of risk, managing and controlling the risks and keeping records of maintenance and control routines (HSE, 2013). Although hot water systems in large buildings carry the highest risk of bacteria colonisation, the risk of proliferation in the cold systems of buildings or in the distribution system cannot be ruled out. Therefore, *Legionella* and *Pseudomonas* control in non-domestic buildings is of paramount importance because a single exposure to pathogens from a contaminated shower, could lead to a serious illness.

Commercial buildings have extensive hot and cold water distribution systems, with lengthy pipework and they are either supplied by a cold water tank or directly from the incoming cold water main. A schematic of a gravity water system with recirculation, typically found in a commercial building, can be seen in Figure 2.3 (HSE, 2013). The water tank supplies cold water directly to baths, basins, showers, sinks and WCs at 20°C, whilst replenishing the calorifier. As shown in the schematic, the water in the calorifier is either heated by exchanger coils containing water previously heated by external heat sources such as boilers, or by electric heating. A pump is used to recirculate the hot water across the whole system to provide instant hot water at all outlets. Hot water is stored at a minimum of 60°C and the pump is seized to maintain the return water to the calorifier at 50°C. Water at such temperatures can cause scalding injuries; thereby sinks, baths and showers are fitted with thermostatic mixers that provide water at temperatures between 41 and 48 °C.

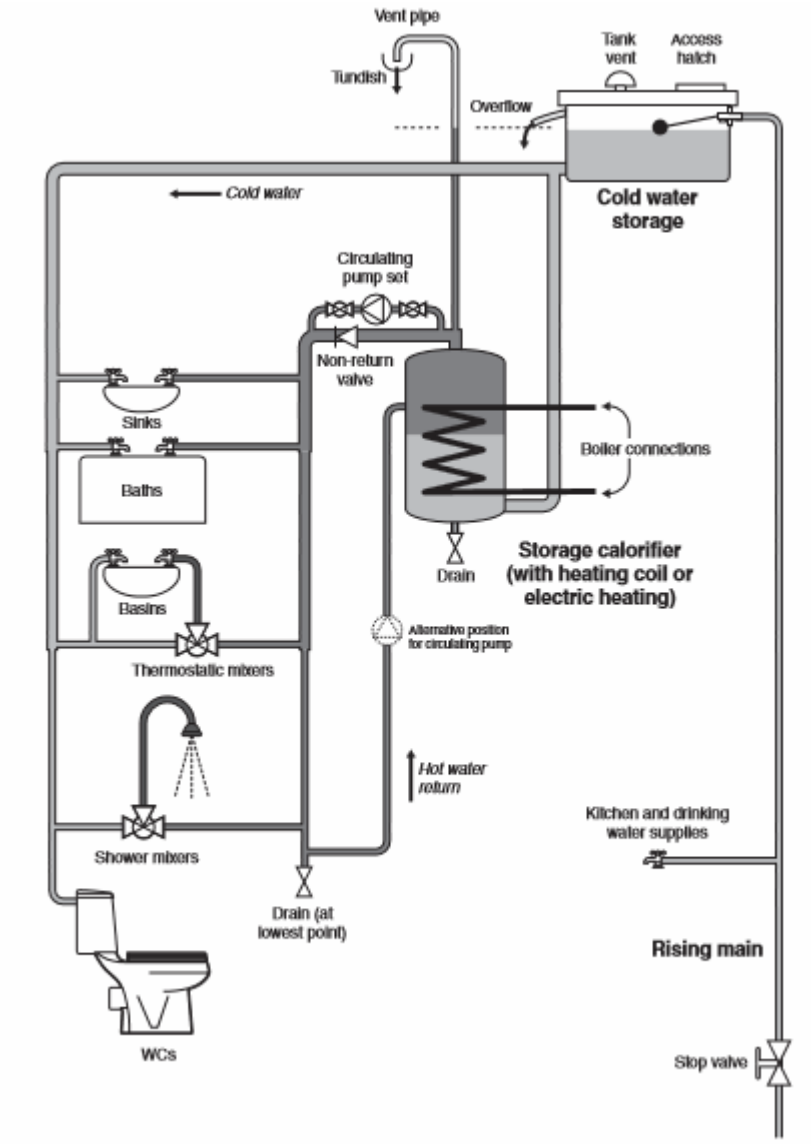

Figure 2.3: Schematic of a typical gravity system with recirculation (Image taken from HSE, 2013).

These buildings not only include aerosol-producing systems such as showers, pools, air-conditioning and hot water taps but they also deliver water to elderly, and immuno-compromised users, the groups most at risk of contracting diseases. The pipework may also include several areas where bacteria can proliferate and form biofilms. These include under-used outlets, dead-legs where water stagnates and water temperature is not maintained, and shower heads, water taps, tanks and calorifiers where nutrients such as scale and sediments accumulate (HSE, 2013).

The Health and Safety Executive (HSE, 2013) defines a dead-leg or blind end as “a length of pipe closed at one end through which no water passes”. Although dead-legs can be reduced by good design, frequently they are the result of poor remedial work; often, when pipes are inaccessible, the contractors might not have any other choice but to create a dead-leg. The Water Regulations Advisory Scheme (2015) advises that any unused pipe should be completely removed but when not possible that the length of dead-leg created is no longer than twice the pipe diameter. Figure 2.4 shows an example of a sink used for storage where water might stagnate for long periods of time, and Figure 2.5 is a photograph of an unnecessary dead-leg.

Figure 2.4: Photograph of a sink used for storage (Ibbotson, 2011).

Figure 2.5: Photographs showing a dead-leg (Ibbotson, 2011).

Other areas at great risk of bacteria proliferation are areas where stratification of water occurs; for example, at the bottom of a calorifier where the cold water comes into contact with the hot water, or in twin coil storage cylinders in which the coil at the base of the tank pre-heats the water to 45˚C whilst the coil at the top of the tank heats the water to 60˚C. In both these examples the water demand might exceed the required water residence time for complete disinfection.

Poorly designed/installed water systems may include hot and cold water pipes in close proximity, resulting in elevated or reduced water temperatures. Even in well-designed Domestic Hot Water (DHW) systems, some outlets may be under-used as a result of changes in the way the building is utilised. For example, schools and universities are closed for long periods of time and hotels occupancy varies with seasons; in addition, it is not unusual for a building to be sold or leased to another company affecting the water usage.

Another problem facing the building services industry is the microbially influenced corrosion (Lewandowski and Beyenal, 2009, Li et al., 2013, Chen et al., 2014) and blockages associated with the growth of biofilm in the pipework, decreasing the flow and therefore the efficiency of the systems (Flemming, 2002). During the pre-commissioning period of a building, when it is unoccupied and water stagnates, *Pseudomonas* might find the conditions to proliferate and forming biofilm that may harbour other pathogens. Biofilm formation such as the one shown in Figure 2.6 can lead to water back flows and loss of pressure in distribution systems, further enhancing bacterial growth.

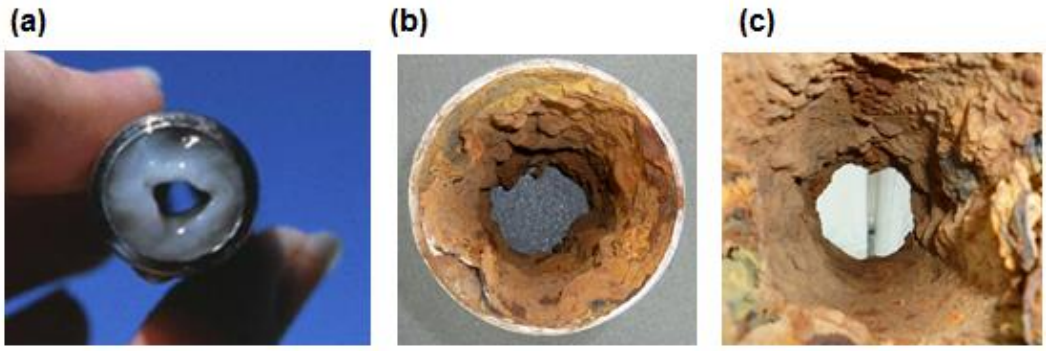

Figure 2.6: (a) Biofilm in pipe section (University of Montana, Centre for Biofilm Engineering); (b) and (c) Photographs of biofilm formation, corrosion and scale deposits in a pipe taken out of a building during maintenance work (photographs taken by the author).

2.2.2 Disease outbreaks in non-domestic buildings

Large Legionnaires' disease (LD) outbreaks are often associated with cooling towers as they can spread the bacteria far and wide in small water droplets. However, according to the HSE, in a study published in 2012 investigating all the outbreaks of LD in the last ten years in the UK, 39% of known outbreaks are due to hot water systems and spas whilst cooling towers are the cause of 16%. The results can be seen in Figure 2.7 (Health and Safety Laboratory, 2012). Similarly, the European Legionnaires' disease surveillance network (ELDsNet) found that 77% of the environmental samples positive for *Legionella* related to LD cases in 2011 were from water systems, whilst only 1% was associated to cooling towers, confirming the HSE findings (European Centre for Disease Prevention and Control, 2013). So, the more frequent smaller outbreaks are associated with hot water systems that can provide ideal breeding grounds for *Legionella,* and include aerosol-producing equipment that can enable transmission. However, the true number of LD cases related to DHW systems is far greater as individual cases are more likely to be missed than outbreaks (European Centre for Disease Prevention and Control, 2013). Recently, the Basildon and Thurrock University Hospital was fined £350,000 for failing to safeguard the seven people who contracted LD from the hospital's hot water system (HSE, 2013) and the water system of a three-year old hospital in Brisbane was

identified to be the source of the Legionnaires' disease outbreak that took place in June 2013 (Queensland Health, 2013). This outbreak confirms that outbreaks are not only limited to buildings with old pipework and aged equipment, but can occur in new buildings with modern plumbing.

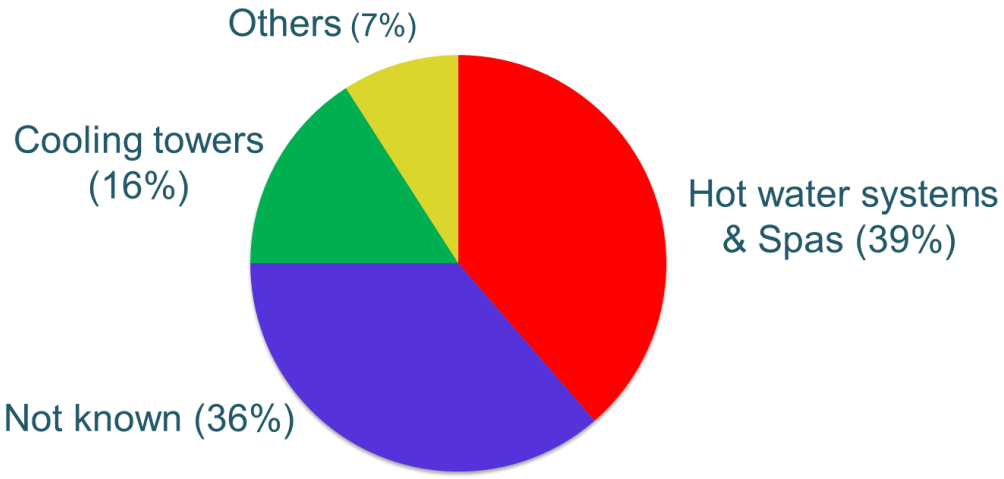

Figure 2.7: Legionnaires' disease outbreaks by system type (Adapted from Health and Safety Laboratory, 2012).

2.3 *Legionella*

2.3.1 History

On 27th of July 1976, members of the American Legion gathered in a hotel in Philadelphia for the American Bicentennial. Within two days, 221 attendees reported chest pain, developed pneumonia and 34 died (Diederen, 2007). Speculations about the cause of the outbreak ranged from lethal chemicals to terrorists attacks, and panic spread around the globe. Finally, in January 1977, the United States Center for Disease Control claimed that the cause of the outbreak was a previously unrecognised bacterium, which they consequently named *Legionella pneumophila*; *Legionella* after the affected Legionnaires, and *pneumophila* based on the Greek word for lung. A colorized scanning electron micrograph of the bacterium can be seen in Figure 2.8.

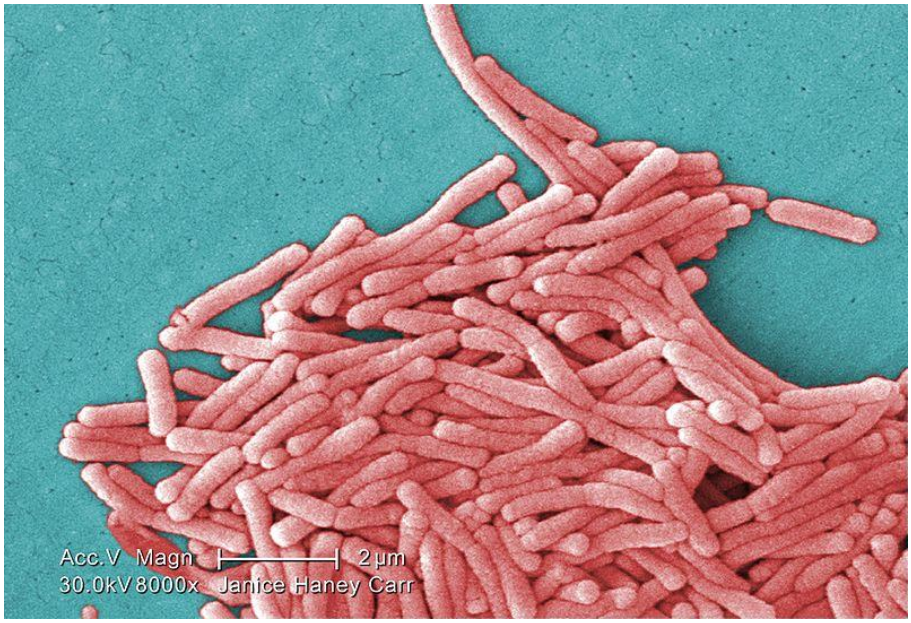

Figure 2.8: Colorized scanning electron micrograph depicting a large grouping of *L. pneumophila* bacteria (Williams et al., 2009).

This discovery prompted scientists to analyse samples of previous outbreaks of pneumonia, and they found *Legionella* to be the cause of several others. These include the 1965 pneumonia outbreak in a psychiatric hospital in Washington DC., during which 81 people contracted the disease and 15 died (Zonderman, 2006). Equally, the bacterium was found to be the cause of the 1968 epidemic of febrile myalgia that affected 144 people in Pontiac, Michigan (Glick et al., 1978). These findings led to the conclusion that *Legionella* could cause two distinct clinical conditions: the deadly Legionnaires' disease, a serious lung infection; and Pontiac fever, a flu-like illness that usually resolves without treatment. Collectively the two are known as legionellosis.

2.3.2 The bacterium

Legionella species are widespread in the environment and can be found suspended in small quantities in the fresh water of lakes and rivers. They can also be found in man-made water reservoir such as hot and cold water systems, cooling towers and other water storage systems. *Legionella* are aerobic bacilli measuring 0.3-0.9 µm in width and 2-20 µm in length

(Diederen, 2007). There are 48 species of the bacteria divided into 70 serogroups, but it is *L. pneumophila* serogroup 1 that accounts for most of the reported legionellosis cases. According to the European Surveillance Network for Legionnaires' Disease, *L. pneumophila* serogroup 1 was the cause of 88% of reported cases of LD in 2011 (European centre for Disease Prevention and Control, 2011). Requirements for their growth include temperatures between 25°C and 42°C and nutrients including cysteine found in protozoa and iron. The presence of scale, sediments, organic matter and nutrients (metals) makes pipes the ideal environment for the bacteria to thrive, whilst water stagnation due to low water demand and poor flow sustains the formation of biofilms, where *Legionella* can persist in a latent state (Murga et al., 2001).

The difficulties encountered when growing *Legionella* in laboratory media confirms that the bacteria requires other microorganisms to survive in water systems. *Legionella* is found living within biofilms where there is a provision of nutrients and where they are protected from high temperatures and disinfectants. In their study, Murga et al. (2001) demonstrated that *Legionella* persisted in biofilm up to 15 days, and that it was even able to multiply in the presence of amoebae, confirming that the latter play a crucial role in the bacteria life cycle as suggested by Rowbotham in the 1980s (Rowbothan, 1980). *Hartmannella* and *Acanthamoeba* are two of the amoebae known to support *Legionella* species intracellularly (Winiecka-Krusnell, Linder, 1999). During the active stage, amoebae feed on bacteria as shown in Figure 2.9; and if there is a change in temperature and/or nutrients are lacking, they are capable of entering a dormant stage by enclosing themselves in a cyst. In this form, they do not replicate and can withstand high temperatures and biocides. *Legionellae* are some of the bacteria amoebae feed upon, the bacteria having evolved to survive, replicate and exist in their amoebae host. This symbiotic relationship, where the amoeba acts as a reservoir as shown in Figure 2.10, might explain the persistence of *Legionella*e in the environment (Greub and Raoult, 2004).

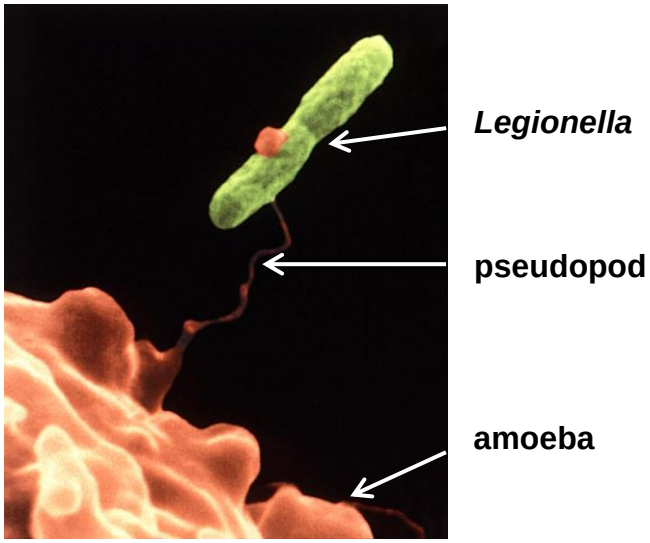

Figure 2.9: Electron micrograph depicting an amoeba, *Hartmannella vermiformis* entrapping a *Legionella* bacterium with a pseudopod (Barry and Fields, 2011).

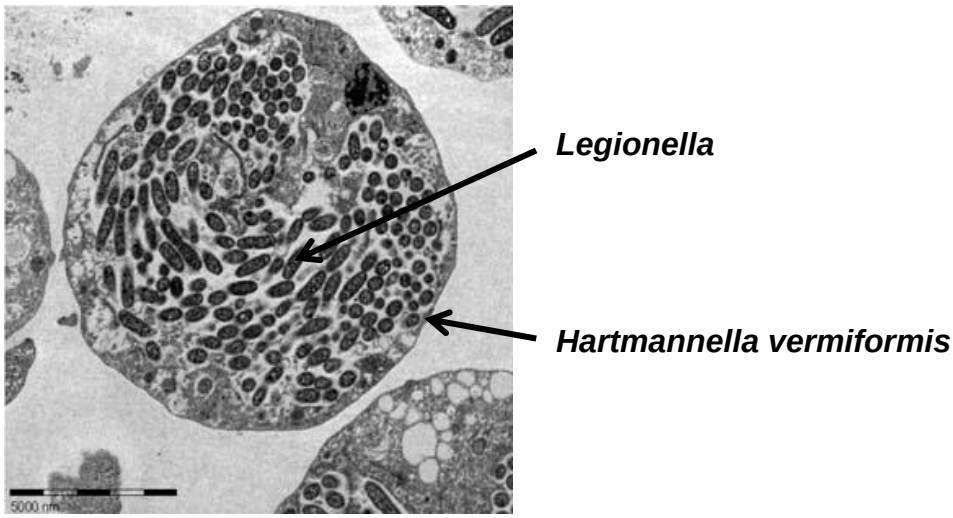

Figure 2.10: *Hartmannella vermiformis* filled with *Legionella* bacteria (Holland, G.).

2.3.3 Legionnaires' disease

Both Legionnaires' disease and Pontiac fever are contracted by inhaling a water aerosol or droplet contaminated with *Legionella* bacteria or with amoebae infected by *Legionella* (Rowbotham, 1980, Brieland et al., 1996). The disease is not transmissible from person to person and the incubation time is 2 to 14 days after exposure. On the other hand the incubation period for Pontiac fever is between 24 and 72 hours. Symptoms include cough, high temperature, muscle pains, shivers, sweats and headaches. Respiratory

failure, sepsis and kidney failure are possible complications of the disease, whilst the fatality rate varies from 5-30% (Centers for Disease Control and Prevention, 2013).

LD cases are classified into three categories identifying the source of exposure. Cases who have travelled abroad during the incubation period prior to the onset of the symptoms are defined as travel-associated LD cases. Cases whose source can be traced back to hospitals or other healthcare facilities are defined as nosocomial cases. The remaining cases associated with other sources are defined as community acquired; sources might include spas, hot/cold water systems in leisure centres, cooling towers, hotels, schools, offices, hot/cold domestic water systems.

The elderly, those with existing respiratory problems and those who are immuno-compromised are most susceptible to the bacterium. Figure 2.11 shows the cases of Legionnaires' disease reported in the European Union/ European Economic Area (EU/EEA) in 2011 by age and gender group. It is clear that the most vulnerable age group is the 65+ and that there is a significant difference between men and women contracting the disease with a ratio of 2.5:1. The reason for the discrepancy between men and women is not known (European Centre for Disease Prevention and Control, 2013).

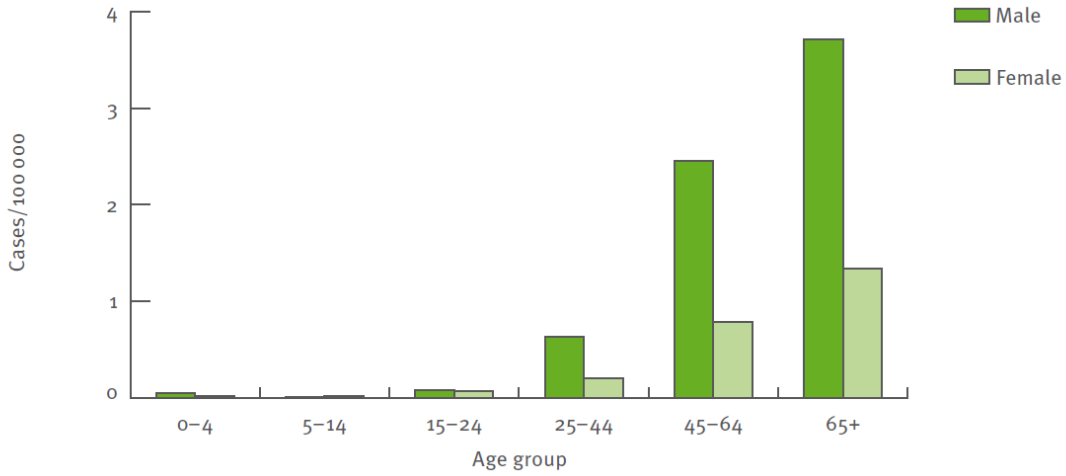

Figure 2.11: Cases of Legionnaires' disease reported in EU/EEA, by age and gender, 2011 (ELDSNet, 2013).

2.3.4 Detection and treatment

The initial detection of LD is by chest x-rays as for any other type of pneumonia. At this stage, the clinician should prompt the appropriate microbiological testing to detect if the illness has been caused by *Legionella* bacteria.

The isolation and growth of bacteria by culture of phlegm and lung tissue on charcoal yeast extract agar can confirm the presence of *Legionella*. The advantage of this method is that clinical isolates can be compared to environmental isolates, enabling the identification of the source of exposure. The prolonged incubation time and the absence of patient sputum are the main reasons why culture of isolates should be performed concurrently with other diagnostic tests (Maywald, 1998). These include Polymerase Chain Reaction (PCR), Direct Fluorescent antibody staining (DFA), serologic diagnosis and urine antigen test. PCR permits the detection of *Legionella* DNA and it is highly specific, enabling the identification of all strains of *Legionella* species. In contrast, DFA staining of sputum enables the detection of *L. pneumophila*, and it is limited by the low number of organisms present in the initial stage of the disease. This can result in false negatives, causing the test sensitivity to be as low as 25%. Other diagnostic tests include serologic analysis of blood samples to detect the antibodies formed in response to the disease; the main limitation of this technique is that the rise in antibodies levels has been recorded for only 70-80% (Edelstein, 1997) of patients. The most frequently used diagnostic method is the urine antigen test that enables the rapid detection of the antigens discarded by the bacteria in the urine of the patient. Although results can be obtained within 30 minutes, and the sensitivity is high at 70-80%, this test is specific to *L. pneumophila* sg. 1 (Diederen, 2007).

Legionnaires' disease can be treated with antibiotics such as erythromycin, clarithromycin, doxycycline and azithromycin that can be administered for 10 days in the form of a pill, or through an intravenous infusion. Although the treatment is relatively rapid, LD is associated with long recovery periods, with

patients experiencing fatigue, shortness of breath, cough, muscle pain and memory loss. A study of 31 survivors of the Philadelphia LD outbreak revealed that 18 patients were not fully recovered after 2 years (Lattimer et al., 1979). Similarly, a more recent follow-up study of 122 LD survivors in the Netherlands confirmed that the majority of people had not completely recovered after 17 months, and that 15% of the patients suffered from posttraumatic stress disorder (Lettinga et al., 2002).

2.3.5 Incidence

According to the ELDsNet, the number of detected LD cases has increased gradually over the years; the reported cases for Europe and the UK from 1993 to 2011 can be seen in Figure 2.12. More than 4,800 cases of LD were reported in Europe in 2011, 281 of which were in the UK. The majority of cases were community-acquired, 24% were travel-associated and 7% were nosocomial (related to hospital stays); the average notification rate was 1 per 100,000 inhabitants; the fatality rate was 9%.

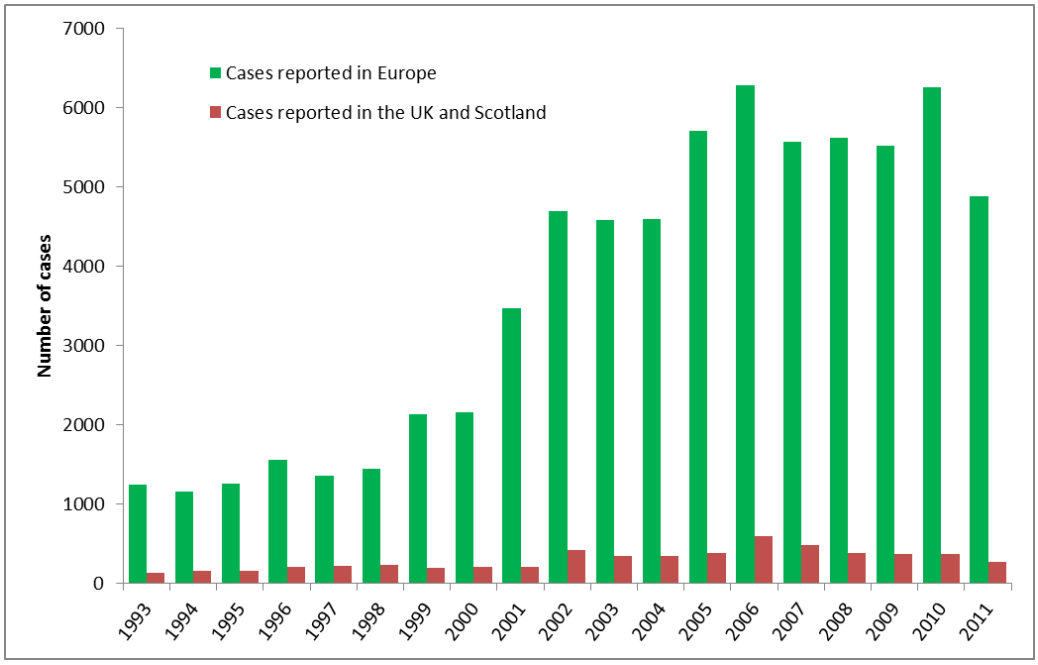

Figure 2.12: Number of reported cases of Legionnaires disease in the UK and Europe 1993-2011. Data for LD cases in Scotland is only available from 2000 (Adapted from Public Health England, 2014, Health Protection Scotland, 2013, European Centre for Disease Prevention and Control, 2013).

2.3.6 The problem with underreporting

83% of notified cases were reported by Spain, the UK, Italy, France, The Netherlands and Germany, confirming underreporting could be a problem in other European countries (European Centre for Disease Prevention and Control, 2013). However, the number of confirmed cases could be further underestimated for a number of reasons. To begin with, it is not possible to distinguish the disease from other forms of pneumonia with chest x-rays, and further tests should be prompted by the clinician. A recent American study reported that microbiological tests for the identification of *Legionella* are performed in less than half of patients with pneumonia (Hollenbeck et al., 2011). It is often difficult to obtain sputum for analysis, and despite its rapidity, the urine antigen test can only identify *L. pneumophila* sg. 1. The high percentage of urinary antigen detections (77% of all the notified cases) confirms possible underestimation of cases caused by other *Legionella* species and serogroups (European Centre for Disease Prevention and Control, 2013). Other reasons include the tendency of clinicians to administer antibiotics prior to diagnosis, preventing the identification of the pathogen; the lack of record on number of patients treated at home may further increase underestimation. In addition, in the UK, Legionellosis became a reportable disease only in April 2010 (Joseph et al., 2010).

L. pneumophila sg. 1 was the cause of the outbreak of Legionnaires' disease in South West Edinburgh, Scotland in June 2012 where 101 people are known to have contracted the disease, and four of whom died (McCallum, 2013). More recent episodes include the Renfrew outbreak in June 2013 where 6 people contracted LD, and the major outbreak in Warstein, Germany where 165 people contracted the disease and two people died. The sources of the outbreaks in Edinburgh, Renfrew and Warstein remain unknown. Outbreaks such as these, are brought to attention through the large number of people infected that facilitates the identification of the bacterium responsible. However, it is believed that a large number of cases of legionellosis go unreported as these do not result in widespread illnesses. Seeing that hospital admissions due to pneumonia have increased over the

years, and that in 90% of episodes the specific bacterium is not identified, it is highly possible that many cases of LD are treated as individual and unrelated cases of pneumonia. Figure 2.13 presents the trend of hospital admission due to pneumonia for the period of 2008-2013. In the EU as a whole the ELDSNet data suggests as many as 50,500 cases or about 100 per million of the population (European Centre for Disease Prevention and Control, 2011). Welte et al. (2012) carried out a detailed review of several years worth of data on pneumonia cases throughout the EU and concluded that in the UK some 9% of all hospitalised pneumonia cases might be LD. According to the Health and Social Care Information Centre (HSCIC), there were nearly 193,000 hospital admissions due to pneumonia in the UK in 2012-13 (HSCIC, 2014) indicating the possibility of 17,370 cases of LD – some 33 times as many as those reported.

In the UK, it is estimated that the aging population will double to 19 million by 2050 (Cracknell, 2007). Therefore, the incidence of Legionnaires' disease and pneumonia is likely to increase with the rise in the number of elderlies, the most susceptible group. This trend is clearly shown in Figure 2.13.

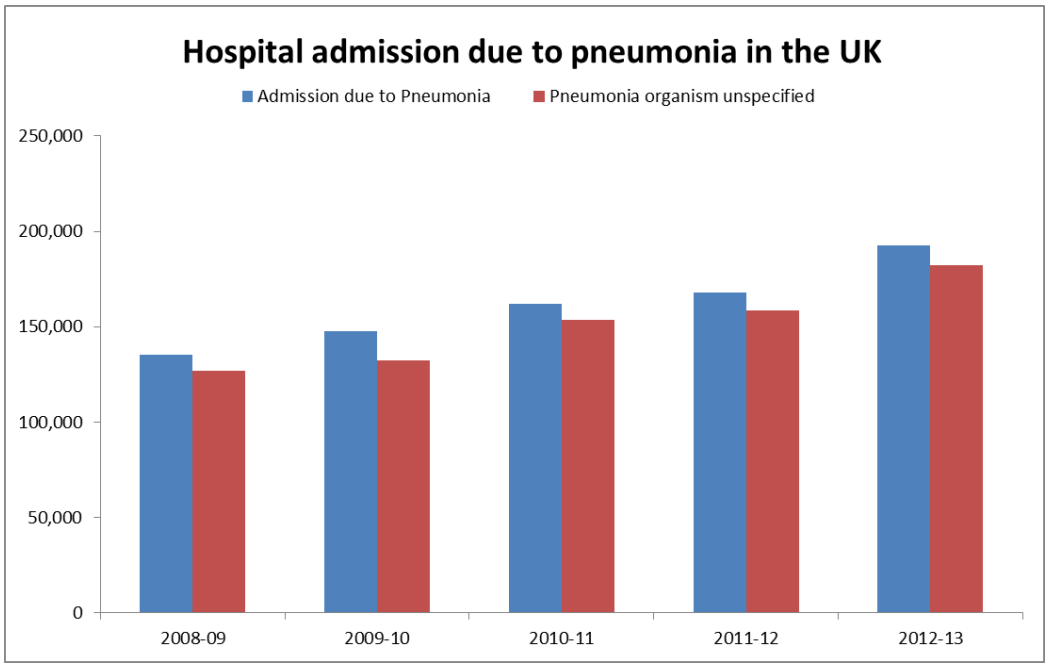

Figure 2.13: Hospital admission due to pneumonia in the UK (Data adapted from HSCIC, 2014).

2.4 *Pseudomonas*

2.4.1 History

Pseudomonas aureginosa was first isolated in 1882 by Carle Gessard, a French chemist and bacteriologist, who noticed the green and blue pigments produced by the bacteria in soldiers' wound bandages. The microbe was later named *Pseudomonas* from the Greek word for "false" (pseudo) and monas for "single unit"; *aeruginosa* is derived from the Latin "copper rust" and it describes the green pigment typical of the bacterium.

2.4.2 The bacterium

Pseudomonas species are found in water, soil and moist surfaces and they can survive at temperatures between 4˚C and 43˚C; an electron micrograph of the bacteria is shown in Figure 2.14. The species most associated with diseases in humans is *Pseudomonas aeruginosa (P. aeruginosa)*, an aerobic bacterium, measuring 0.5-0.8 μm in width and 1.5-3.0 μm in length. *P. aeruginosa* can persist on minimal nutrition, multiply in distilled water, utilise oxygen or nitrate for respiration, and survive a range of unfavourable conditions. Hence, the bacterium has been isolated from aviation fuels, cosmetics, on fresh vegetables, on wet surfaces, on skin, on contaminated equipment, in faeces, in hospital and domestic taps. (Todar, 2012)

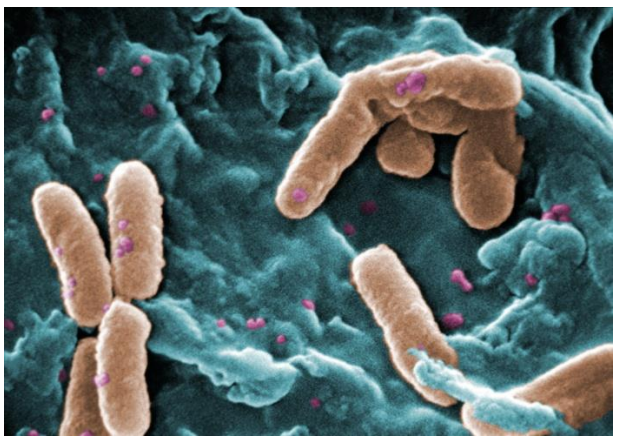

Figure 2.14: Scanning electron micrograph of a number of *P. aeruginosa* bacteria (Carr, 2014).

P. aeruginosa have a flagellum that allows them to be motile and swim freely in water, and it is during this mode of life, called the planktonic mode that bacteria are able to cause infection in the host cell. In response to host defence or hostile conditions, P. *aeruginosa* can also enter a sessile mode of life attaching to surfaces and forming biofilm. Reasons for the bacteria to develop into biofilm include low nutrition, pH changes and the stagnant nature of the flow adjacent to surfaces (Costerton et al., 1995). In *Pseudomonas* the formation of biofilm is aided by the secretion of Extracellular Polymeric Substances (EPS), commonly referred to as slime, including nucleic acid, lipids, proteins and polysaccharides which account for 90% of the biofilm (Flemming & Wingender, 2010). EPS protect bacteria from biocides, protozoa and host defence, enabling them to endure sudden changes in the surrounding environments; EPS also play an important role in the structure of the biofilm, assisting adhesion between cells and providing nutrition. Within the EPS matrix, bacteria are able to multiply, developing biofilm micro-colonies containing billions of bacteria that compete and cooperate for survival. Once the biofilm is developed, cells are dispersed into the surrounding environment for the purpose of colonising new sites; for pathogenic bacteria such as *P. aeruginosa* and *Legionella*, biofilm detachment is crucial to the transmission of disease to humans (Harmsen et al., 2010). Furthermore, there is evidence to suggest that *Pseudomonas* assists *Legionella* in the form of biofilm, providing protection and nutrients (Yun, 2007, Murga et al., 2001). The schematic representation of biofilm formation in Figure 2.15 shows the three steps necessary for biofilm formation. In the initial attachment, motile bacteria adhere to a surface and enter the sessile mode; the bacteria then start to duplicate forming surface-attached communities. Finally in the detachment step, the biofilm releases clumps of bacteria into the surroundings.

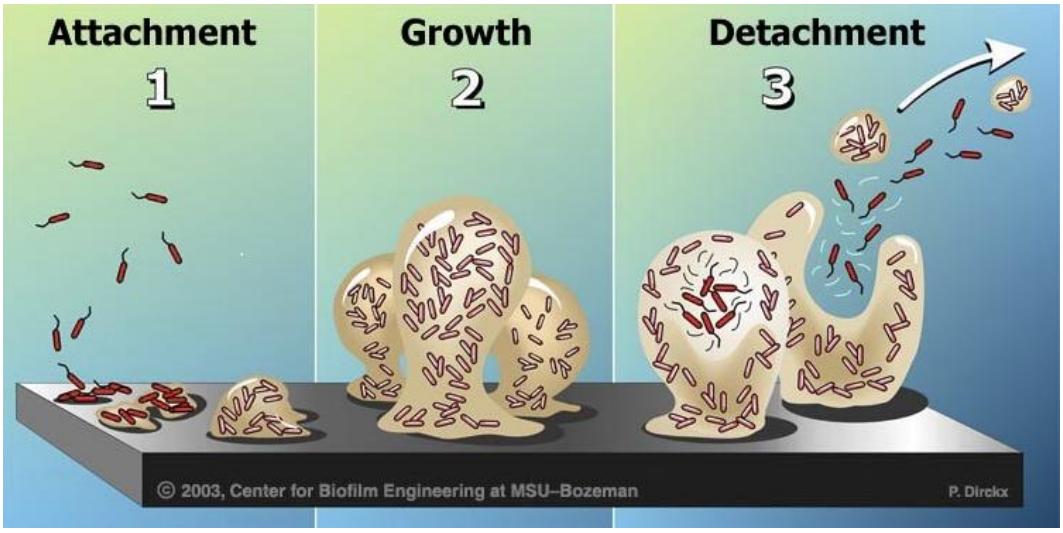

Figure 2.15: The biofilm life cycle illustrated in three steps: initial attachment, growth of complex biofilms, and detachment of clumps of bacteria, resulting in seeding. Reproduced with permission (Stoodley and Dirckx, 2003).

2.4.3 Infections, detection and treatment

P. aeruginosa infections can develop in bones, skin, ears, urinary tract, eyes, blood, heart valves, respiratory tract, lungs and wounds. Being an opportunistic bacterium, *P. aeruginosa* causes infections in people who are immune-compromised, rarely affecting healthy people. It is responsible for several life-threatening infections including blood poisoning, which starts with fever, chills and fast heart beating and progresses into organ failure, septic shock and death. The bacteria can also cause joints and bones infections in post-surgical patients, people using intra-venous drugs and patients with diabetes; meningitis and brain abscess in patients who undertook neurological procedures; cardiovascular infections that can result in heart failure; and pneumonia in patients with existing respiratory problems and cystic fibrosis. Mild illnesses include skin rushes, ears and toe-web infections contracted in swimming pools or hot tubs and eye infections in people wearing contact lenses, with a possible complication being loss of vision. (Chen and Rudoy, 2014) Photographs of infections due to *Pseudomonas* can be seen in Figure 2.16. A foot with toe web infection and a close-up of infected hair follicles in the skin (folliculitis) are shown in (a) and (b), respectively.

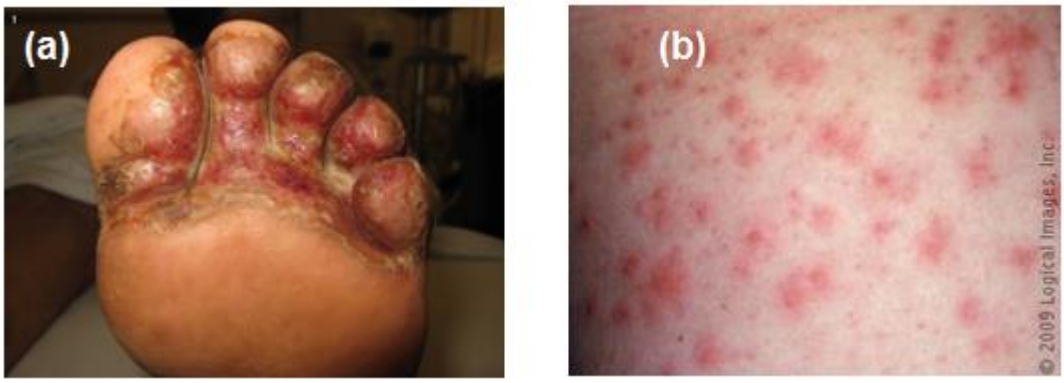

Figure 2.16: Photographs of infection due to *P. aeruginosa.* (a) Toe web infection (Fang, 2013); (b) Folliculitis (Skinsight, 2008).

Transmission pathways include inhalation, ingestion, contact with contaminated water and person to person contact. *Pseudomonas* is particularly problematic in hospitals where staff and equipment can become the vehicle of transmission if hygiene is poor. Mortality varies from 15% to 68%, and managing infections can be difficult, due to the increasing pathogen resistance to antibiotics. (Exner et al., 2005, Kerr and Snelling, 2009, Loveday et al., 2014) Moreover, P. *aeruginosa* has the ability to develop resistance to antibiotics during the course of treatment (Lister, 2009).

The symptoms of illnesses associated with P. *aeruginosa* are similar to the one caused by any other bacteria, making diagnosis without laboratory culture almost impossible. Immediate combination therapy with two different antipseudomosal antibiotics is crucial, as treatment delays of life-threatening infections, caused by the bacterium, are associated with high mortality rate. Once the culture results are available, therapy is reviewed accordingly and the appropriate antibiotic is selected depending on the specimen identified. (Chen and Rudoy, 2014)

2.4.4 Incidence

Due to the numerous infections caused by P. *aeruginosa*, it is not possible to quantify the number of cases related to the bacterium. In the UK, the HSCIC (2014) reported 882 pneumonia hospital admissions due the bacterium in 2012-2013 - twice as many as those reported for *Legionella*. Also, the European Centre for Disease Prevention and Control (ECDC) (2013) reports that in Europe, P. *aeruginosa* is the most frequently isolated microorganism in Intensive Care Units (ICU) acquired pneumonia, the fifth in ICU-acquired bloodstream infections and the fourth in ICU-acquired urinary tract infections. Moreover, Chen (2014) suggests that in the USA, the bacterium is the most common cause of ear, cornea and heart infections, and the 5th most isolated hospital pathogen. Recent outbreaks include the one in Northern Ireland in January 2012 where four babies lost their lives to *P. aeruginosa* infections and the one in Southmead Hospital, Bristol where 12 babies contracted a waterborne disease caused by the bacterium and one baby sadly died.

2.5 The economic burden of LD and pseudomonal infections

Burden of disease calculations are important because they may help in policy-making, in developing preventative actions, in setting priorities in health research and in planning for future needs. The value for the prevention of Legionnaires' disease and pseudomonal infections include the following elements of cost:

- Cost of hospital treatment
- Loss of output due to inability to work
- Cost of mortality and eventual disability
- Out-of-court settlements

This section assesses the costs arising from healthcare and lost working days due to contracting Legionnaires' disease. A case study approach was used to calculate the likely cost to both health services and to the economy for each person who contracted Legionnaires' disease in the 2012 Edinburgh

outbreak. This outbreak was chosen because the details of hospitalised people were readily published by National Health Service (NHS) Lothians (2012). The results were then extrapolated to obtain an approximation of the cost of LD for the UK and for the whole of Europe. Given that *P. aeruginosa* can cause several life-threatening infections, and that the median age of people contracting an infection from the bacterium is between 40 and 73 years (Hota et al., 2009, Vitkauskiene et al., 2010), we used the same cost per person we estimated for LD. The results for *P. aeruginosa* infections were extrapolated for the whole of Europe only, as data for the UK were not found. In this study, no adjustment for under-reporting was made and the cost of disability associated with the infections was not accounted for.

2.5.1 The Edinburgh Outbreak

L. pneumophila sg. 1 was the cause of the outbreak of Legionnaires' disease in South West Edinburgh, Scotland in June 2012, where 92 people are known to have contracted the disease. 56 were confirmed cases, 36 were probable cases and four people sadly died (NHS Lothians, 2012). Some cases were hospitalised in intensive care or general ward, whilst others were treated in the community. Although the source of the outbreak was never identified, it is believed that cooling towers in the area were the cause.

Based on the average number of patients days obtained from the NHS (NHS Lothians, 2012) and daily typical treatment costs of £2,478 if the patient was in intensive care, £1,800 if in high dependency and £350 if in general ward (NHS 2012), it was possible to estimate the likely direct and immediate costs to the health service of this outbreak. This excludes the costs of treating patients who were not hospitalised, the costs of drugs used and the costs of diagnostic tests. Total health service costs will therefore be higher. The calculations are set out in Table 2.1.

	Average number of patient days	Cost per patient per day	Cost
Intensive care unit	260	£2,478	£644,280
High Dependency	80.6	£1,800	£25,200
General ward	613.6	£350	£21,250
Total			**£884 730**

Table 2.1: Estimated treatment costs to the health service due to the Edinburgh *Legionella* outbreak.

There are also costs to the country's economy due to lost working days. Symptoms include cough, fatigue, memory loss, headaches and loss of concentration, muscle/joint pain, muscle weakness and tingling in feet fingers and arms. There have been cases where patients improved after seven months, cases of permanent disability and cases where people were not capable of returning to full-time employment. (McCoy, 2005) Unfortunately, no data on long-term illness is available for the Edinburgh outbreak. It is possible however to make an order of magnitude estimate of cost to the economy through lost productivity. It is thought that for the UK this is about £312/man-day lost or £6,500/man-month, assuming that the total revenue per employee is three times the average salary of £26,000 and that each employee works 250 days a year (Office for National Statistics, 2011).

So, for the 92 survivors of the Edinburgh outbreak, assuming that half were in full-time employment and were off work for three months on average, the cost to the UK economy was about £900,000, comparable to the health service costs. Overall, the Edinburgh outbreak probably cost about £1.8M – or about £18,000 per person who contracted the disease, similar to the cost of $33,366 or £21,000 estimated for each LD episode by Collier et al. (2012).

We can also estimate the cost of the 4 people who died during the outbreak by using the figure of £1,860,801 per person taken from the Highways Economics and adjusted for the 2013/2014 prices. This figure includes both,

the element of lost output and of human cost. The latter representing “the pain to the casualty and relatives and the loss of enjoyment of life over and above the consumption of goods and services”. (Department of Transport, 2007)

Therefore, the total cost of the Edinburgh outbreak, is 4-fold higher at £9.2M if we include the cost of mortality. However, the cost is likely to be even higher as it is not possible to estimate the time and money spent investigating the case or to assess long–term disability costs without knowing the victims.

2.5.2 Legionnaires’ disease in the UK and Europe

The ELDsNet collects and disseminates information on the incidence of LD as reported by member states. Over the last few years, the number of cases reported has been about 12 per million of the population per annum (European Centre for Disease Prevention and Control, 2013). In 2012, 5,852 cases were reported, 401 of which were in the UK – roughly 6 cases per million of the population. However, it is considered that under reporting occurs in most member states for the reasons discussed in section 2.3.6 of this thesis. The ELDSNet estimates that in the EU as a whole, there could be as many as 44,650 unreported cases.

In a detailed review, Welte et al. (2012) concluded that in the UK some 9% of all hospitalised pneumonia cases might be LD. In the UK, there were nearly 193,000 hospital admissions due to pneumonia in 2012-13 (HSCIC, 2014). This implies 17,100 cases of LD – some 42 times as many as those reported.

Table 2.2 shows the combined health care and days lost costs for the UK and the EU based on the costs derived from the Edinburgh study using the reported numbers, the numbers suggested by the ELDSNet and the ones suggested by Welte et al. (UK only). The estimation includes reported cases from all sources, including cooling towers, pools, water systems and other sites. The table also presents the cost of mortality for LD. In their annual

report, ELDSNet (2013) confirmed 419 deaths associated with LD in Europe, 36 of which were in the UK. Based on this data, the cost of mortality is nearly £7M for the UK and £780M for the whole of Europe.

Country	Number of cases in 2012	Cost per person	Cost
United Kingdom (reported)	401	£18,000	£7.2 M
United Kingdom (ELDSNet)	6,200	£18,000	£111.5 M
United Kingdom (Welte)	17,100	£18,000	£307.8 M
Europe (reported)	5,433	£18,000	£97.8 M
Europe (ELDSNet)	50,500	£18,000	£909 M
Mortality UK	36	£1.8 M	£66.7 M
Mortality Europe	419	£1.8 M	£780 M

Table 2.2: Reported and estimated numbers and cost of LD in the UK and Europe.

2.5.3 Out-of court settlements and bad publicity

LD cases where the source is identified can result in very expensive out-of court settlements. The Melbourne Aquarium payout for the outbreak of LD in 2000, is believed to be in the region of $AU 40-70 million (McCoy, 2005). The Basildon and Thurrock University Hospitals NHS Foundation Trust was ordered to pay £35,000 to each of the family of the two men who died from contracting LD whist at the hospital, between 2007 and 2010 (Donaghy, 2013).

Bad publicity may also arise from someone contracting LD and this can result in a great deal of damage to the business. Although the cost of out-of-court settlements cannot be estimated and bad publicity cannot be

measured, they both should be acknowledged when defining the burden of the disease.

2.5.4 *Pseudomonas* associated infections

The number of people contracting Healthcare-acquired Infections (HAIs) each year in Europe amounts to 4,000,000, but only for half of these cases, the bacterial source is known (European Centre for Disease Prevention and Control, 2012). *P. aeruginosa* is the cause of 10.1% of HAIs reported with a microbiological result, corresponding to 202,000 infections. We can estimate that of the 2,000,000 HAIs without a known source, the same percentage is due to *P. aeruginosa*. Mortality from *Pseudomonas* infection is reported to be between 39% and 58.8% (Kang et al., 2003, Hota et al., 2009, Vitkauskiene, 2010), so the number of deaths due to the bacterium can be estimated to be between 79,000 and 119,000 in Europe.

Table 2.3 presents the combined health care and loss of productivity costs for the EU, based on the costs derived from LD. The estimation includes reported pseudomonal infections and the minimum and maximum cost of mortality based of the data from Kang et al. (2003), Hota et al. (2009) and Vitkauskiene et al. (2010).

	Number HAIs due to *Pseudomonas*	**Cost per person**	**Cost**
Europe (reported)	202,000	£18,000	**£3.6 billion**
Mortality cost min.	78,780	£1.8 M	**£146 billion**
Mortality cost max.	118,776	£1.8 M	**£221 billion**

Table 2.3: Estimation of healthcare, economy costs and mortality due to pseudomonal infections.

2.6 *Legionella* control in non-domestic buildings

2.6.1 Legislation and prevention

In the UK, *Legionella* control is driven by Legionnaires' disease: the control of *Legionella* bacteria in water systems. Approved Code of Practice (ACoP) by the HSE (2013). The guide gives technical details on how to manage, control and prevent the risk in water systems. The Chartered Institution of Building Services Engineers (CIBSE) guide TM13: Minimising the risk of Legionnaires' disease (2013) gives details of engineering and design criteria. It also underlines the importance of managerial and operational procedures for the prevention of risk. The ACoP L8 (2013) suggests a number of ways of preventing the growth of *Legionella* in hot water systems. This includes temperature control, dosing with chemicals, copper/silver ionisation and UV irradiation. L8 makes no recommendations regarding the control of *Pseudomonas*.

Legionella control in health premises is ruled by the Health Technical Memorandum 04-01 guidance document: the control of *Legionella*, hygiene, "safe" hot water, cold water and drinking water systems (Department of Health, 2006). In the memorandum, the Department of Health gives recommendations on the management and operation of hot and cold water systems, and it summarises the legal requirement of estate managers, healthcare managers and engineers. It also underlines the importance of designing adequate water systems providing sufficient water supply in case of a disruption, whilst ensuring water turnover for the purpose of minimising water stagnation and bacteria proliferation.

The outbreak of infections related to *P. aeruginosa* in Northern Ireland in 2012, where four babies died in a neonatal unit, prompted the publication of an addendum concerning *P. aeruginosa* in hospitals. The addendum underlines the link between infections, biofilms and water outlets and it stresses the importance of training, sampling of outlets, control and management of the water system. It also stresses the importance of forming

a Water Safety Group (WSG) including the director of infection prevention and control, the infection prevention and control team, consultant medical microbiologists, the Estates and Facilities Team and senior nurses from the relevant augmented unit. The WSG is in charge of developing a water safety plan and advising on remedial actions if the water system is found to be contaminated. (Department of Health, 2013)

2.6.2 The cost of controlling *Legionella* and pathogens in DHW

The Approved Code of Practice L8 emphasizes the monitoring and maintenance of non-domestic water systems. Suggestions include scale treatments to prevent calcium deposits, removal of water outlet not in use and regular flushing of under-used outlets. All maintenance work and remedial actions should be logged and water temperatures should be monitored according to the recommended routine in L8. The installation of Thermostatic Mixing Valves (TMVs) is also recommended where a significant risk of scalding has been identified (HSE, 2013).

Although extensive research has been carried out on the efficacy of disinfection methods, no single study exists which attempts to estimate the likely cost of monitoring DHW systems in non-domestic buildings for *Legionella*. Therefore, this section estimates the cost of managing *Legionella* and other pathogens in the UK, in non-domestic buildings that contain aerosol-producing equipment such as showers, spas, pools, water fountains and hot/cold water taps. These include universities, hospitals, schools, health clubs, leisure centres and hotels. The costs of installing and maintaining TMVs to prevent the risk of scalding are also discussed.

2.6.2.1 Universities in the UK

In the last financial year, the cost incurred by the Estates of a UK university for monitoring and sampling domestic hot water systems in academic/administrative buildings and the halls of residences was £74,000 and £80,000 respectively (Brunel University, 2013). These costs exclude the risk assessments and any remedial work. This equates to £4.4 per person

per annum using the academic/administrative buildings (students and staff) and £17.7 per resident per annum in the halls. Since the university in question is an average-sized UK university, the above costs have been extrapolated to cover all UK universities (see Table 2.4).

UK universities	Number of student/staff	Yearly Cost/person	Cost
Academic/administrative buildings	2.88 million	£4.4	£12.7 M
Halls of residence	960,000	£17.7	£17 M
Total cost for monitoring and sampling			£29.7 M

Table 2.4: Estimated costs of *Legionella* control in the UK universities (Higher Education Statistics Agency, 2013).

2.6.2.2 Hospitals and schools in the UK

The chief executive of Basildon and Thurrock University Hospitals reported £3 million were invested in *Legionella* control in the last decade (BBC News, 2012) equivalent to £356 per bed per annum. NHS data give a total of 140, 000 beds available in NHS hospitals in England (Department of Health, 2012). Extrapolating this number of beds to the whole of the UK and assuming that Basildon and Thurrock University Hospitals is typical, leads to a total cost of £59M per annum. This excludes private health hospitals.

The management of *Legionella* in schools is not as complex as in hospitals or universities but it should not be overlooked. Risk assessments, temperature monitoring, descaling of showers and inspection of water tanks are required to comply with Health and Safety. It is thought that the cost of attending the site by a water treatment contractor is £80- £100. So, each of the 28,916 (National Statistics for Wales, 2009, Department for Education, 2012, The Scottish Government, 2013) schools in the UK could spend up to £1,000 a year, excluding remedial work. That is about £29 million in *Legionella* control.

2.6.2.3 Health Clubs, Leisure Centres and Hotels in the UK

Leisure centres and health clubs present higher risks of exposures than schools because of the presence of showers, pools and spas, and would be expected to carry out more frequent inspections, monitoring and sampling. Assuming that each of the 5,900 leisure facilities in the UK (The Leisure Database Company, 2012) could spend double the amount of a school, the total cost could be as high as £12 million per annum. The yearly expenditure for the management of *Legionella* in hotels is assumed to be comparable to the one for the universities halls of residences. So, assuming there are 650,000 rooms (Office for National Statistics, 2011), the yearly estimated cost for hotels is also in the region of £11.5 million.

2.6.2.4 The cost of diminishing the risk of scalding with TMVs

On April 6, 2010, the amendments to The Building Regulations 2000, Sanitation, hot water safety and water efficiency (HM Government, 2010), came into effect, meaning that all new-builds are now fitted with TMVs to limit the temperature of the water to 48°C to lower the risk of scalding. Hospitals, schools, nursing homes, leisure centres and even households could also save by reducing the installation of thermostatic mixing valves, costly to install at £22.50 per valve and to maintain (CAPT, BBA et al., 2008). The Child Accidents Prevention Trust (CAPT) and the British Burn Association (BBA) (2008) have estimated the cost of installing TMVs in new dwellings to amount to £5.4M. A further advantage of lowering temperatures in hot water systems is the lower risk of scalding, preventing costly hospital treatments associated with burn injuries.

2.7 Typical water treatments

Water in the distribution system, may be treated either by physical or chemical means or by a combination of both. Physical methods include filtration, heat treatment and UV lights whilst chemical methods comprise chlorination, chloramination, chlorine dioxide, copper-silver and ozone.

2.7.1 Physical Methods

2.7.1.1 Temperature control and pasteurisation

The first evaluation on the efficacy of temperature regimes in controlling *Legionella* was carried out in 1986, when Stout et al. (1986) found that 19 members of the *Legionella*ceae family could be inactivated in vitro by elevating temperature above 60°C. However, in complex water systems complete eradication is not possible, and in their study, Stout et al. (1986) underline the importance of an adequate flushing regime to disinfect distal sites where elevated temperatures may not be achievable. This study also found that at temperatures of 60, 70 and 80°C, the microorganisms can be killed in 1.3-10.6 min, 0.7-2.6 min and 0.3-0.7 min respectively, concluding that flushing at 60°C at each outlet should be for a minimum of 30 minutes to be effective, as this will enable the all of the water in the pipework reach the temperature. They also suggested for hot water tanks temperatures to be maintained above 60°C.

The study by Stout et al. (1986) was followed by the publication of the ACoP L8 in 1991, where the HSE recommends water to be maintained at temperatures unfavourable to bacteria proliferation (ACoP, 2013). Hot water should be stored at a minimum of 60°C with a flow temperature of 50°C (55°C in healthcare premises) at every hot water outlet within one minute of opening the outlet, whilst cold water should be below 20°C after running the cold outlet for two minutes. Such high temperatures increase the risk of scalding so that in healthcare premises, TMVs are required at each outlet. For water treatments to be effective, routine monitoring of temperatures in the first and last tap in the recirculation loop should be carried out monthly,

and water demand should be monitored. Moreover, visual inspection of the system will ensure its cleanliness. Routine sampling for *Legionella* is not required, unless temperatures and/or biocide levels are not maintained, an outbreak is suspected and in hospitals wards where patients could be at risk. (ACoP, 2013)

Pasteurisation, also known as heat and flush, heat shock treatment or thermal disinfection, should be performed if high positive counts of *Legionella* are detected, if the monitored temperatures at outlets have not been achieved according to the HSE or HTM recommendations, or if the system has been unused for some time. The water in the cylinder is heated up to a sufficiently high temperature so that a flow temperature of 60˚C can be achieved at each hot water outlet in turn for at least 5 minutes. This is in contrast to the study of Stout et al. (1986), where it is recommended to flush each outlet for at least 30 minutes.

A considerable amount of literature has been published in the last 30 years showing that the recommended flushing time is not effective on eradicating *Legionella* (Steinert et al., 1998, Mouchtouri et al., 2007, Farhat et al., 2010). Steinert et al. (1998) reported that the water system of a hospital was re-contaminated with *Legionella* within two months of a thermal disinfection procedure, whilst Mouchtouri et al. (2007) found *Legionella* 2 to 7 days after heat shock treatment in more than half of the 27 buildings monitored. Similarly, in a recent study by Farhat et al. (2010), the pilot system was subjected to two thermal disinfections at 70°C for 30 minutes and *Legionella* was not eradicated. The results of the resistance test undertaken, suggested that the bacteria became thermo-acclimated to the heat-shock treatment and consequently they concluded that thermal disinfection should not be used as a remedial treatment (Farhat et al., 2010).

Another problem associated with maintaining hot water at high temperatures is the increased risk of scalding, see Table 2.5 . In 2008, it was estimated that the cost of scalding incidents to the UK economy was in the region of £266 million pound a year (Child Accidents Prevention Trust, British Burn

Association et al., 2008). Such shocking evidence prompted an amendment of the Building Regulations making a requirement that from April 2010 all new dwellings must be fitted with TMVs which provide hot water at temperatures within 41 and 48 °C. However, a growing body of literature comparing the performance of TMVs to conventional taps has reported that TMVs are often contaminated with bacteria including *Legionella* and *Pseudomonas* and they become a risk to the user, especially in hospitals (Halabi et al., 2001, Curran, 2012, Macken et al., 2012, Walker et al., 2014). Indeed, the residual warm water present within a tap and the local pipework provides the favourable conditions for bacterial growth. These findings have led the Department of Health (2013) to advise the removal of TMVs if water systems in hospitals are found to be contaminated.

Temperature ° C	1st degree burn	2nd degree burn
43.9	270 min	300 min
45.0	120 min	180 min
46.7	20 min	45 min
51.1	2 min	4.2 min
55.0	17 sec	30 sec
60.0	3 sec	5 sec
66.1	instant	2 sec

Table 2.5: Water temperature effect on adult skin. Adapted from Moritz and Henriques (1947).

Finally, maintaining water at 60°C increases maintenance costs and lowers the performance of water systems because the higher the temperature, the greater the accumulation of scale on heat exchange surfaces and in pipes. In order to achieve the 2019 zero carbon targets for new non-domestic buildings set by the UK government (HM Government, 2011), the energy demand associated with DHW needs to be addressed, but maintaining systems at such high temperatures renders difficult the use of greener technologies such as solar thermal energy, that could reduce the CO_2 impact

of heating water.

2.7.1.2 Point-of-use filtration

Pathogen-free water can be achieved by fitting filters to water taps and showers as the one shown in Figure 2.17 to remove any particle or microorganisms greater than a defined size. The filters are fitted with a 0.1, 0.2 or 0.45 µm membrane, and the filter casing is often coated with nano-silver for additional antibacterial effect. Filters are mostly fitted on water outlets of intensive care units and hospital wards with high-risk patients, where the risk of contracting Legionnaires' disease is significantly higher.

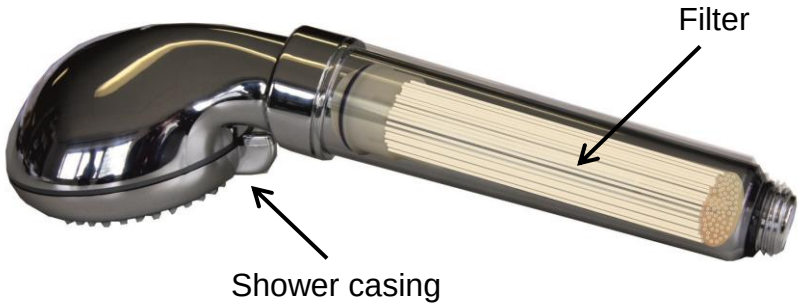

Figure 2.17: Photographs of a point-of-use filter in hospital showers for *Legionella* control (Prime water membranes, 2013).

Several studies have demonstrated the benefits of point-of-use filters in hospitals (Salvatorelli et al., 2005, Sheffer et al., 2005, Vonberg et al., 2005, Daeschlein et al., 2007, Cervia et al., 2010). Salvatorelli et al. (2005) investigated the efficacy of a new showerhead filter (Pall-Aquasafe Shower Head Filter, AQL3) and found that the outlets previously positive for *Legionella*, tested negative in the nest sampling routine. Similarly, in a study conducted by Sheffer et al. (2005), *Legionella* was completely eliminated after using filters on water outlets. Further, after the 9 months period with filters in place in a bone marrow transplant unit, Cervia et al. (2010) concluded that the rates of infections caused by Gram-negative bacteria were considerably reduced from 0.4 to 0.09 per 100 patient days.

Although the above mentioned investigations have demonstrated the efficacy of filtration, they all fail to report on cost. Considering that filters need replacement every 1 to 4 weeks depending on their quality, each tap may need between 12 and 52 filter changes a year. The filters cost (≈ £80) may be justified in outlets used by high risk patients or in an emergency situation by the financial advantage of patients not contracting an often fatal disease, but this is not the case in other areas of the hospital and other commercial buildings. Therefore, the author believes that filtration at water outlets cannot be the solution to the problem of pathogenic bacteria in non-domestic buildings because they are simply too expensive.

2.7.2 Chemical methods

National and international guidelines also recommend the use of chemical processes such as chlorination and chloramination for the treatment of hot water. The water temperature may be lower than with temperature control, but the level of chemicals should be thoroughly controlled as any lapses on biocide dosing may leave the system exposed to risk. In the eventuality that contamination persists, hyperchlorination (or shock chlorination) treatments should be undertaken.

2.7.2.1 Chlorination

The introduction of chlorination in the mid to late 1800's led to a significant decrease in morbidity and mortality associated with waterborne diseases such as typhoid, dysentery and cholera in developed countries. The guideline value for free chlorine in drinking water suggested by World Health Organisation (WHO) (1997) is 5 ppm with a minimum residual of 0.2-0.5 ppm, needed to reduce the risk of microbial growth in the water distribution system. The minimum residual is measured after the "chlorine demand" of the water has been fulfilled. The chlorine demand is defined as the difference between the amount of chlorine added to the water and the residual chlorine measured in the water after some time. Part of the chlorine reacts with Hydrogen Sulfide (H_2S), Iron (Fe^{2+}), Manganese (Mn^{2+}), Ammonia (NH_3), phenols, amino acids, proteins and carbohydrates naturally present in the

water, fulfilling the so called “chlorine demand”. This must be taken into consideration when adding chlorine to the water, so that the correct concentration of chlorine is achieved to obtain the desired chlorine residual.

In high-risk buildings, chlorine may be added to the water in a sustained basis to achieve levels of 2-6 ppm and if the bacteria counts are not within safe limits, a shock chlorination treatment may be undertaken. During the latter, high levels of chlorine (20-50 ppm) are introduced into the water and the chlorine is maintained at such levels for the time necessary to inactivate *Legionella* (1-2 h); the water is then drained and the system is re-filled (Lin et al., 2001).

Although chlorination is widely used in water systems disinfection, numerous published studies have reported its inefficacy (Muraca et al., 1987, Garcia et al., 2008, Casini et al., 2014). In all of the water systems investigated, *Legionella* species were detected within a few months of the shock chlorination treatments, when the levels of chlorine returned to normal. In a recent review, Lin et al. (2011) went so far as to point out chlorination as the most unreliable water treatment because the 17 hospitals monitored using chlorination, were forced to convert to different disinfection methods. Reasons explaining its inefficacy in controlling *Legionella* species include its failure in penetrating biofilm in highly resistant areas (De Beer et al., 1994) where *Legionella* can shelter until the conditions are favourable. In addition, *Legionella* is able to colonise amoebae that can resist chlorine levels of 50 ppm (Kilvington and Price, 1990). Other possible explanations are the increasing resistance of the bacteria to chlorine as a result of continuous treatments (Garcia et al., 2008) and the chlorine effectiveness in eliminating other bacteria with inhibitory effect on *Legionella* species. In a study investigating bacteria contamination in the hot water systems of 40 Italian hotels, it was found that *L. pneumophila* sg. 1 survived in hotels with chlorine residual between 0.1 ppm and 0.5 ppm, but it was not detected in hotels with chlorine residual below 0.1 ppm. The opposite was observed for both *L. pneumophila* sg. 2-14 and *Pseudomonas* spp. as they both persisted in hotels with low chlorine residual but they were not present in the ones with

high chlorine residual (Borella et al., 2005). This suggests that *Legionella* serogroup 1 may thrive in environments with higher chlorine residual, not having to compete with other microbial flora.

2.7.2.2 Chloramination

The discovery that adding chlorine to water results in disinfection by-products (DPBs) such as Trihalomethanes (THMs) and Haloacetic acid (HAAs), and the subsequent publication of regulations limiting their levels in water, prompted researchers to consider alternative water disinfectants (United States Environmental Protection Agency, 1978). A disinfection method showing promise was the addition of ammonia to water containing chlorine to form monochloramine (NH_2Cl), dichloramine ($NHCl_2$) and trichloramine (NCl_3). Although by mixing ammonia and chlorine in water all of the three compounds can form, the most desirable disinfectant is monochloramine and only careful dosage of chemicals causes monochloramine to be the dominant species with guidelines values of 3 ppm (WHO, 2011). Oversupplying chlorine prompts the formation of NH_2Cl and NCl_3 often associated with bad odour and taste whilst excessive ammonia induces nitrification. During this process, oxidizing bacteria consume the ammonia, causing loss of chloramine and leaving the water system at risk of contamination (WHO, 2011). Other disadvantages of chloramination include leaching of lead from lead pipes and pinhole pitting in copper pipes, that could lead to costly plumbing repairs (Postlethwaite, 2012).

Monochloramine is a weaker biocide than chlorine, but being more stable, it provides longer lasting residual. A substantial field study evaluating the conversion from chlorine to chloramine in 53 buildings confirmed that the use of chloramines results in lower THMs and in significantly higher residuals. Monochloramine also reduced the level of *Legionella* and it was found to be more persistent in hot water systems. (Flannery, 2006) Further field evaluations confirmed that monochloramine could be a promising alternative to chlorination, particularly in controlling *Legionella* (Moore et al., 2006, Marchesi et al., 2012). By contrast, monochloramine was found to be less

efficient than chlorine in laboratory experiments (Thomas et al., 2004, Loret et al., 2005), probably because being less stable, chlorine dissipates more rapidly in buildings. Another possible explanation for the discrepancy between field and laboratory results could be the length of field monitoring. *Legionella* persistence is due to its capability of developing resistance to the same disinfectant over time (Garcia et al., 2008), but the buildings were only monitored for a maximum period of a year. Monitoring should have been carried out for a longer period of time to confirm the effectiveness of the disinfectant over time.

2.7.2.3 Chlorine Dioxide

Chlorine dioxide (ClO_2) was discovered at the beginning of the 1800 by Sir Hamphrey Davy. It is an oxidant added to the water. Its levels should not exceed 0.5 mg/L (BSI, 2009), and it is most effective in systems with continuous hot water demand, due to its short life in hot water above 50˚C. Being explosive under pressure, it is dangerous to transport and it is usually made in situ by mixing Sodium chlorite ($NaClO_2$) and hydrochloric acid (HCl). Another reason for not transporting ClO_2 is because it dissociates into chlorine and oxygen if stored for long periods.

The use of chlorine dioxide to control *Legionella* in hot water has had mixed reviews. Hosein et al. (2005) found that ClO_2, at the maximum permissible level of 0.5 ppm, was not effective in eradicating *Legionella* in a hospital hot and cold water system. Conversely, another study reported that samples positive for *Legionella* decreased from 41% to 4% in the 17 months monitoring period after the installation of a chlorine dioxide unit (Srinivagan et al., 2003). Confirming this, a study from Zhang et al. (2007) reporting that the number of samples positive for *Legionella* decreased from 60% to 10% in 30 months but that the time needed to achieve significant reduction is specific to the site (Zhang et al., 2009). In another study comparing the efficacy of various control strategies, it was found that, although chlorine dioxide did not completely eradicate *Legionella*, it maintained *Legionella* below 100 CFU/L and it is the most economic method of disinfection when

compared to hyperchlorination, thermal shock, monochloramine, electric boilers and point of use filters (Marchesi et al., 2011).

Collectively these studies suggest that chlorine dioxide is efficacious at controlling *Legionella* in the buildings monitored. However, strict control of chlorine dioxide injection is necessary to diminish the formation of chlorite and chlorate, disinfection by-products that may not exceed 0.7 mg/L (WHO, 2005). In addition, levels of ClO_2 residual should be measured periodically as any lapse in biocide might allow bacteria levels to increase within days.

2.7.2.4 Copper/Silver ionisation

Copper-silver ionisation disperses silver and copper ions into the water systems. By passing a current through electrodes close together, positively charged silver and copper ions (Ag^+, Cu^+, Cu^{2+}) are generated and released into the water, where they react with negatively charged particles such as bacteria (see Figure 2.18. Copper ions affect bacteria cell permeability, allowing silver ions to penetrate the cell and inhibit DNA replication. The levels of both ions are regulated in water in the ACoP L8 with permissible quantities of 0.4 mg/L for copper and 0.04 mg/L for silver in hot water systems and 0.4 mg/L for copper and 0.02 mg/L for silver in soft water systems (HSE, 2013).

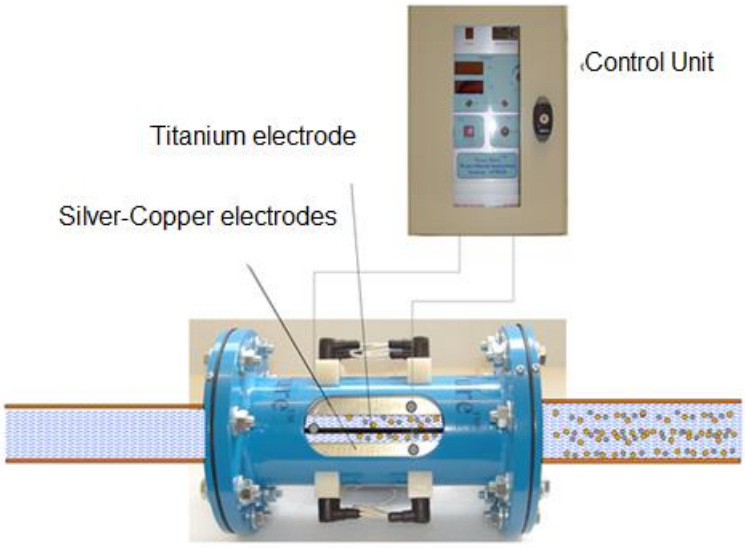

Figure 2.18: Schematic of a copper-silver unit installation (Tarn-pure, 2012).

Successful disinfection depends on water flow rate, water conductivity, pH and the volume of water in the system so it is important to assess the water system prior to installation. Ag^+ and Cu^+ levels in the water should be strictly monitored because silver ions may react with nitrate and chloride contained in water, inhibiting its anti-bacterial properties. The concentration of ions might decrease in areas of hard water with scaling up of the electrodes (HSE, 2013).

As for chlorine dioxide, the application of copper-silver ionisation for *Legionella* control has had mixed reviews. An early study by Rohr et al. (1999) reported that copper silver ionisation was effective during the first year of monitoring with a 3.8 log reduction of *Legionella* (nearly 99.99% reduction) at Ag^+ concentration of 0.01 mg/L. In spite of the initial positive results, after 4 years monitoring, they found that ionisation was not effective in the long term. In 2005, another study reported the inefficacy of the disinfection method, due to the low levels of copper and silver ions and the high pH (Blanc et al., 2005).

On the other hand, Stout et al. (2003) demonstrated that copper silver was effective in 12 of the 16 hospitals monitored. Modol et al. (2007) found that with silver and copper concentration above 0.03 mg/L and 0.3 mg/L respectively, samples positive for *Legionella* significantly decreased over time. In a more recent study monitoring 10 hospitals, Bedford (2012) reported a decrease in samples positive for *Legionella* and *Legionella* counts within 3 months of having used copper silver ionisation. In 4 of the hospitals monitored *Legionella* was not detected after 4 years.

The evidence presented above suggests that copper-silver can eradicate *Legionella* from water systems when the necessary levels of silver and copper are maintained. Despite copper-silver ionisation having proved effective, the use of elemental copper as a biocide has been banned in most of the European countries from February 2013 because of its toxicity (HSE, 2014). The UK, Spain, Poland and The Netherlands have been granted essential use derogation allowing the sale of biocidal products containing

copper for the purpose of *Legionella* control. Those countries have been asked to submit a dossier for the approval of copper by the 31st of December (The European Commission, 2014). For this reason, alternative water treatments should be investigated.

2.7.2.5 Ozone

According to ACoP L8 (2013), ozone provides disinfection locally to the point of applications but it is not effective in the rest of the water system because it fails to provide a residual. Ozone is a powerful oxidizing agent made by passing a high voltage across a gas containing oxygen (O_2). The discharge divides the oxygen molecule into atoms (O) that then recombine with O_2 molecules to form ozone (O_3), see Figure 2.19.

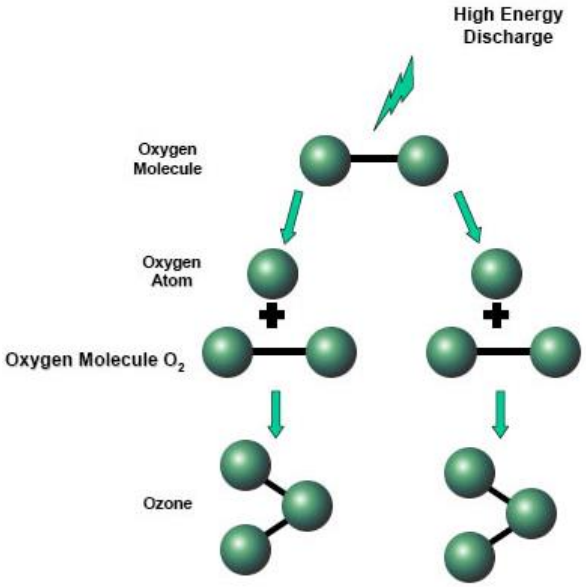

Figure 2.19: Schematic of ozone generation. The discharge divides the oxygen molecule into atoms (O) that then recombine with O_2 molecules to form ozone (ESG Waterwise, 2014).

Ozone is efficacious in eliminating bacteria, viruses, fungi and algae by allowing the necessary contact time. When exposing a microorganism to ozone, an oxidative burst occurs, which breaks the cell wall (Leusink, 2010). The ozone continues to burst the cell wall until the cell membrane is disrupted and the microorganism is dead, see Figure 2.20. Due to its high

reactivity and short half-life, it is difficult to store or transport, so it must be created in situ.

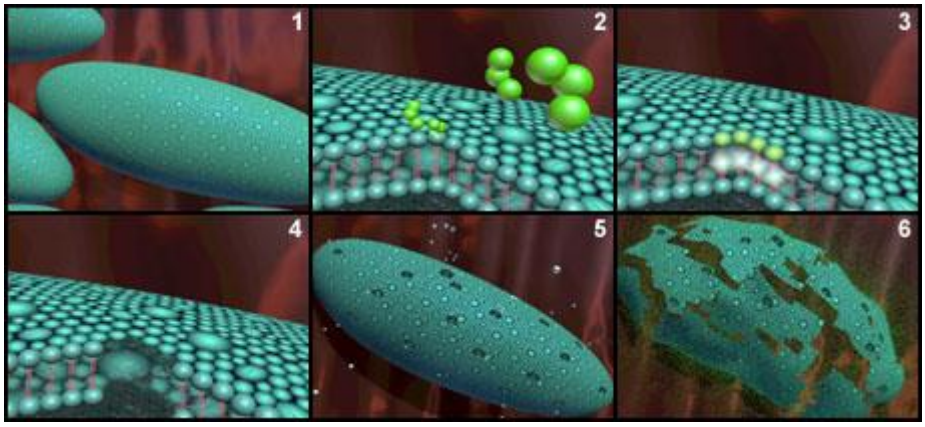

Figure 2.20: Effect of ozone on bacteria. 1) Healthy bacteria; 2) Ozone coming into contact with cell wall; 3) Ozone creating a hole in the cell wall; 4) Close-up of effect of ozone on cell wall; 5) Bacteria after having been in contact with ozone; 6) Lysis of cell (Ozonesolutions, 2014).

Although ozone has been found to produce fewer by-products and to be more effective than chlorine, it should not be used as the sole method of disinfection in extensive water systems, as it decomposes rapidly, failing to provide residual disinfection (Muraca et al., 1986, Domingue et al., 1988, McGrane, 1995, Li et al., 2011). Not surprisingly, one recent study has confirmed that ozone is not efficient in controlling *Legionella* in a hospital hot water system (Blanc et al., 2004).

2.7.2.6 Ultraviolet treatment (UV)

Ultraviolet lights with wavelengths in the range of 200-300 nm can inactivate most organisms by hindering DNA replication. It is broadly agreed that UV treatment does not provide residual disinfection for *Legionella* eradication and that it is better used as a supplement to other disinfection methods (Liu et al., 1995, Franzin et al., 2002, Triassi et. al., 2006). In another study UV treatment combined with temperature control prevented *Legionella* colonisation of a new hospital water system during the 13 years of monitoring. Although UV treatment is not effective on contaminated systems, this study demonstrated that it is effective at preventing *Legionella*

contamination of new water systems. Therefore, installing a UV treatment unit could be beneficial at the time of construction of a new building. (Hall et al., 2003)

2.7.3 Conclusions

From the literature review, it is clear that *Legionella* control in hot water systems continues to remain a challenge. Even in well-designed DHW systems, some outlets may be under-used as a result of changes in the way the building is utilised. Communication between the members of the team in charge of the building and the gathering of information is therefore critical to the identification of under-used taps, and to the understanding of water usage patterns. In buildings of the size of hospitals or universities, communication between maintenance, monitoring and sampling teams can be slow. For example, it might take two days before management is informed of a temperature out of range and it might take as long before a remedial action is undertaken by maintenance. Furthermore, contractors are often changed every couple of years and information regarding the building might not be passed on. Attempts at solving these issues include the implementation of bar code recording systems, less vulnerable to human errors. They enable staff to monitor and log temperatures at water outlets, alerting immediately the maintenance team to take a remedial action.

The obvious advantage of preventing any disease is the improvement of the quality of people's lives, but there are also other economic benefits to the NHS and employers. These include the reduction of healthcare costs and the increase of productivity.

The authors estimated that in Europe, the annual cost associated with LD arising from healthcare and absence from work was in the region of £1 billion and in the UK, £100M, but the actual costs may be higher due to under-reporting and misdiagnosis. The cost of mortality was estimated at £66.7M for the UK and £780M for Europe. The cost to the healthcare and the economy for patients contracting pseudomonal infections is greater, at £3 billion for the whole of Europe, whilst mortality could be between £146 and

£221 billion. These figures are underestimates because it is not possible to include data on the costs of patient disability, outpatients care and out-of-court settlements. Indeed, the burden of diseases caused by *Legionella* and *Pseudomonas* to the healthcare and economy is substantial, and these figures are bound to rise with the increase of the aging population which is estimated to double by 2050.

Chapter 2 also estimates the costs associated with controlling *Legionella* and other pathogens in DHW systems in different types of non-domestic buildings, because no single study exists attempting to do so. It was estimated that in the UK, the cost of controlling *Legionella* could amount to around £140M per annum. This review has highlighted the need for better understanding the cost of monitoring and controlling *Legionella* to identify areas for improvement. An evaluation of the costs associated with LD and *Pseudomonas* infections in Europe, arising from healthcare and lost working days through the illness, provides a measure of the burden to society of LD and *Pseudomonas* infections.

Overall, there seem to be evidence that temperature control, heat and flush and chlorination continue to be the most common disinfection methods, and their inefficacy in eradicating and controlling *Legionella* has been demonstrated time and time again. It is difficult if not impossible to maintain water temperatures of 55°C in all the parts of an extensive pipework system. Furthermore high temperatures result in high costs and CO_2 emissions.

Disadvantages associated with chlorination include the transport and storage of dangerous chemicals and the production of toxic by-products such as carcinogenic THMs and HHAs. Water chlorination can also lead to costly repairs associated with the failure of pipes due to corrosion. Moreover, the regular increase of chlorine levels to 50 ppm is not only dangerous to human (U.S. Department of Health and Human Services, 1993), but it can be harmful to organisms living in the receiving waters of wastewater treatment plants.

Based on these findings, chloramination, chlorine dioxide and copper-silver

ionisation are viable options for the disinfection of recirculating hot water systems. However, the author believes that growing awareness of the negative health effects and environmental impact of chemical disinfection is likely to lead to stringent laws regulating the levels of chemicals allowed to be added to water. For example, the use of copper as a biocide in water treatment has been banned in 2013 in most of the European countries. UV treatment has been proved to be effective in new buildings but not in contaminated systems. Finally, point-of-use filters and ozone provide disinfection locally to the point of applications and might not be effective in the rest of the water system, and for this reason, they should not be used as the main water treatment.

The evidence presented in this section suggests that a chemical-free disinfection method capable of maintaining pathogen-free hot water at lower temperatures is needed, not only to lower the energy demand of buildings, but also to lower the risk of scalding. An estimate of the energy and CO_2 savings that might arise from operating DHW systems at lower temperatures is lacking.

2.8 Electrochemical Disinfection as a solution to water disinfections in buildings

2.8.1 Introduction

In recent years, considerable attention has been paid to ED, as a promising method for both the control of hot water systems (Furuta et al., 2004, Nakajima et al., 2004, Delaedt et al., 2008) and for the inactivation of bacteria in process wash water (Feng et al., 2004, Lopez-Galvez et al., 2012, Gomes-Lopez et al., 2013). Disinfectants are generated on site, eliminating the handling, storage and transport of hazardous chemicals.

In ED, current is applied to electrodes immersed directly into the contaminated water or in a bypass. Inactivation of bacteria is achieved by electrosorption of the microorganisms on the anode surface, as bacteria are mostly negatively charged, and by the simultaneous action of electrochemically formed oxidants and electric fields; see Figure 2.21 (Ghernaout and Ghernaout, 2010).

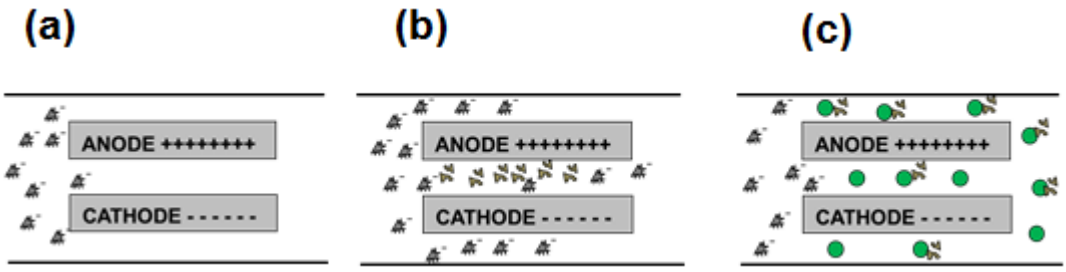

Figure 2.21: Schematic of electrochemical disinfection stages. (a) Live bacteria in water flowing through the unit; (b) Bacteria are first inactivated by elctrosorption on the electrode surface; as electrolysis continues, bacteria are inactivated by the oxidants formed. Adapted from Ghernaout and Ghernaout (2010).

Oxidants cause cellular damage whilst electric fields induce the formation of permanent pores through which oxidants have free access to the interior of the microorganisms (Drees et al., 2003). The ED reaction mechanism has been widely investigated and the efficacy of electrochemical inactivation of various microorganisms is well documented (Bergmann et al., 2002, Drees et

al., 2003, Li et al., 2004, Kerwick et al., 2005, Liang et al., 2005, Fang et al., 2006). Researchers have attributed the higher performance of ED to the formation of short-lived oxidants such as ozone (O_3), atomic oxygen (O^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) produced according to equations 2.1 and 2.2 below (Bergmann et al., 2008, Diao et al., 2004, Furuta et al., 2004). In water containing chloride, the generation of active chlorine species such as chlorine (Cl_2), hypochlorous acid (HClO) and hypochlorite ions (OCl^-) can provide the reservoir disinfection (Kraft et al., 1999). These are produced according to equations 2.3, 2.4 and 2.5. Furthermore Bergmann and Koparal demonstrated that chlorine dioxide (ClO_2), a strong oxidising agent, is also generated according to equations 2.6 and 2.7 (Bergmann and Koparal, 2005).

$$O_2 + 2H_2O + 2e^- \rightarrow H_2O_2 + 2OH^- \quad (2.1)$$

$$3H_2O \rightarrow O_3 + 6e^- + 6H^+ \quad (2.2)$$

$$2Cl^- \rightarrow Cl_2 + 2e^- \quad (2.3)$$

$$Cl_2 + H_2O \rightarrow HClO + HCl \quad (2.4)$$

$$HOCl + H_2O \rightarrow H_3O^+ + OCl^- \quad (2.5)$$

$$Cl^- + 2H_2O \rightarrow ClO_2 + 4H^+ + 5e^- \quad (2.6)$$

$$Cl_2 + 4H_2O \rightarrow 2ClO_2 + 8e^- \quad (2.7)$$

2.8.2 The problem of scale deposits

The increase of OH^- ions in the vicinity of the cathode leads to a local pH increase, causing the calcium carbonates ($CaCO_3$) and Magnesium Hydroxide ($Mg(OH)_2$), naturally found in the water, to precipitate according to equations 2.8 and 2.9.

$$CaCO_3 + 2H^+ \rightarrow Ca^{2+} + CO_2 + H_2O \quad (2.8)$$

$$Mg(OH)_2 + 2H^+ \rightarrow Mg^{2+} + 2H_2O \quad (2.9)$$

The film of calcareous deposits formed on the cathode is not desirable,

because with time, it can affect electrodes performance, see Figure 2.22. The most commonly used routine to dispose of scale is the reversal of the electrode polarity because dissolution of scale occurs at the anode after reversal (Kraft et al.,2002).

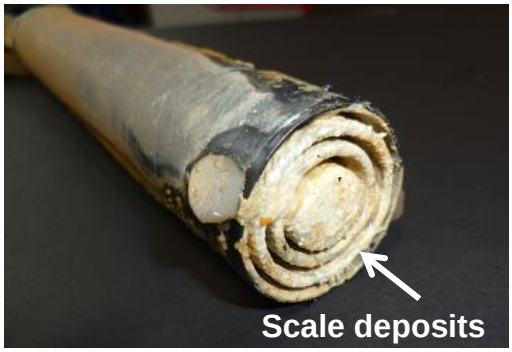

Figure 2.22: Photograph of electrode fouled with scale during monitoring of a building installation.

2.8.3 Parameters affecting electrolysis and inactivation rate of bacteria

It is broadly agreed that the effectiveness of the ED process is dependent on current density, water composition, flow rate, temperature, electrode material and cell configuration (Kraft et al., 1999, Bergmann and Koparal, 2005, Kerwick et al., 2005, Jeong et al., 2007, Polcaro et al., 2007, Jeong et al., 2009).

2.8.3.1 Electrode material

Electrodes participate in the electrochemical process by assisting in the transfer of electrons but also by increasing and modifying the chemical reactions taking place. Hence, the electrode material directly influences the rate of generation of the oxidants necessary to inactivate the bacteria. A variety of electrodes materials have been used in ED of water including platinum (Jeong et al., 2007), boron doped diamond (Furuta et al., 2004, Polcaro et al., 2007, Lopez-Galvez et al., 2012) and ceramic (Reimanis et al., 2011). Although the use of boron doped diamond electrodes is well established in the area of wastewater treatment, their use in electrochemical drinking water disinfection was halted by the discovery that perchlorate, a by-

product with high health implications, is generated (Bergmann and Rollin, 2007).

During the past twenty years, a considerable amount of literature has been published on the use of titanium based electrodes coated with mixed metal oxide (Sarkka et al., 2008), platinum, iridium oxide (Kraft et al., 1999,) ruthenium oxide, zirconium dioxide or titanium dioxide (Diao et al., 2004, Bergmann and Koparal, 2005, Delaedt et al., 2008). These electrodes combine the anti-corrosion qualities of titanium with the enhanced electrochemical properties of the coating. The above mentioned studies reported on the efficacy of each distinct electrolytic device investigated, and they are limited to having used either a single or two electrode materials, making it difficult to compare the effect of materials on the generation of oxidants.

Kraft et al. (1999) compared the performance of electrodes coated with platinum and electrodes coated with IrO_2, and found that at lower current densities Pt electrodes produced more active chlorine (Cl_2, HClO and ClO^-) than the IrO_2 electrodes, but that at values of current densities above 37mA/cm^2 IrO_2 produced significantly higher levels. They also observed that the polarity reversal of the electrodes greatly shortened the IrO_2 electrodes lifetime, concluding that Pt electrodes were the most suitable for applications with lower current densities.

The first systematic study on the effect of electrode material on the generation of oxidants was reported by Jeong et al. (2009) a decade later. They found that the boron doped diamond (BDD) electrode was 10 times more efficient in generating ozone and $^{\cdot}OH$ radicals than Ti/RuO_2 and Pt. Conversely, BDD was not as efficient in the production of active chlorine which was in the order of Ti/RuO_2>Ti/Pt-IrO_2>BDD>Pt, leading to the conclusion that the material employed significantly affected the efficiency of the electrolytic process. The substantial difference in the rate of production of $^{\cdot}OH$ and active chlorine for the same material was attributed to the enhanced material catalytic activity towards a chemical reaction rather than another

reaction. Although the Pt electrode was not efficient neither in the production of ·OH nor active chlorine, when the experiment was repeated with the addition of *E.coli*, the researchers observed that the Pt efficiency in inactivating the microorganisms was only second to the BDD electrode.

2.8.3.2 Current density

Current density has a significant effect on the production of disinfectants and the inactivation of bacteria. Kraft et al. (1999) performed experiments to investigate the dependence of active chlorine generation on current density, and found that as the latter increased, more chlorine was generated, due to more electrons being transferred, see Figure 2.23. Active chlorine was detected at current densities of 11 mA/cm^2 for tap water at 30°C and of 23 mA/cm^2 at 60°C, followed by a linear increase with the rise in current density. At lower current densities, the active chlorine produced could not be detected because it reacted with organic and inorganic components and suspended particles in the water, fulfilling the so called “chlorine demand” of the water. The amount of chlorine consumed by the water varies depending on the water quality, water temperature and flow velocities (Kraft et al., 1999).

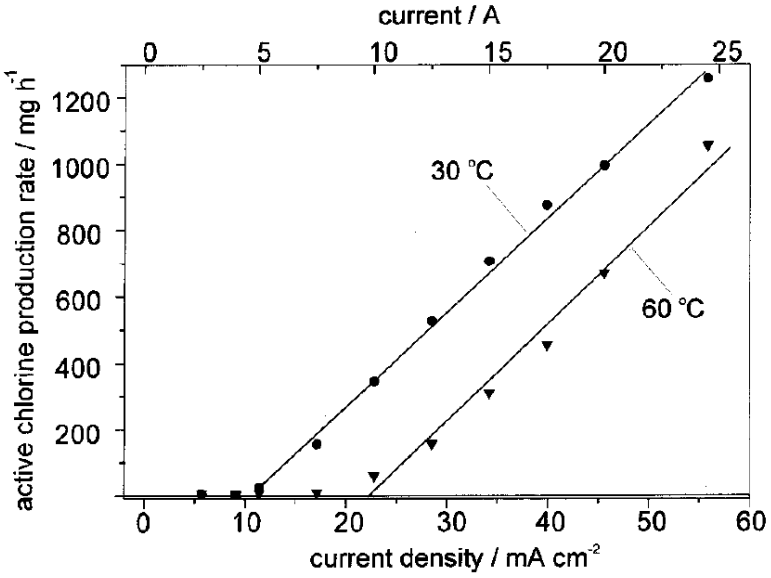

Figure 2.23: Dependence of the active chlorine production rate on current density at 30 and 60 °C on IrO2 sheet electrodes (Flow rate 120 l/h) (Kraft et al., 1999).

Patermarkis and Fountoukidis (1990) reported that a more rapid inactivation rate can be achieved with the increase in current density, consistent with more recent reports. (Drees et al., 2003, Jeong et al., 2007). Drees et al. (2003) exposed *E.coli* and *P. aeruginosa* bacteria to electric current of various magnitudes for 5 seconds, and confirmed that higher current led to a larger reduction of bacteria population. Jeong et al. (2007) conducted electrolysis of a chloride-free solution in a cylindrical reactor investigating the effect of hydroxyl radicals on the inactivation rate of *E.coli* and also reported on the effect of applied current density. They also concluded that the higher the current density, the higher the percentage of bacteria eliminated. As seen in Figure 2.24, by applying 0.1 mA/cm^2 most of the *E. coli* is alive after 180 minutes treatment, whilst with the application of 100 mA/cm^2, 90% of the bacteria has been inactivated after the same treatment time.

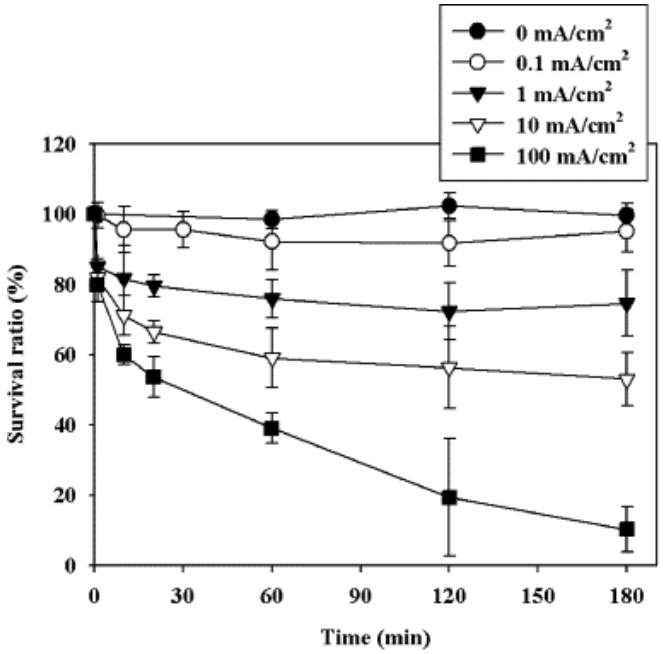

Figure 2.24: Effect of the anodic current density on *E. coli* inactivation in the electrochemical disinfection using a Pt anode ([*E. coli*] $_0 = 10^3$ CFU ml^{-1}; $[KH_2PO_4]_0$ = 0.2 M) (Jeong et al., 2007).

In a study where current density was varied from 5 to 65 mA/cm^2, Sarkka et al. (2008) found that effective inactivation of three bacteria species was achieved at current densities higher than 25 mA/cm^2 with exposure time of 3 minutes. They observed that the difference in inactivation between 25-65

mA/cm^2 was insignificant, leading to the conclusion that after the threshold value of 25 mA/cm^2, the energy is used to form oxygen and not oxidants.

2.8.3.3 Flow rate

Few studies discussed the effect of water flow rate on the performance of an electrolytic device (Kraft et al., 1999, Hsu, 2005, Yao et al., 2011). Kraft et al. (1999) measured the amount of chlorine produced by IrO_2 electrodes sheets at different current densities with the variation of flow rate, and found that only a minor increase in chlorine generation could be observed with the increase of flow rate. The results can be seen in Figure 2.25. Increasing the flow-through velocity, not only results in an increase in chlorine production, but also in an increase in chlorine demand, due to more water passing through the reactor and consuming the chlorine produced.

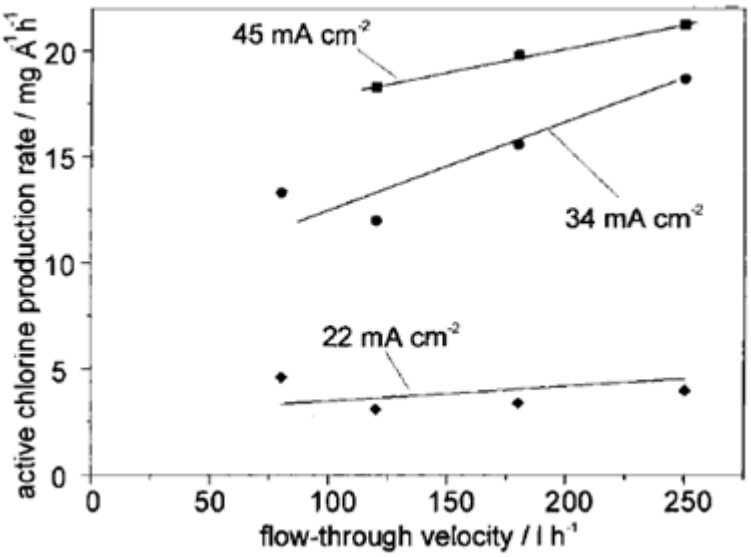

Figure 2.25: Dependence of the active chlorine production rate on flow through velocity for three different current densities (22, 34 and 45 mA/cm^2), using IrO_2 sheet electrodes (60 °C) (Kraft, 1999).

In a study investigating the effect of flow rate, temperature and salt (NaCl) concentration on the chemical and physical properties of electrolyzed oxidizing water, Hsu (2005) found that the increase of flow rate caused a decrease of chlorine production. One possible reason for the decrease is that the progress of any electrochemical reactions requires the appropriate

supply of reactants to the electrode surface combined with the adequate removal of products from the surface. However, if the flow is too rapid, the reactants might not have enough time to generate the products (Allen and Larry, 2001).

More recently, Yao et al. (2011) investigated the effect of current density, flow rate and NaCl concentration on the inactivation efficiency of an electrochemical disinfector. The water was contaminated with 10^7-10^8 CFU/ml concentration of *E.coli*, *L. pneumophila* and *P. aeruginosa* respectively, but the experiments investigating flow rate were carried out with *E.coli*. The sampling was carried out after the contaminated water went through the ED device twice (every two cycles). It was found that the flow rate was a significant parameter affecting the survival rate of *E.coli* and the results can be seen in Figure 2.26. The inactivation efficiency improved with the increase of flow rate from 100 mL/h to 250 mL/h due to the higher production rate of disinfectants. However, they observed that increasing the flow rate to 500 mL/h caused the performance of the reactor to decrease. This is because the higher flow rate leads to short contact time with the electrodes and causes the production of fewer oxidants. We can conclude that for any electrolytic device there must be a threshold value for flow rate depending on the geometry of the cell, above which the inefficiency of the cell decreases. Therefore flow rate plays a significant role along with the current density.

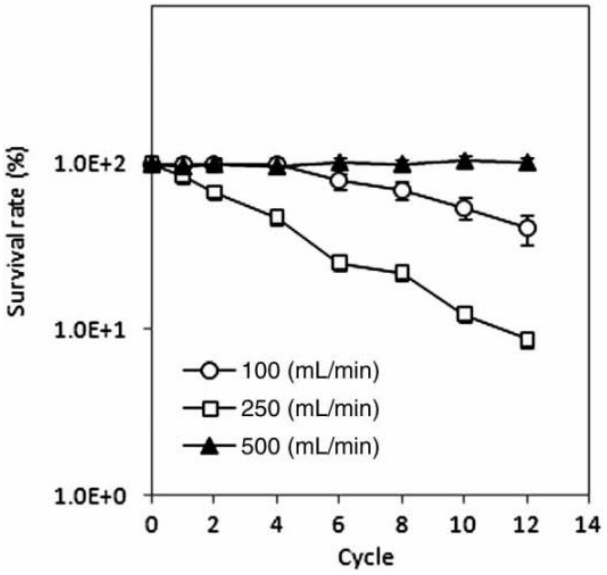

Figure 2.26: Effect of flow rate on the bactericidal properties of Boron Doped Diamond reactor against *E. coli*. The concentration of NaCl solution was controlled at 0.1% and a voltage of 3 V was used in the electrolytic process (Yao et al., 2011).

2.8.3.4 Temperature

Temperature influences the rate of reaction; as temperature increases, diffusion and solubility increase, increasing the rate of reaction. Kraft et al. (1999) investigated the effect of temperature on the production of active chlorine at current densities of 22 mA/cm^2 and 34 mA/cm^2 with IrO_2 electrodes; the results can be seen in Figure 2.27(a). They found that chlorine production decreases with temperature and that the chlorine produced significantly decreases at both current densities at 60°C, the temperature of hot water in water systems. They further investigated this trend by measuring the water chlorine consumption rate at different temperatures, and they observed an exponential increase of chlorine consumption with rising temperature, concluding that this phenomenon is responsible for the decrease in chlorine production. Also, they observed that the decrease of chlorine production was significantly different between the two electrode materials tested. Figure 2.27 (b) presents the temperature dependence of chlorine production rate with titanium electrodes either coated with iridium oxide or platinum. With Pt coated electrodes there was a gradual

decrease whilst with IrO_2 coated electrodes, there was a notable decrease between 23 and 30°C. The authors were not able to explain why.

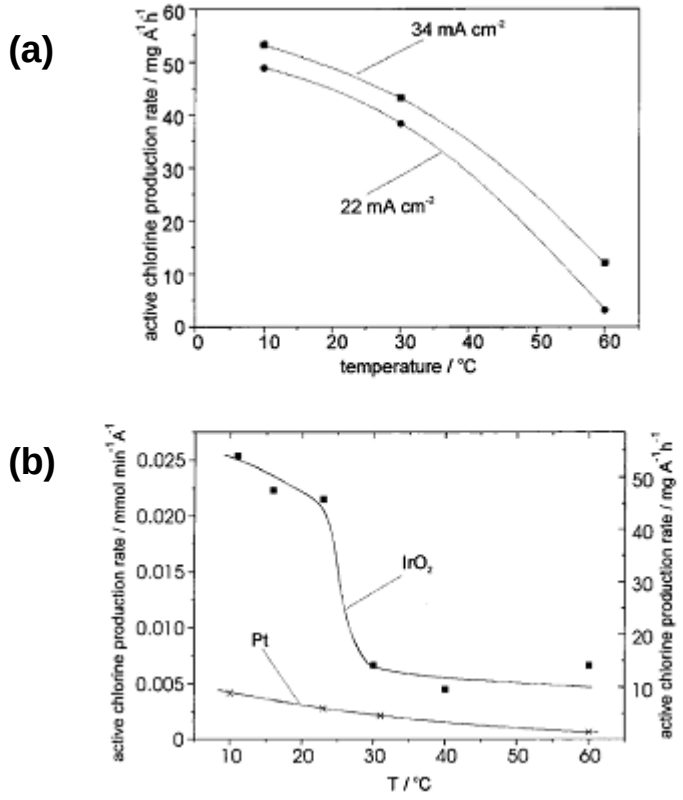

Figure 2.27: a) Dependence of the active chlorine production rate on temperature for two different current densities (22 and 34 mA/cm^2), using IrO_2 sheet electrodes (120 l/h). b) Comparison of the temperature dependence of active chlorine production rate with iridium oxide and platinum coated titanium expanded metal electrodes with current density 15 mA/cm^2 and chloride concentration 150 mg dm^{-3} (Kraft et al., 1999).

2.8.3.5 Chloride concentration

The chloride content of the water is the parameter that defines the quantity of chlorine generated. Kraft et al. (1999) observed that with the rising of chloride concentration there is an increase in active chlorine generated, but they also noticed that the production rate differs considerably between the two materials considered. Figure 2.28 shows that IrO_2 electrodes produce more chlorine than Pt electrodes at any chloride concentration, but above 5,000 mgdm^{-3} the difference in production rate lowers with the increase of chloride content.

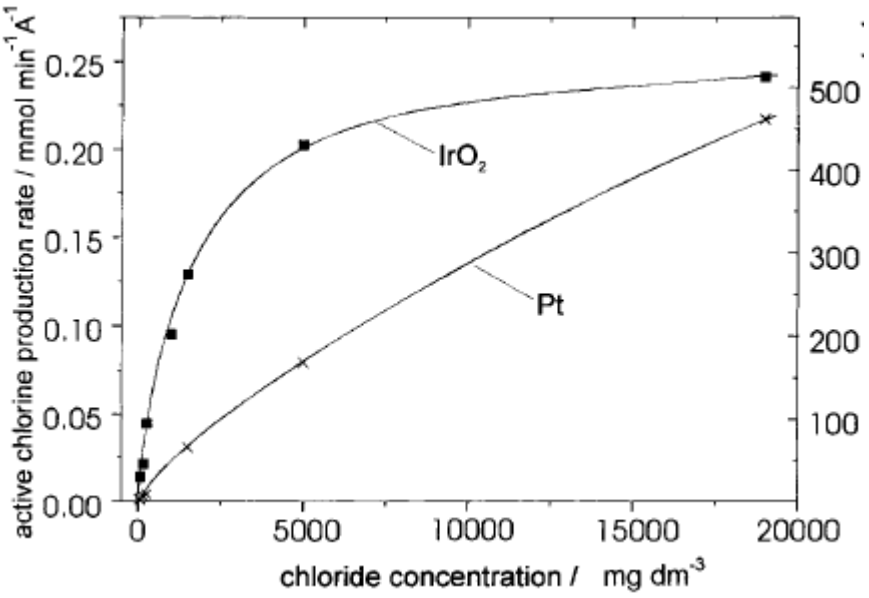

Figure 2.28: Dependence of active chlorine production rate per A with iridium oxide and platinum coated titanium expanded metal electrodes on the chloride concentration (current density 15 mA/cm^2, temperature 23°C) (Kraft et al., 1999).

Comparison of two studies investigating the electrochemical disinfection of *E. coli* shows that chloride accelerates *E. coli* inactivation. Jeong et al. (2007) obtained 1-log inactivation of *E. coli* in 180 min with a current density of 100 mA/cm^2 in an electrolyte not containing chloride whilst Drees et al. (2003) achieved the same log-reduction with 5 mA/cm^2 in 5 minutes in an electrolyte containing chloride. These results were confirmed by another study carried out by Furuta et al. (2004) testing the ability of *L. pneumophila* to withstand different concentration of oxidants at temperatures between 22 and 26 °C, see Figure 2.29. The graph clearly shows that with higher concentration of oxidants (0.71 ppm and 0.67 ppm as Cl_2) inactivation was immediate. However, with 0.18 ppm of Cl_2 90% inactivation was only achieved after 60 minutes, whilst with 0.19 ppm and 0.11 ppm of Cl_2 after 60 minutes, 30% and 20% of the *Legionella* was inactivated, respectively. They also investigated the role of bicarbonates and sulphates on the disinfection capacity of the reactor and found that only bicarbonates enhance disinfectants production.

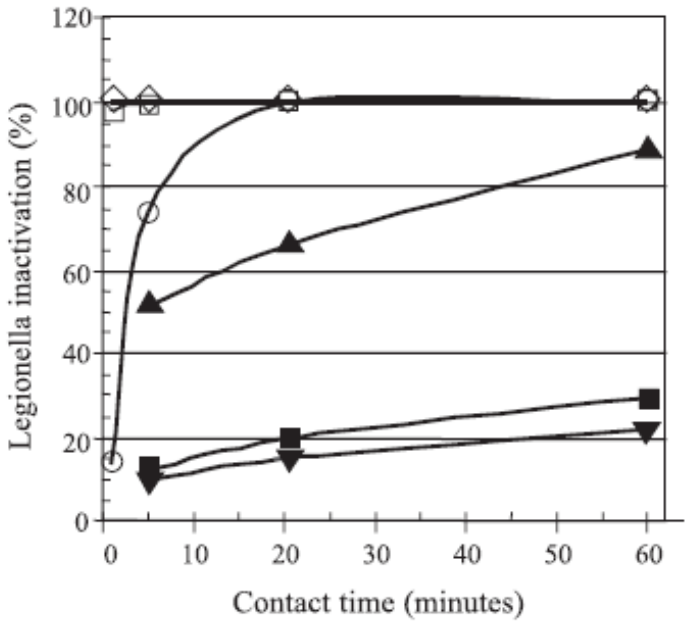

Figure 2.29: *L. pneumophila* inactivation versus contact time after injection, oxidant concentration immediately after *Legionella* injection and type of water, temperature between 22 and 26 °C; (◊) 0.71 ppm oxidant as Cl_2 (tap+NaCl—50 mA/cm^2), (□) 0.67 ppm oxidant as Cl_2 (tap+NaOCl), (○) 0.18 ppm oxidant as Cl_2 (tap+NaOCl), (▲) 0.19 ppm oxidant as Cl_2 (tap—150 mA/cm^2), (■) 0.13 ppm oxidant as Cl_2 (tap—100 mA/cm^2) and (▼) 0.11 ppm oxidant as Cl_2 (tap—50 mA/cm^2) (Furuta et al., 2004).

Sarkka et al. (2008) investigated the efficacy of ED on the bacterium *Deinococcus geothermalis* at various chloride concentrations and found that without chloride they could obtain a 3-log reduction in 15 minutes whilst with 130 mg/L of chloride content, 6 log reduction could be achieved in 5 minutes. Figure 2.30 (a) shows the inactivation rate dependence on chloride concentration, and Figure 2.30 (b) the amount of oxidants generated with different initial chloride content.

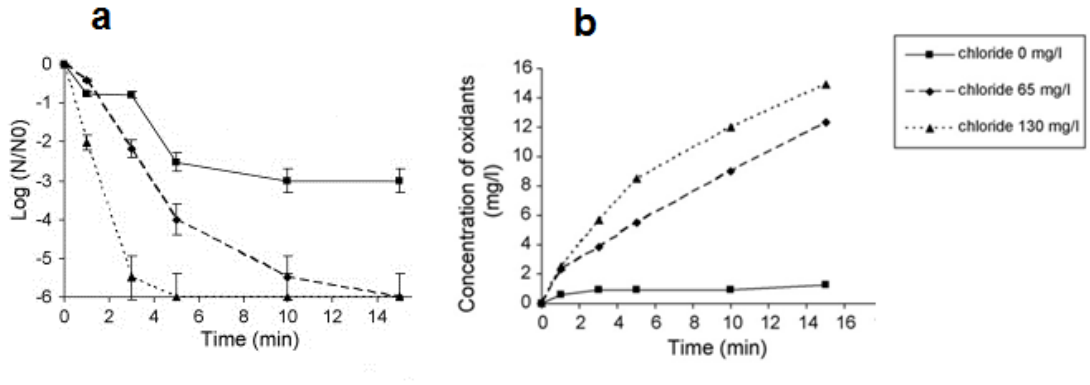

Figure 2.30: a) inactivation of *Deinococcus geothermalis* using MMO electrode and different initial chloride concentrations during the galvanostatic electrolysis (pH 7, current density 50 mA/cm^2); b) amounts of electrochemically generated oxidants on MMO electrode during the galvanostatic electrolysis using different initial chloride concentrations (pH 7, current density 50 mA/cm^2) (Sarkka, et al., 2008).

2.8.4 Disinfections by-products (DBPs)

Thrihalomethanes, haloacetic acids, chlorite and bromate are the disinfections by-products for which regulations have been established as they may pose health risks. They are formed when chlorine or other disinfectants added to the water for pathogen control, react with organics and inorganics in the water. Although the purpose of this study is not to investigate the DBPs, it is important to acknowledge that in ED, chlorine and other disinfectants are generated, thus the formation of disinfection by-products is expected. Currently, DBPs are not mentioned in the ACoP L8 and hence they are not measured nor controlled in non-domestic hot water.

The studies investigating DBPs formation with ED are few. In 1997, Venczel et al. published a study comparing the inactivation of *Cryptosporidium parvum* oocysts and *Clostridium perfringens* spores either by electrochemically produced mixed-oxidant disinfectants or by free chlorine. They found that not only the mixed oxidants were more efficient but also that their use would reduce the production of THMs by 50%.

Conversely, in a later study comparing the performance of disinfectants on biofilm and *L. pneumophila* control, Loret et al. (2005) found that in the

experimental rig used, chlorine dioxide, chlorine, ED and ozone, all produced disinfection by-products in excess of the regulatory limits. However, most of the disinfectants investigated have been used in different systems for many years without exceeding the limits, suggesting that DBPs are system dependent. (Loret et al., 2005) In a more recent study, Bergmann et al. (2014) found that THMs formation in inline electrolysis was comparable to chemical chlorination but that Halogenated organic compounds (AOX) generation was significant.

2.8.5 Conclusions

From the review, it is clear that ED could be an alternative disinfection process against waterborne pathogenic microorganisms, providing residual disinfection that potentially could eliminate biofilms with prolonged use.

Although several reports indicate that configuration of the electrolytic cell determines the overall efficacy of ED (Bergmann et al., 2008, Polcaro et al., 2007), to the best knowledge of the author, a systematic study of the cell geometrical parameters on bacteria inactivation rate is lacking. Careful knowledge of the relationship between geometrical parameters and bacteria killing may lead to the development of cells adaptable to different environmental or operational conditions. Such innovations would cater for variations in water quality, changes in water use for both buildings and disinfection processes and different building sizes.

While several studies, using a range of cell configurations, have also confirmed ED to be effective in eliminating various pathogens under controlled laboratory conditions (Patermarkis and Fountoukidis, 1990, Furuta et al., 2004, Kerwick et al., 2005, Fang et al., 2006, Jeong et al., 2007, Polcaro et al., 2007, Delaedt et al., 2008, Sarkka et al., 2008), a long-term real-world evaluation of the efficacy of electrolytic devices to control bacteria has not been performed.

Although DBPs formation is not investigated in this thesis, the literature review has highlighted the need to further understand the generation of by-

products associated with ED. This would enable the safe applicability of ED also to drinking water disinfection.

Chapter 3 : A fundamental study of the electrolytic process and the effect of control parameters

‘All interest in disease and death is only another expression of interest in life’.

Thomas Mann

3.1 Introduction

Electrochemical disinfection of hot water in non-domestic buildings continues to remain a challenge today and any attempts to implement the technology have not yet been successful for long-term practical use (Kraft, 2008). The author believes that one of the reasons why this technology has failed to progress in this application is because ED cells are designed without taking into consideration that each building is significantly different from the next. In the past, devices have been wrongly dimensioned, causing mixed reviews on the use of such technology in hot water recirculation systems (Kraft, 2008). The understanding of the effect of geometrical and operational parameters on the rate of elimination of bacteria during ED, may lead to the development of ED devices adaptable to different conditions.

In this chapter, we provide a detailed description of the controlled laboratory experiments undertaken in a flask where platinum coated electrodes were immersed in 3.5 litre of tap water contaminated with *E. coli* (NCT10418) or *L. pneumophila* serogroup 1 (NCTC12821). The effect of voltage, area and spacing of electrodes, volume of contaminated water, spiking time, different initial microorganism density and speed of mixing on the rate of bacteria elimination is discussed in details.

3.2 Material and methodology

3.2.1 Experimental apparatus

The experimental set up was comprised of a 3.5 litres cell culture Bellco Pyrex glass flask and a purpose built lid fitted with a variable speed electric glass stirrer used to maintain the water homogeneous during the entire experiment, see Figure 3.1 (a). Electrolysis was performed using two platinised (platinum thickness was between 2-5 μm) titanium mesh electrodes with a thickness of 0.5 mm, both for cathode and anode (Metakem GmbH, Germany). The geometrical surface area of each electrode immersed in the tap water was 27 cm^2 (135 mm x 20 mm). The surface factor G=1.8, defined as the actual surface area over the projected

area, provided by the manufacturer was used to calculate the current densities according to the following equation:

$$J = \frac{I}{A \times G} = \frac{Current}{Projected\ area \times Surface\ factor} \quad (3.1)$$

The inter-electrode distance tested were 2 mm and 5 mm, whilst the electrochemically effective area was increased by stacking electrode sheets as shown in Figure 3.1 (b). The electrode stack consisted of between two and five identical titanium mesh electrodes. The apparatus was connected to a dc power supply (Thurlby Thandar Instruments CPX400A) and a 6 or 12 V voltage applied to give current densities ranging from 2.8 and 13.5 mA/cm^2 depending on the area employed. The experimental parameters can be seen in Table 3.1.

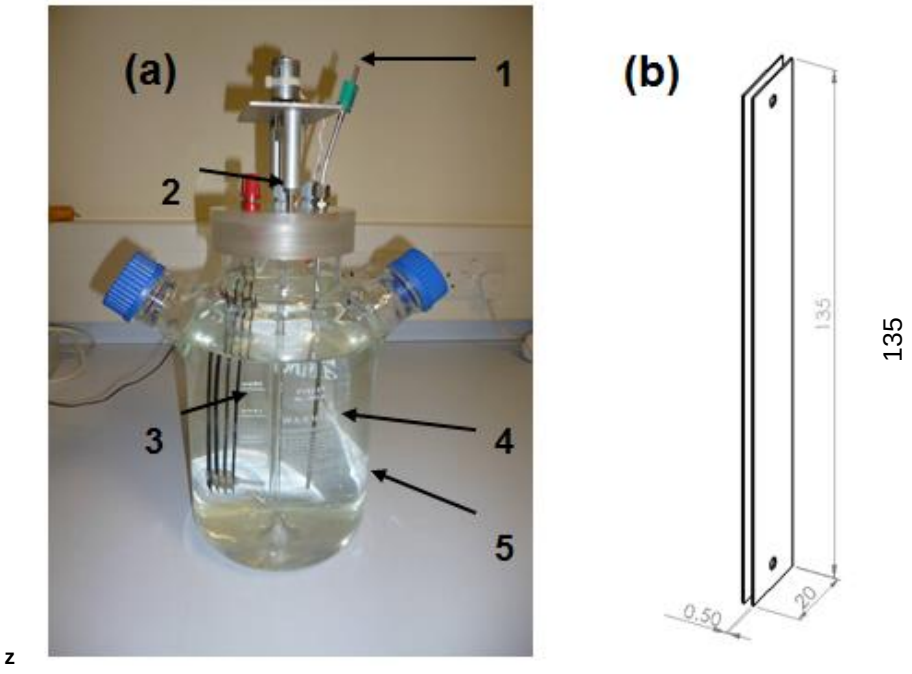

Figure 3.1: (a) Photograph of the experimental flask. (1) Motor, (2) Electrode terminals, (3) Electrode stack, (4) Glass stirrer, (5) Thermocouple; (b) Schematic showing the electrode sheets (dimensions in mm).

The minimum voltage of 6 V was applied to drive nonspontaneous redox reactions to occur in a reasonable time scale. The maximum value was selected to exceed the maximum value suggested by the electrode manufacturer (7 V). The distance between the electrodes was varied from 2

to 5 mm, because of space restrictions. The choice of temperature was driven by the fact that in control experiments at 35 and 40°C, *E.coli* could not be detected.

Parameters	Min	Max
Voltage	6 V	12 V
Current	0.14 A	1.13 A
Current Density	2.8 mA/cm^2	13.5 mA/cm^2
Electrode Distance	2 mm	5 mm
Electrode Area Projected	27 cm^2	135 cm^2
Electrode Area Actual	48.6 cm^2	243 cm^2
Stirrer speed	2,000 rpm	3,000 rpm
Temperature	Constant at 30˚C±1˚C	

Table 3.1: Geometrical and operational experimental parameters.

3.2.2 Culture preparation

The bacteria used in the experiments were *Escherichia coli* (non-pathogenic strain) NCT10418 and *L. pneumophila* (serogroup 1) NCTC12821. E.coli is the most commonly used indicator organism to detect faecal contamination in process wash water whilst routine sampling for Legionella is recommended in hot water systems of commercial buildings. The culture of *E. coli* was prepared by growing the bacteria in 100 ml sterile nutrient broth (Oxoid CM001B) for 18 hours at 37˚C at 180 rpm in a Gallenkamp shaking orbital incubator. 1 ml was taken from the overnight broth culture and added to another 100 ml sterile nutrient broth previously warmed in the same shaking incubator. The spiked flask was placed in the shaking incubator for a further 2 hours at which time the bacteria were in the exponential stage of growth. The initial population of each flask experiment ranged from 1.6x10^5 CFU/ml to 3x10^5 CFU/ml. *L. pneumophila* was purchased in the form of

lenticules from the National Collection of Type Culture (NCTC12821); a culture collection of Public Health England (PHE). Each lenticule disc consisted of a known quantity of bacteria contained in a solid water-soluble matrix stored at -20°C ± 5°C. Lenticules were allowed to reach room temperature for 10 minutes before use, after which they were rehydrated in a 1 ml volume of Maximum Recovery Diluent (Oxoid CM0733), allowed to stand for 15 minutes and shaken vigorously for 5 minutes. The initial flask population of each experiment ranged from 180 CFU/ml to 244 CFU/ml.

3.2.3 Experimental procedure

The experimental flask was filled with 3.5 litres of tap water after 1 minute of flushing to ensure that the sample was part of the main body of water. The water chemical composition can be seen in Table 3.2. The flask was subsequently autoclaved to remove any bacteria present in the tap water, and after cooling to 30˚C the stirrer was switched on. During experiments the temperature of the water was kept constant at 30˚C±1˚C by immersing the experimental flask in the water bath (Grant Instruments, type SB1, 1.5 KW). The experimental set up can be seen in Figure 3.2.

Properties	**Mean Value**
pH	7.4
Chloride	53±7 mgCl/L
Sulphate	67±7 mgSO_4/L
Calcium	130±1 mgCa/L
Sodium	32.7±6 mgNa/L
Total hardness	325 mg$CaCO_3$/L
Alkalinity	249 mgHCO_3/L

Table 3.2: Chemical composition of the water in Uxbridge (Data taken adapted from Veolia Water).

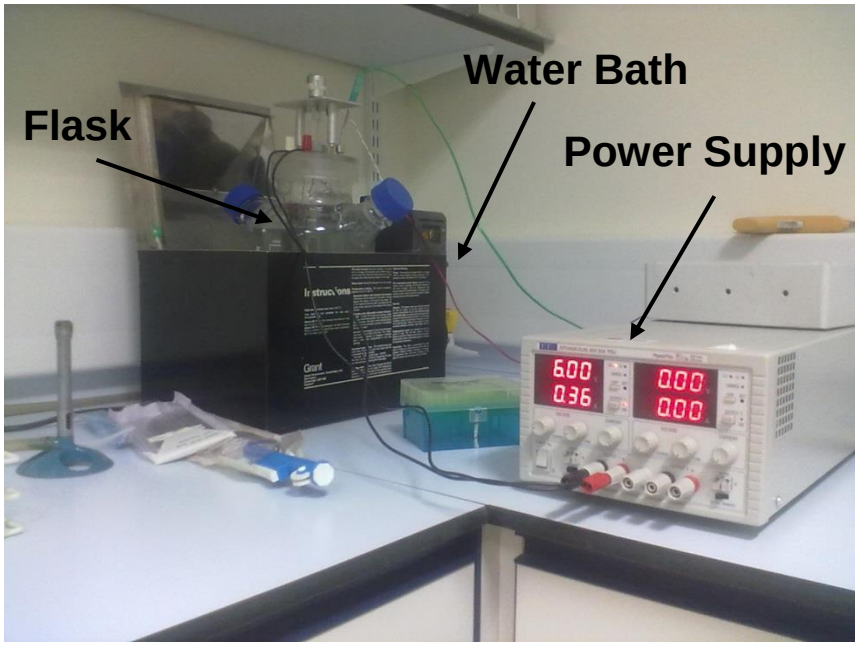

Figure 3.2: Photo of the experimental setup.

After 10 minutes stirring, either an aliquot of *E. coli* suspension (equivalent to $5.6x10^8$-$1.05x10^9$ CFU) or 4 lenticules of *L. pneumophila* hydrated in Maximum recovery diluent (equivalent to $11.1x10^5$ CFU) were introduced into the flask and the spiked water was mixed for a further 10 minutes prior to commencement of electrolysis. In the experiments with *E. coli*, 0.1 ml samples were taken at timed intervals of 5 minutes and diluted 1/10 and 1/100 in sterilised deionised water. 0.1 ml replicates of each diluted sample was then plated in triplicate onto nutrient agar (Oxoid CM0003). The number of viable cells was determined by counting the colonies after 18-24h incubation time at 37 ˚C.

In the experiments using *L. pneumophila*, 0.5 ml of the sample were plated in triplicates directly on ready-poured Buffered Charcoal Yeast Extract agar plates (BCYE) (Oxoid PO5072A). The plates were placed in a supporting rack inside a sealable polythene bag with a dampened piece of absorbent paper to provide a moist environment. The number of viable cells was determined by counting the colonies after 72 hours incubation time at 37 °C. Each experiment was repeated twice to confirm the reproducibility of the results. Prior to every experiment the electrodes and the flask were washed

thoroughly to eliminate the dead cells deposited on the anode.

Free Chlorine and Total Chlorine in the water were measured with a photometer (Palintest 7100) and tablet reagents (DPD 1 and 3). Control measurements for disinfectants were performed prior to treatment during each experiment.

3.2.4 Log inactivation and CT determination

The results were plotted in a semi-log graph with the log of survival ratio [log (N/N_0)] on the y-axis as a function of sampling time plotted in the x-axis. N being the bacteria concentration at a given time and N_0 being the initial concentration. The Log of survival ratio of Log-reduction is a method developed to quantify the level of reduction in the number of bacteria by a factor of 10. Log-reduction expresses the percentage of bacteria removed:

1-Log reduction = 90%

2-Log reduction = 99%

3-Log reduction = 99.9%

4-Log reduction = 99.99%

5-Log reduction = 99.999%

In order to determine the efficiency of ED at eliminating the target microorganisms, the results were expressed in term of CT values (Concentration-Time value). It is defined as the product of the residual disinfectant concentration, in mg/L, and the contact time between the disinfectant and the target microorganism, in minutes (WHO, 2004). The Chick-Watson disinfection model states that the rate of inactivation of any microorganism $\ln(\frac{N}{N^0})$ is dependent upon an empirical organism, temperature and pH dependent constant (-k), the concentration of disinfectant (C^n) and the contact time (t) between the disinfectant and the microorganism, see equation below:

$$\ln(\frac{N}{N^0}) = -kC^n t \quad (3.2)$$

So, for given pH and temperature conditions, a weaker disinfectant would require longer contact with the organism to achieve the required level of inactivation. CT tables exist for the various disinfectants such as chlorine, chloramine and ozone. In our experiments, the CT values were calculated for a 5 log inactivation, the time needed to inactivate 99.999% of the target microorganism.

For data analysis, the log of the survival ratio (N/N_0) was estimated as a function of sampling time by non-linear regression modelling (logit function, least-squares). All statistical analysis was performed using GraphPad Prism version 6.04 for Windows (GraphPad Software, San Diego California USA).

3.2.5 Limitations and uncertainties

Components of uncertainty in microbiology include the variation of the bacteria number in the water due to random mixing, the volume of the inoculum, the accuracy and precision of the count and sample dilution (Niemela, 2002).

The combined uncertainty of the test result is given by the relative standard deviation (RSD) of the Poisson scatter of the total count (ω_z), the uncertainty of the total volume (ω_v) and the uncertainty of the dilution factor (ω_f) (Niemela, 2002). Dilution errors may include variation in the volume of media during autoclaving and loss of part the sample adhering to the wall of the dilution tubes after having mixed the sample. However, the variability introduced by dilution is relatively small compared to the variation of the bacteria number in the water source.

The Poisson scatter was calculated for random groups of replicate plates to vary:

ω_z^2= from 0.039 to 0.06 or 3.9% to 6%

The uncertainty of the total volume was computed by taking the standard

uncertainty of a semi-automatic pipette to be 0.0051 as suggested by Niemela (2002).

$\omega_v^2 = 0.029$ or 2.9%

In this experiment all results are based upon counting the number of viable cells on the plates, so the observation depends on the human eye and mind. Because the experiments were undertaken by the author, it was possible to estimate the uncertainty of the count by counting the same plates three times during the same day. This enabled the calculation of the relative individual repeatability standard deviation to be 0.0105 or 1%, well within the ideal repeatability standard deviation (RSD) of 2% (BSI, 2001).

The samples were diluted and plated immediately, so the errors arising from transportation and storage of the sample were reduced. Finally, incubation temperature fluctuations and media quality cannot be quantified.

The combined uncertainty (u_c), given by the equation below, was found to be between 0.049 and 0.067, meaning that each test result has a 4.9% to 6.7% relative uncertainty.

$$u_c = \sqrt{(\omega_z)^2 + (\omega_v)^2 + (\omega_f)^2} \quad (3.3)$$

3.3 Results and Discussion

A control test (no electrochemical disinfection) over two hours confirmed that in water at 30°C the *E. coli* concentration remained constant for 60 minutes after which viability started to decline. After 120 minutes, in the absence of ED, the colony forming units were one third of the initial concentration. For *Legionella*, a viability test (no electrochemical disinfection) over a period of 90 minutes showed the bacteria viability remained stable.

3.3.1 Effect of voltage applied on *E. coli* inactivation

A rapid inactivation rate, seen in Figure 3.3 A was achieved with voltage/current increase, consistent with previous reports (Patermarkis and Fountoukidis, 1990, Gomes-Lopez et al., 2013). More electrons are

transferred and more oxidants are generated, as current density increases. Complete inactivation was achieved by 105 minutes at 6 V and in 35 minutes at 12 V, (with inter-electrode spacing of 5 mm and area of 48.9 cm^2 in both cases).

Electrochemical reactions are described by the Faraday's law of electrolysis, so the decomposition of chloride is proportional to the electrical charge (Q) according to the following equation.

Q = I * t = Current x time

Higher input of electric charge Q generates a higher amount of free chlorine as shown in Fig. 3 B. After 20 minutes, in the experiments at 12 V, the amount of free chlorine present in the water is double the amount of chlorine generated at 6 V.

A comparison of the results shows a lag phase at 6 V, where no inactivation seems to take place, as the amount of free chlorine is not sufficient to initiate inactivation. The time lag may be due to the tendency of *E. coli* to aggregate in clumps, which prolongs the time needed to inactivate all of the cells in the cluster (Xiong et al., 1999). Moreover, it has been reported that in tap water at temperatures of 30°C, the linear increase of chlorine production starts at current densities of 11 mA/cm^2 because part of the chlorine reacts with organic and inorganic components and suspended particles in water (Kraft et al., 1999). This phenomenon (defined as the “chlorine demand” of the tap water) varies depending on the water quality, water temperature and flow velocities. Therefore, at 6V and a current density of 4.3 mA/cm^2, the chlorine produced in our tests initially reacts with the water, but once the chlorine demand of the water is satisfied, the ED products become effective against the bacteria. At 12 V the current density is in the region of 11 mA/cm^2 so the chlorine produced is sufficient to satisfy the chlorine demand of the water and to inactivate the bacteria simultaneously. The immediate increase of chlorine level in the 12 V experiments can be clearly seen in Figure 3.3 B, confirming the results obtained by Kraft et al. (1999).

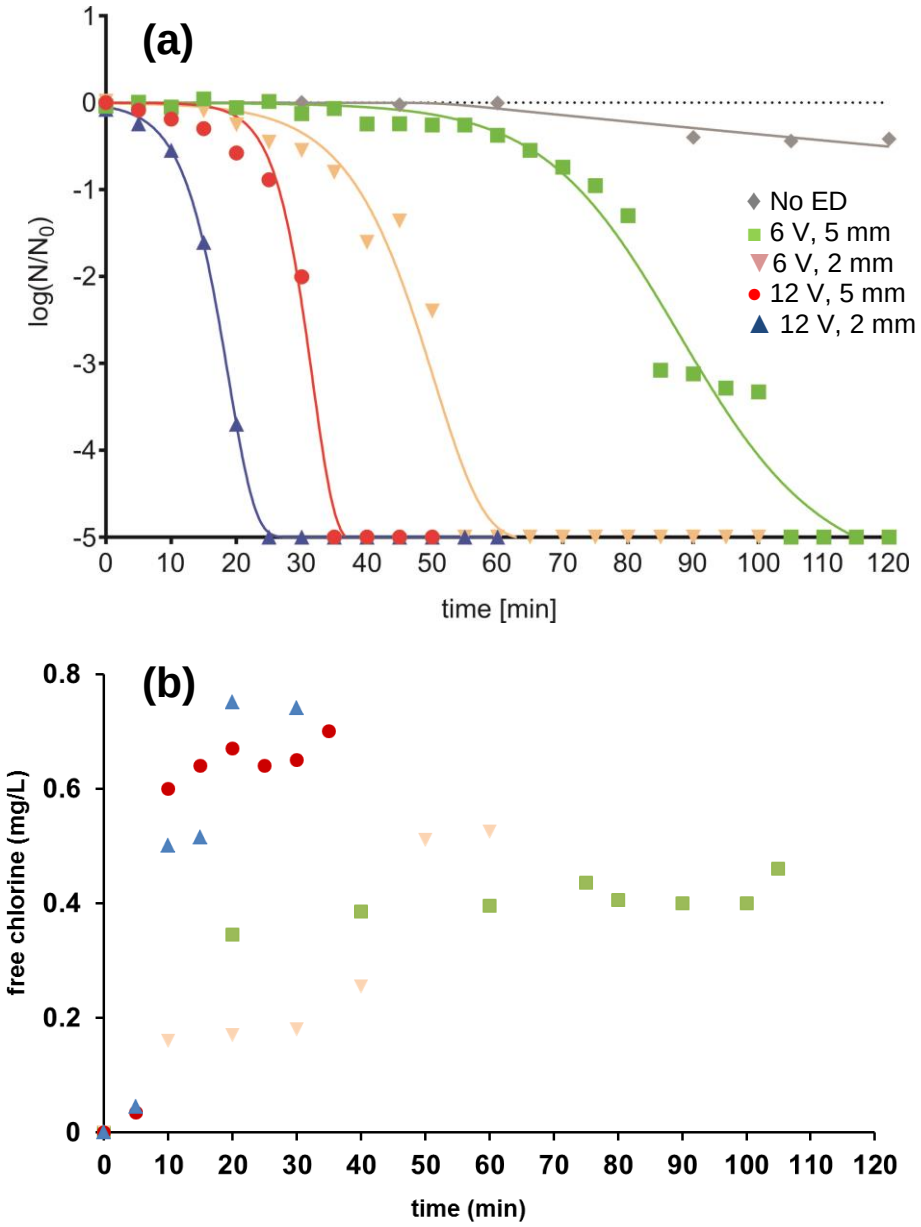

Figure 3.3: a) Inactivation rate of *E.coli* in dependence on the applied voltage/current in tap water at 30˚C, with electrode area of 48.6 cm^2, inter-electrode spacing of 2mm and 5mm and 3.5 L volume. b) Free chlorine production rate in dependence on applied voltage/current. No Electrochemical Disinfection (♦);6 V, 5 mm distance (■);6 V, 2 mm distance (●);12 V, 5 mm distance (▼);12 V, 2 mm distance (▲).

In the experiments shown in the graph, the level of residual chlorine measured to achieve 99.999% inactivation, was between 0.4 and 0.7 mg/L giving the CT values presented inTable 3.3. The CT values calculated in this experiment are the product of contact time (in minutes) to obtain 5-log

inactivation and the free chlorine residual detected (mg/L). As expected, the increase in voltage and current causes the CT value to decrease because the chlorine necessary for inactivation is produced more rapidly.

Experiment	Electrode spacing	Contact time	Free Chlorine residual	CT value
6 V	2 mm	55 min	0.52 mg/L	28.6 mg/Lmin
6 V	5 mm	105 min	0.4 mg/L	42 mg/Lmin
12 V	2 mm	25 min	0.5 mg/L	12.5 mg/Lmin
12 V	5 mm	35 min	0.7 mg/L	24.5 mg/Lmin

Table 3.3: **CT values, contact times and free chlorine residual for 5 log inactivation of E.coli at 30°C with electrode area of 48.6 cm2, inter-electrode spacing of 2mm and 5mm and voltage applied of 6 V and 12 V.**

3.3.2 Effect of electrode spacing on *E. coli* inactivation

Increased inactivation at both 6 V and 12 V voltage was achieved with a constant area by reducing the inter-electrode spacing from 5 mm to 2 mm, see Figure 3.3 A. At 6 V, the *E. coli* was inactivated in 55 minutes with 2 mm spacing compared to 105 minutes with the 5 mm spacing. The reduction of lag phase can be explained by lowered electrical resistance associated with a decreased distance between the electrodes and a rise in current density. At 12 V, the bacteria were also inactivated faster as electrodes were moved closer together but the relative size of the reduction was not as pronounced as at 6 V. This supports the statement by Sarkka et al. (2008) that after a certain threshold value for current density, the energy used will mainly generate oxygen and not oxidants.

3.3.3 Effect of electrode area on *E. coli* inactivation

The effect of an incremental change in electrode area on bacteria elimination area and on free chlorine production are shown in Figure 3.4 A and B, respectively (single area=48.6 cm^2 and triple area=145.8 cm^2). At 6 V, the lag phase duration was similar using the two electrode areas because

despite a rise in current flow increasing electrode area, current density (which is inversely proportional to the area) remains constant. However, once the chlorine water demand is fulfilled, the linear killing rate assumes a steeper slope because more water is in contact with the increased electrode area, and complete inactivation is achieved more rapidly at 85 minutes compared to the 105 minutes observed with the smaller electrode area, even if the amount of chlorine produced is in the region of 0.5 mg/L for both areas. The results with 12V applied are similar, as the linear killing rate has a steeper slope with the increased area; 100 % inactivation is achieved in 35 minutes with the single area and in 20 minutes with the triple area, see Figure 3.4 A. The amount of chlorine generated with the single area and 12 V applied was in the region of 0.7 mg/L whilst with the triple area and 12 V, it was considerably higher at 3 mg/L, confirming that the increment of area and voltage/current increases the electrical charge input, causing the generation of more oxidants (Panizza et al., 2001).

A more in-depth investigation of the effect of the area at 6 V was undertaken using the 2 mm electrode spacing; the results are depicted in Figure 3.4 C and D. Successive increase in the area of the electrode resulted in a step-wise increase in the free chlorine production and in the rate of inactivation of bacteria. A reduction in the lag phase which decreased considerably from 20 to 5 min can also be observed.

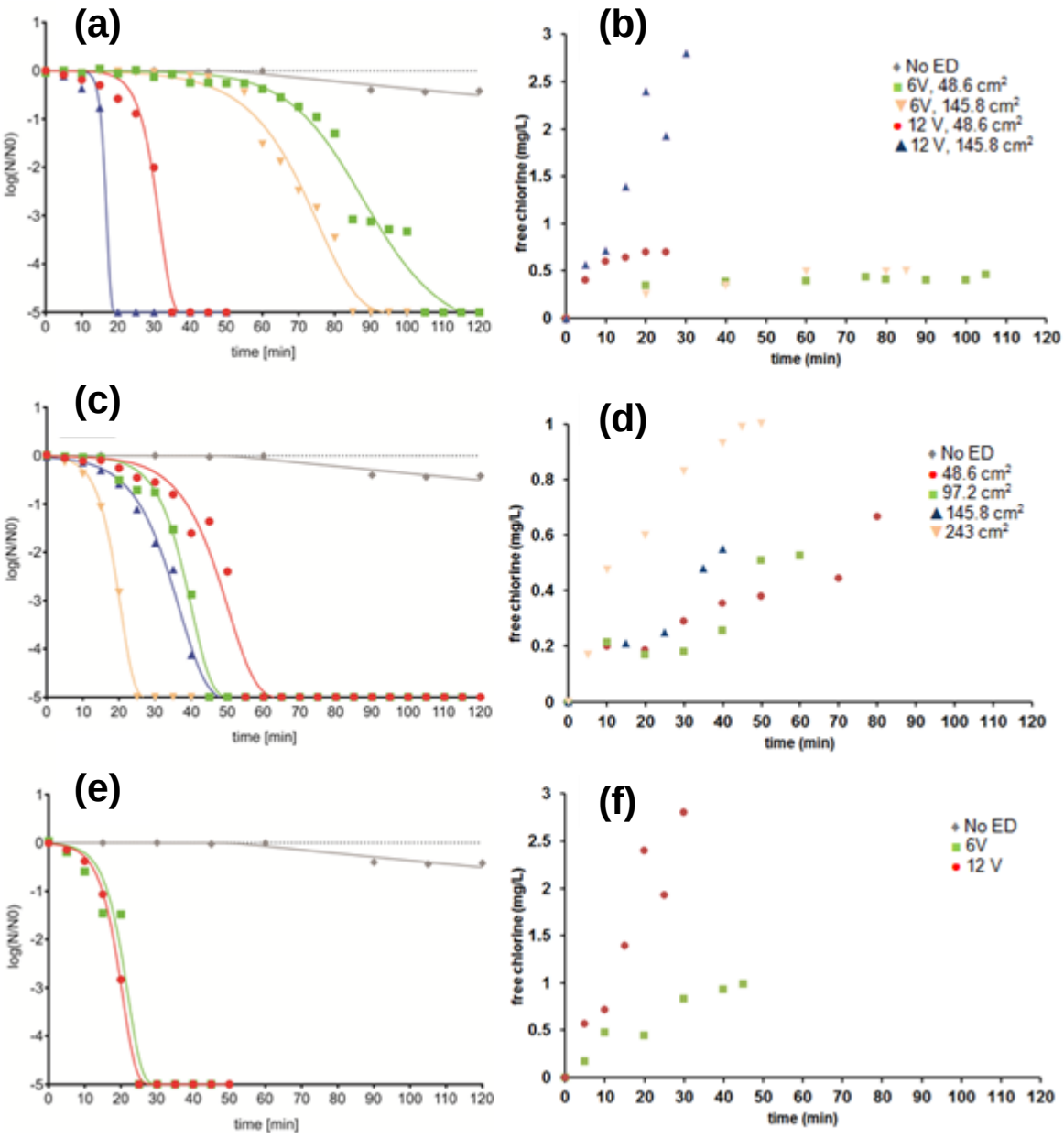

Figure 3.4: (a) Inactivation rate of *E.coli* in dependence on electrode area and (b) Free chlorine production rate in dependence on electrode area in tap water at 30˚C with 5 mm electrode spacing and 3.5 L volume. No Electrochemical Disinfection (♦); 6 V, 48.6 cm^2 area (■); 6 V, 145.8 cm^2 area (▼); 12 V, 48.6 cm^2 area (●);12 V, 145.8 cm^2 area (▲). (c) Inactivation rate of *E.coli* in dependence on electrode area and (d) Free chlorine production rate in dependence on electrode area in water at 30˚C with 6V, 2 mm electrode spacing and 3.5 L volume. No Electrochemical Disinfection (♦); 48.6 cm^2 area (●); 97.2 cm^2 area (■); 145.8 cm^2 area (▲); 243 cm^2 area (▼). (e) Comparison of the inactivation curves and (f) Free chlorine production rate when applying 1A. No Electrochemical Disinfection (♦); with 6 V (●); with12 V (■).

Notably, the 25 minutes killing time achieved at 12 V with 2 mm distance and single electrode area was also achieved with the application of 6 V, with the 2 mm distance and five times the electrode area. In both cases the current recorded was in the region of 1 A, and the comparison graph illustrates the almost identical inactivation rates, see Figure 3.4 E. Indeed, with a current density of 4 mA/cm^2 at 6 V, the performance of the electrolytic cell is analogous to use at 12 V with current density of 11 mA/cm^2 producing 3 mg/L free chlorine, see Figure 3.4 F. These facts indicate that by increasing the electrode surface area and decreasing the current density, the inactivation rate can be enhanced to match the performance obtained with higher current densities, even if a reduced amount of free chlorine is generated. This is an important result considering that the concentration of THMs increases with the increase of chlorine dosed (Saidan et al., 2013). In addition, where space considerations are not an issue, the use of larger electrolytic cells with large surface area are advisable, as disinfection will proceed with the benefit of reduced energy demand and prolonged electrode service life.

Table 3.4 shows the dependency of the CT value on the electrode area with 6 V applied and 2 mm electrode spacing. The quantity of free chlorine residual measured was between 0.38 and 0.52 mg/L, similar to the previously found values. Because more disinfectants are generated at the same time, the necessary contact time was expected to lower with the increase of area.

Area (cm^2)	Contact time	Free Chlorine residual	CT value
48.6	105 min	0.52 mg/L	28.87
97.2	45 min	0.38 mg/L	17.1
145.8	40 min	0.44 mg/L	17.8
243	25 min	0.45 mg/L	11.25

Table 3.4: CT value, contact time and free chlorine residual for 5 log inactivation of *E.coli* at 30°C with different areas, inter-electrode spacing of 2mm and 6 V applied.

In Figure 3.5, the calculated CT values plotted versus the area and the line of best fit [R^2= 0.96] are shown. Given that $CT \rightarrow 0$ for $A \rightarrow \infty$, the area should be increased to lower the CT number and hence the time of contact.

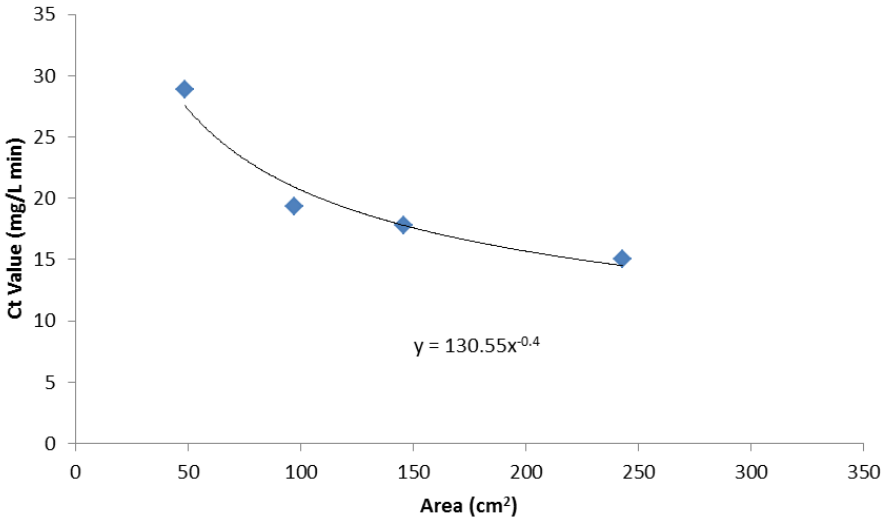

Figure 3.5: Ct value for 5 log inactivation of *E.coli* at 30°C as a function of area with 6 V applied and inter-electrode spacing of 2 mm.

3.3.4 Effect of different spiking time on *E. coli* inactivation

Inactivation curves at 6 V were characterized by an initial lag phase due to the water chlorine demand and the gradual generation of oxidants in the flask. However, in a recirculation system, the water entering the disinfection unit will immediately come into contact with the oxidants generated by the

electrolysis of the water up stream. Therefore, the experimental procedure was changed to simulate what happens in a recirculation system. The experimental flask was spiked with bacteria, either prior to, 5 minutes or 10 minutes after turning on the electrodes. Bacteria were inactivated rapidly once introduced into the water that had previously undergone ED, as expected, see Figure 3.6. Measurements taken 5 minutes after introduction of bacteria to the flask, showed the percentage of live cells to be 72% of the initial value when spiking occurred prior to ED, 51% when spiking occurred 5 minutes after electrolysis, and 15% when spiking occurred 10 minutes after electrolysis, confirming that the water had accumulated more oxidants over time which where necessary for bacterial elimination.

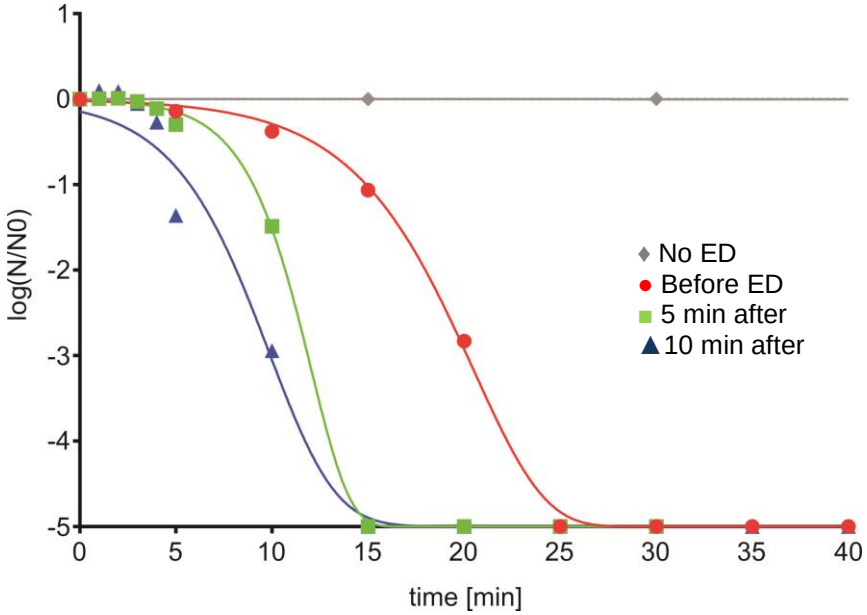

Figure 3.6: Inactivation rate of *E.coli* in dependence on pre-ED treatment time of tap water (prior to introduction of bacteria) with 6 V, 2 mm electrode distance, 243 cm^2 area and 3.5 L volume.

3.3.5 Changing the volume of contaminated water

To assess the effect of changing the volume of contaminated water in the flask on *E. coli* inactivation rate, the experiment parameters were kept constant at 6 V, electrode area of 243 cm^2 and inter-electrode spacing of 2 mm. As depicted in Figure 3.7 A, a >5-log inactivation was achieved in 15 minutes with 2.5 L and in 25 minutes with 3.5 L of water, with a similar

starting density of bacteria. At 10 minutes, only 7% of the bacteria are viable with the lower volume, compared to 42% in the larger volume. Figure 6 B confirms that with a lower volume, the free chlorine is generated more rapidly confirming that the size of the cell and the water residence time are important parameters to take into consideration during the design process.

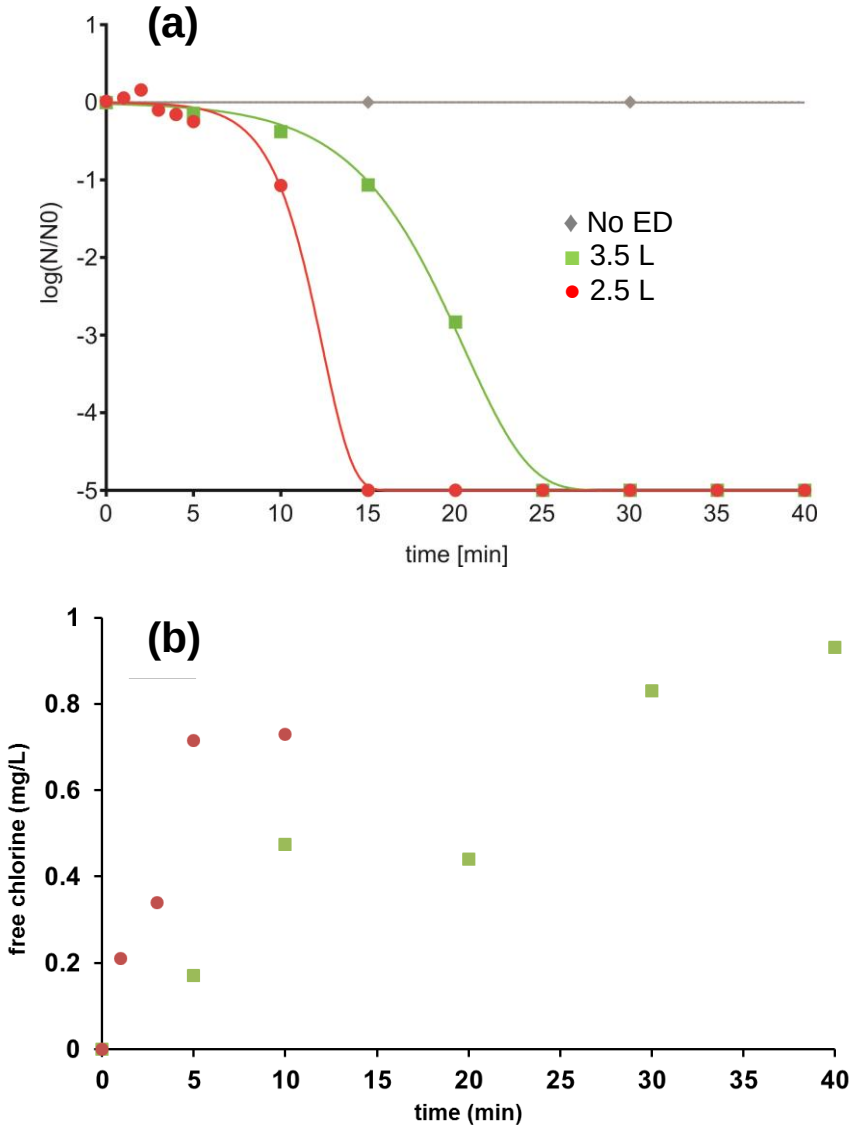

Figure 3.7: (a) Inactivation rate of *E.coli* in dependence on contaminated water volume and (b) Free chlorine production rate in dependence on contaminated water in tap water at 30˚C at 6 V with electrode area of 243 cm^2 and inter-electrode spacing of 2 mm. No ED (♦); 3.5 litres (■); 2.5 litres (●).

3.3.6 Changing initial bacteria concentration

To assess the effect of decreasing the initial density of bacteria in the flask on *E. coli* inactivation rate, the experiment parameters were kept constant at 6 V, electrode area of 243 cm^2 and inter-electrode spacing of 2 mm. The low concentration of bacteria was between $2.4x10^3$ and $2.8x10^3$ CFU/ml; the results are shown in Figure 3.8. With lower initial concentration, 5-Log inactivation was achieved in 10 minutes and after 5 minutes, only 5% of the bacteria remain detectable. In comparison, with high initial concentration of 10^5 CFU/L, 5-Log inactivation is achieved in 25 minutes and after 5 minutes 72% of the bacteria can be detected. With less bacteria, inactivation starts immediately after the electrodes are turned on, making the initial bacteria concentration an important parameter. So, the treatment duration is 5 times higher for an initial concentration 10^2 higher (i.e. 5 minutes for $2.4\text{-}2.8x10^3$ CFU/ml compared to 25 minutes for $1.6\text{-}3x10^5$ CFU/ml).

The HSE L8 code of practice (2013) requires buildings to monitor the bacteria levels in the water and record the results of the samples for evidence. So, the history of bacteria levels of a building, coupled with these findings would enable the appropriate sizing of the electrolytic cell. If a building has a history of high bacteria levels, the water residence time in the device should be increased. On the other hand, if the bacteria are typically present in lower numbers, the water residence time can be reduced.

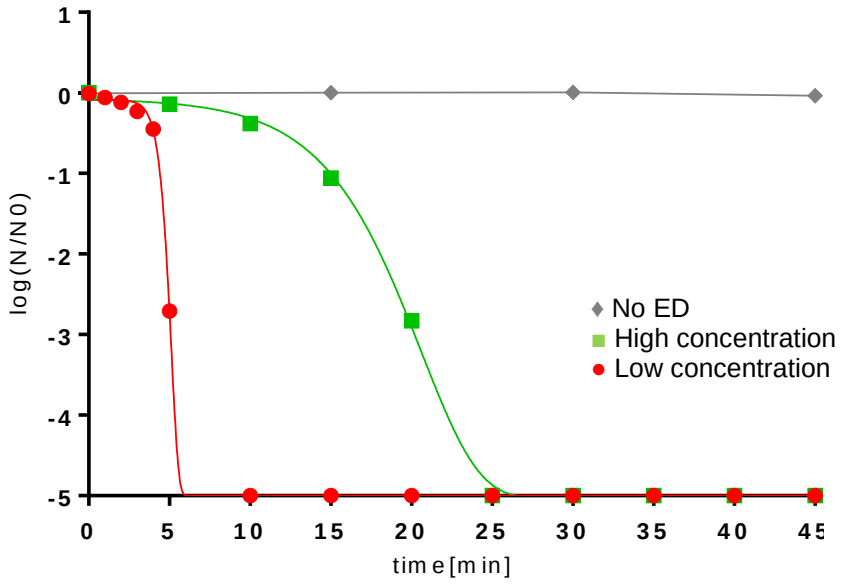

Figure 3.8: Inactivation rate of *E.coli* in dependence on initial bacteria concentration in tap water at 30˚C at 6V with electrode area of 243 cm^2, inter-electrode spacing of 2 mm and 3.5 L volume. No ED (♦); 1.6-3x10^5 concentration (■); 2.4-2.8x10^3 concentration (●).

3.3.7 Enhancing mixing

The final operational parameter assessed in this study was the effect of increasing mixing in the flask; the results are shown in Figure 3.9 A and are obtained using the same parameters described in section 4.5 and a volume of 3.5 L. Disinfection started immediately (no time lag) once the motor speed was increased from 2,000 to 3,000 rpm, with only 2% of bacteria remaining viable after 5 minutes. This result indicates that the flow velocity is crucial to the process to provide the adequate amounts of reactants supply to the electrode and removal of products from it, but also to meet the water chlorine demand more rapidly. After 5 minutes the free chlorine concentration is 3 times higher, see Figure 3.9 B, so for the purpose of incrementing the performance of the electrolytic device, the cell and electrodes surfaces could be designed to increase mixing or the pipes at the inlet of the cells could be fitted with a mixing enhancer.

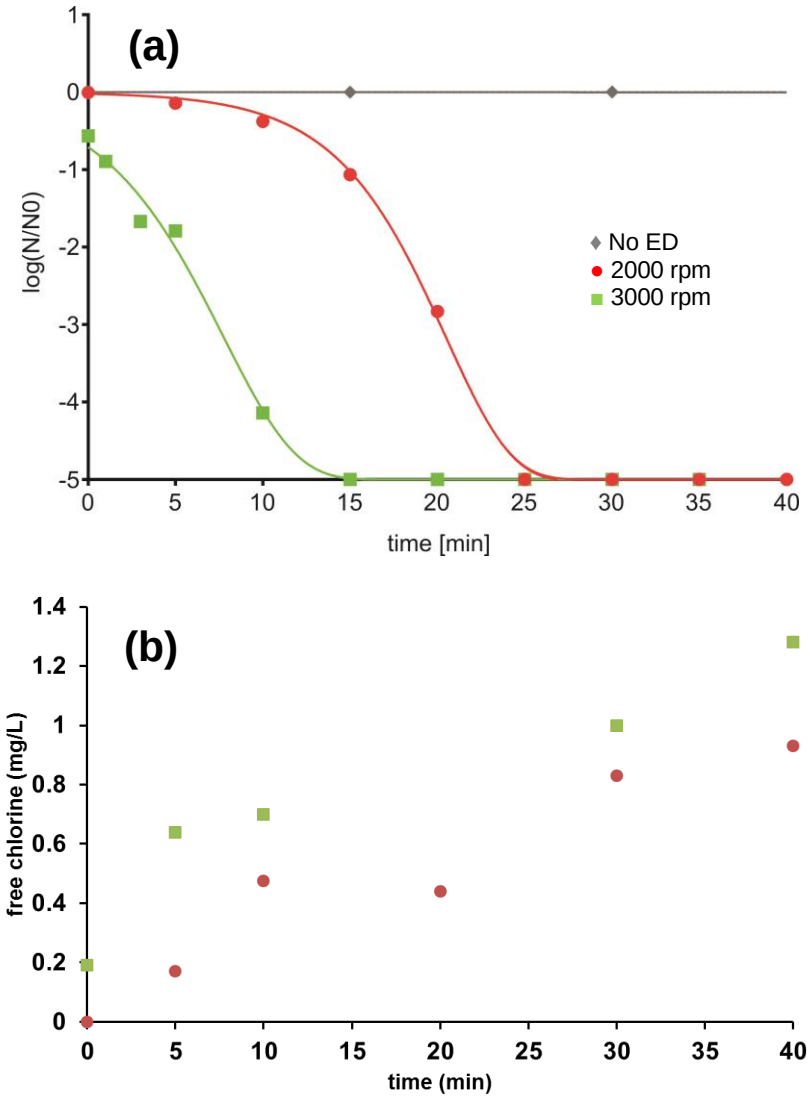

Figure 3.9: (a) Inactivation rate of *E.coli* in dependence on mixing speed in tap water and (b) Free chlorine production rate in dependence on mixing speed in tap water at 30˚C at 6 V with electrode area of 243 cm^2, inter-electrode spacing of 2 mm and 3.5 L volume. No ED (♦); 2000 rpm (●); 3000 rpm (■).

3.3.8 Comparison between *Legionella* and *E. coli* inactivation

Figure 3.10 compares the inactivation curves of *E. coli* and *L. pneumophila* sg. 1 in tap water at 30°C, 6 V applied, electrode area of 243 cm^2, inter-electrode spacing of 2 mm, 3.5 L volume and 2,000 rpm stirring speed. A >5 log inactivation of *Legionella* was achieved after 45 minutes compared to 25 minutes for *E. coli*, demonstrating that *L. pneumophila* sg. 1 is more resilient to ED. A 25 minutes time lag can be observed in the *L. pneumophila* sg. 1

inactivation curve despite the chlorine demand of the water having been fulfilled. This result indicates that *L. pneumophila* sg. 1 requires higher levels of disinfectants for inactivation to occur, as reported previously (Kutchta et al., 1983). In our experiment the level of free chlorine after 5 minutes was 0.6 mg/L whilst at 30 minutes (when the levels of *Legionella* start to diminish) the free chlorine was 1 mg/L, suggesting that these are the values necessary to achieve inactivation. Increasing the electrode active surface area, decreasing the volume of contaminated water around the electrodes and increasing turbulence will result in a decrease in the time lag, and ultimately improve the performance of the electrolytic device in inactivating *L. pneumophila* sg. 1 and other more resilient bacteria.

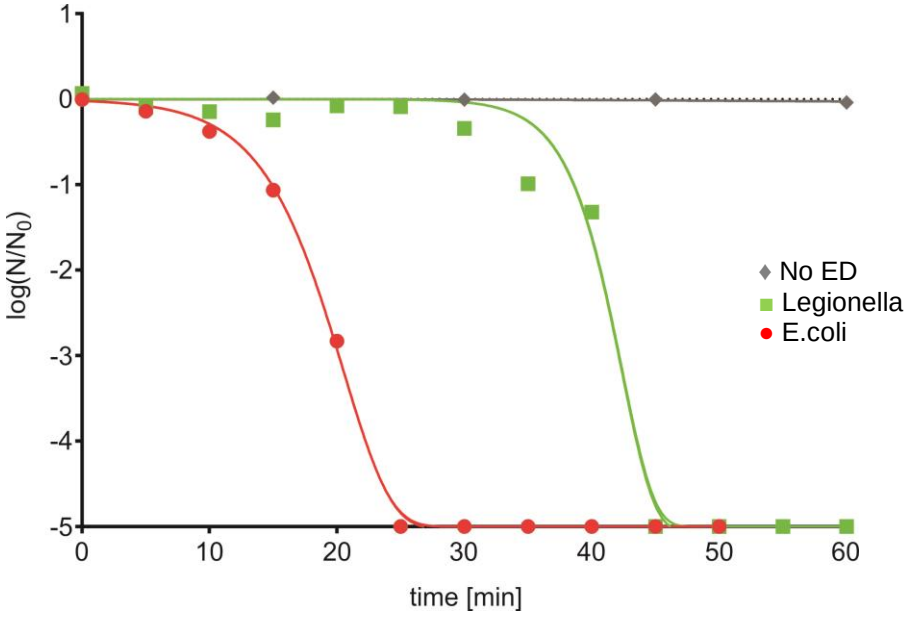

Figure 3.10: Comparison between *L.* pneumophila sg. 1 and *E. coli* inactivation curves in tap water at 30˚C at 6 V with electrode area of 243 cm^2, inter-electrode spacing of 2 mm and 3.5 L volume. No ED (*Legionella*) (♦); *Legionella* curve (■); *E. coli* curve (●).

3.4 Conclusions

This was a systematic study on the effect of geometrical and operational parameters in an ED device on bacteria inactivation rate. These results are needed to inform engineers, researchers and scientists charged with developing electrochemical cells for bacterial disinfection.

This investigation has confirmed that electrochemical disinfection has potential. Concentrations between $1.6x10^5$ CFU/ml and $3x10^5$ CFU/ml of *E. coli* were inactivated effectively (>5 log) in 25 minutes with current density of ≈ 4 mA/cm^2 whilst a > 5 log inactivation of 180 CFU/ml to 244 CFU/ml of *L. pneumophila* sg. 1 was achieved in 45 min under the same conditions. This confirms that Legionella is more resilient and needs more time to be inactivated. However, the results of this investigation show that increasing the surface area improves the performance of the electrolytic process without the application of a higher potential which is detrimental to the service life of the electrodes. It also demonstrated that the time necessary to achieve 4-log inactivation of *E. coli* was decreased from 110 minutes to 10 minutes, by manipulating the geometrical and operational parameters of the cell. The same method can be used to reduce *L. pneumophila* sg. 1 inactivation time.

In building applications, the residence time of the water in the ED device is likely to be lower than in the flask experiment. Therefore, the necessary CT value cannot be achieved with the same surface area. The investigation has demonstrated that by increasing the surface area and lowering the volume of the reactor, the necessary CT value to obtain 5-log inactivation could be achieved in building applications. Having devices of different sizes would be expensive and unsustainable and these findings have confirmed the possibility of designing modular intelligent devices adaptable to different environmental and operational conditions. However, from the literature (Kraft et al., 1999, Hsu, 2005, Yao et al., 2011), it is clear that flow rate plays an important role in the production of chlorine and it should be investigated further.

Chapter 4 : Long-term assessment of electrochemical disinfection in buildings hot water systems

Think of the earth as a living organism that is being attacked by billions of bacteria whose numbers double every forty years. Either the host dies, or the virus dies, or both die.

Gore Vidal

4.1 Introduction

The review of the literature identified that although several studies have confirmed ED to be effective in eliminating pathogens under controlled laboratory conditions (Patermarkis and Fountoukidis, 1990, Furuta et al., 2004, Kerwick et al., 2005, Fang et al., 2006, Jeong et al., 2007, Polcaro et al., 2007, Delaedt et al., 2008, Sarkka et al., 2008), long-term evaluations of the efficacy of electrolytic devices in controlling pathogens in the hot water systems of buildings, are limited. It is important to study the effect of a treatment on site because the different pipe materials used the temperature variation, dead-legs and under-used taps, in addition to the presence of other flora or amoeba may increase the persistence of the pathogenic bacteria, something that cannot be easily simulated in a laboratory. Therefore, two prototype devices were installed in the hot water recirculation systems of two different buildings: the Multiple Sclerosis (MS) Centre in Wendover, a small size medical centre and the Halsbury and Bragg/ETC complex at Brunel University, a medium size university building. The presence of pathogens was monitored by analysing water samples taken from carefully selected locations in the hot water systems. The device in the Halsbury and Bragg/ETC complex was monitored for a year in 2013, whilst the device in Wendover continues to be monitored.

4.2 Multiple Sclerosis Centre, Wendover, Buckinghamshire

4.2.1 Materials and Methods

The electrolytic device supplied by Environmental Scientific Group (ESG) was installed by contractors in the hot water recirculation system of the Chiltern Multiple Sclerosis centre located in Wendover, Buckinghamshire. The building was opened in October 2012 and it is used by 200+ patients every week. There are 29 hot water outlets, including showers and all of the hot water outlets are fitted with thermostatic mixing valves (TMVs) with the exception of the cleaner room and the kitchenette. TMVs are fitted to lower the risk of scalding so that the hot water (60˚C) is pre-mixed with cold water

to achieve the temperature of 45˚C. A photograph of the building is shown in Figure 4.1.

Figure 4.1: Photograph of the MS centre in Wendover, Buckinghamshire.

Because the water hardness in the area is 293 mg$CaCO_3$/L, classified high in the hardness level, the DHW system has been fitted with an ion exchange resin water softener, replacing calcium and magnesium ions with sodium ions. A photograph of the installation is shown in Figure 4.2 (a). The installation is such that the cold water entering the hot water system and the water in the recirculation system are both disinfected by the device before entering the hot water calorifier, as shown in the schematic in Figure 4.2 (b). This ensures that the bacteria naturally present in the cold water are inactivated and that residual disinfectants are generated to control the bacteria released by biofilm forming in pipes. Cold water comes directly from the mains to feed the BFC Cyclone III condensing glass-lined heater. The hot water is then pumped across the whole system by a stainless steel Grundfos UPS 15-50N (130) pump.

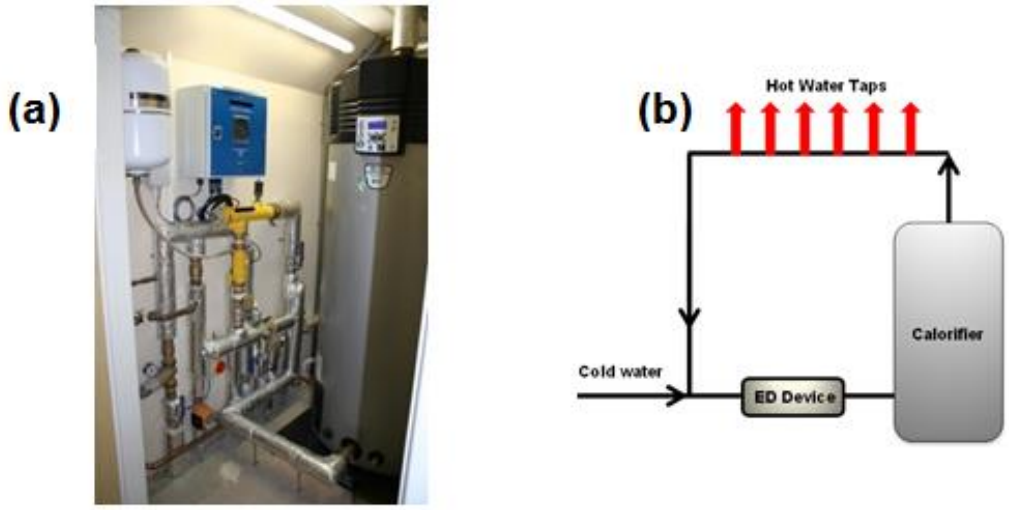

Figure 4.2: (a) Photograph of the device installation in the Chiltern MS centre; (b) Schematic of the installation.

The standard equipment comprises of a control panel (electronics), an oxidation chamber encasing the electrode package and a flow sensor, as indicated in the schematic of a typical installation in Figure 4.3. Table 4.1 and Table 4.2 provide the technical data for the electronics and the oxidation chamber, respectively. The voltage input to the electrodes and the reversal of electrode polarity to eliminate the calcareous deposit every 10 minutes, are regulated by the electronics. The oxidation chamber is made of polypropylene-R and it is fitted with an automatic air vent (Honeywell, 6 bar) so any gas created during the electrolysis can be vented out of the system. The flow sensor (TURCK Banner FCS-G1/4A4-NA-K11141) ensures that the device is immediately switched off if there is no flow of water in the event of pump malfunction.

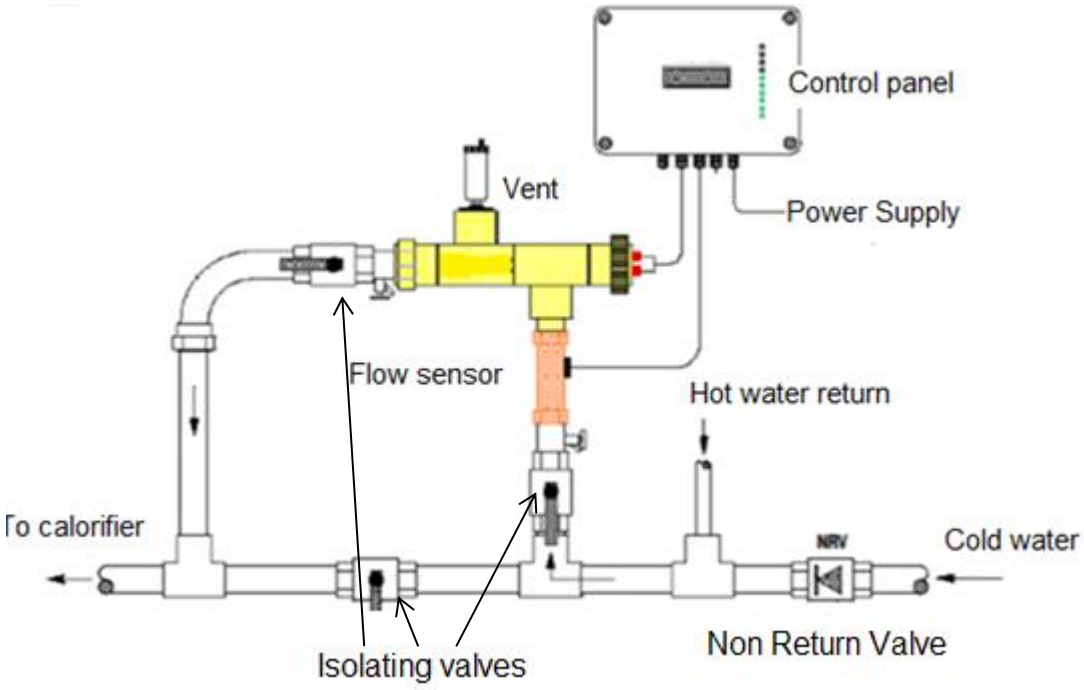

Figure 4.3: Schematic of the ED device installation in Wendover.

Technical Data - Electronics	
Casing	Glass Reinforced Polyester (GRP)
Enclosure Rating	International Protection Marking IP65
Power Supply	230 V / 50Hz
Input Power	Automatic according to settings Max. 150W
Display	LED
Connection electrode pack	Galvanic isolation
Allowable ambient temperature	0-30°C

Table 4.1: Control panel technical data.

Technical Data - Oxidation Chamber	
Casing, socket welding	Polypropylene-R
Maximum Continuous Temperature	65°C
Allowable Operation Excess Pressure	8 bar / 65°C
Maximum Flow	8 m^3/h
Electrode Package	Pipe-in-pipe system
Connection Dimension	1 ¼ " BSP
Automatic air vent	Honeywell 6 bar

Table 4.2: Oxidation chamber technical data.

The electrode package consisted of a 12 mm rod surrounded by concentric 19 mm, 25 mm and 30 mm outside diameter cylindrical electrodes, 200 mm in length and with thickness of 1 mm. The electrodes were made of platinised titanium with platinum thickness between 2-5 µm (made by Metakem GmbH, Germany). The schematic of the configuration can be seen in Figure 4.4 (a) and (b). The electrode projected area was 351.68 cm^2 and the oxidation chamber volume 0.726 litres, in line with the findings presented in Chapter 4. The device was connected to a dc power supply and 5 V applied to give current densities ranging from 9.4 to 11 mA/cm^2 depending on the quality of the water. The distance between the electrodes was maintained within 2 and 2.5 mm depending on manufacturing tolerances.

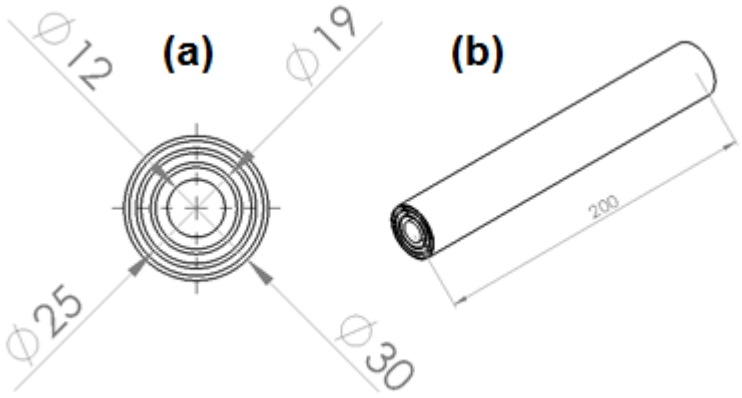

Figure 4.4: Chiltern Multiple Sclerosis Centre installation. (a) Perspective of the electrode package (dimensions in mm); (b) Isometric view of the electrode package.

Starting in May 2013 1-litre water samples were taken at monthly intervals by the author from each of four sampling locations into sterile plastic containers with screw-top lid. The sample bottles supplied by Latis Scientific contained sodium thiosulphate for neutralising the chlorine and preventing continuation of bacterial action during transportation. 'Pre-flush' samples were collected immediately as soon as the tap was opened, representing the water held in the tap and pipework local to the tap. 'Post-flush' samples were collected after the water was run for a minute, representing the quality of the water supplied in the recirculation loop (BSI, 2008). A schematic of the recirculation system, indicating in red the water local to the pipes and in black the water in the main recirculation, is shown in Figure 4.5. Chemical parameters of the water relevant to ED, supplied by Thames Water Utilities in the area, can be seen in Table 4.3.

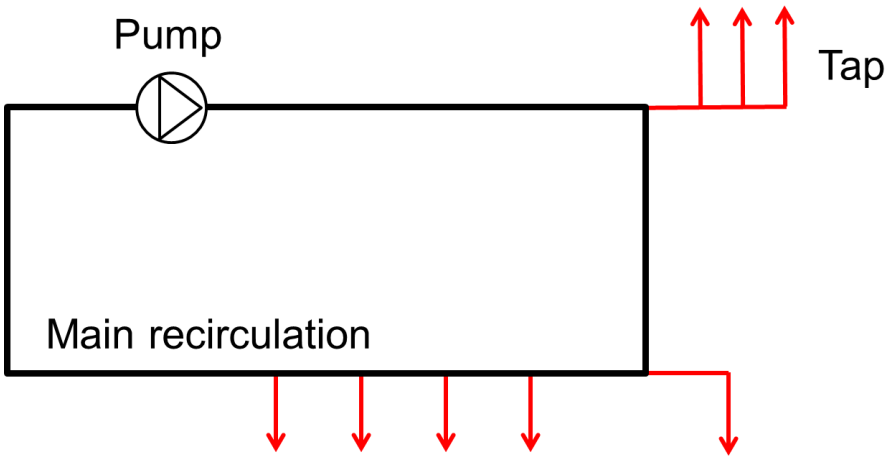

Figure 4.5: Schematic of the recirculation system. Highlighted in red is the water held in the tap and in the pipes local to the tap, in black is the water in the recirculation.

Properties	Mean Value
pH	7.3
Chloride	20.9±1.7 mgCl/L
Sulphate	16.1±2.3 mgSO_4/L
Sodium	10.8±1.2 mgNa/L
Total hardness	293mg $CaCO_3$/L

Table 4.3: Chemical composition of the water in Wendover (Data taken adapted from Thames Water Utilities).

In July 2014, it was decided to stop taking post-flush samples from Tap A and to take a monthly post-flush sample from another tap located in the Cleaners room instead. The decision was taken because the latter is the only tap in the whole recirculation system not fitted with a TMV. This enabled a comparison between the taps fitted with a TMV and the conventional tap.

Labelled bottles containing water samples were stored at ambient temperature and processed within 24 hours at Latis Scientific laboratory in Barbican, London (http://www.latisscientific.co.uk/). Each sample was

analysed to determine the total number of microorganisms (Total Viable Counts or TVCs) and the density of *Legionella*, *Pseudomonas* species and *P. aeruginosa*. *Legionella* detection and enumeration were performed according to British Standards (BSI, 1998). *Pseudomonas* and Total Viable Count (TVC) detections and enumerations were performed in accordance with the practices and procedures listed in the Microbiology of Drinking Water, Part 7 and 8, by the Environment Agency.

4.2.2 Results and Discussion

The sampling locations and the sample types taken at each outlet are shown in Table 4.4. Also shown are the TVCs, *Pseudomonas* species and *P. aeruginosa* results of the samples taken at the four location points prior to turning on the ED device in May 2013. Samples labelled with Not Tested (NT), were not processed due to administration errors at the laboratory. When the symbol – appears, samples were not taken.

Sample Locations	**Sample Type**	**TVCs 22˚C 72 hours**	**TVCs 37˚C 48 hours**	***Pseudomonas* spp. 30˚C**	**P. *aeruginosa***
		CFU/ml	**CFU/ml**	**CFU/100 ml**	**CFU/100 ml**
Tap A, Toilets	Pre-flush	NT	NT	NT	NT
Tap A, Toilets	Post-flush	8,400	8,400	11,000	0
Tap B, Toilets	Pre-flush	15,000	9,400	13,000	0
Kitchen	Post-flush	8,400	7,400	11,000	11
Cleaners	Post-flush	-	-	-	-

Table 4.4: Results from the first sampling routine (May 2013) prior to Electrochemical Disinfection.

Table 4.5 shows the TVCs results of the samples taken in all of the location points since the device has been turned on, whilst Table 4.6 presents the *Pseudomonas* species results. Table 4.7 shows the *P. aeruginosa* results for all of the sample locations and the *Legionella* results for Kitchen post-flush.

The other samples were also analysed for *Legionella* but none was detected during the monitoring period. Highlighted in red are the sample results above the control limits of 10,000 Colony Forming Unit (CFU)/ml for TVC, 10 CFU/100 ml for *P. aeruginosa* and 1,000 CFU/L for *Legionella*. Although a comprehensive research of the regulations concerning the standards for non-potable water was undertaken, it was not possible to find a control limit for *Pseudomonas* species.

		Pre	← Electrochemical Disinfection →													
Sample Location	**Results (CFU/ml)**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**May**	**Jun**	**Jul**	**Aug**	**Sep**
Tap A, pre-fl.	Mean of TVCs	NT	13150	6 680	577	535	1622	5455	2970	3465	4235	209	163	550	24	272	144
Tap B, pre-fl.	Mean of TVCs	15000	1705	1045	852	1387	1100	3217	277	4207	1430	8415	82	142	2227	578	133
Tap A, post-fl.	Mean of TVCs	8400	478	162	34	60	97	85	48	935	187	201	425	30	-	-	-
Kitchen, pre-fl.	Mean of TVCs	-	-	-	-	-	-	10223	-	-	-	-	-	-	-	-	-
Kitchen, post-fl.	Mean of TVCs	8400	825	427	295	247	524	289	16	550	675	880	381	42	79	99	23
Cleaners, post-fl.	Mean of TVCs	-	-	-	-	-	-	-	-	-	-	-	-	-	80	0	3

Table 4.5: Means of Total Viable Counts results of samples taken over the monitoring period June 2013 to September 2014. Highlighted in red are TVCs results >10,000 CFU/ml.

		Pre	← Electrochemical Disinfection →														
Sample Location	**Results (CFU/100ml)**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**May**	**Jun**	**Jul**	**Aug**	**Sep**
Tap A, pre-flush	*Pseud.* spp.	NT	0	NT	780	17000	0	605	0	0	0	0	0	18	6	7	11
Tap B, pre-flush	*Pseud.* spp.	13000	200	NT	350	0	0	0	0	0	0	10	0	16	2	8	53
Tap A, post-flush	*Pseud.* spp.	11000	0	NT	110	170	0	0	0	0	0	6	33	18	-	-	-
Kitchen pre-flush	*Pseud.* spp.	-	-	-	-	-	-	1000	-	-	-	-	-	-	-	-	-
Kitchen, post-flush	*Pseud.* spp.	11000	160	NT	3	0	5	12	0	14	160	67	50	13	4	120	110
Cleaners, post-flush	*Pseud.* spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0

Table 4.6: *Pseudomonas* species results for samples taken over the monitoring period June 2013 to September 2014.

		Pre	← Electrochemical Disinfection →														
Sample Location	**Results (CFU/100ml)**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**May**	**Jun**	**Jul**	**Aug**	**Sep**
Tap A, pre-flush	*P. aeruginosa*	NT	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0
Tap B, pre-flush	*P. aeruginosa*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tap A, post-flush	*P. aeruginosa*.	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-
Kitchen pre-flush	*P. aeruginosa*.	-	-	-	-	-	-	30	-	-	-	-	-	-	-	-	-
Kitchen, post-flush	*P. aeruginosa*.	11	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
Cleaners, post-flush	*P. aeruginosa*	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
Kitchen, post-flush	*Legionella spp.*	0	0	0	0	0	0	0	0	0	0	0	0	30	0	0	0

Table 4.7: *P. aeruginosa* and *Legionella* spp. results for samples taken over the monitoring period June 2013 to September 2014.

Since the device has been on to supplement temperature control, the samples results for both TVCs and *Pseudomonas* have been within the control limits with the exception of Tap A pre-flush. On two occasions the counts have been high, for TVCs in June 2013 and for *Pseudomonas* in Sept. 2013 confirming the tap is under-used. Every building incorporates under-used outlets where water stagnates and chlorine dissipates and the most efficacious way of control is flushing regardless of the water treatment in place.

Figure 4.6 and Figure 4.7 present the means of pre-flush and post-flush sample results of TVCs and *Pseudomonas* species, respectively. On average, there was a 2-log (pre-flush) and 4-log (post-flush) reduction in the number of counts measured in water samples once ED has started. There was a marked 3-log decrease in Pseudomonas spp. counts in both pre and post-flush samples over time with commencement of ED. The total bacteria counts in the same tap were up to 60-fold higher in pre-flush samples than in post-flush samples, confirming that bacteria proliferate at outlets where water stagnates and scale accumulates. Bacterial proliferation at taps may also be a result of the local temperature, as water is pre-mixed with cold water to achieve 45˚C.

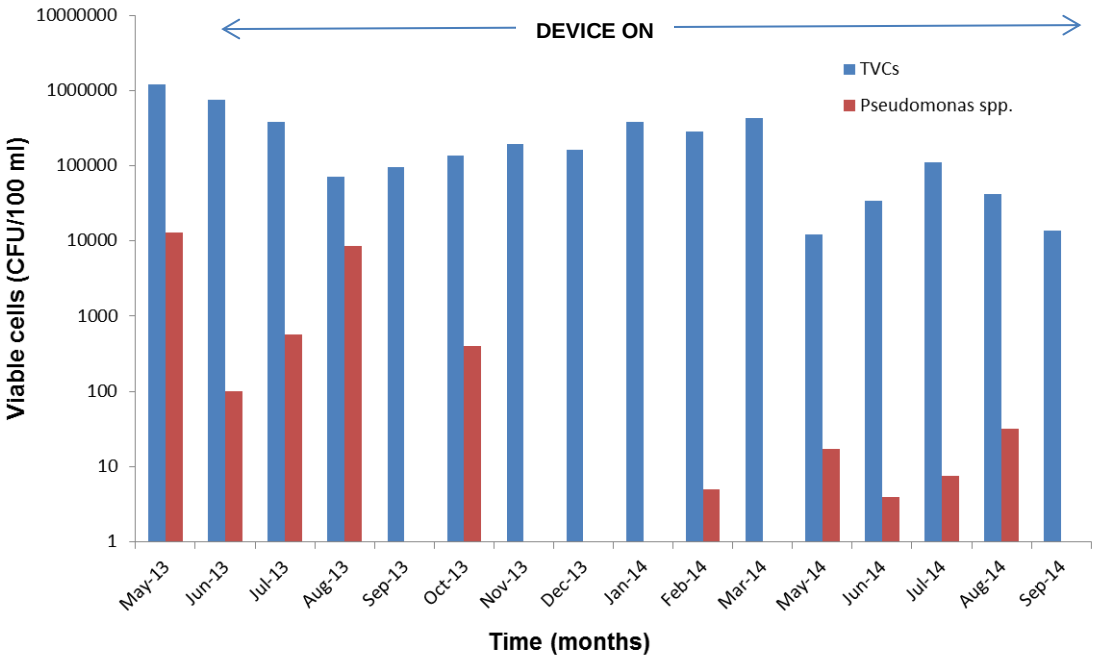

Figure 4.6: Average of pre-flush results for *Pseudomonas* species grown at 30°C, TVCs grown at 22°C for 72 hours and TVCs grown at 37°C for 48 hours.

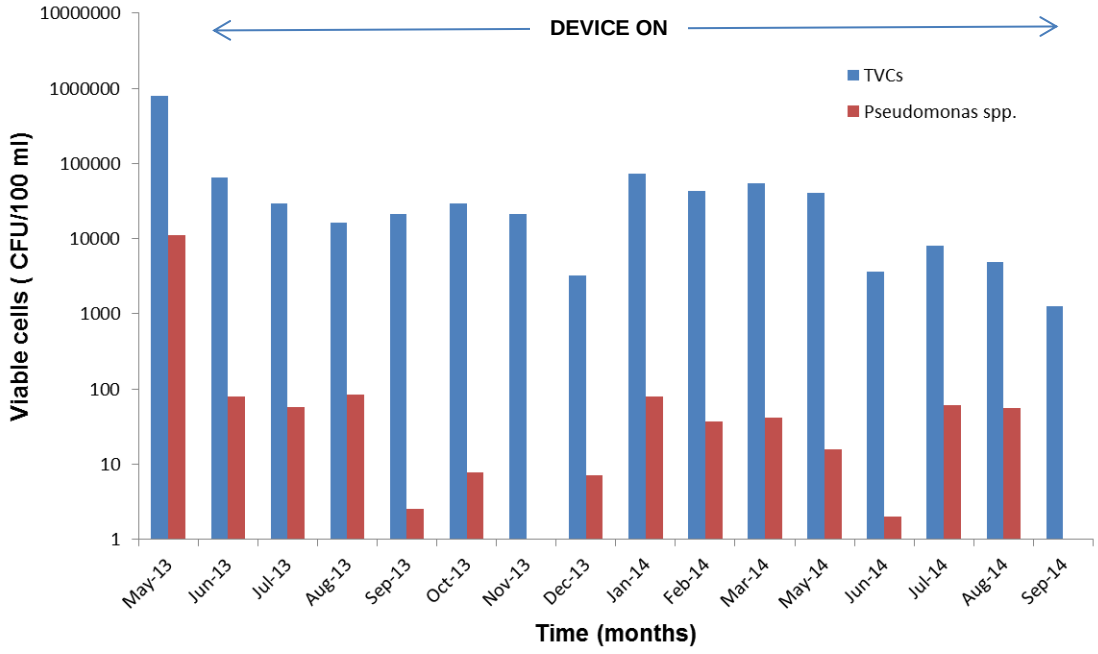

Figure 4.7: Average of post-flush sample results for *Pseudomonas* species grown at 30°C, TVCs grown at 22°C for 72 hours and TVCs grown at 37°C for 48 hours.

Bacteria levels in the recirculation loop have been reduced significantly (t-test, $p<0.01$) by ED and these levels were maintained throughout the monitoring period, see Figure 4.7. Despite almost continuous operation from May 2013-June 2014, the device was intentionally turned off for 1 week in October 2013 (to determine if bacterial levels would respond to removal of ED), and accidentally for a few days in April 2014 due to electrical failure. The system was sampled in October prior to switching ED back on, and routine monthly samples continued from November 2013 and July 2014. Although there are no apparent changes to TVCs and *Pseudomonas* species counts in pre and post-flush samples after ED was turned off, pathogenic bacteria (*Legionella* species and *P. aeruginosa)* were detected above control limits at these times, see Figure 4.8.

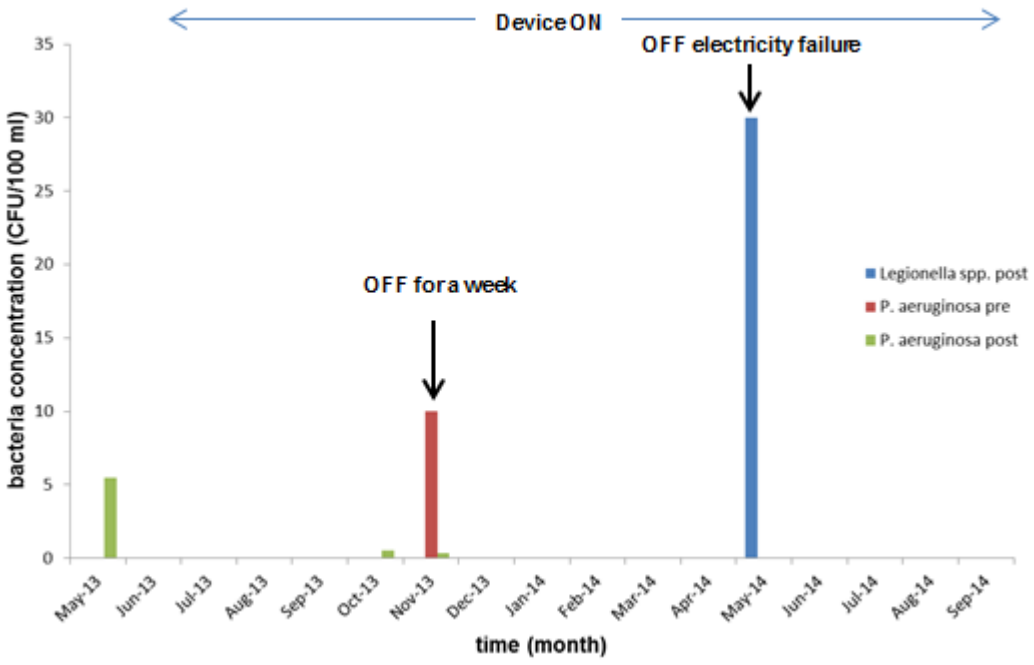

Figure 4.8: Pathogens detected during the monitoring period including *Legionella* species and *P. aeruginosa* (CFU/100 ml).

As mentioned in the materials and methods section, from July 2014, it was decided to take samples from a conventional tap not fitted with a TMV. Taking samples from the conventional tap enabled the microbiological analysis of the hot water without having been mixed with the cold water in the TMV. The results for post–flush samples of the conventional tap are

shown in Figure 4.9, whilst the results for post-flush samples of the tap fitted with the TMV are presented in Figure 4.10.

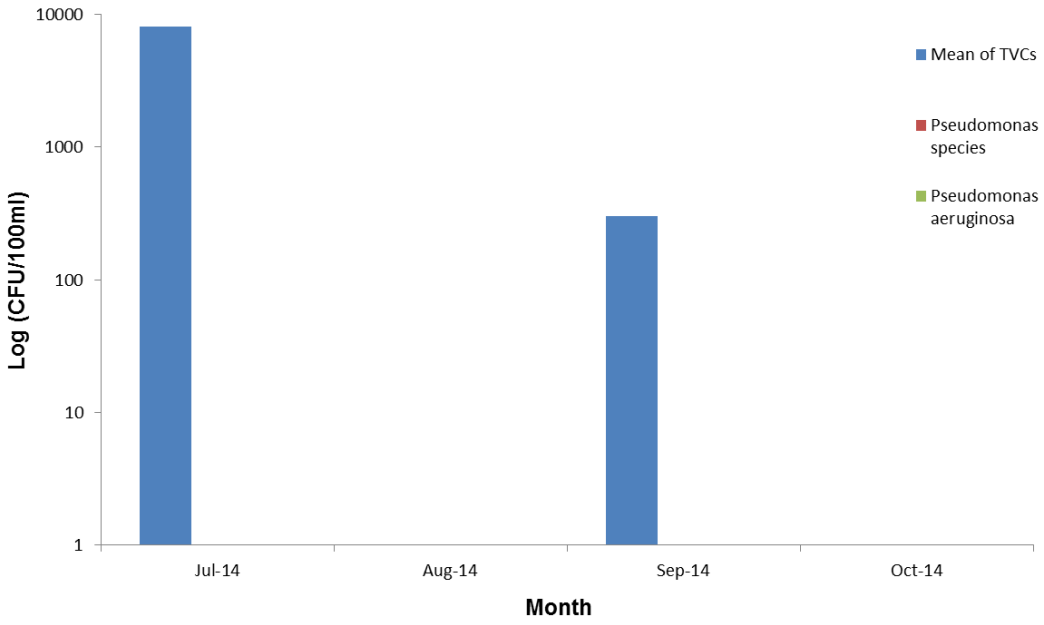

Figure 4.9: Conventional tap (no TMV) post-flush sample results for mean of TVCs, *Pseudomonas* species and *P. aeruginosa.*

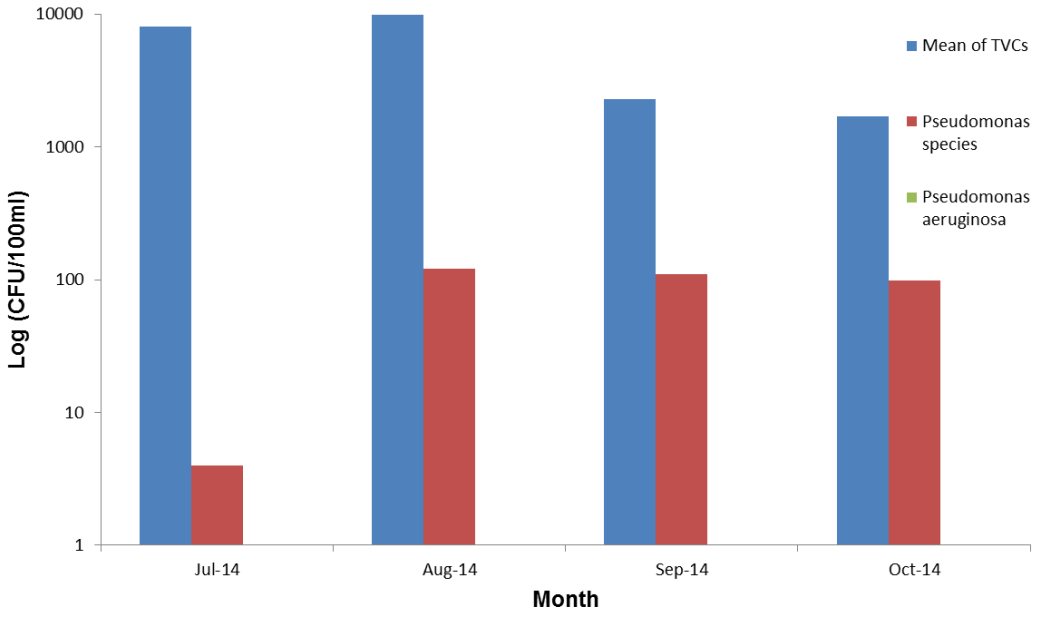

Figure 4.10: TMV fitted tap post-flush sample results for mean of TVCs, *Pseudomonas* species and *P. aeruginosa.*

Interestingly, the conventional tap presents a lower number of bacteria in all of the samples taken to date. In contrast, the tap fitted with the TMV appears to have a problem with *Pseudomonas* contamination. This is confirmed by the pre-flush sample taken in November 2013 where both the TVCs and *Pseudomonas* levels are considerably higher than the respective post-flush sample. The high readings and the detection of *P. aeruginosa* in the sample prompted the cleaning of the TMV in June 2013, but the samples results have shown *Pseudomonas* species were still present and increasing month by month. Possible explanations for the bacteria presence are that the bacteria have been introduced from the cold water with which the hot water is mixed in the TMV, or that the tap has been contaminated by external sources such as surface cleaning equipment. The bacteria colonised the components of the TMV, the pipe between the TMV and the tap and the tap itself, thriving under the temperature conditions and forming biofilms. The TMV mixes the hot and cold water to achieve 45°C, and if the tap is not used for a certain time, the water within the TMV and the tap can lower in temperature, providing favourable conditions for growth, 4-43°C for *Pseudomonas* (Todar, 2012).

These findings support previous research (Pottage et al., 2012, Walker et al., 2014) which was undertaken after the tragedy in Northern Ireland where 4 babies died from *P. aeruginosa* infection between December 2011 and January 2012. Pottage et al. (2012) found that the highest colony counts were associated with the TMV whilst Walker et al. (2014) that it was associated with the flow straightener.

4.2.3 Lowering the water temperature: how much could be saved?

By knowing the shape and size of the building, it is possible to make a conservative estimate that the hot water recirculation comprises about 120 metres of copper pipes. The building schematic of the hot water system indicates that the pipe size distributing hot water is 28 mm and the pipes on the return leg are 15 mm in diameter; hence, it can be assumed that 60 metres of the pipes are 28 mm in size and 60 metres are 15 mm in size.

The values for heat loss from insulated pipes per unit length and per unit temperature difference are taken from CIBSE Guide C (2007). Hence, the heat loss for 60 metres of 15 mm size pipes with 25 mm insulation and 0.070 W/mK stated thermal conductivity is 744 W, and for 60 metres of 28 mm size pipes with the same conditions is 1,032 W. This gives a total of 1.776 KW of heat loss.

The centre is open weekdays from 9 am to 5 pm for a total of 40 hours a week, so for the remaining 128 hours of the week the calorifier uses the energy solely to compensate the heat loss from the pipes. Most certainly, some heat is also lost during opening hours, but it is not considered in this calculations. Therefore, it can be estimated that 11,820 KWh are used yearly to compensate heat loss in this building.

Using the gas readings recorded by the maintenance team at Wendover, it is possible to make an estimate of how much hot water is used each month in the building. During the summer months, the gas consumed by the building is used solely to heat the water serving the taps and showers of the recirculation system; the heating is off and no other devices use gas. Using the summer readings, it can be estimated that 16,080 KWh are used each year to heat non-domestic hot water and that 73% of the total energy is used to compensate for the pipe heat loss.

An estimation of the reduction in energy consumption if the water temperature of the building was decreased to 45°C has been done, and the results are set in Table 4.8.

Month	Energy used (KWh)	Energy saved if water at 45°C (KWh)	Emission Reduction ($kgCO_2$)
August	1545	282	56
September	1136	207	41
Average	**1340**	**245**	**48.5**

Table 4.8: Estimate of the reduction in energy consumption and emission reduction if the temperature was lowered to 45°C.

By reducing the temperature from 55°C to 45°C, the site at Wendover could save 18% of the total energy used in heating water. Moreover, the energy consumption could decrease by 2,940 KWh yearly and the total emission by 582 $kgCO_2$ per annum, generating savings of £132 per year. If the water temperature could be lowered further to 40°C, 27 % of the energy used could be saved and the total emission could be reduced by 871.2 $kgCO_2$/annum, generating savings of £198 per year.

Although the reduction in energy and CO_2 emissions may seem small, further reductions can be achieved by heating the water with green technologies that become more efficient if one demands water at a lower temperature. For example, the hot water system could be isolated from the primary heating system and it could be partly powered by solar panels.

In addition, the condensing boiler in the building could work more efficiently at lower water return temperatures. Condensing boilers are usually more efficient than conventional boilers because they extract additional heat from the waste gases but the dew point is at 55°C, as shown in Figure 4.11. In non-domestic recirculation systems, the return water temperature is within 50-55°C, preventing significant condensation in the heat exchanger. However, if the return water temperature was decreased, the boiler efficiency would increase, further reducing gas consumption and CO_2 emissions.

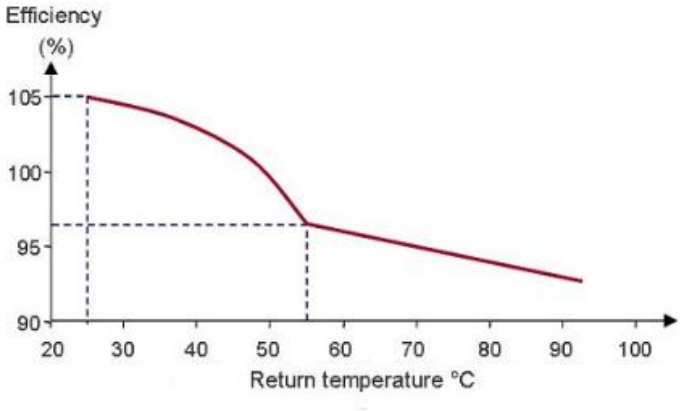

Figure 4.11: Condensing boiler efficiency vs return water temperature (Image adapted from HVAC Learning).

4.2.4 Lowering the water temperature: estimation of savings in DHW systems

DHW systems in hospitals, hotels, leisure centre and schools often comprise central hot water storage with extensive pipework, as discussed in section 2.2 of this thesis. Heat losses for this pipework leads to very poor efficiency, particularly at times of low hot water demand. Reducing the temperature at which the water is circulated would lead to a significant decrease in energy consumption and resultant CO_2 emissions. If new non-domestic buildings are to achieve zero carbon from 2019, the energy demand associated with heating water needs to be addressed. An estimation of this reduction has been made by using energy statistics data for the UK and the results are set out in Table 4.9. The energy (E) required to raise the mass of water (m) with specific heat capacity © by a certain temperature difference T_f-T_i is given by the following equation:

$$E = mc(T_f - T_i) \quad (4.1)$$

Using the equation and the data set out in the column DHW consumption Natural Gas (Department of Energy and Climate Change (DECC), 2012), the mass of water heated from 20 to 60°C can be calculated. Substituting this value into the same equation, the energy required to heat the same amount of water from 20 to 45°C is found. Assuming that 73% of the heat in DHW

system is lost during recirculation, the potential energy savings are identified. The emission reductions are then calculated by using the conversion factor given by the Department for Environment, Food and Rural Affairs (DEFRA) (2007), and the monetary savings by assuming an average cost of 3 p/KWh of natural gas (Department of Energy and Climate Change (DECC), 2013).

End user	DHW consumption Natural Gas (toe)[1]	Energy Savings (GWh)	Emission reduction (tCO_2)	Energy savings (£)
Commercial offices	58,000	184	36,562	£5.5 M
Education	198,000	630	124,814	£18.9 M
Government	73,000	232	46,017	£7 M
Health	111,000	353	69,972	£10.6 M
Hotel and catering	256,000	815	161,376	£24.5 M
Sport and leisure	97,000	308	61,146	£9.3 M
Total	793,000	2,525	499,887	£75.7 M

Table 4.9: Estimated CO_2 emission reduction if DHW is circulated at 45˚C (adapted from reference Department of Energy and Climate Change, 2012).

By reducing the temperature of DHW systems from 60˚C to 45˚C, there could a reduction of 0.63 $MtCO_2$. The energy consumption could be reduced by 2,525 GWh per annum, generating savings of £75.7 million.

For new buildings, the percentage savings would be considerably larger as space heating, cooling and lighting demands are considerably reduced. The energy statement of a planning application for a new hotel examined by the author claims DHW system is responsible for 62% of the energy demand of the building (AE Building Services Consultancy, 2012). However, if the

[1] Tonnes of equivalent oil (toe)

temperature is decreased, the emissions associated with heating water could also be decreased.

4.3 Halsbury and Bragg/ETC complex, Brunel University, London

4.3.1 Materials and Methods

The Halsbury and Bragg/ETC buildings at Brunel University London are supplied by the same water system from two tanks located in the Halsbury building. The larger tank can hold up to 16,800 litres and feeds both the domestic cold water services and the DHW system calorifiers. Another 2400 litre tank was used to feed the domestic hot water system, but it has now been isolated. The pipe work feeding the smaller tank was undersized and when the hot water demand was high, the tank was not filled rapidly enough, causing air to enter the system. Hot water is re-circulated throughout the buildings by a pump located on the secondary return pipe. There are 107 hot water outlets within the buildings and these include basins and laboratory sinks. There are also 3 emergency showers and one emergency eyewash, which are flushed weekly, being rarely used. Routine *Legionella* samples in the hot water system had tested positive for *L. pneumophila* sg. 2-14 for a number of years (Ibbotson, 2011) and in several occasions, *L. pneumophila* sg. 1 was also detected. This suggested that heat alone had not been sufficient to control the pathogens, either because the water temperature was not maintained above 50 ˚C throughout the system, or the bio-load was sufficiently resilient and protected by the condition of the system to overcome the temperature. In addition, a chlorine dioxide unit installed for one year had not been effective in eliminating the bacteria.

An ED device was installed in the hot water recirculation system by contractors. The installation was such that the cold water entering the system and the hot water returning from the recirculation loop were mixed before entering the electrolytic unit. A photograph and a schematic diagram for the installation can be seen in Figure 4.12 and Figure 4.13, respectively.

Figure 4.12: photograph of the device installation in the Halsbury Building, Brunel University.

The installation consists of the following components: (a) standard equipment including control panel, oxidation chamber and flow sensors; (b) electromagnetic flow meter; (c) redox controller; (e) thermocouples.

The equipment was identical to the one installed in Wendover but the potentiometer has been modified to enable voltage variation to 12 V. The electromagnetic flow meter mag-flux A and the transmitter mag-flux M1 supplied by MECON Flow Control System were used to measure the rate of cold water entering the hot water system. The flow meter withstands maximum temperature of 90°C, maximum pressure of 10 bar, and a range of 0-10 m^3/h. The Redox controller, model BR93 with a range ±1,000 mV, supplied by LHT Electronics Limited was installed to measure the reduction potential of the water and hence to determine the quality of the water. The temperature was measured at the exit of one of the calorifiers and at the outlet of the ED device with mineral insulated type-K thermocouples with miniature flat pin plug sourced from TC Direct (3.0 mm diameter x 100 mm long). The voltage and current was measured at the connections of the cell.

The data were collected using a TC-08 Thermocouple Pico data logger (manufactured by Pico technology) and viewed and analysed with the Picolog data acquisition software. The USB TC-08 Single-Channel Terminal Boards (manufactured by Pico technology) were used to adapt the data logger to measure 4-20 mA current signal outputs from the instrumentation.

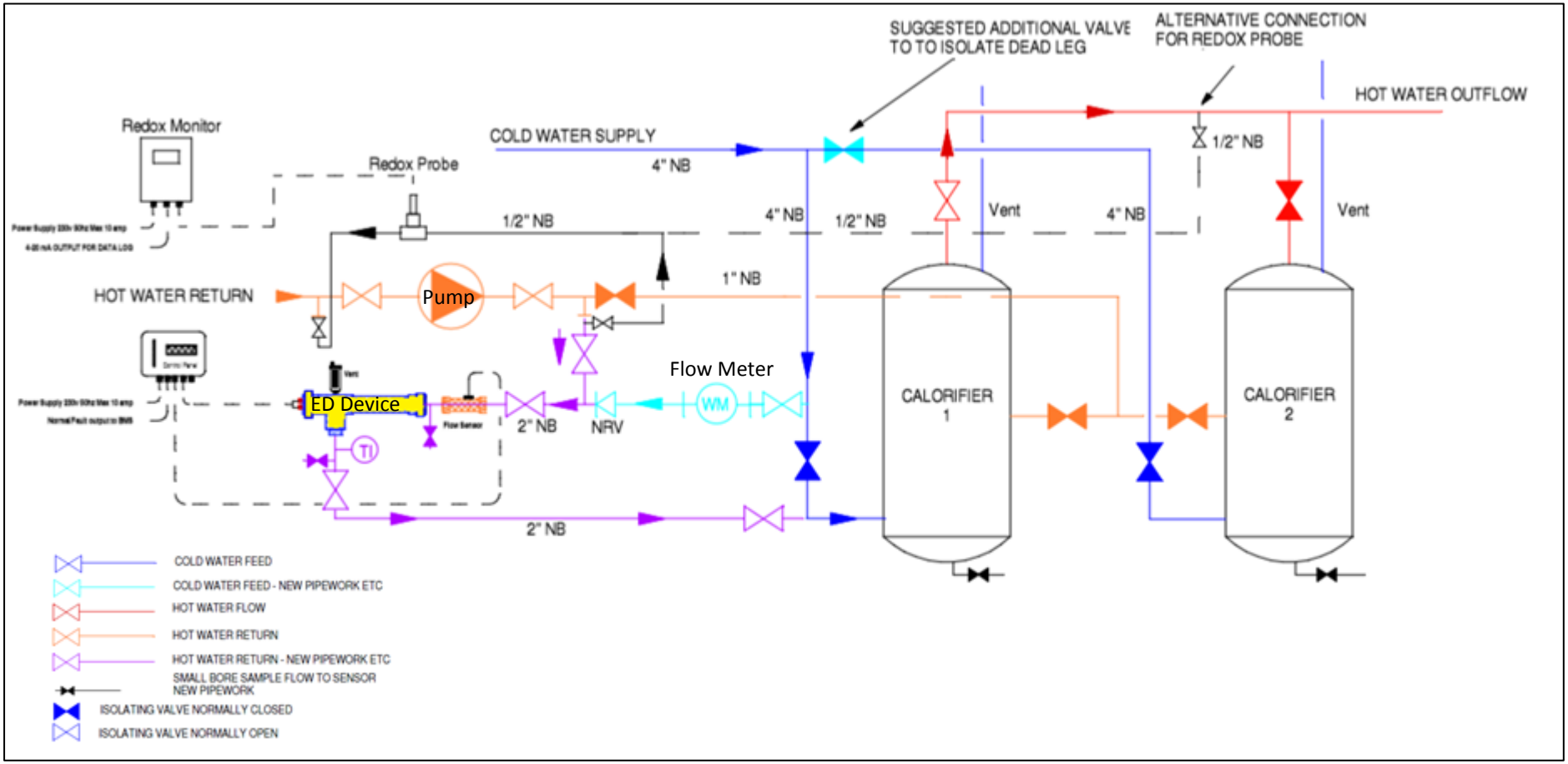

Figure 4.13: Schematic diagram of the ED device installation in the hot water system of the Halsbury/Braggs/ETC (made by ESG Ltd).

The device was installed by contractors between March 2012 and May 2012 and during the installation one of the calorifiers was isolated having identified that the building hot water demand was lower than initially believed. Valves were positioned ensuring dead-legs were not created. Sampling started in February 2012, to obtain a baseline period with the Chlorine dioxide unit, given the changes in the pipework. The ED device was switched on in June 2012 and turned off in August 2012 when the Estates at Brunel decided to replace the 30 years old calorifiers. The results of this period are not discussed in this chapter because the calorifiers and the recirculation pump were often out of service.

The new calorifiers F21 Modul Plus have a capacity of 230 litres each, peak flow of 0.675 l/s and each unit is set to deliver 1,750 L/hr of hot water. After the installation of the new calorifiers, starting in October 2012, post-flush samples were taken for three months before turning on the ED device. In January 2013, the ED device was turned on, and weekly 1 litre post-flush samples were taken from two location points, the calorifier and the last tap in the system in the Braggs/ETC building. The samples were taken by the water treatment company in charge of all the sampling at Brunel University and analysed for *Legionella* species, *Pseudomonas* species and Total Viable counts. The author went along to take the samples, monitored the device, maintained and cleaned the electrodes. In October 2013 the water treatment company in charge of the samples was changed, and the Estates team decided not to analyse for *Pseudomonas* and TVCs any further. To begin with, the device was set at 8 V giving current densities ranging from 11 to 12.5 mA/cm^2, but in July 2013 the voltage was decreased to 6 V, with current densities between 9 and 11.3 mA/cm^2.

In November 2013, the device was turned off and removed from the system, as more maintenance work was carried out. Sampling of the hot water system continued and in March 2014 a chlorine dioxide unit was installed to control *Legionell*a. In this chapter, we report the results from January 2013 to May 2014, and Table 4.10 summarises the important dates of the monitoring period.

Date	
22nd of October 2012	Sampling started
15th January 2013	**ED device ON at 8V**
13th to 22nd of March 2013	Pump not working
18th July 2013	Voltage decreased to 6V
29th July 2013	Pasteurisation of system
5th November 2013	Started pasteurisation every Tuesday
30th November 2013	**ED device OFF**
10th March 2014	New Chlorine Dioxide Unit ON
15th March 2014	Hyperchlorination with Chlorine dioxide

Table 4.10: Table summarising the important dates of the monitoring period.

4.3.2 Results and Discussion

4.3.2.1 Bacteria levels prior to ED

Monitoring of the hot water system was started three months before the ED device was turned on, as soon as the new calorifiers were commissioned. The results for *Legionella* species can be seen in Figure 4.14 whilst the results for *Pseudomonas* species and TVCs are shown in Figure 4.15.

The hot water return temperature recorded at the outlet of the ED device with the old calorifiers in use was on average 47°C±1˚C, but after the installation of the new calorifiers it increased to between 53±1˚C. The author ensured that the temperatures analysed were the ones recorded during the night, when the water at the outlet of the ED device was not blended with the cold water because the building is not in use.

As the overall temperature of the water system was increased by the more efficient calorifiers, a decrease in bacteria numbers was expected. The results in Figure 4.14 confirm that there was an initial decline in the number

of *Legionella* species in the hot water, but within two months the system appeared to be re-contaminated. This is in agreement with the studies discussed in the literature (Steinert et al., 1998, Mouchtouri et al., 2007, Farhat et al., 2010), reporting that in systems subjected to thermal disinfection, *Legionella* was not eradicated. The results also suggest that the bio-load was sufficiently resilient or protected by the condition of the system to overcome the temperature.

The results presented in Figure 4.15 show that *Pseudomonas* spp. are also present in the system and that the level of TVCs is relatively high but within control limits.

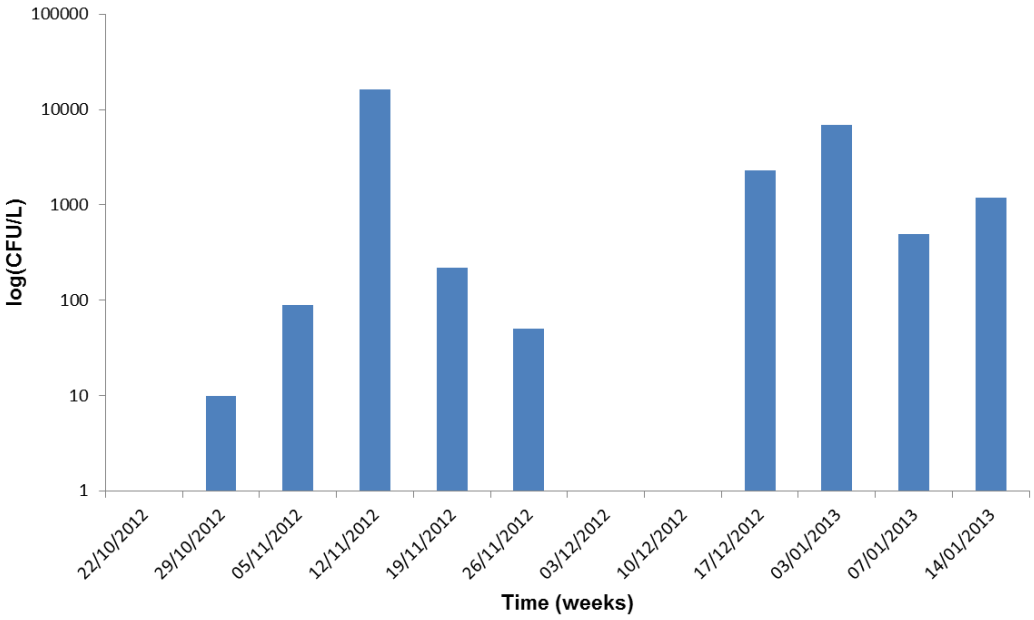

Figure 4.14: Average of post-flush sample results for *L. pneumophila* sg. 2-14 during the baseline period October 2012 to January 2013 with ED device OFF.

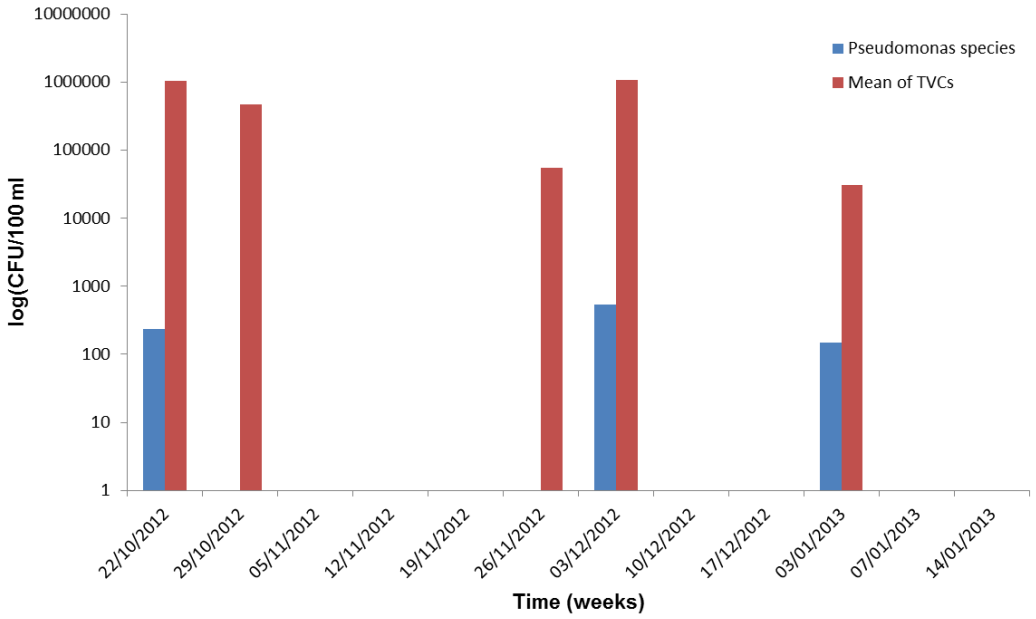

Figure 4.15: Average of post-flush sample results for *Pseudomonas* species and TVCs during the baseline period October 2012 and January 2013 with ED device OFF.

4.3.2.2 Monitoring of ED device performance

The ED device was turned on the 15th of January 2013 and the results for *Legionella* spp., *Pseudomonas* spp. and TVCs can be seen in Figure 4.16 and Figure 4.17, respectively. Given the findings of the flask experiment and the consistent problem with *Legionella*, it was initially decided to apply voltage in the region of 8 V. Due to scaling of the electrodes which will be discussed later in this Chapter, the voltage was decreased to 6 V in July 2013.

Figure 4.16 presents the sample results for *Legionella* for the monitoring period when the device was turned on. With 8 V applied, in 5 occasions the readings for *Legionella* were negative in all of the samples but overall, a noticeable decrease in the level of *Legionella* was not observed. Although the findings were rather disappointing, it could not be concluded that the device was not efficacious for a number of reasons. To begin with, any water treatment needs time to achieve significant reduction in the bacteria numbers and it is clear that 11 months was not enough for this particular site. As mentioned in the literature review, Zhang et al. (2007) found that it took 30

months to reduce *Legionella* levels with a chlorine dioxide unit.

Secondly, continuous operation of the device is critical to the successful disinfection of the water, as it was observed in the Wendover site, where pathogens were detected in both the times the device was off. Not only did the recirculation pump fail a number of times, but the device was also turned off on a few occasions without consulting the author. Between the 6th and the 7th of February, the 13th and the 22nd of March and the 27th of September and the 1st of October there was no recirculation due to pump failure (indicated in Figure 4.16 by arrows), and lapses in the disinfectants might have allowed bacteria to proliferate. An initial decrease in *Legionella* levels was observed as soon as the voltage was decreased, but a significant increase ($p<0.01$; t-test) of 1-log was noticed, following the pump failure in September. During this time the water was not circulated for a week, allowing bacteria to proliferate.

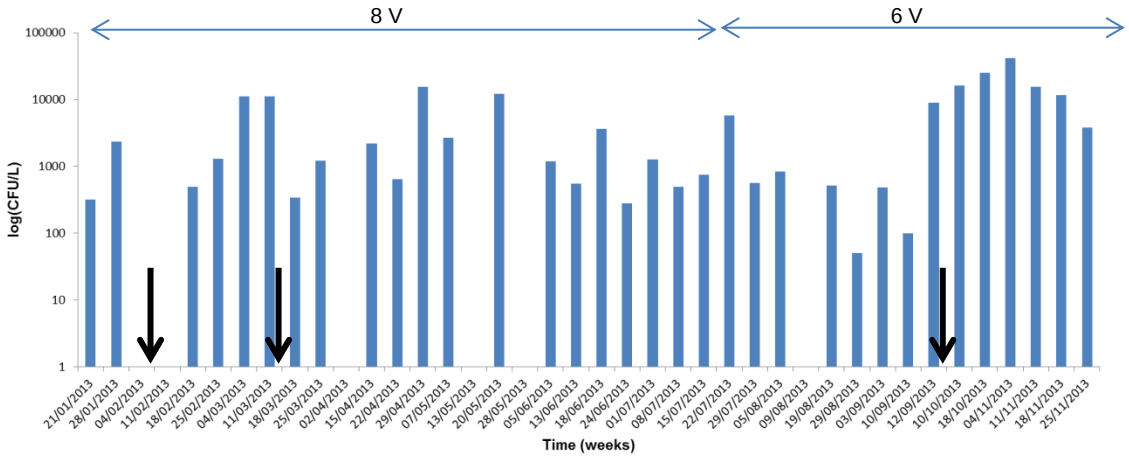

Figure 4.16: Average of post-flush sample results for *Legionella* species during the monitoring period January 2013 to November 2013 with ED device ON. Arrows indicate period when the recirculation pump had failed.

Another possible explanation for the inefficacy of the device may be the detrimental effect of the scale deposits to the current efficiency of the cell. The discontinuous operation of the device for de-scaling is also unfavourable because it causes a lapse in the production of disinfectants which may cause

the bacteria to grow.

With the ED device in operation, *Pseudomonas* bacteria were never detected and TVCs readings were only positive in two occasions; the results are presented in Figure 4.17. *Pseudomonas* bacteria were detected at least once every month of sampling and while these findings should be interpreted with caution, given that sampling for TVCs and *Pseudomonas* was interrupted in September 2013, they suggest that the ED device was effective. Moreover, the contamination of *Legionella* could have been such that more time was needed to eradicate it from the system. Having continued sampling when the device was turned off in November 2013, might have enabled a firm conclusion.

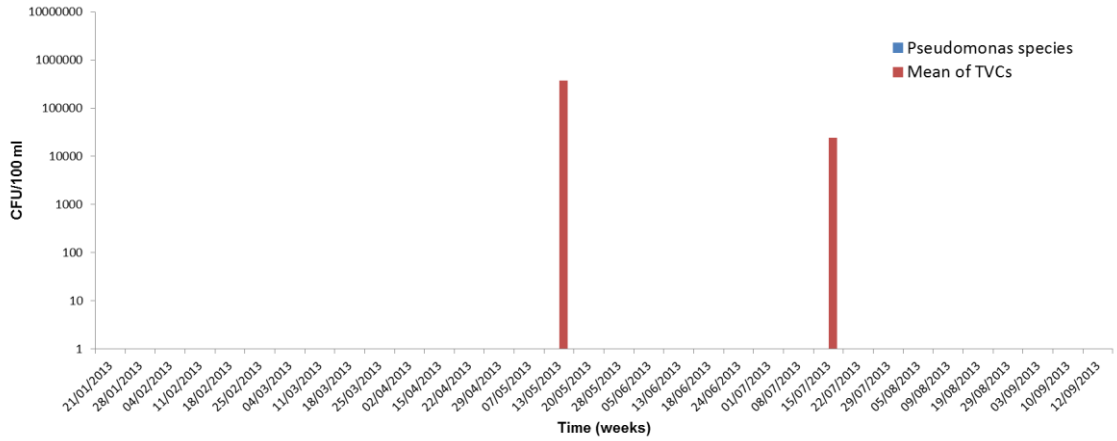

Figure 4.17: Average of post-flush sample results for *Pseudomonas* species and TVCs with ED device ON.

4.3.2.3 Scale deposits

Finally, it was also found that, although polarity reversal was in place every 10 minutes, scale accumulated on the cathode. The increasing thickness of the deposits on the cathode caused the electric current as well as the flow of water through the cell to diminish, inhibiting the performance of the device. A photograph of the scaled electrodes can be seen in Figure 4.18, Figure 4.19 whilst shows the comparison of the 20 minutes electrode current cycle at different times. The graph clearly shows that within 3 weeks, the current dropped by 20%, rendering de-scaling of the electrodes necessary.

Figure 4.18: Photograph of the scaled electrodes after use at 8 V.

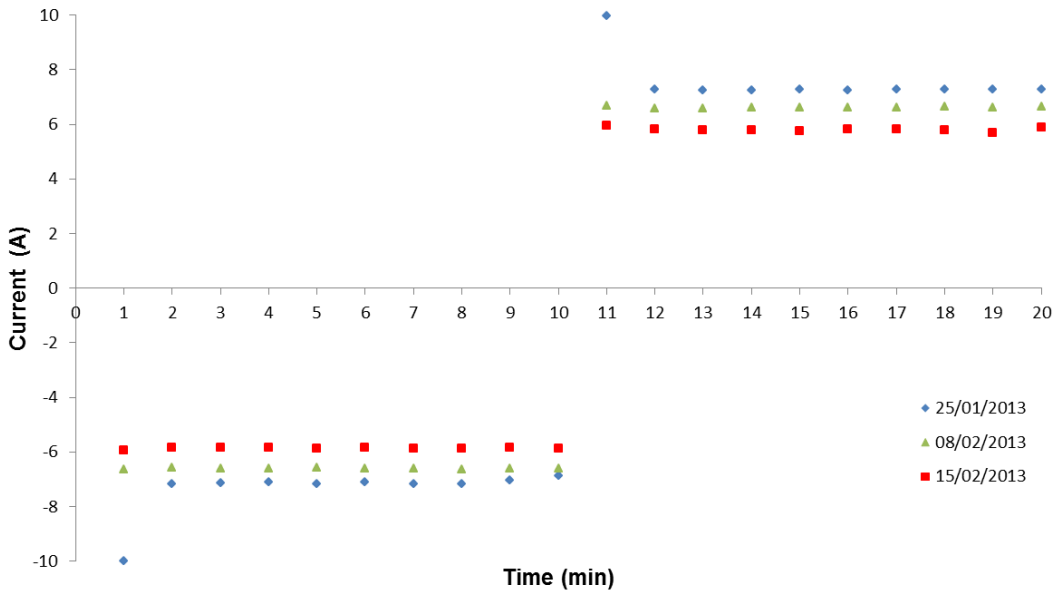

Figure 4.19: Recording of the 20 minutes cycle taken weekly from the 25/01/2013.

During this study, it was also observed that scaling-up of the electrodes occurred more rapidly each time, prompting analysis of the electrode surface with Scanning Electron Microscopy (SEM). This was carried out at the Experimental Techniques Centre (ETC) at Brunel University. Figure 4.20 is

one of the images obtained after routine cleaning of the electrodes, clearly showing the presence of scale on the electrode surface. Although the operator might believe that the scale deposits have been removed, these crystals not visible to the naked eye, initiate additional nucleation and crystal growth is accelerated. So, from May 2013, it became necessary to de-scale the electrodes every three days.

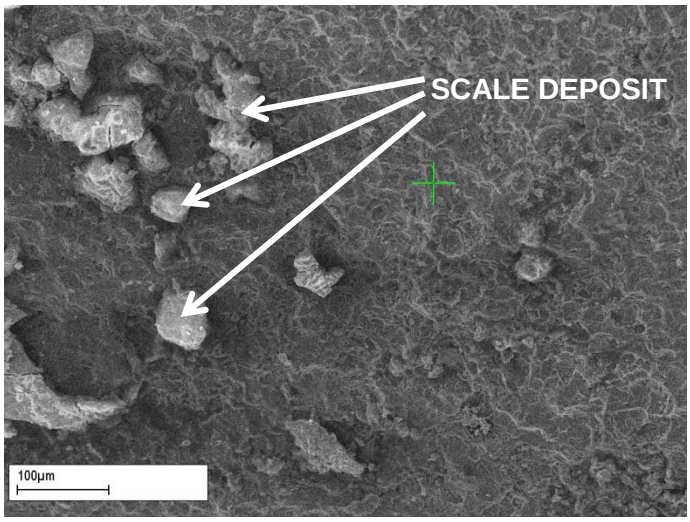

Figure 4.20: Image obtained with the Scanning Electron Microscopy of the electrodes after de-scaling procedure.

4.3.2.4 Monitoring of the chlorine dioxide unit performance

Although the maintenance team decided to remove the ED device in November 2013, they continued to provide the results of the *Legionella* sampling to date. Figure 4.21 presents the results for *Legionella* in January and February 2013 with a thermal disinfection procedure taking place every Tuesday. In spite of the thermal disinfection, the counts of *Legionella* have significantly increased ($p<0.01$; t-test) an order of magnitude compared to the same time the previous year with ED (in Figure 4.16) , with 10,000 CFU/L averages being consistently recorded each week.

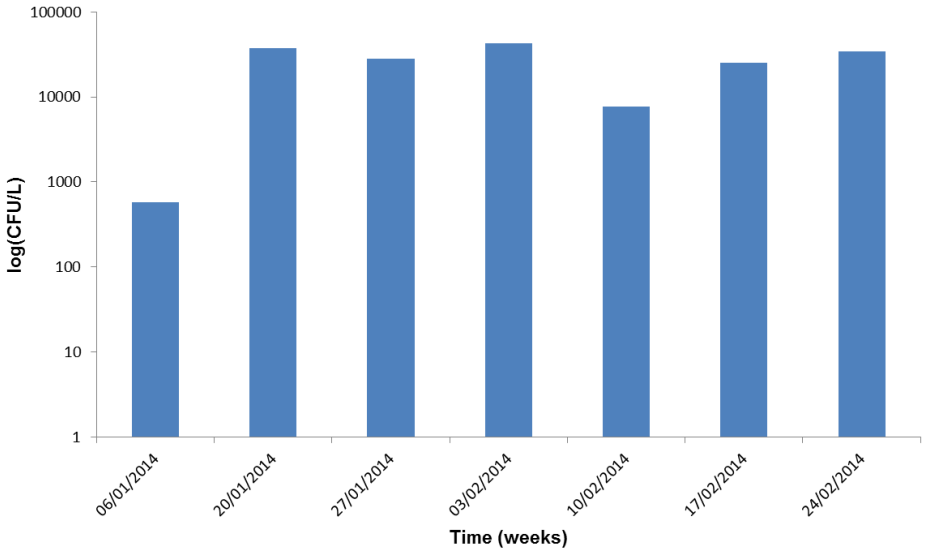

Figure 4.21: Averages of post-flush sample results for *Legionella* with thermal disinfection in place (January and February 2014).

In March 2014, a chlorine dioxide unit was installed on the main water pipework and Figure 4.22 shows the results for *Legionella* readings to date. A marked decrease in bacteria levels was not observed but overall, with time, the number of bacteria have significantly decreased ($p<0.01$; t-test) 1-log compared to when only temperature control was in place (in Figure 4.21). The *Legionella* counts in Figure 4.21 trend appears similar to the readings obtained when the ED device was on (see Figure 4.16). These finding confirmed that the system is highly contaminated with *Legionella* bacteria, and that any disinfection method would need time to achieve substantial reduction in bacteria levels.

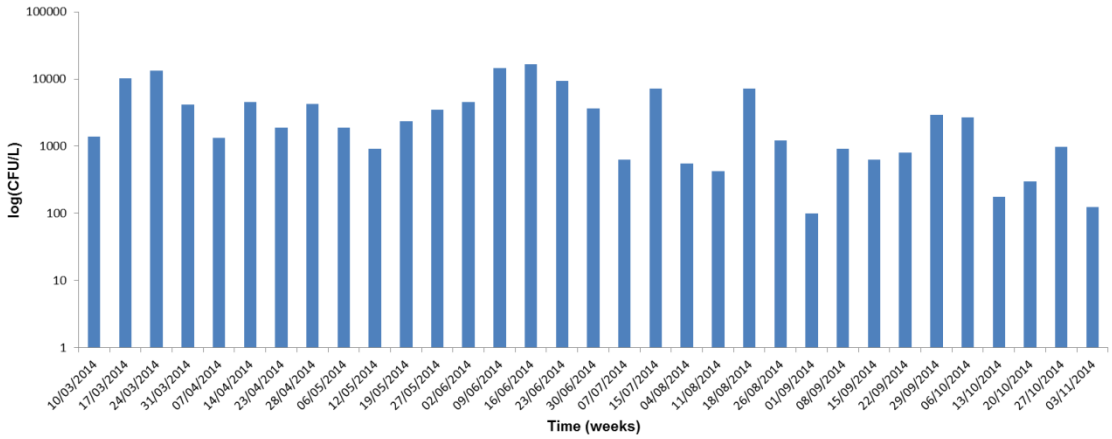

Figure 4.22: Averages of post-flush sample results for *Legionella* since the installation of the chlorine dioxide unit (March to November 2014).

4.3.3 Operational issues

During the monitoring period, it was possible to identify some issues with the design of the electrode package. Disassembling the electrode package to empty the scale trapped within the electrode gaps, was found to be very difficult and time-consuming. Therefore, the electrodes could be designed to ease assembly and disassembly. Moreover, if the operator does not pay attention when fastening the electrode nuts indicated by red arrows in Figure 4.23, the electrodes could be twisted. This can cause the electrodes to touch, hence shorting the circuit and stopping the electrolysis taking place. Again, the design could be adapted to stop this from happening. It would also be beneficial to add an alarm informing the maintenance team if the device is not in operation, so that they can take remedial actions.

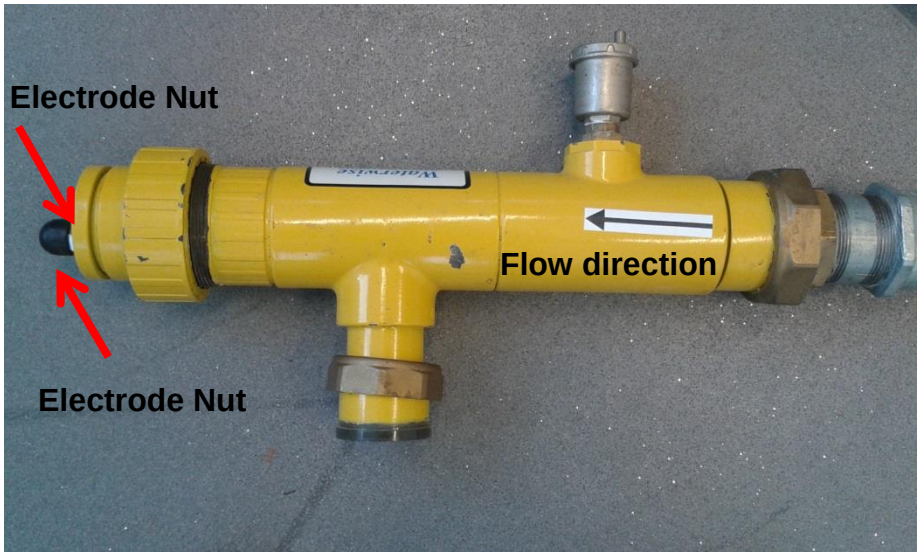

Figure 4.23: Close-up photograph of the ED device.

The cylindrical electrodes are also costly to manufacture and by using flat plates, the cost could be reduced to one tenth (as discussed with the electrode manufacturer Metakem GmbH, Germany). Moreover, the use of mesh electrodes instead of flat sheet would not only increase the geometrical area and current, but it would also enhance mixing of the water in the device.

During these investigations, maintenance of the electrodes was carried out on a regular basis and during the routine, the ED device was not in operation, resulting in the water system to be vulnerable for up to two days. It is suggested that the unit is sold with two electrode packages so that when de-scaling is necessary, the electrode package can be replaced, without turning off the device for a prolonged amount of time.

4.4 Conclusions

These case studies are the first to document the long-term use of electrochemical disinfection in the hot water recirculation systems of two operating buildings. Although the ED device was efficacious in controlling pathogens in the Wendover MS centre, the apparent failure to control *Legionella* in the Halsbury/Braggs/ETC complex was more likely due to the inadequate operation of the recirculation pump during the monitoring period.

Despite the DHW system in the new health-care building in Wendover maintained the water circulation at around 60˚C, high concentrations of microorganisms and in particular *Pseudomonas* were detected. Although *Legionella* species are considered a common inhabitant of hot water systems (Sabria and Yu, 2002), they were only detected once in the samples analysed in the monitoring period so far. This investigation has highlighted that *Pseudomonas* is an issue that if not addressed could lead to the formation of biofilm where *Legionella* species could eventually thrive. 15 months after activating the ED device, *Pseudomonas* species counts were reduced 3 log in both pre-and post-flush samples, whilst TVCs were reduced 2 log in pre-flush samples and 4-log in post-flush samples. Although the pre-flush samples counts have remained higher than the post-flush, they decreased over the monitoring period, suggesting that if the water in the recirculation loop is controlled, the pathogens reaching the taps are fewer. In spite of the reduction in bacteria counts, monitoring of the building should continue to evaluate the use of electrochemical disinfection long term, and to determine the electrodes service life.

Although the findings in the Halsbury/Braggs/ETC complex were inconclusive, this installation enabled the identification of issues with the ED device design. The most crucial issue is that in areas of hard water, the electrodes need more maintenance because of the scale build-up. Our monitoring shows that the current density decreases with the build-up of scale, so methods of scale prevention should be investigated. The monitoring also revealed that the average hot water demand of the building was 0.8 m^3/ day, suggesting that most of the taps in the building are under-used and that they may be causing the problem with *Legionella*. This confirms that the system was grossly overestimated having been sized for peak demand, with the result that for most of the time the system is oversized and the draw off rates are only a small fraction of design capacity.

Overall, the case studies indicate that ED has potential for use in the disinfection of hot water systems and given these results, we recommend the long-term monitoring of ED devices in other buildings. The long-term

assessment of the device in operating buildings and the careful knowledge of the effect of geometrical parameters may lead to the development of cells adaptable to different environmental or operational conditions. Such innovations would cater for variations in water quality, changes in water use for both buildings and disinfection processes and different building sizes.

The field installations have also highlighted some operational issues that need to be resolved. The electrode package should be re-designed for ease of assembly and disassembly, the design of the connections should be re-evaluated to prevent twisting of the electrodes and an alarm could be added to inform the operator that a remedial action is needed, such as descaling. Moreover, the use of flat plates would decrease the device manufacturing costs.

Chapter 5 : Laboratory evaluation of the efficacy of electrochemical disinfection at reduced hot water temperatures

‘The water was not fit to drink. To make it palatable, we had to add whiskey. By diligent effort, I learned to like it.’

Winston Churchill

5.1 Introduction

It is clear that there could be considerable potential for the use of ED devices in hot water systems. There are some 2 million hot water installations in non-domestic buildings in the UK alone where an electrolytic device might offer effective water hygiene protection at reduced energy consumption and carbon emissions, together with simpler and cheaper maintenance.

The device has been used both in the flask experiment with water at temperatures of 30 °C and in the field at temperatures of 60 °C, and much useful data has been gathered relating to the design of the electrodes. However, the substantial energy savings identified in Chapter 4 can be achieved by decreasing the hot water temperatures in non-domestic buildings to 40-45°C, so another objective of this thesis is to evaluate the efficacy of the prototype device at those temperatures. For this purpose, an experimental rig simulating a scaled-down hot water system similar to the ones found in large buildings was developed. The experimental rig simulates the irregularities in temperature, water velocities and water usage characterising large buildings, whilst enabling repeatability of results, not always achievable in field studies. In addition, the rig has been fitted with purposely-built section where biofilm can be grown on microscope slides for observation.

This chapter presents a detailed description of the experimental facility and discusses the results of the experiments aimed at evaluating the performance of the ED device on *L. pneumophila* sg. 1 at lower temperatures and its efficacy on preventing biofilm growth.

5.2 Materials and methods

5.2.1 Experimental apparatus

Figure 5.1 and Figure 5.2 show a photograph and a schematic of the experimental apparatus designed and built by the author at Brunel University. The experimental facility consisted of the following systems: (a)

tank system and (b) scaled-down hot water recirculation system with 40 m of pipes. Unless stated otherwise, all pipes in the system are made of 15mm diameter copper Yorkex Plain copper (EN1057-R250), the material typically used in water lines. All valves and connections were sourced from RS components UK.

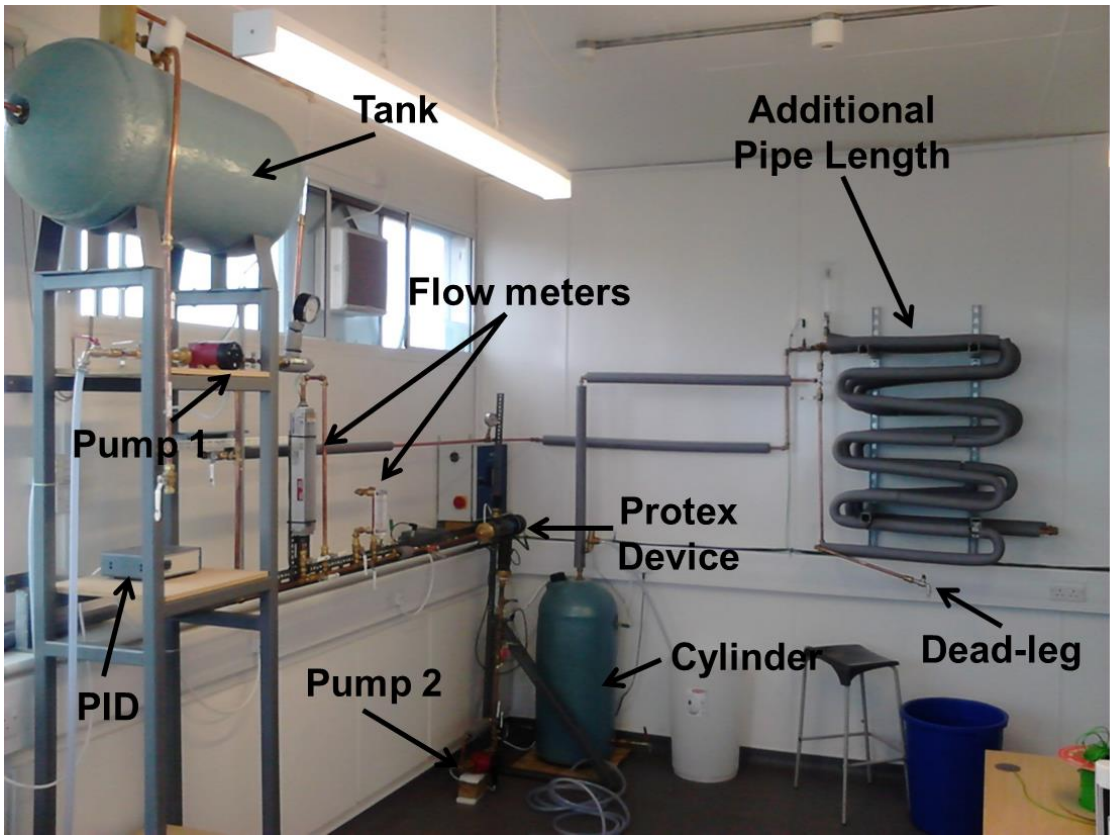

Figure 5.1: Photograph of the test rig facilities at Brunel University.

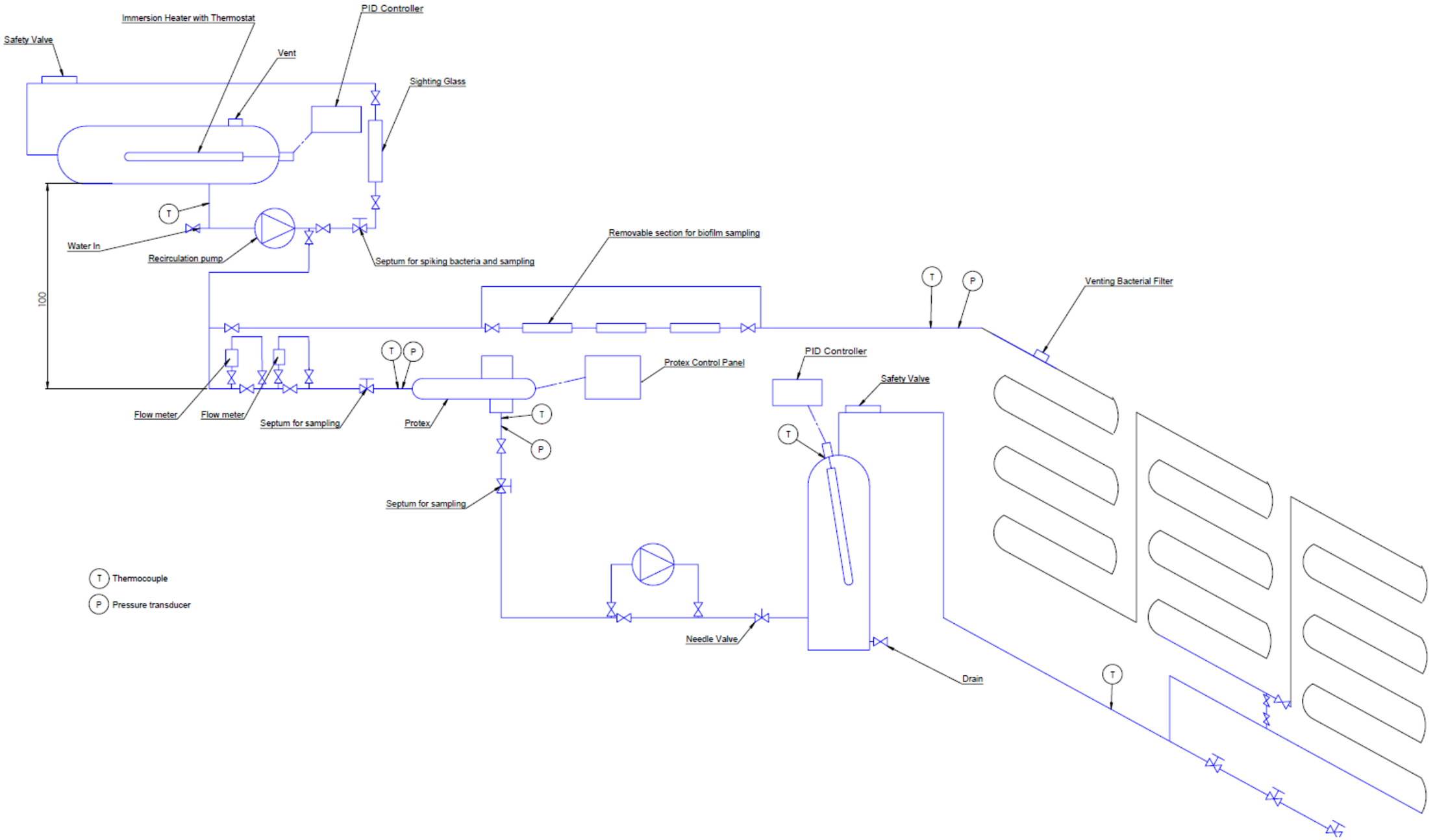

Figure 5.2: Schematic diagram of the experimental rig.

The rig was designed to achieve a maximum temperature of 90°C to enable disinfection of the contaminated water before disposal into the drain. The operational flow velocity ranged between 0 and 1 m/s because the flow velocity in the problematic Halsbury installation was found to be within 0.18 and 0.74 m/s. In addition the British Standards suggest the flow velocity in pipes should not exceed 3 m/s to avoid noise and wear of pipes (BSI, 2009). The Reynolds numbers were between 2,000 and 26,000, enabling experimentation with both laminar and turbulent flows.

5.2.2 Tank system

The tank system comprises an (a) elevated tank fitted with a (b) pump. A photograph of this system is shown in Figure 5.3. The tank system could be isolated from the rest of the rig and it serves the purpose of filling the scaled-down hot water recirculation system. Initially, the tank was filled with water and once the required temperature was reached, the operator could introduce the bacteria through a septum. The pump enabled the bacteria to be mixed through the whole volume of water before the water was released into the hot water recirculation system. The elevated tank was a direct copper cylinder foamed for insulation, and it was held by a purposely built frame. It had a total volume of 98 litres and it was filled with water by a hose (Codeflex 2000 PVC) connected to the water mains. Inside the tank, there was a 3 kW, 11 inches titanium immersion heater (Heatrae Sadia model 95:110:400R) controlled by a Proportional-Integral-Derivative (PID) controller built with components bought at RS Components UK (Cat. N. 208-2739, Cat. N. 321-2931, Cat. N. 455-9742, Cat. N. 455-9764, Cat. N. 222-020). It was also fitted with a DN10 enclosed discharge safety relief valve (Seetru Part N. 67010A1273) set to a relieving pressure of 3 bar and a vent. The tank system was also equipped with a sighting glass to observe the liquid level in the tank, and a mineral insulated type-K thermocouple with miniature flat pin plug sourced from TC Direct (3.0 mm diameter x 100 mm long) used to measure the temperature of the water, and thus to send a signal to the PID controller to increase or decrease the temperature to the preset value. The pump ALPHA 2L (Model N. 950475M) manufactured by Grundfos with a flow

rate of up to 3 m^3/h and maximum head of 6 m, was fitted below the tank to circulate the water.

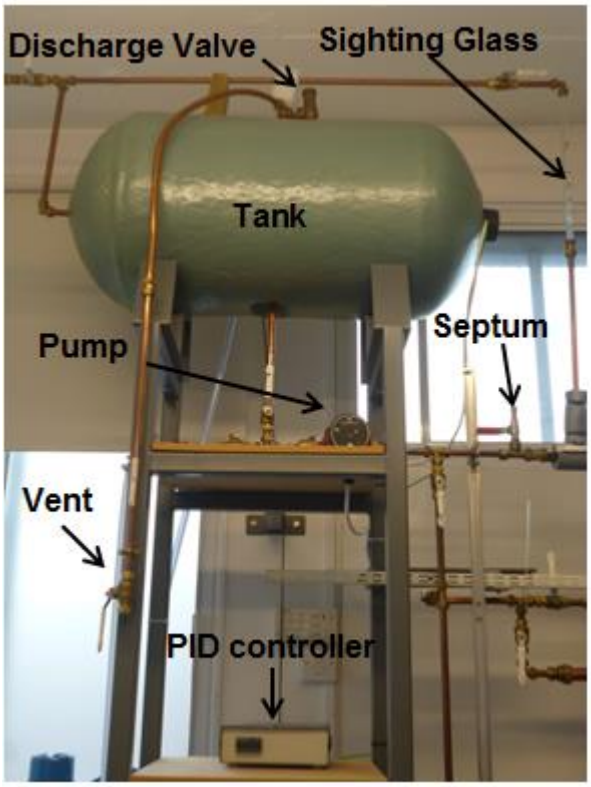

Figure 5.3: Photograph of the tank system.

The tank system included a septum through which bacteria can be added to the water by means of a syringe. The septum was purposely built with a brass DN8 ball valve (CW617N made by Integplumbing) and a stainless steel compression fitting straight coupler (M12MSC1/2N-316 made by Parker) fitted with a 12.5 mm solid disc (Thermogreen LB-1 Septa 20661 made by Supelco). A photograph of the septum is shown in Figure 5.4.

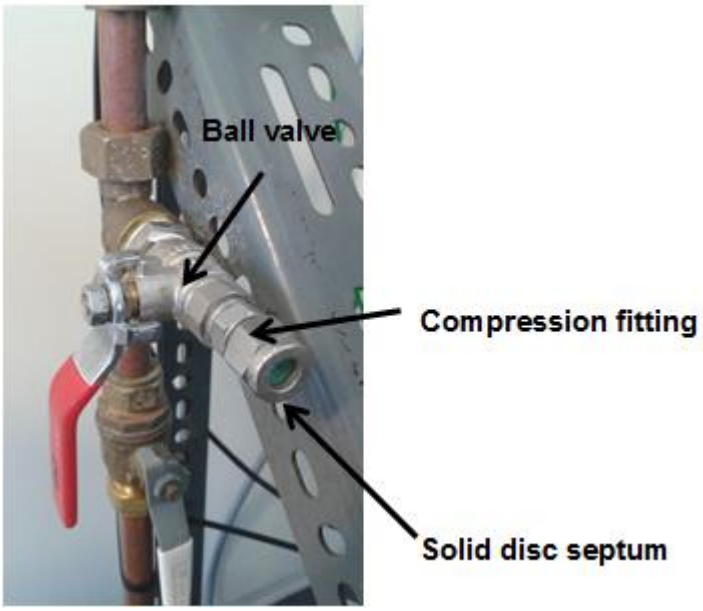

Figure 5.4: Photograph of the purpose-built septum.

5.2.3 Scaled-down hot water recirculation system

The scaled-down hot water system consisted of (a) two flow meters; (b) the ED device; (c) the recirculation pump; (d) the hot water cylinder; and (e) some additional pipe length. Two models of rotometer flow meters were used to measure the flow rate. The Brooks acrylic flow meter (Model 2520 A 4L53 N VT) was capable of measuring flow within the range of 0 and 1.5 l/m and the OMEGA flowmeter (Model FL-1504A) within 0 and 23.77 l/m. Isolating valves were fitted before and after the rotometers in order to isolate one while using the other for measurements. After leaving the flow meter, the water passed through an ED device identical to the ones installed in the buildings described in the previous chapter. A type-K thermocouple with miniature flat pin plug sourced from TC Direct (3.0 mm diameter x 100 mm long) and a pressure transducer (Gems sensors and controls 3100 with max pressure of 10 bar) were installed before and after the ED device to confirm the water temperature and pressure at the inlet and outlet. Two septums were also fitted at the ED device inlet and outlet to enable sampling of the water before and after disinfection. Another ALPHA 2L pump with the same specification of the tank system pump was used to circulate the water. The flow rate was controlled by a stainless steel needle valve manufactured by

Swagelok (Model SS-18RS8) located downstream from the pump. After the needle valve, the water entered a 76 litre vertical direct copper cylinder foamed for insulation. The cylinder was fitted with a 3 kW 27 inch titanium immersion heater (Heatrae Sadia model 95:110:401R) controlled by another PID controller identical to the one described previously. It was also equipped with a thermocouple, a DN10 enclosed discharge safety relief valve (Seetru Part N. 67010A1273) set to 3 bar, and a drain to empty the whole system when the experiment was complete. After exiting the cylinder the water was pumped through a purposely built serpentine coil pipe assembly, designed to add length to the pipe system, hence simulating floors in a building. A pipe bender was used by the author to make the coil with 15 mm diameter Kuterlon coil range BS EN1057-Annealed (R220) copper pipe. Part of the coil assembly could be isolated from the rest of the pipe system to simulate the variation in water demand in a building, where some taps are not used for long periods of time. For the same purpose, the coil assembly was also fitted with a 50 cm long dead leg. The flow temperature at the exit of the coil assembly was measured with a type-K thermocouple. The vent fitted with a 0.2 μm bacteria filter (Isovent PTFE venting filter Part N. FH0555) located in the highest point of the recirculation system enabled the safe replenishment of the system without any remaining bacteria escaping in the eventual aerosol produced. The remaining pipe was fitted with 3 purposely-built biofilm sampling sections shown in the photograph in Figure 5.5(a). Each section contained two glass microscope shown in Figure 5.5 (b) slides where the biofilm established given optimal conditions.

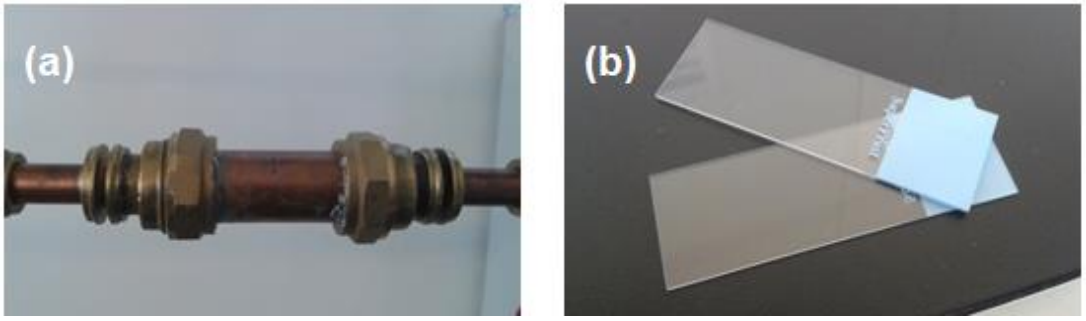

Figure 5.5: Photograph of the biofilm sampling section and photograph of the microscope slides contained in the section.

The data were collected using a TC-08 Thermocouple Pico data logger and viewed and analysed with the Picolog data acquisition software. The USB TC-08 Single-Channel Terminal Boards were used to adapt the data logger to measure 4-20 mA current signal outputs from the pressure transducers.

5.2.4 Culture preparation

The bacteria used in the experiments were *L. pneumophila* (serogroup 1) NCTC12821 purchased in the form of lenticules from the National Collection of Type Culture (NCTC12821); a culture collection of PHE. Each lenticule disc consisted of a known quantity of bacteria contained in a solid water-soluble matrix stored at -20°C ± 5°C. Lenticules were allowed to reach room temperature for 10 minutes before use, after which they were rehydrated in a 1 ml volume of Maximum Recovery Diluent (Oxoid CM0733), allowed to stand for 15 minutes and shaken vigorously for 5 minutes. Each lenticule should contain 277,500 CFU. Masks Pro 3 (FFP3 Masks CE certified FFP3 respirator face) were worn during each experiment.

All of the 60 ml experimental water samples were taken with disposable syringes and then transferred into a sterile glass vial with screw cap, which then was mixed with a vortex-mixer. 0.05 ml and 0.1 ml of each sample were plated in triplicates directly on ready-poured BCYE agar plates (Oxoid PO5072A). The plates were placed in a supporting rack inside a sealable polythene bag with a dampened piece of absorbent paper to provide a moist environment. The number of viable cells was determined by counting the colonies after 72 hours incubation time at 37 °C.

The bacteria growing on the BCYE were stained with safranin to enhance visualisation under the microscope and to ensure only *Legionella* was growing on the media. Photographs of the plated *Legionella* before electrochemical disinfection and of the stained bacteria are shown in Figure 5.6 and Figure 5.7, respectively.

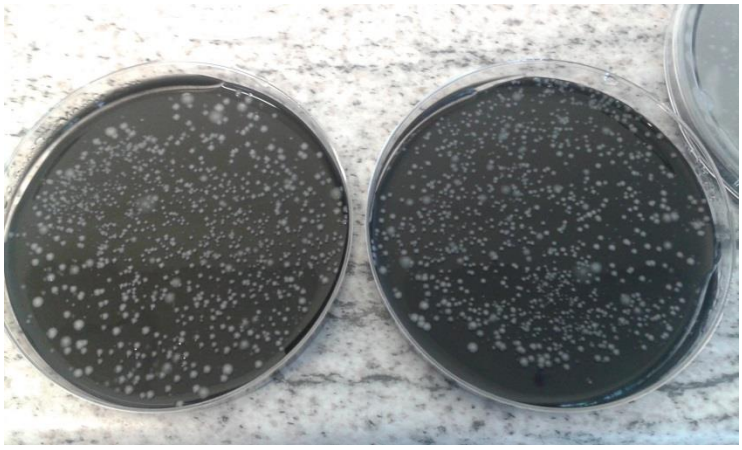

Figure 5.6: Photograph of a plated sample of *L. pneumophila* sg. 1 before treatment.

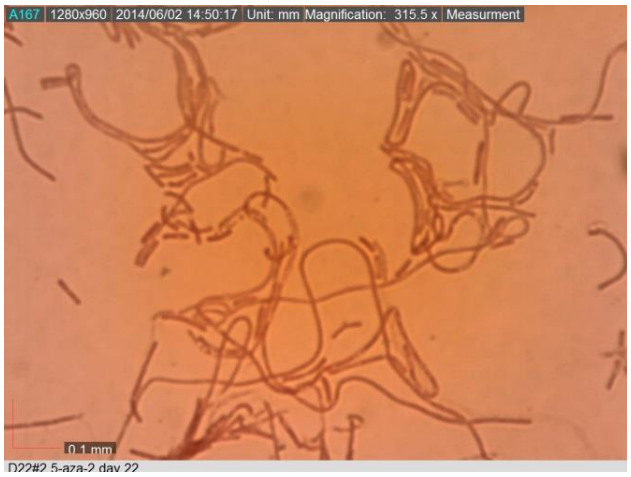

Figure 5.7: *L. pneumophila* sg. 1 stained with safranin.

5.2.5 Electrochemical device

The device was identical to the ones installed in the buildings with the electrode package consisting of a 12 mm rod surrounded by concentric 19mm, 25 mm and 30 mm outside diameter cylindrical electrodes, 200 mm in length and with thickness of 1 mm, see Figure 4.4. The electrodes were made of platinised titanium with platinum thickness between 2-5 μm (Metakem GmbH, Germany). The electrode projected area was 351.68 cm^2 and the oxidation chamber volume 0.726 litres. The device was connected to a dc power supply and 6 V applied to give current densities ranging from 11.4 to 16 mA/cm^2, depending on the temperature of the water. The distance between the electrodes was maintained within 2 and 2.5 mm depending on

manufacturing tolerances.

Free Chlorine and Total Chlorine in the water were measured with a photometer (Palintest 7100) and tablet reagents (DPD 1 and 3). Control measurements for disinfectants were performed prior to treatment during each experiment.

5.2.6 Types of experiments

The experimental rig was used to carry out two sets of experiments which for ease of understanding were named:

- Once-through experiments and
- Biofilm experiments (Experiment 1 and 2)

The purpose of the once-through experiment was to define the concentration of bacteria inactivated in the device when the water was passed through once, at set flow rates and temperatures. In the biofilm experiment, water contaminated with *L. pneumophila* sg. 1 was circulated at temperature of 37°C for two months until biofilm was detected on the microscope slides. The ED device was then turned on and its effect on biofilm evaluated.

5.2.6.1 Once-through experiment

As mentioned in the introduction, one objective of this thesis was to evaluate the performance of the device at lower temperatures; hence, the experiments were performed at 30°C, 38°C and 45°C. It was not possible to experiment at 50°C because *Legionella* bacteria were not viable at that temperature in the laboratory conditions. The flow rates of 1.3 l/min and 10 l/min were chosen to achieve laminar and turbulent flow in the pipes, respectively. Therefore, the maximum flow velocity of 1 m/s was within the recommended value of 3 m/s (BSI, 2009). The experimental parameters for the once-through experiments are summarised in Table 5.1.

The initial *L. pneumophila* sg. 1 population density varied from 0.14×10^6 to 15.1×10^6 CFU/L. Each experiment was repeated twice to confirm the reproducibility of the results.

Parameters	Values tested
Temperatures	30°C 38°C and 45°C
Flow rate	1.3 l/m and 10 l/m (78 l/h and 600 l/h)
Flow velocity	0.12 m/s and 1m/s
Reynolds number	Between 2,000 and 26,000 depending on temperature
Water residence time	1.67 s and 0.2 s

Table 5.1: Experimental parameters for the once-through experiment.

The schematic of the once-through experiment is shown in Figure 5.8 and the experimental procedure was as follow:

- The elevated tank and the pipes up to valve 15 were filled with water from the mains.
- Valve 8 was closed to isolate the elevated tank system from the rest of the pipes and the ED device was turned on.
- Pump 1 was turned on and water heated to the required temperature.
- *Legionella pneumophila* sg. 1 was introduced via Septum 1 with a 2.5 ml syringe containing a known quantity of bacteria.
- Water was circulated by the pump for 30 minutes to mix bacteria in the volume of water.
- After having disinfected Septum 1 with the bunsen burner, three 60 ml samples were taken to confirm initial concentration at different times. This is to enable the sampling of the whole body of water.
- Valve 15 was opened to release the water.
- The flow rate was set by turning the needle valve.
- The contaminated water flowed from the tank to the collection cylinder through the ED device.
- Three independent samples were taken at 5, 15 and 30 minutes from the start of experiment at low flow rate (1.3 l/min), from septum 3

located at the ED device outlet, ensuring the whole body of water was sampled. Once the experiment was finished, valves 17 and 18 were open and valve 16 closed and the water in the cylinder was circulated with Pump 2 for 20 minutes. Three samples were taken from valve 20 after having flushed the tap.

- Tank 2 was then heated to 90°C for 60-90 minutes.
- In the meanwhile, the elevated tank was also re-filled with tap water and heated to 90°C for the same amount of time.
- Once the system had been disinfected, the water was released to the drain.
- The procedure was repeated at temperatures of 30, 38 and 45 °C.
- The whole process was then repeated at a higher flow rate of 10 L/min for the three temperatures. The three samples were taken at 4, 5 and 6 minutes after the beginning of the experiment, because the total length of the experiment was 7.4 minutes.

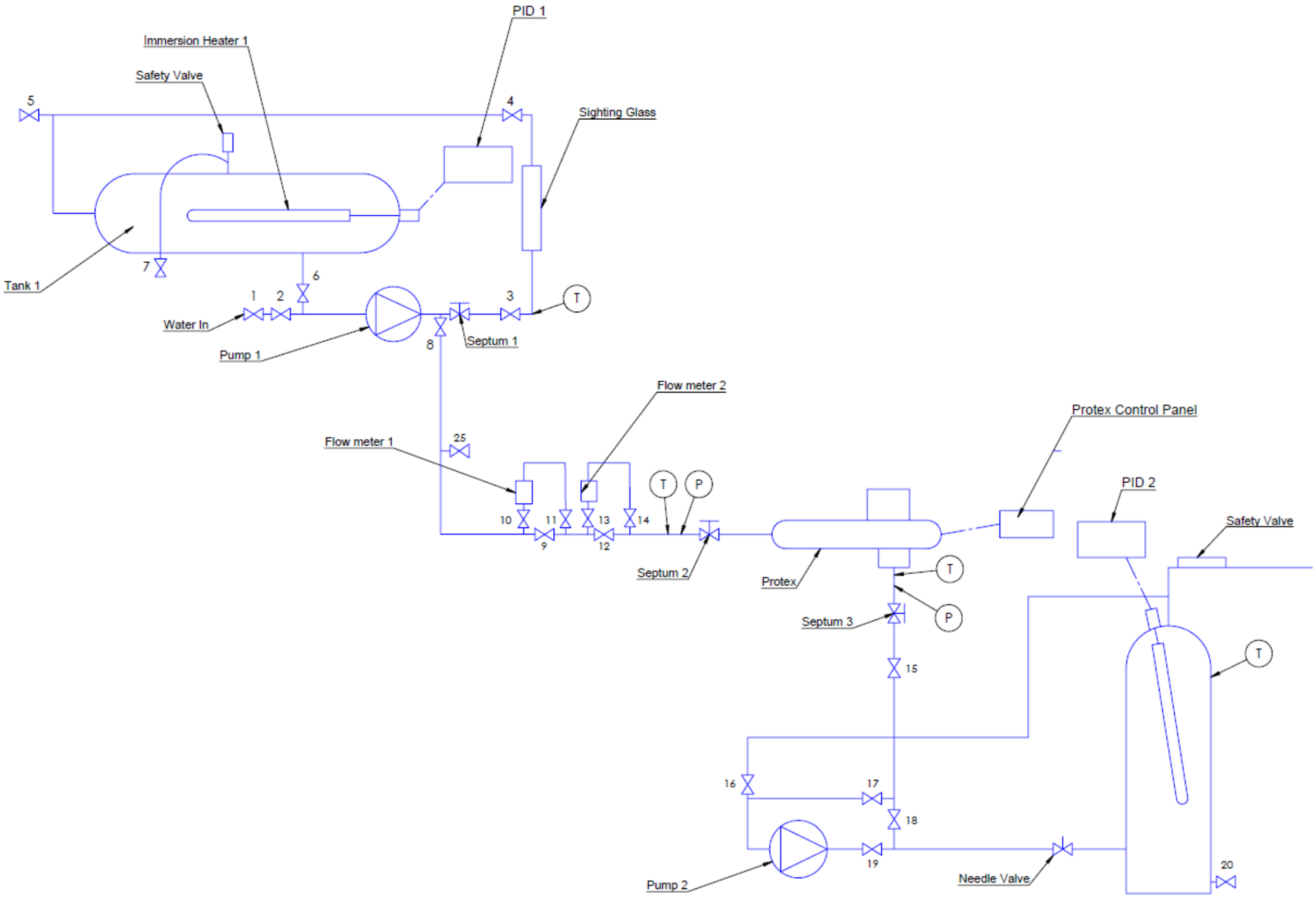

Figure 5.8: Schematic diagram of the experimental rig in use for the once-through experiment.

5.2.6.2 Biofilm experiment

The objective of the biofilm experiment was to observe if treatment of the water with the ED device would produce any visible changes on the biofilm. For this purpose, 6 microscope slides were introduced in the purposely-built biofilm sections. The tap water in the experimental rig was maintained at 37°C to optimise bacteria seeding and biofilm growth. This also ensured that the water temperature would not have any impact on the bacteria viability. The flow rate was chosen to be 5.22 L/min, giving a turbulent flow with Reynolds number of 11,450, and water residence time in the ED device of 8.3 seconds. Such value was selected to assist biofilm settlement, as a more turbulent flow could disrupt growth. The water in the rig was changed regularly to simulate the random usage in a building, and *L. pneumophila* sg. 1 lenticules were introduced frequently to accelerate biofilm growth. After two months, three of the microscope slides were taken out and the biofilm was heat-fixed by passing each slide through the flame of a Bunsen burner 3 or 4 times, ensuring the bacteria were properly affixed to the slide. The microscope slides were then stained with 4% crystal violet, a stain used to colour the biofilm biomass, including bacteria and EPS. Once stained with crystal violet, the bacteria fixed to the microscope slides were viewed by bright-field microscopy with a 100x objective (Olympus BX41, Leica DM4000) to identify any changes in the biofilm. The experimental parameters for the biofilm experiments are shown in Table 5.2. The experiment was repeated twice to confirm the reproducibility of the results. The initial *L. pneumophila* sg. 1 population was 1.6×10^6 CFU/L in Experiment 1 and 4.6×10^6 CFU/L in Experiment 2. The ED device was set at 6 V with current density between 11.6 and 14 mA/cm^2.

Parameters	Values tested
Temperatures	37 °C
Flow rate	5.22 l/m (313 l/h)
Flow velocity	0.5 m/s
Reynolds number	11450
Water residence time	0.4 s

Table 5.2: Experimental parameters for the biofilm experiment.

In the biofilm experiment the rig shown in the schematic in Figure 5.9 was used and the experimental procedure was as follow:

- The three purposely-built biofilm sections were each fitted with two new glass microscope slides.
- The experimental rig was filled with water from the mains ensuring that no air was left in the system.
- Pump 2 was turned on, the desired flow rate achieved and the water was heated to 37°C.
- The system was left to run in this condition for two months.
- During the two months, 10 or 20 litres of water were flushed out of the system, and replaced with water from the mains, to simulate the random water usage of a building.
- The system was regularly spiked with 2, 4 or 6 lenticules of *Legionella* and samples were taken to count the bacteria concentration in the bulk of water.
- After the two months, three of the glass microscope slides (one from each biofilm section) were taken out of the system and stained with crystal violet for observation under the microscope. The three microscope slides were replaced with new ones.
- The ED device was turned on and the water was treated for two weeks.

- During the two weeks, 10 litres of water were flushed and replaced with water from the mains every two days to simulate the random water usage of a building. The systems was spiked with 4 lenticules of *L*. *pneumophila* sg. 1 to simulate bacteria seeding.
- After the two weeks, the device was turned off. The microscope slides were stained with crystal violet for observation under the microscope and samples of the treated water were taken to determine bacteria viability.

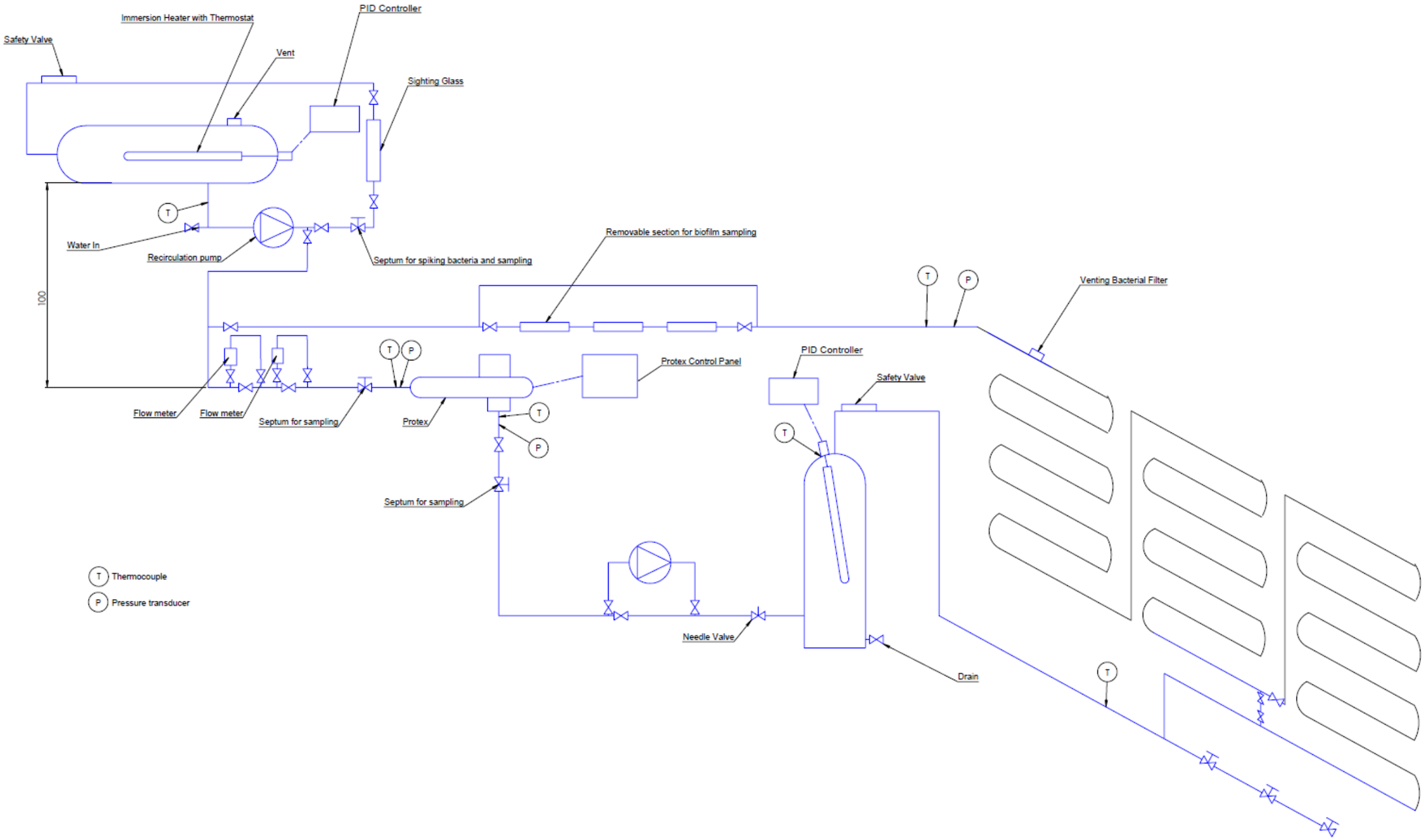

Figure 5.9: Schematic diagram of the experimental rig in use for the biofilm experiment.

5.3 Results and Discussions

5.3.1 Once-through experiment

During the monitoring of the installation in the Halsbury building, discussed in Chapter 5, it was noticed that the higher the voltage, the faster the scale accumulation on the cathode. For the purpose of minimising scale formation, the ED device was set at 6 V in this study. The current achieved varied with the water temperature, as shown in Table 5.3, where the experiments completed in this study are listed. The higher the temperature, the higher the current achieved with the same applied voltage; as the water is heated, the kinetic energy increases, increasing the molecular motion and the electrolysis efficiency (Nicolik et al., 2010). For each temperature, experiments at 1.3 l/m and 10 l/m were performed.

Experimental conditions	Temperature	Flow rate
Viability (device off)	30°C	1.3 L/min (78 l/h)
Viability (device off)	38°C	1.3 L/min (78 l/h)
Viability (device off)	45°C	1.3 L/min (78 l/h)
6 V and 4 A	30°C	1.3 L/min (78 l/h)
6 V and 4 A	30°C	10 L/min (600 l/h)
6 V and 5 A	38°C	1.3 L/min (78 l/h)
6 V and 5 A	38°C	10 L/min (600 l/h)
6 V and 5.5 A	45°C	1.3 L/min (78 l/h)
6 V and 5.5 A	45°C	10 L/min (600 l/h)

Table 5.3: List of experiments performed.

Table 5.4 shows the results of the experiments performed at 30°C, whilst Table 5.5 presents the results for the experiments at 38°C. Table 5.6 shows

the results with water at 45°C. The tables summarise the experimental conditions, the concentration of *L. pneumophila* sg. 1 pre-treatment, the concentration of *L. pneumophila* sg. 1 post-treatment, the percentage of viable bacteria and the quantities of free and total chlorine residual detected at the outlet of the ED device.

In all of the experiments, the concentration of free chlorine and total chlorine residual in the untreated water were below 0.15 mg/L and 0.34 mg/L, respectively. The level of free chlorine was below the recommended point of delivery residual guideline of 0.2 mg/L for potable water (WHO, 2011).

At 30°C±1 °C (Table 5.4) and 45°C±1°C (Table 5.5) there is a slight reduction in viability (97% mean); at 38°C ±1°C (Table 5.6) there was a slight increase in viability (106% mean), confirming that the *L. pneumophila* sg. 1 bacteria survive.

Experimental conditions	Initial concentration (CFU/L)	Viable bacteria (CFU/L) post-treatment	Percentage live bacteria post-treatment	Free Chlorine (mg/L)	Total Chlorine (mg/L)
Viability	$8.68x10^6$	$8.86x10^6$	102%	0.08	0.21
Viability repeat	$12.6x10^6$	$11.7x10^6$	93%	0.15	0.26
1.3 l/min	$1.4\ x10^6$	0	0%	2.8	3.2
1.3 l/min repeat	$13.4x10^6$	0	0%	2.65	2.9
10 l/min	$15.1x10^6$	$6.1x10^6$	40%	0.61	0.86
10 l/min repeat	$6.27x10^6$	$3.68x10^6$	58.8%	0.46	0.58

Table 5.4: Impact of electrochemical treatment of tap water at 30°C on *L. pneumophila* sg. 1.

Experimental conditions	Initial concentration (CFU/L)	Viable bacteria (CFU/L) post-treatment	Percentage live bacteria post-treatment	Free Chlorine (mg/L)	Total Chlorine (mg/L)
Viability	$11.8x10^6$	$12.7x10^6$	107%	0.12	0.34
Viability repeat	$5.19x10^6$	$5.53x10^6$	106%	0.09	0.27
1.3 l/m	$2.4x10^6$	0	0	2.27	3.36
1.3 l/m repeat	$0.14x10^6$	0	0	2.75	3.5
10 l/m	$8x10^6$	$6.3x10^6$	79%	0.4	0.6
10 l/m repeat	$0.5x10^6$	$0.3x10^6$	58.9%	0.42	0.71

Table 5.5: Impact of electrochemical treatment of tap water at 38°C on *L. pneumophila* sg. 1.

Experimental conditions	Initial concentration (CFU/L)	Viable bacteria (CFU/L) post-treatment	Percentage live bacteria post-treatment	Free Chlorine (mg/L)	Total Chlorine (mg/L)
Viability	$3.98x10^6$	$3.86x10^6$	97%	0.06	0.13
Viability repeat	$6.6x10^6$	$6.58x10^6$	98%	0.08	0.17
1.3 l/m	$5.6x10^6$	0	0	2.2	2.50
1.3 l/m repeat	$0.8x10^6$	0	0	2.35	2.76
10 l/m	$0.6x10^6$	$0.44x10^6$	73%	0.30	0.37
10 l/m repeat	$0.27x10^6$	$0.15x10^6$	57%	0.47	0.56

Table 5.6: Impact of electrochemical treatment of tap water at 45°C on *L. neumophila* sg. 1.

At all temperatures and flow rate of 1.3 L/min, no living *L. pneumophila* sg. 1 could be detected neither at the outlet of the ED device, nor in the cylinder. Similar results were obtained by Delaedt et al. (2008) in a study where water at 21°C was spiked with higher concentration of *Legionella* and passed through and ED device at the same flow rate of 1.3 L/min but with lower

currents between 0.3 and 0.5 A. Conversely, at the higher flow rate of 10 l/min, 40-60 % of the bacteria were found to be viable at 30°C, 58.9-70% at 38°C and 57-73% at 45°C. This difference can be explained by the amount of chlorine detected, consistently higher at the lower flow rate, and above the value of 1 mg/L, found to inactivate concentrations of *Legionella* between $1.8x10^5$ and $2.4x10^5$ CFU/L in the flask experiment. The distinct change in colour due to chlorine content of the water samples before and after treatment is shown in Figure 5.10. The photograph was taken during one of the experiment at 1.3 L/min flow rate and 45°C water temperature.

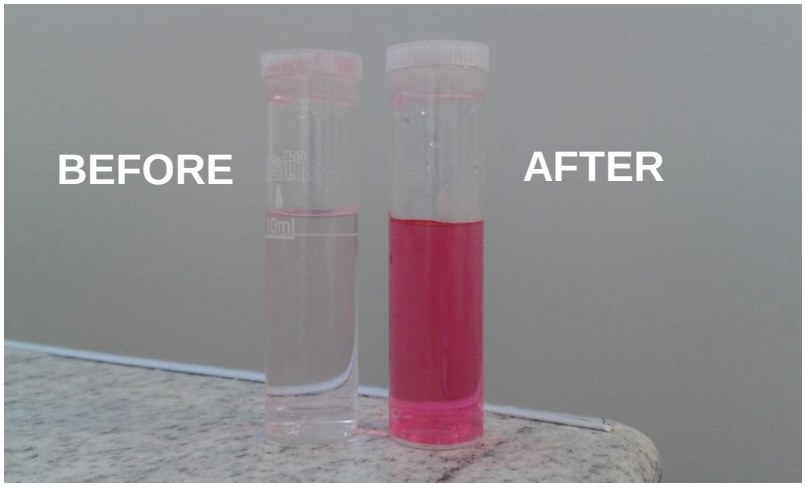

Figure 5.10: Photograph of the water samples taken before and after ED and treated with of a DPD tablet for the purpose of measuringe chlorine concentration.

When the flow rate was increased to 10 L/min, the level of free and total chlorine residual are up to 6 times lower because of the shorter water residence time in the ED device. The short contact time between the water and electrodes lowers the efficiency of the ED device, consistently with previous studies (Ezeike and Hung, 2004, Hsu, 2005, Yao et al., 2011). Conversely Kraft et al. (1999) found that the level of chlorine increased with flow through velocity but the highest flow rate they investigated was 4.2 L/min, half of the flow rate investigated in this study. Regardless of temperature, at the flow rate of 1.3 L/min, concentrations of *L. pneumophila* sg. 1 ranging between $0.14x10^6$ CFU/L and $13.4x10^6$ CFU/L are inactivated. These results are promising as the bacteria concentration inactivated are

within the levels of *Legionella* found in problematic buildings. In the Halsbury complex described in Chapter 5, the maximum value recorded for *Legionella* during the monitor period was never above 10^5 CFU/L.

Figure 5.11 shows the free chlorine residual concentration as a function of temperature. A decrease in chlorine production is noticeable at both flow rates but it is more accentuated at 10 L/min. In a similar study investigating the performance of IrO_2 sheet electrodes and platinum electrodes Kraft et al. (1999) analysed the temperature dependence of the free chlorine production at a flow rate of 2 L/min. However, they only analysed IrO_2 electrodes at current densities of 22 and 34 mA/cm^2 and they did not report on the performance of platinum plated electrodes at different temperatures. It is important to take into consideration that the actual level of chlorine produced in our experiments is higher because the chlorine reacting with the bacteria cannot be measured. On the other hand, Kraft et al. (1999) do not use bacteria in their investigation.

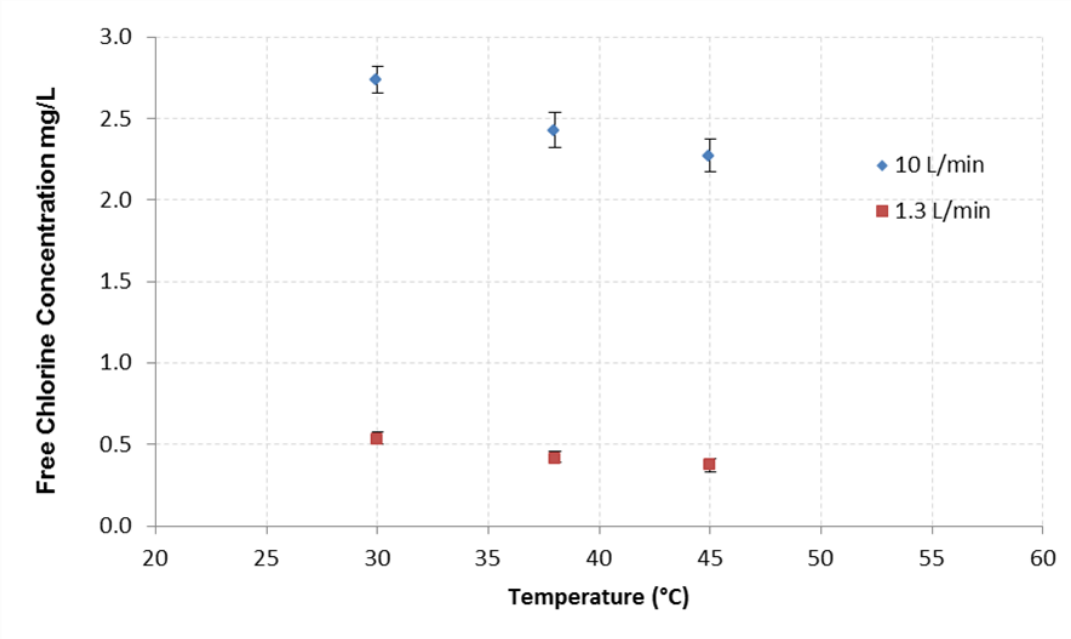

Figure 5.11: Free chlorine residual concentration as a function of temperature for flow rates of 1.3 L/min and 10 L/min.

The results obtained by Kraft et al. (1999) normalized on the current are plotted in Figure 5.12 for comparison with the results obtained in this

investigation. Interestingly they show that at current densities between 11 and 16 mA/cm^2, the level of chlorine residual produced by the platinum electrodes in this study is higher than the chlorine produced by the IrO_2 electrodes at 22 mA/cm^2. In the same study, Kraft et al. (1999) found that IrO_2 electrodes at 22 mA/cm^2 produced similar amount of free chlorine at both flow rates of 1.3 L/min and at 2 L/min. This suggests that the platinum plated electrodes will produce more chlorine than IrO_2, at the same current density and that they are the best choice for low current applications.

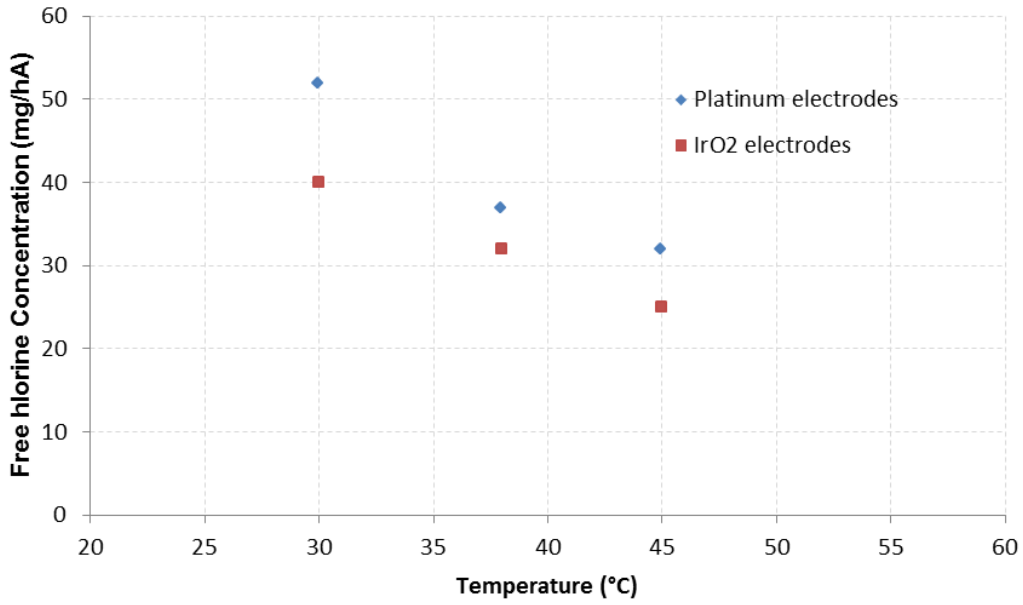

Figure 5.12:Comparison of the results obtained by Kraft at el. (1999) at current densities of 22 mA/cm^2 and IrO_2 electrodes and the results of this investigation with current densities varying between 11 and 16 mA/cm^2 and platinum plated electrodes.

As explained in the experimental procedure, after the completion of each experiment, the cylinder filled with the water from the elevated tank, was isolated from the rest of the rig and the water was circulated in the cylinder by pump 2 for 20 minutes to mix the body of water. Samples were taken for bacterial analysis and the level of free chlorine was measured; the results are set out in Table 5.7.

Experimental conditions	Temperature	*Legionella* CFU/L	Free Chlorine Residual (mg/L)
6 V and 4 A 1.3 L/min	30°C	0	1.9
6 V and 4 A 10 L/min	30°C	0	0.45
6 V and 5 A 1.3 L/ min	38°C	0	1.77
6 V and 5 A 10 L/min	38°C	0	0.35
6 V and 5.5 A 1.3 L/min	45°C	0	1.36
6 V and 5.5 A 10 L/min	45°C	0	0.29

Table 5.7: Results of the samples taken 20 minutes after treatment. The samples were analysed for *Legionella* density and the level of residual free chlorine was also measured.

In all of the experiments, no *L*. *pneumophila* sg. 1. was detected in the samples taken 20 minutes after the completion of each test, confirming that the electrolyzed water has a residual effect. Even with the flow rate of 10 L/min, the low residual free chlorine inactivated the remaining *Legionella* (50-70% of original quantity), having failed to be inactived in the passage through the ED device. Delaedt et al. (2008) performed a similar experiment by adding a 107 CFU/L concentration of *E. coli* bacteria dowstream a disinfection unit to investigate the residual effect of the electrolyzed water. They obtained similar results by inactivating the *E. coli* bacteria in 15 minutes but they did not perform the experiments with *L*. *pneumophila* sg. 1.

5.3.2 CT value

The results obtained in the experiments described previously enabled the determination of the CT numbers for the ED device to obtain between a 5 log or 99.999% inactivation of *L. pneumophila* sg. 1 at temperatures between 30 and 45°C with water pH of 7.4. The CT values determined in this study can be seen in Table 5.8. Previous research on the dependence of CT number found that the effectiveness of the disinfectants increases with the increase in temperature, (WHO, 2004). Hence, when selecting a CT value, it is

advisable to decide on the one at the lowest temperature because it will provide a margin of safety.

Temperature	Residence time	Free chlorine residual	CT Value
30°C	0.03 min	2.73 mg/L	0.082 mg/L min.
38°C	0.03 min	2.43 mg/L	0.073 mg/L min.
45°C	0.03 min	2.27 mg/L	0.068 mg/L min.

Table 5.8: Experimentally determined CT value necessary to achieve 5 log inactivation of *L. pneumophila* sg. 1 at different temperatures with residence time equivalent to 0.03 min.

The results indicate that a CT value of 0.082 mg/L minutes achieves a 5 log inactivation of *L. pneumophila* sg. 1 at temperatures above 30°C. Hence, the value can be used in scaling the device to the particular application, to obtain the same performance at different flow rates. The experiments carried out in the flasks and presented in Chapter 3, indicated that by increasing the volume of the device and the area of the electrodes, a larger amount of free chlorine can be produced. Therefore, using the CT value of 0.082 mg/L minutes and increasing the level of free chlorine residual at the device outlet by altering the geometrical parameters of the device, would enable 5 log inactivation of *L. pneumophila* sg. 1, with lower water residence time.

5.3.3 Biofilm experiments

5.3.3.1 Planktonic *Legionella*

The concentration of free-floating *L. pneumophila* sg. 1 achieved in the water after two months was 1.6×10^6 CFU/L for Experiment 1 and 4.6×10^6 CFU/L for Experiment 2 (see p. 143 for description of experiments). No residual chlorine was detected in the tap water introduced in the experimental rig whilst the level of free chlorine residual generated by the device was 4.9 mg/L, a 5 fold increase on the initial value. Figure 5.13 (a) and (b) are photographs of the water samples plated in BCYE agar plates, taken before

the ED device was turned on and two days after treatment. The photographs clearly show that the planktonic *Legionella* bacteria were eliminated within the two days.

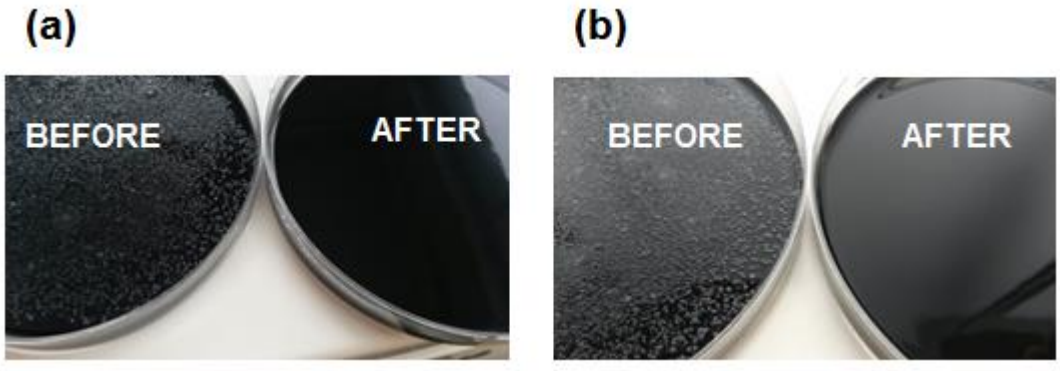

Figure 5.13: Photographs of water samples plated before and after treatment.

5.3.3.2 Biofilm

Figure 5.14, Figure 5.15, Figure 5.16 and Figure 5.17 are photographs of the biofilm formed in the microscope slides after the two months of biofilm growth period during experiment 1. Although the system was spiked with *L. pneumophila* sg. 1 bacteria during the two months, the tap water introduced in the system would have also contained other bacteria and microorganisms. So, the biofilm formed in the microscope slides is likely to contain a variety of bacteria. The purpose of the experiment was not to determine the bacteria species growing in the biofilm, but to identify any eventual change in the biofilm after treatment.

Undoubtedly the fouling procedure led to the establishment of biofilm on the slides, in some areas more than others. Rod-shaped bacteria and large colonies of bacteria can be clearly seen in all of the photographs taken and some have been identified by white arrows. Even in areas where large colonies have yet to establish, rod-shaped bacteria are clearly visible, see Figure 5.17.

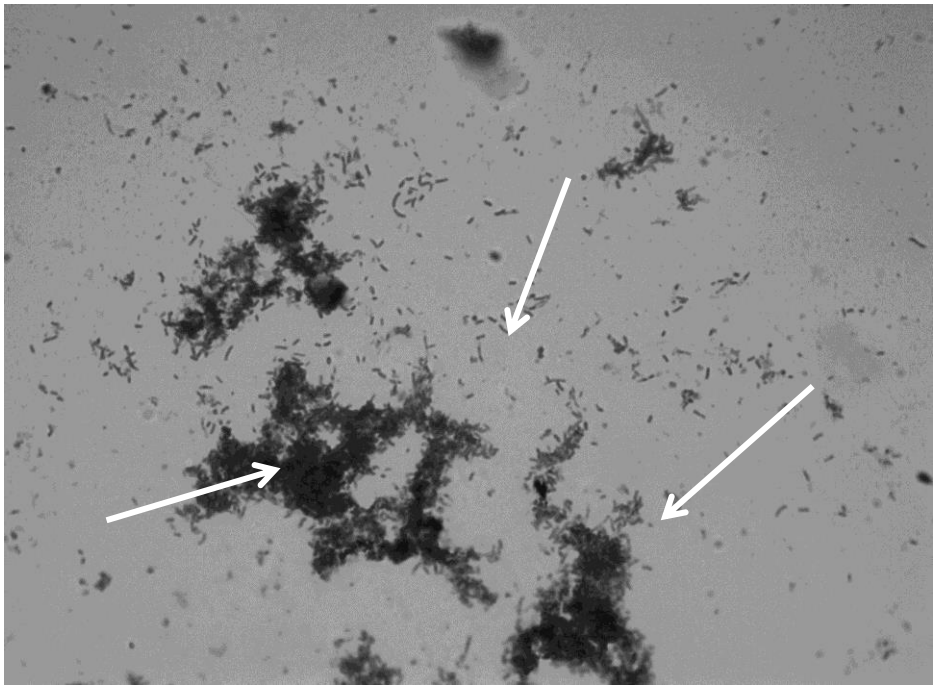

Figure 5.14: Photograph of the large colonies of biofilm in slide 1 Experiment 1 (100x objective).

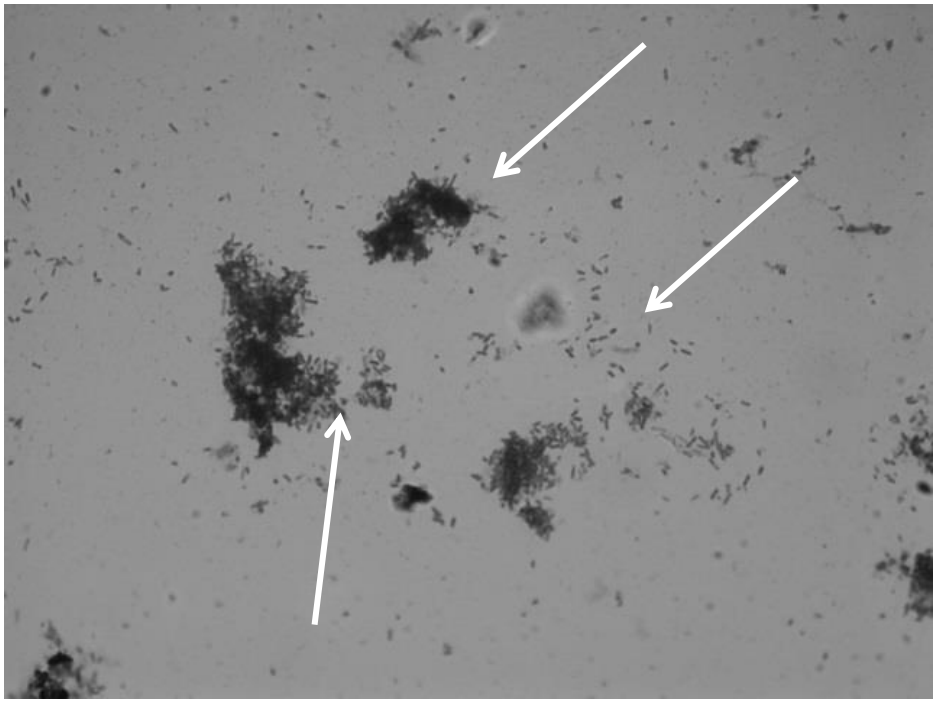

Figure 5.15: Photograph of the biofilm in slide 2 Experiment 1 (100x objective).

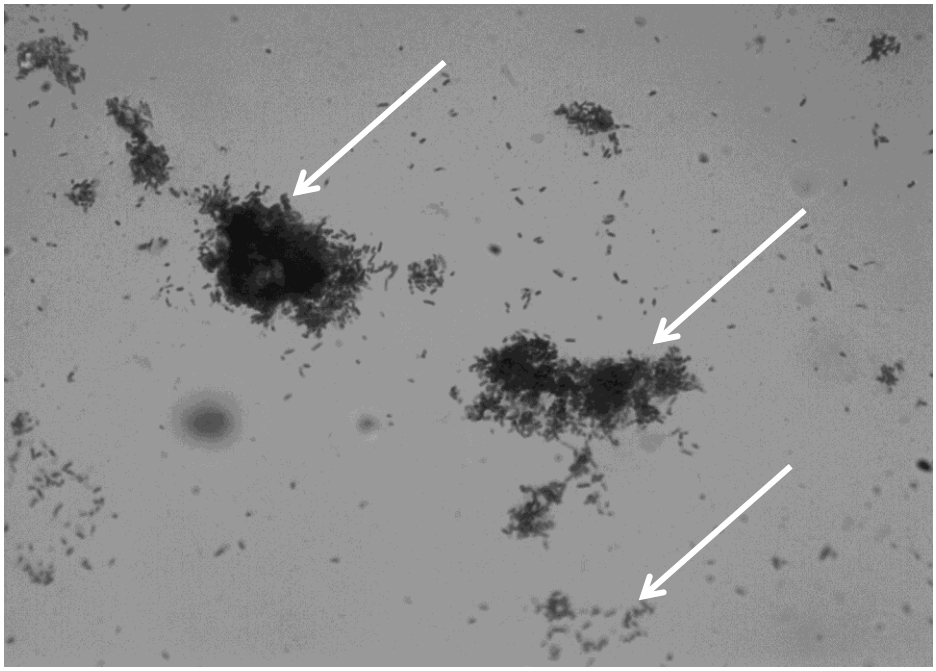

Figure 5.16: Photograph of the biofilm in slide 3 Experiment 1 (100x objective).

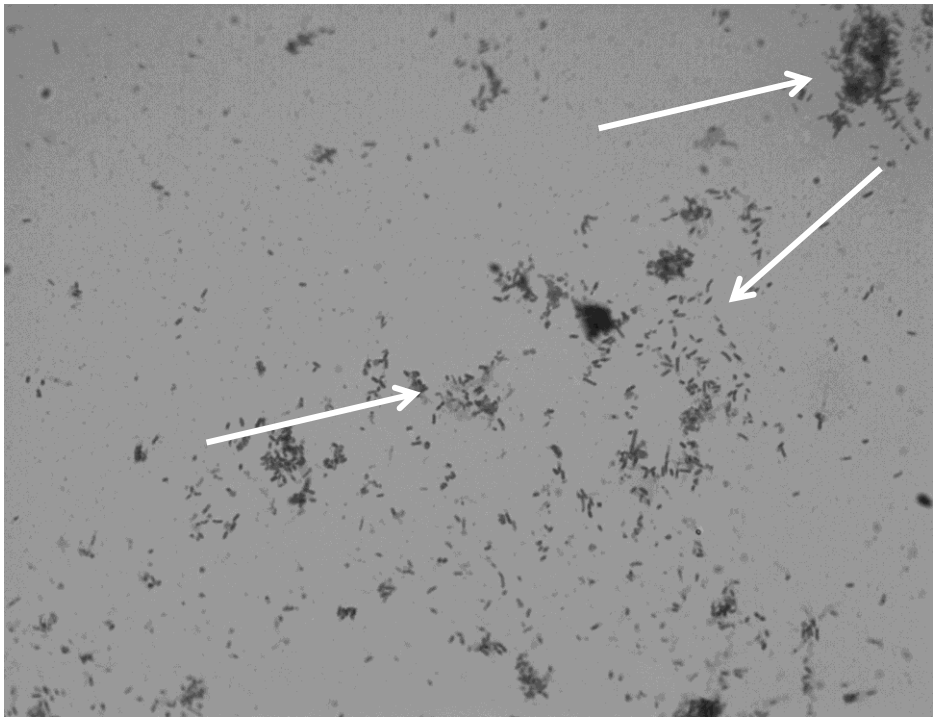

Figure 5.17: Photograph of the biofilm in slide 1 Experiment 1 (100x objective).

Microscopy of the biofilm after treatment revealed changes in the morphology of the bacteria colonies; see Figure 5.18, Figure 5.19, Figure 5.20 and Figure 5.21. Neither rod-shaped bacteria nor large agglomerates of cells are observed in any of the slides. These photographs provide vivid evidence that the disinfectants generated by the ED device have caused damage to the cell membranes. The outlines of the large colonies are still visible, but their appearance is deformed and they do not appear as dark as in the photographs prior to treatment. Most of the microorganisms have been lysed during disinfection releasing a large amount of cellular material. The few dark areas present in the slides could be thicker parts of the biofilm that have not been damaged or dead cells that have remained intact.

These results are in agreement with previous studies (Diao et al., 2004 and Loret et al., 2005). Diao et al. (2004) employed SEM to visualise the surface changes of planktonic *E. coli* after ED treatment and found that the disinfectants attacked the cell membranes causing large cellular leakage. Although they did not publish any microscope images, Loret et al. (2005) obtained similar results in a study comparing the efficiency of different disinfectants in eliminating *Legionella*, protozoa and biofilm. They found that 2.75 mg/L of electro-generated chlorine was sufficient in eliminating *L. pneumophila* sg. 1 concentrations of 2.6×10^5 in 3 days and to reduce biofilm thickness in one week.

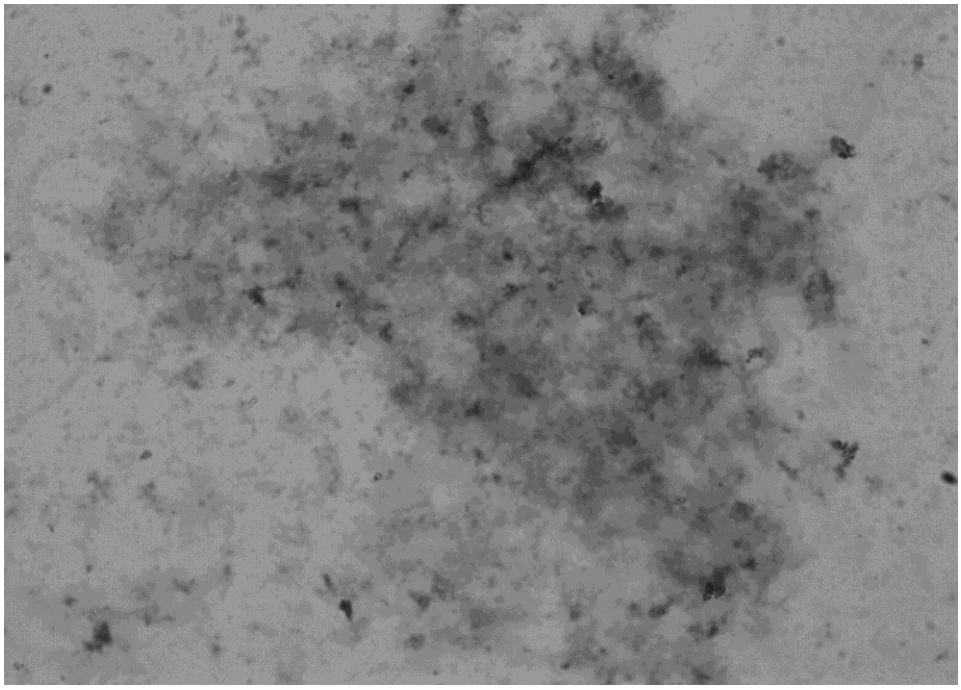

Figure 5.18: Photograph of the biofilm after treatment in slide 4 Experiment 1 (100x objective).

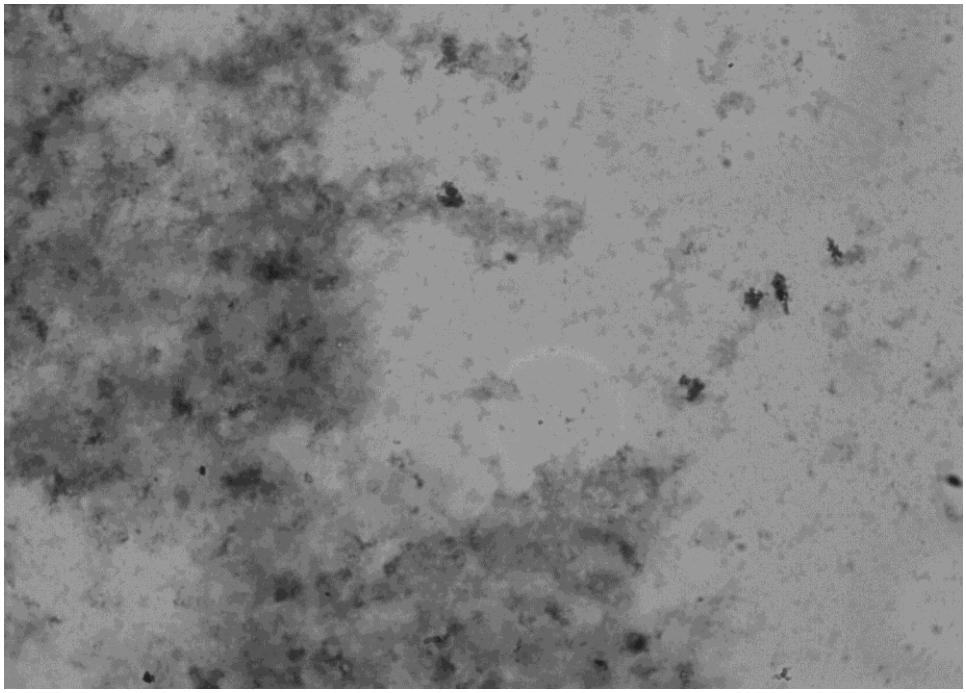

Figure 5.19: Photograph of the biofilm after treatment in slide 5 Experiment 1 (100x objective).

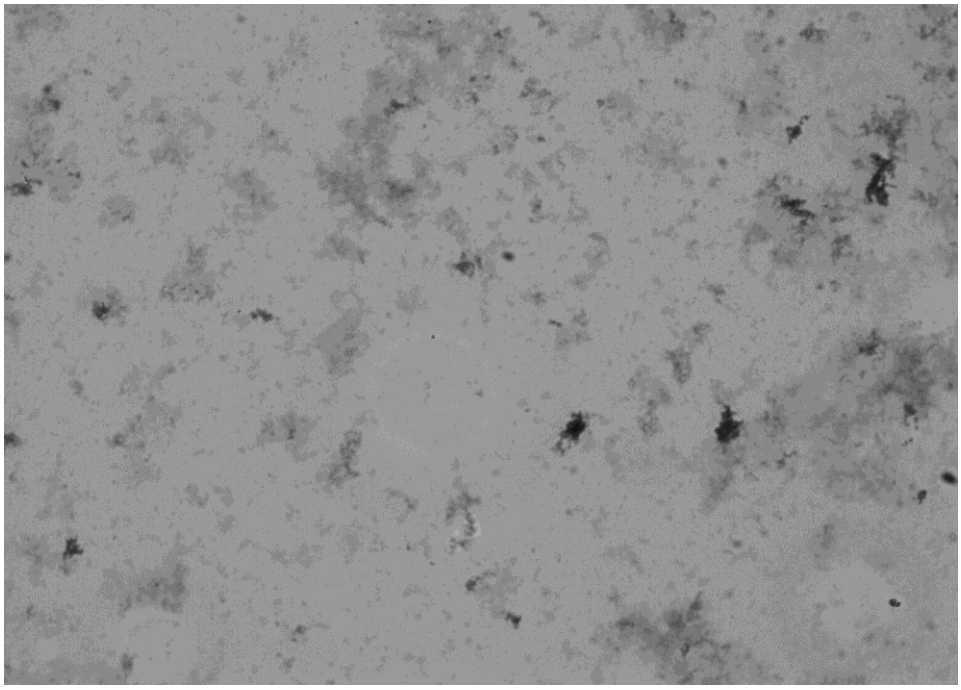

Figure 5.20:Photograph of the biofilm after treatment in slide 6 Experiment 1 (100x objective).

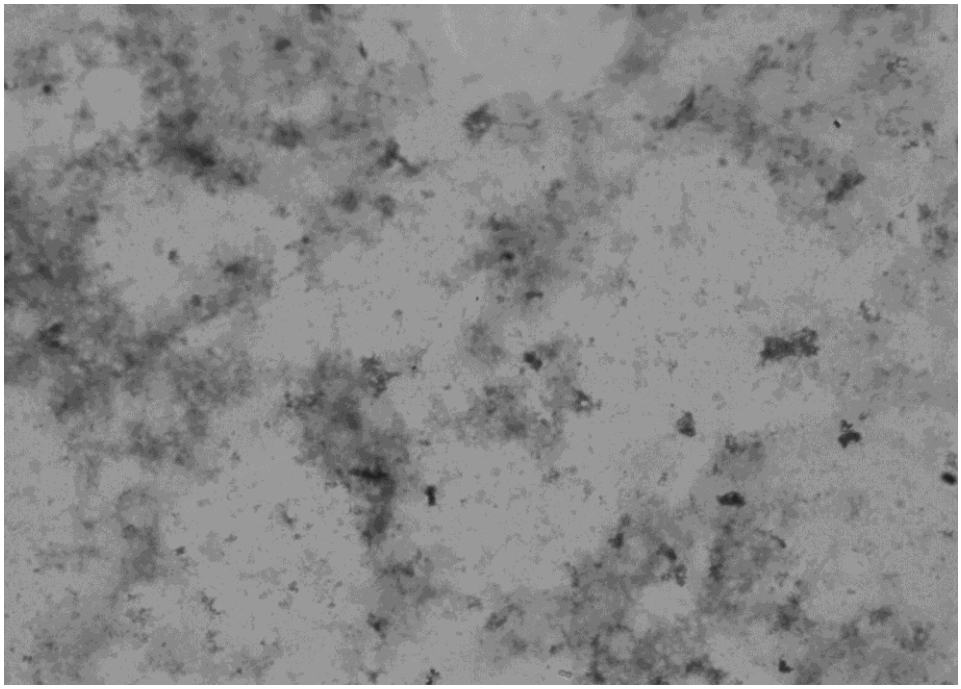

Figure 5.21: Photograph of the biofilm in slide 6 Experiment 1 (100x objective).

The experiment was repeated and the biofilm microscopy before and after treatment are shown in Figure 5.22 (a,b,c and d) and Figure 5.23 (a,b, c and d), respectively. As in the previous experiment, the biofilm has grown in the slides since rod-shaped bacteria and agglomerates of cells are clearly visible. Microscopy of the biofilm after treatment shows deformation of the once smooth bacteria colonies.

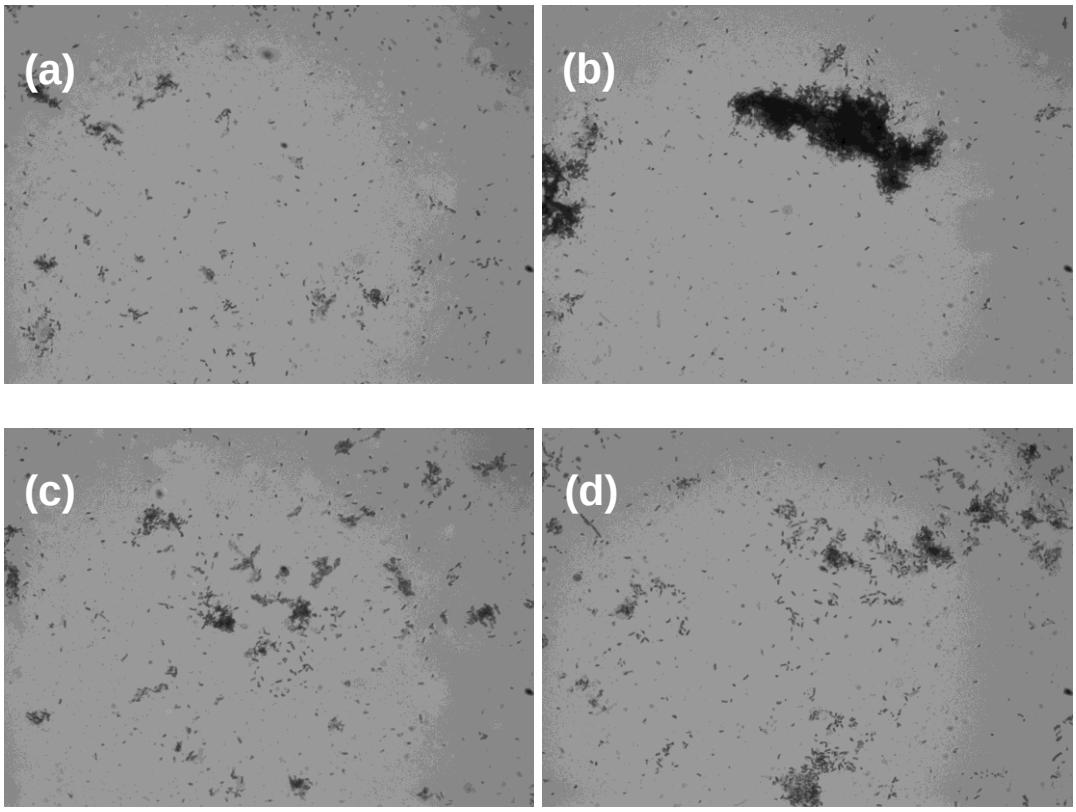

Figure 5.22: Photograph of the biofilm formation on the slides in Experiment 2 prior to treatment (100x objective). (a) slide 1; (b) slide 2; (c) slide 3; (d) slide 1.

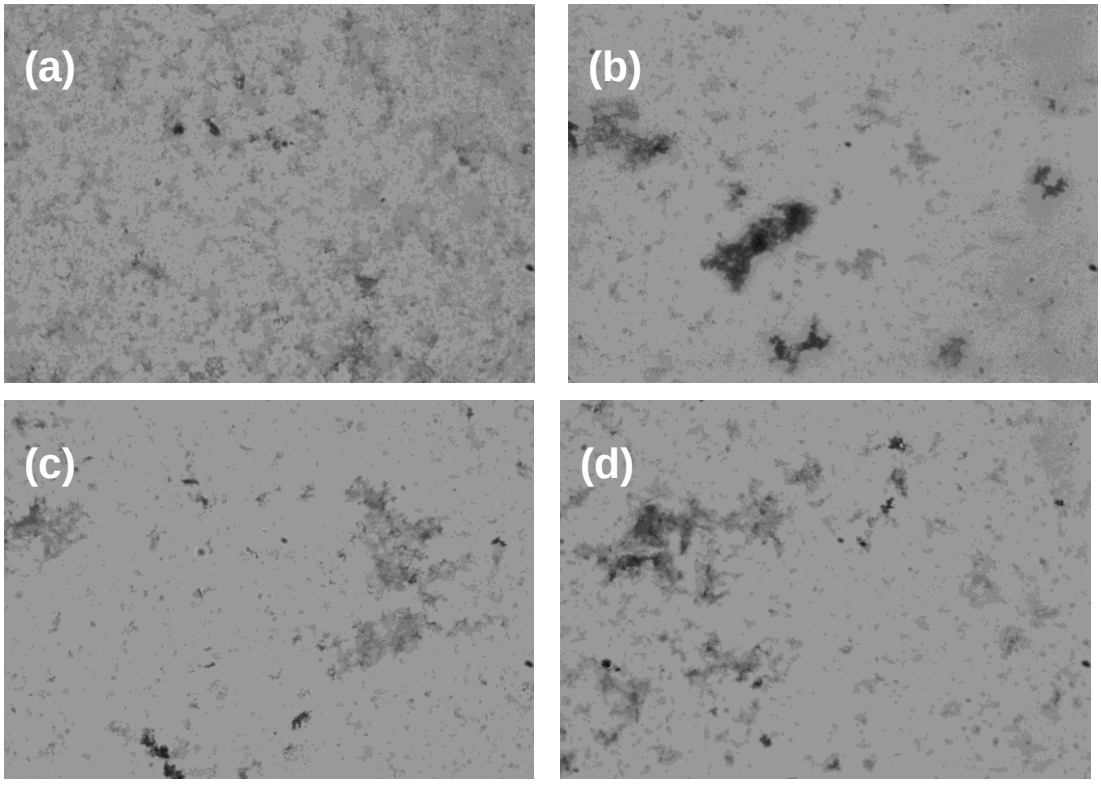

Figure 5.23: Photograph of the biofilm formation on the slides in Experiment 2 after treatment (100x objective). (a) slide 4; (b) slide 5; (c) slide 6; (d) slide 4.

5.4 Conclusions

This investigation has confirmed that ED is effective at lower hot water temperatures and that it has potential in the disinfection of hot water systems in non-domestic buildings. Hence, ED could play an important role in the energy reduction strategies necessary to meet the ambitious non-domestic zero carbon target the UK government has set for 2020.

5 log inactivation of *L. pneumophila* sg. 1 was achieved with CT values between 0.068 and 0.082 mg/L minutes in water at temperatures between 30° and 45°C and pH equal to 7.4. Flow rate was also identified as a critical parameter in the formation of disinfectants as it defines the residence time of the water in the device.

It is also concluded from this study that ED has the capacity of removing laboratory formed biofilm with chlorine levels of 4.9 mg/L. These results are promising because in periods of low hot water demand (i.e: during school

holidays or low seasons in hotels), the level of chlorine residual could be increased to remove biofilm or inhibit its growth. Hence, the results are a baseline for further more in-depth investigation on the effect of ED on biofilm.

Chapter 6 : Conclusions and suggestions for further work

‘Let there be work, bread, water and salt for all.’

Nelson Mandela

6.1 Conclusions

Water systems in buildings have resulted in disease outbreaks associated with opportunistic water-borne pathogens, such as *Legionella* and *Pseudomonas*. Although regulations and codes of practice suggest ways of preventing the growth of pathogens in water systems, not only has the number of disease cases continued to increase each year but it is also believed to be greatly underestimated, due to misdiagnosis and under-reporting (European Centre for Disease Control and Prevention, 2013). The smaller more frequent outbreaks have been associated with hot water systems because they provide ideal breading conditions for the pathogens together with aerosol producing systems such as showers, spas and hot water taps. However, current hot water treatments are not always effective in maintaining the level of bacteria within the control limits, are often energy-intensive and require expensive maintenance routines. Evidently, there is a need for the development of an alternative more effective water treatment technology.

This research was set out to evaluate the potential use of electrochemical disinfection in eliminating pathogens, to understand the fundamental physical phenomena and the effect of control parameters. This was followed by the evaluation of the use of a prototype in real building applications. This research also aimed at assessing the performance of the ED process at lower temperature (40-45°C) as this could result in energy savings and CO_2 emission reductions, lowering the carbon footprint of buildings.

This project aimed to address the following questions:

- What is the extent and cost of LD and *Pseudomonas* infections in the UK and Europe?
- How do geometrical and operational parameters influence the performance of ED?
- How can the electrochemical process be optimized in terms of control parameters?

- Is the prototype device effective in commercial buildings with water at temperatures of 60°C?
- Is the prototype device effective at lower water temperatures between 30 and 45°C?
- Do the electrochemically generated residual disinfectants inhibit biofilm growth?

The objectives were met by:

- Performing a systematic study of the cell geometrical and operational parameters under controlled laboratory conditions in a flask where platinum coated electrodes were immersed in 3.5 litre of tap water contaminated with *E.coli* or *Legionella*. Understanding of the effect of each parameter on the rate of bacteria elimination is crucial for the optimization of the ED process to be adaptable to different environmental and operational conditions in building applications.
- Installing prototype devices in the hot water systems of two different buildings operating at 60°C, as required by the regulations (HSE, 2013). Although many studies have confirmed the potential of ED in laboratory conditions, these case studies are the first to document the long-term monitoring of electrolytic devices in buildings. These studies have confirmed that the device in its present form is not efficacious in all buildings and that it should be re-designed taking into consideration the flask experiments results. It also highlighted that adequate operation and monitoring of the hot water system is crucial to the successful use of the ED device.
- Evaluating the efficacy of the prototype device at lower temperatures (40-45°C) in a purposely built rig, designed to simulate a scaled-down hot water system. Lowering the temperature of hot water will not only decrease energy consumption and CO_2 emission, but it will also increase the efficiency of the water system and it would enable the use of greener technologies to heat the water.

6.1.1 The burden of LD and *Pseudomonas* infections

- The annual cost associated with Legionnaires' disease could be in the region of £1 billion in Europe and £100 M in the UK.
- The annual costs associated with pseudomonal infections could be up to £3 billion in Europe.
- The cost of mortality due to LD and pseudomonal infections in Europe were estimated to be £780 million and between £146 and 221 billion, respectively.
- The burden of the diseases associated with *Pseudomonas* and *Legionella* is substantial but it is still underestimated, as it was not possible to consider the costs of patient disability, outpatients care and out-of-court settlements.
- In the UK alone, the cost of controlling *Legionella* in non-domestic buildings hot water systems could amount to around £140M per annum.

6.1.2 How do geometrical and operational parameters influence the performance of the electrochemical processes?

- The increment of area and higher potential, decreasing distance between the electrodes and lowering the volume of contaminated water improve the performance of the electrolytic process.
- The electrode manufacturer suggests the maximum voltage input should not be over 7 V as the life of the electrodes will decrease significantly.
- With current density of ≈ 4 mA/cm^2, >4 log inactivation of *Escherichia coli* and > 5 log inactivation of *L. pneumophila* sg. 1 were achieved in 10 min and 45 min, respectively.
- The time necessary to achieve 4-log inactivation of *E. coli* was decreased from 110 minutes to 10 minutes, by manipulating the geometrical and operational parameters of the cell.

6.1.3 How can the device be sized/optimized?

- The greatest challenge for the application of ED for pathogen control in commercial buildings is the diversity of hot water systems as each building presents a different arrangement.
- One device might be efficacious in one building but not in another either because of the different size of the water system, the variation in water use and water quality or because the bacteria inhabiting the system are different.
- In water treatment CT tables can be used to determine the necessary disinfection dosages to achieve the required removal of microorganisms for a known disinfectant at constant temperature and pH.
- The CT number is the product of the residual disinfectant concentration, in mg/L, and the contact time between the disinfectant and the target microorganism, in minutes.
- The device can be sized to each building by using the CT number found to be effective in obtaining 5 log inactivation of *Legionella*, in the experiments undertaken in the experimental rig.
- The device can be adapted to obtain the necessary CT value for the building by adjusting the volume of the reactor, by increasing the area of the electrodes, by varying the voltage/current applied, increasing mixing and/or decreasing the distance between the electrodes.

6.1.4 Is the device effective in commercial buildings with water at temperatures of 60°C?

- The case studies are the first to document the long-term use of ED in the hot water recirculation systems of operating buildings.
- The ED device was efficacious in controlling pathogens in the Wendover MS centre.
- 15 months after activating the ED device, TVCs counts were reduced by 2-log in pre-flush samples and 4-log in post-flush samples. *Pseudomonas* species counts were reduced 3-log in pre- and post-

flush samples.

- The failure to control *Legionella* in the Halsbury/Braggs/ETC complex was more likely due to the inadequate operation of the recirculation pump during the monitoring period.
- The Halsbury installation enabled the identification of issues with scale build-up, so methods of scale prevention should be investigated.

6.1.5 Is the device effective at lower water temperatures between 30 and 45°C?

- ED was effective in eliminating *L. pneumophila* sg. 1 at temperatures between 30 and 45 °C in the test rig.
- It was also effective in eliminating laboratory grown biofilm.
- 5 log inactivation of *L. pneumophila* sg. 1 was achieved with CT values between 0.068 and 0.082 mg/L minutes in water at temperatures between 30° and 45°C and pH equal to 7.4 in the test rig.
- The author suggests the temporary installation or the incorporation of a flow meter to measure the system flow rate prior to switching on the ED device, enabling the operator to calculate the necessary size of the device to achieve a CT value of 0.082 mg/L minutes.
- The level of chlorine necessary to inactivate a 10^6 concentration of *Legionella* was found to be 2.7 mg/L.
- A chlorine detector could be fitted at the outlet of the device and connected to an alarm informing the operator if the level of chlorine falls below the set value.
- In problematic buildings the level of chlorine can be maintained higher to eradicate the higher number of bacteria. This should not be above the guideline value of 5 mg/L residual (WHO, 1997).
- The use of ED for disinfection of non-domestic hot water at lower temperature of 45°C could result in savings of £62 million in energy consumption and 0.41MtCO_2.

- ED could play an increasingly important role in renewable energy because further reductions can be achieved by heating the water with green technologies such as solar panels that become more efficient if one demands water at a lower temperature.
- The Government plan of new non-domestic buildings achieving zero carbon emission from 2019 can only be fulfilled if the energy demand associated with heating water is addressed (HM Government, 2011).
- Using water at lower temperatures would reduce the risk of scalding and would reduce the installation of costly TMVs, estimated to be £5.4M.

6.2 Suggestions for further work

The research results described above have demonstrated the ability of electrochemical disinfection and the ED device in killing *Legionella*. We recommend that additional research be considered to improve the efficacy of the device when used in hot water services. These include:

- Optimize the device in terms of design for the purpose of reducing manufacturing costs, prevent scale build-up and improve serviceability.
- Investigate the effect of pipe material on device performance.
- Evaluate the performance of the ED device in cold water.
- Determine whether the optimal electrode configuration is able to kill a range of pathogenic bacteria, including *Pseudomonas* and amoebae.
- Investigate further the effect of biofilm in laboratory conditions with the use of LIVE/DEAD BacLight Bacterial Viability Kit which allows the visualisation and enumeration of dead and live cells.

Additionally, ED could be used for the delivery of safe water in other applications such as in domestic hot water, hospital taps, process wash water, paper production and dental chairs. Finally, developing countries could benefit from the use of ED in the provision of safe drinking water, given the low energy input required to power the cell.

6.2.1 Optimisation of the device

The successful performance of the device in a building depends on sizing the device for the particular application. Hence, there is a need for a unit that can be adapted to the building size, the actual water demand of the building and the water flow rate. The cell could be re-designed so that it can be adapted to the building without changing its overall size, for example by fitting it with more electrodes to increase disinfection capability. Sizing the ED device according to the building flow rate will remain a challenge because it is very unusual for the flow rate in hot water systems to be measured. Simply looking at the size of the tank and calorifiers could cause mistakes in sizing the ED device. Hence, the author suggests either the temporary installation or the incorporation of a flow meter to measure the system flow rate prior to switching on the ED device. This will enable the operator to calculate the necessary size of the device in order to achieve a CT value of 1.52 mg/L minutes, either by adding more electrodes or by installing two or more units in series or parallel to boost the level of disinfectants.

The installations in buildings have also highlighted problems with the design of the electrode package. The latter could be modified by using flat plates instead of using rod shaped electrodes, decreasing the cost of the electrode package by ten times (as discussed with the electrode manufacturer). The use of mesh electrodes instead of flat sheet would not only increase the geometrical area and current, but it would also enhance mixing of the water in the device. The electrode package could also be re-designed to ease maintenance and to simplify assembly and disassembly.

To demonstrate effectiveness, the device should be installed in other buildings of different size and type and case data produced. The careful selection of buildings would also enable the evaluation of the device performance with different pipe materials. In addition, given the promising results obtained in the experimental rig at lower temperatures, the company should seek advice from the HSE to safely lower the water temperature in a low risk building fitted with the device. Proof of the successful

implementation of the device with low water temperatures could provide a powerful marketing tool for the company.

The level of chlorine necessary to inactivate a 10^6 concentration of *Legionella* was found to be 2.7 mg/L. So, to maintain such level of residual in a building, a chlorine detector could be fitted at the outlet of the device and connected to an alarm informing the operator if the level of chlorine falls below the set value. Remedial actions will include either electrode de-scaling or the activation of another unit to increase the level of disinfectants. In order to achieve effective disinfection, it is advisable that a problematic building maintains higher level of chlorine residual to eradicate the higher number of bacteria present.

In order to achieve effective disinfection in areas with hard water, the problem of scale should also be addressed by investigating ways of preventing scaling up of the electrodes. These include examining regular change in polarity and/or integrating a scale inhibiting device prior to the system. The latter could form an integral part of the device.

Another avenue for optimisation includes investigating other electrodes material for the purpose of enhancing performance, decreasing production cost and increase electrode life.

6.2.2 Evaluation of the device in cold water and other bacteria like *Pseudomonas*

The results obtained so far have indicated that electrochemical disinfection could be applied to other areas such as the disinfection of cold water. Further research should also include the monitoring of the device performance either for the disinfection of reclaimed water or rainwater for toilet flushing.

Future studies on the capability of the device on inactivating *Pseudomonas* species, an emerging problem in new buildings and in hospital water systems, are also recommended.

6.2.3 Hot water in dwellings

According to the National Statistics there are 26.4 million households in the UK (The Office for National Statistics, 2012) whilst according to the European Environment Agency (2011) there are 151 million households in Europe. As in non-domestic buildings, hot water should be stored at 60˚C to prevent *Legionella* proliferation. However, the owner has the control of the temperature, and it cannot be assumed that temperatures are maintained as suggested. Having a device that ensures the delivery of safe hot water at lower temperatures in households, not only would lower the carbon footprint of each household and lower the risk of contracting diseases associated with opportunistic bacteria, but it would also decrease the risk of scalding. In the UK alone 2,500 young children are scalded in bath water every year (The Royal Society for the Prevention of Accidents, 2014).

6.2.4 Hospital taps

A smaller device could also be used in hospital taps instead of the expensive point of use filters designed to remove pathogens. The main advantage of using an electrolytic device is the ease of maintenance, as electrodes need to be cleaned quarterly while points of use filters are disposable and need to be changed monthly. The market is considerable as there are 140,000 hospital beds in the UK (Department of Health, 2012), and it is estimated that there are nearly 4 million hospital beds in Europe (Organisation for Economy Cooperation and Development, 2012).

Further research is needed to scale the product for these applications to ensure water free from *Legionella* and *Pseudomonas* is delivered for people in their homes and for patients in hospitals.

6.2.5 Process wash water and paper production

The increased consumption of fruit and vegetables in the last twenty years has not only been linked to the year-round availability of fresh-produce, but also to the health benefits associated to a diet rich of fruit and vegetables (Codex Alimentarius Commission,2010). Unfortunately, the rise in demand

has caused an increase of foodborne outbreaks. In the UK, 88 outbreaks and 3435 illnesses related to fresh produce were reported between 1996 and 2006 (WHO, 2008). Fruit and vegetables can be contaminated with pathogens during growth, harvest, post-harvest treatment, distribution and storage so, the disinfection and sanitation of water in the food-production industry for washing vegetables is crucial to the delivery of safe food.

In recent years considerable attention has been paid to Electrochemical Disinfection (ED), as a promising method for the inactivation of bacteria in process wash water Feng et al., 2004, Lopez-Galvez et el., 2012, Gomes-Lopez et al., 2013). There are over 6,000 food and drink manufacturing businesses in the UK (Focus Management Consultants, 2013) that, according to the Environmental Agency (2013), are the third largest industrial users of water.

6.2.6 Dental chairs

Dental chair also present the risk of contracting diseases, as rinsing jets and the water supply to cool drills generate aerosols and droplets that could be inhaled by the patients. The water system in the dental chair has valves and pipe restriction that could aid the formation of biofilm if the water is not treated. For these reasons the use of water in dentistry must comply with a series of regulations including L8 and the Health Technical Memorandum 01-05: Decontamination in primary care dental practices (2013). Therefore, a scaled-down ED device could provide a solution; the water could be continually re-circulated through the device without the need of disinfectants that often give the water an unpleasant taste. There are in the region of 10,000 dental practices in the UK.

6.2.7 Drinking water disinfection in under-developed countries

The World Health Organisation (2008) claims that “10% of the disease burden in the world could be prevented by improving the provision of drinking-water, sanitation, hygiene and water resources management”. Currently 780 million people do not have access to drinking water causing

one child to die every 21 seconds from a water-related disease (Water.org, 2014). With further research, an ED device could provide a solution to this problem. The flask experiment has revealed that electrochemical disinfection has the potential to be utilised to inactivate pathogens in drinking water. The device could be powered with a solar panel to disinfect water in boreholes or it could be powered with a battery for use in time of disaster.

In order to use such a device for drinking water disinfection, further research is needed to investigate and quantify the production of by-products.

Chapter 7 : References

"I went to sleep on May 4, hardly able to breathe. That's pretty much all I remember until I woke up on June 3rd. My wife advised me I was on life support for almost a month and it looked very grim at times. This disease almost killed me."

T.C. *(http://www.hcinfo.com/quotes-from-legionnaires-disease-survivors)*

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Cossali G., Routledge E. J., Ratcliffe M. S., Karayiannis T. G., Fielder J (2015). Electrochemical disinfection: the effect of cell geometrical parameters on the inactivation of E.coli and Legionella. Submitted to Journal of Environmental Engineering.

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Presentations and Awards

Cossali G., Ratcliffe M. S., Routledge E. J., Karayiannis T. G., Fielder J (2014). Eliminating those harmful bacteria. 7th Annual Student Research Conference 2014, Brunel University. 1st Prize award for oral presentation.

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