# Inactivation of *E. coli*, *Legionella*, and *Pseudomonas* in Tap Water Using Electrochemical Disinfection

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**Abstract:** Disinfection of hot water systems is critical in reducing the incidence of disease outbreaks caused by pathogenic bacteria. Electrochemical disinfection (ED) has been identified as an economical, low-maintenance, and chemical-free alternative in the fight against waterborne pathogenic microorganisms. It also provides the residual disinfection needed to inactivate the planktonic bacteria released by the biofilm. The work presented here includes fundamental small-scale laboratory optimization experiments in a flask where platinum-coated electrodes were immersed in 3.5 L of tap water contaminated with *Escherichia coli* (NCT10418) with an initial population density between $3 \times 10^5$ and $1.6 \times 10^5$ colony forming units/mL (CFU/mL) or *Legionella pneumophila* serogroup 1 (NCTC12821) ranging from 180 to 244 CFU/mL. Voltage, electrode area, interelectrode distance, spiking time, volume of contaminated water, and mixer speed were varied to determine the optimal geometrical and operational requirements needed to kill bacteria. Experimental results indicate ED to be an effective control method, with a >4-log inactivation of *E. coli* and a >5-log inactivation of *Legionella* in 10 and 45 min, respectively, at a current density of $\approx 4$ mA/cm$^2$. The findings of the flask experiments were translated into real-world conditions by evaluating the long-term performance of an optimized ED prototype device installed in the hot water recirculation system of a small-size healthcare center building. The results showed that ED is effective at minimizing pathogen contamination of the hot water distribution system from initial values, with total bacteria levels and *Pseudomonas* species being reduced in all of the samples over a 15-month period following activation of the ED device. **DOI: 10.1061/(ASCE)EE.1943-7870.0001134.** 

**Author keywords:** Electrochemical disinfection; Water treatment; Hot water systems; *Escherichia coli*; *Legionella pneumophila*; *Pseudomonas* species.

## Introduction

Failure to control pathogens in systems storing water has often been associated with disease outbreaks. In high-risk buildings such as hospitals, universities, schools, leisure centers, nursing homes, and hotels, users in contact with water sprays from showers and spas may get Legionnaires' disease in the absence of adequate control measures. The cost of monitoring *Legionella* in hot water (HW) systems of United Kingdom nondomestic building stock has been estimated to be £140 million per annum (Cossali et al. 2012) with the number of suspected cases of Legionnaires' disease continually increasing in the last 15 years (ECDC 2011). Possible explanations for this trend include improvements in the detection of *Legionella*, an increase in the number of human-made environments favorable to bacterial growth, and inadequate control. Hot water distribution systems are associated with 25% of known Legionnaires' disease outbreaks in the United Kindgom (HSL 2012), but the true number is probably greater as individual cases are more likely to be missed or misdiagnosed. Hot water is typically disinfected with biocides (such as chlorine and chlorine by-products) or by storing water at 60°C. Although these methods are effective, the presence of dead-legs, underused taps, and irregular use/occupation of buildings can cause water stagnation and stratification, promoting the growth of biofilms where bacterial communities comprised of a variety of species arise and become more persistent and resistant to disinfection.

The main disadvantage of water chlorination is the generation of toxic by-products such as trihalomethanes (THMs) and haloacetic acids (HAAs) (Goodburn and Wallace 2013; Sapers 2001). Chlorine action is short-lived as it typically dissipates overnight at high water temperatures or in the presence of organics, thus reducing its effectiveness in controlling the bacteria dispersed from the biofilms. Chlorine dioxide is hazardous, and care must be exercised in its transport, storage, and use. Temperature control is associated with high running costs, considerable $CO_2$ emissions, and risk of scalding, and—despite copper–silver ionization having proved effective—the use of elemental copper as a biocide has been banned in Europe from February 2013 (Health and Safety Executive 2012). Other alternative methods of disinfection such as

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ultraviolet (UV) lights, radiation, ultrasound, and the use of filters do not provide residual disinfection.

## Electrochemical Disinfection

In recent years, considerable attention has been paid to electrochemical disinfection (ED), as a promising method for the control of hot water systems (Delaedt et al. 2008; Furuta et al. 2004; Nakajima et al. 2004); as ED provides both primary and residual disinfection, it is environmentally friendly, inexpensive, and easy to operate and maintain.

In ED, current is applied to electrodes immersed directly into the contaminated water or in a bypass. Inactivation of bacteria is achieved by the simultaneous action of electrochemically formed oxidants such as free chlorine and ozone, electric fields, and electrosorption of the microorganisms on the electrode surface (Ghernaout and Ghernaout 2010). Oxidants cause cellular damage while 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 electrochemical inactivation of various microorganisms is well documented (Bergmann et al. 2002; Drees et al. 2003; Fang et al. 2006; Kerwick et al. 2005; Li et al. 2004; Liang et al. 2005). 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.

During the past 20 years, a considerable amount of literature has been published on the use of titanium-based electrodes coated with lead dioxide (Chen et al. 2009), mixed metal oxide (Sarkka et al. 2008), platinum, iridium oxide (Kraft et al. 1999a, b), ruthenium oxide, zirconium dioxide, or titanium dioxide (Delaedt et al. 2008; Diao et al. 2004; Bergmann and Koparal 2005). These electrodes combine the anticorrosion qualities of titanium with the enhanced electrochemical properties of the coating. The previously mentioned studies report the efficacy of each distinct electrolytic device investigated, but are limited to having used either one or two electrode materials, making it difficult to compare the effect of materials on the generation of oxidants.

However, in a study comparing the performance of $IrO_2$ and Pt coated electrodes, Kraft et al. (1999b) found that, at lower current densities, Pt electrodes produced more active chlorine and that their lifetime was not decreased by regular polarity reversal. In their experiment, the Pt electrodes continued to be in use after 12 months. Therefore, Pt-coated titanium electrodes were selected for application in hot water systems, where it would be cost-prohibitive to utilize materials with short life spans.

Trihalomethanes, haloacetic acids, chlorite, and bromate are the disinfections by-products (DBPs) for which regulations have been established as they may pose known 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, and thus the formation of disinfection by-products is expected. In a recent laboratory study, Bergmann et al. (2014) found that THM formation in inline electrolysis was comparable in concentration to chemical chlorination, confirming ED to be a more advantageous disinfection method, given that disinfectants are generated on-site, thus eliminating the handling, storage, and transport of hazardous chemicals.

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 (Bergmann and Koparal 2005; Jeong et al. 2007, 2009; Kraft et al. 1999a; Polcaro et al. 2007). 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 authors' knowledge, a systematic study of the effect of the cell geometrical parameters on bacteria inactivation rate is lacking. This paper presents the findings of a small-scale laboratory optimization experiments in a flask where platinum-coated electrodes were immersed in 3.5 L of tap water contaminated either with *Escherichia coli* (NCT10418) or *Legionella pneumophila* serogroup 1 (NCTC12821). The effect of voltage, area, and spacing of electrodes, volume of contaminated water, spiking time, and speed of mixing on the rate of bacteria elimination was investigated in repeated experiments. The electrode configuration was optimised to achieve most-effective killing of bacteria in solution, for the purpose of designing an ED device that could be used in the hot water recirculation system of a building.

While several studies, using a range of cell configurations, have also confirmed ED to be effective in eliminating various pathogens under controlled laboratory conditions (Delaedt et al. 2008; Fang et al. 2006; Furuta et al. 2004; Jeong et al. 2007; Kerwick et al. 2005; Patermarakis and Fountoukidis 1990; Polcaro et al. 2007; Sarkka et al. 2008), long-term real-world evaluations of the efficacy of electrolytic devices to control bacteria are currently lacking. To the authors' knowledge, this study is the first to report long-term monitoring of an electrolytic prototype device, named Protex!, installed in the hot water recirculation system of a healthcare center building. It is important to study the effect of ED treatment in a real-world scenario because differences in pipe materials, temperature variations, and number of dead-legs and under-used taps, in addition to the presence of other flora or amoeba, may increase the persistence of the pathogenic bacteria, which cannot be simulated in a laboratory.

## Material and Methods

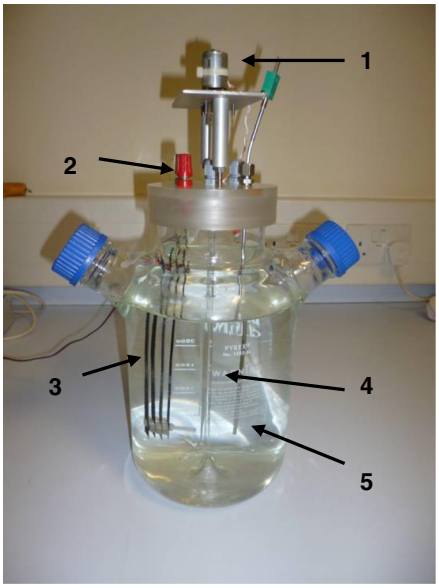
### *Flask Experiments*

#### Experimental Apparatus

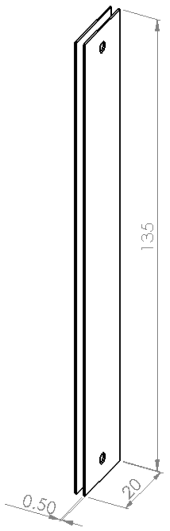
The experimental set up was comprised of a 3.5 L cell culture Bellco (Bellco, Vineland) Pyrex glass flask and a purpose-built lid fitted with a variable-speed electric glass stirrer used to maintain the water homogeneity during the entire experiment [Fig. 1(a)]. Electrolysis was performed using two platinized (platinum thickness was between 2 and 5 $\mu$m) titanium mesh electrodes with a thickness of 0.5 mm, both for cathode and anode (Metakem, Usingen, Germany). The geometrical surface area of each electrode immersed in the tap water was 27 $cm^2$ (135 × 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:

$$J = \frac{I}{A \times G} = \frac{\text{Current}}{\text{Projected area} \times \text{Surface factor}}$$

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




1 Motor
2 Electrode terminals
3 Electrode stack
4 Glass stirrer
5 Thermocouple




(a) (b)

**Fig. 1.** (a) Experimental flask; (b) schematic of electrode sheet (dimensions in mm)

restrictions, while the electrochemically effective area was increased by stacking electrode sheets as shown in Fig. 1(b). The electrode stack consisted of between two and five identical titanium mesh electrodes. A water temperature of 30°C was selected because it did not affect the survival of *E. coli*, whereas in control experiments at 35 and 40°C, *E. coli* could not be detected by culture. The apparatus was connected to a direct current (DC) power supply (T.T.I. CPX400A, Thurlby Thandar Instruments Ltd., Huntingdon, U.K.) and a 6 or 12 V voltage applied to give current densities ranging from 2.8 to 13.5 mA/cm$^2$ depending on the area employed. The experimental parameters can be seen in Table 1.

### Culture Preparation

The bacteria used in the experiments were *E. coli* (nonpathogenic strain) NCT10418 and *Legionella pneumophila* (serogroup 1) NCTC12821. *E. coli* is the most commonly used indicator organism to detect fecal contamination in process wash water while 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 (CM001B, Oxoid, Basingstoke, U.K.) for 18 h at 37°C in a Gallenkamp shaking orbital incubator at 180 rpm in a Gallenkamp (Loughborough, U.K.) shaking orbital incubator. A total of 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 h, at which time the bacteria were in the exponential stage of growth. The initial population of each experiment ranged from $3 \times 10^5$ to $1.6 \times 10^5$ CFU/mL. *Legionella 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 $-20 \pm 5$°C. Lenticules were allowed to reach room temperature for 10 min before use, after which they were rehydrated in a 1 mL volume of Maximum Recovery Diluent (Oxoid CM0733), allowed to stand for 15 min, and shaken vigorously for 5 min. The initial population of each experiment ranged from 180 to 244 CFU/mL.

### Experimental Procedure

The experimental flask was filled with 3.5 L of tap water after 1 min of flushing to ensure that the sample was part of the main body of water. The water's chemical composition prior to autoclaving is given in Table 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 $30 \pm 1$°C by immersing the experimental flask in a water bath (Type SB1, 1.5 kW, Grant Instruments, Royston, U.K., 1.5 kW).

After 10 min stirring, either an aliquot of *E. coli* suspension (equivalent to $5.6 \times 10^8$–$1.05 \times 10^9$ CFU) or *Legionella* bacteria recovered from four lenticules (equivalent to $11.1 \times 10^5$ CFU) were introduced into the flask and the spiked water was mixed for a further 10 min prior to commencement of electrolysis. In the experiments with *E. coli*, 0.1-mL samples were taken at timed

**Table 1.** Geometrical and Operational Experimental Parameters

| Parameter | Values tested |
|---|---|
| Voltage | 6 and 12 V |
| Electrode distance | 2 and 5 mm |
| Electrode area | 48.6, 97.2, 145.8, and 243 cm$^2$ |
| Stirrer speed | 2,000 and 3,000 rpm |
| Temperature | Constant at $30 \pm 1$°C |

**Table 2.** Chemical Composition of the Tap Water Used in the Laboratory Experiments (Data from Affinity Water 2014)

| Property | Mean value |
|---|---|
| pH | 7.4 |
| Chloride | 55 mgCl/L |
| Sulphate | 67 mg$SO_4$/L |
| Calcium | 130 mgCa/L |
| Sodium | 32.7 mgNa/L |
| Total hardness | 325 mg$CaCO_3$/L |
| Alkalinity | 249 mg$HCO_3$/L |

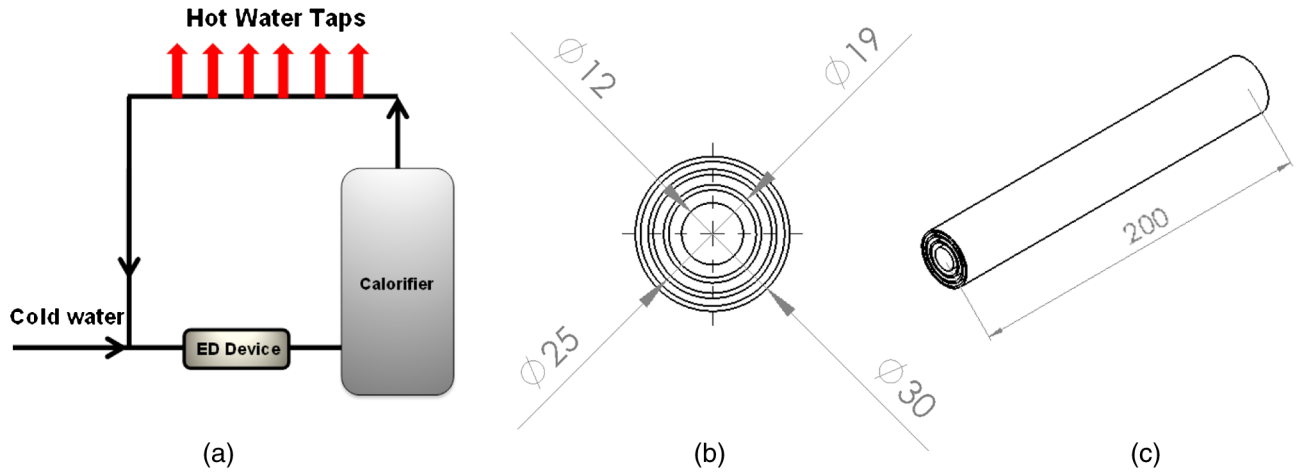



**Fig. 2.** Chiltern Multiple Sclerosis Centre installation: (a) schematic of the installation; (b) perspective of the electrode package (dimensions in mm); (c) isometric view of the electrode package

intervals of 5 min and diluted 1/10 and 1/100 in sterilized deionized water. Then, 0.1-mL replicates of each diluted sample were plated in triplicates onto nutrient agar (CM0003, Oxoid, Basingstoke, U.K.). The number of viable cells was determined by counting the colonies after 18–24 h incubation time at 37°C.

In the experiments using *Legionella*, 0.5 mL of the sample were plated in triplicates directly on ready-poured Buffered Charcoal Yeast Extract agar plates (BCYE, PO5072A, Oxoid, Basingstoke, U.K.). 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 h 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 (7100, Palintest, Gateshead, U.K.) and tablet reagents [diethyl-*p*-phenylenediamine (DPD 1 and 3)]. Control measurements for disinfectants were performed prior to treatment during each experiment.

For data analysis, the log of the survival ratio ($N/N^0$) was estimated as a function of sampling time by nonlinear regression modeling (logit function, least squares). All statistical analysis was performed using *Prism* version 6.04 for Windows.

### Building Installation

An electrolytic cell was designed (using the geometrical and operational data acquired from the flask experiments) to be installed in the hot water recirculation system of the Chiltern Multiple Sclerosis centre located in Wendover, Buckinghamshire, U.K. 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 premixed with cold water to achieve the temperature of 45°C. Because the water hardness in the area is 348 mg$CaCO_3$/L, the HW system has been fitted with an ion exchange resin water softener, replacing calcium and magnesium ions with sodium ions.

The installation is such that the cold water entering the system and the water in the recirculation system are both disinfected by the device before entering the hot water calorifier, as shown in Fig. 2(a). This approach ensured that any bacteria naturally present in the cold water coming into the system would be subjected to ED inactivation, and that any residual disinfectants would prevent system recontamination from dead-legs and underused pipes.

Hydrogen evolution during the electrolysis of water causes the formation of calcareous deposits on the cathode surface due to the increase of pH, while in the vicinity of the anode, the opposite occurs, causing a decrease in pH. For the purpose of eliminating scale deposits, the device has been designed so that the electrodes' polarity is reversed every 10 min. The device is operated in potentiostatic mode to limit the voltage applied to the electrodes to 5 V to give current densities ranging from 9.4 to 11 mA/cm$^2$. When reversing polarity, an increase in current to 11 mA/cm$^2$ is observed immediately after reversal, followed by a steady decrease to 9.4 mA/cm$^2$ within the first 2 min of the cycle.

The electrode projected area was increased to 351.68 cm$^2$ and the reactor volume 0.726 L. The distance between the electrodes was maintained within 2 and 2.5 mm depending on manufacturing tolerances. The electrode package consisted of a 12-mm rod surrounded by concentric 19, 25, and 30 mm outside diameter cylindrical electrodes, 200 mm in length and with thickness of 1 mm. The electrodes were made of platinized titanium with platinum thickness between 2–5 $\mu$m (Metakem, Usingen, Germany). The schematic of the configuration can be seen in Figs. 2(b and c), and the chemical composition of the tap water is given in Table 3. The chlorine generated by the ED device increases with the increase of chloride concentration (Kraft et al. 1999a). Hence, lower quantities of chlorine are expected in the building application due to the lower chloride content.

The building was opened in October 2012 and the hot water recirculation system was operated as recommended in the approved

**Table 3.** Chemical Composition of the Water in the Wendover Area (Data from Thames Water 2013)

| Property | Mean value |
|---|---|
| pH | 7.3 |
| Chloride | 20.9 mgCl/L |
| Sulphate | 16.1 mg$SO_4$/L |
| Sodium | 10.8 mgNa/L |
| Total hardness | 287.7 mg$CaCO_3$/L |

code of practice (ACoP) by the U.K. Health and Safety Executive (2013) to prevent *Legionella* proliferation. HW was stored at 60°C and distributed at 50°C, and temperature monitoring was carried out monthly.

Starting in May 2013 1-L water samples were taken at monthly intervals from each of four sampling locations into sterile plastic containers with screw-top lid. Preflush samples were collected immediately as soon as the tap was opened, representing the water held in the tap and pipework local to the tap. Postflush samples were collected after the water was run for 1 min, representing the quality of the water supplied in the recirculation loop (BSI 2008). Water samples were taken in labeled bottles containing sodium thiosulphate in order to neutralize residual oxidants. They were subsequently stored at ambient temperature and processed within 24 h at Latis Scientific Laboratory in Barbican, London. Each sample was analyzed to determine the total number of microorganisms (Total Viable Counts or TVCs) and the density of *Legionella*, *Pseudomonas* species, and *Pseudomonas 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*, Parts 7 and 8 (U.K. Environment Agency 2010, 2012).

## Results and Discussion

### Flask Experiment

A viability test (no electrochemical disinfection) over 2 h confirmed that the *E. coli* concentration remained constant for 60 min, after which viability started to decline. After 120 min, 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 min showed the bacteria viability remained stable.

### Effect of Voltage/Current Applied on E. coli Inactivation

A rapid inactivation rate, seen in Fig. 3(a) was achieved with voltage/current increase, consistent with previous reports (Gomez-Lopez et al. 2013; Patermarakis and Fountoukidis 1990). More electrons are transferred and more oxidants are generated as current density increases. Complete inactivation was achieved by 105 min at 6 V and in 35 min at 12 V, (with interelectrode 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:

$$Q = I \times t = \text{current} \times \text{time}$$

Higher input of electric charge $Q$ generates a higher amount of free chlorine, as shown in Fig. 3(b). After 20 min, 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 also 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 already present organic and inorganic components and

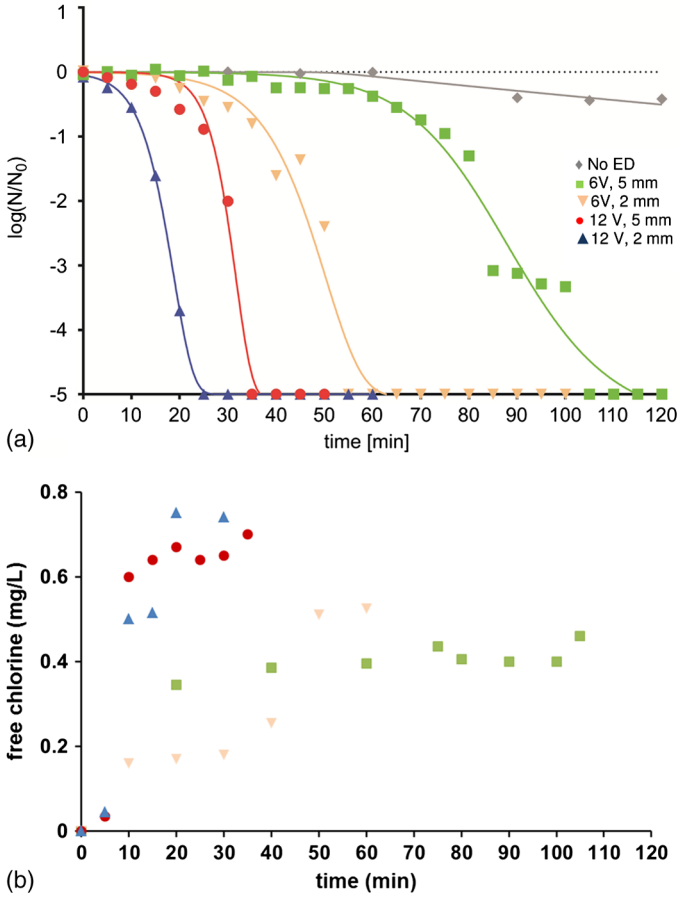



**Fig. 3.** (a) Inactivation rate of *E.coli*; (b) free chlorine production rate in dependence on the applied voltage/current in tap water at 30°C, with electrode area of 48.6 $cm^2$ and interelectrode spacing of 2 and 5 mm

suspended particles in water (Kraft et al. 1999b). This phenomenon (defined as the chlorine demand of the tap water) varies depending on the water quality, water temperature, and flow velocities. Therefore, at 6 V and a current density of 4.3 $mA/cm^2$, the chlorine produced in the tests reported here 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 Fig. 3(b), confirming the results obtained by Kraft et al. (1999b).

### Effect of Electrode Spacing on E. coli Inactivation

Increased inactivation at both 6 and 12 V voltage was achieved with a constant area by reducing the interelectrode spacing from 5 to 2 mm as shown in Fig. 3(a). At 6 V, the *E. coli* was inactivated in 55 min with 2-mm spacing compared to 105 min 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.

### *Effect of Electrode Area on E. coli Inactivation*

The effects of an incremental change in electrode area on bacteria elimination area and on free chlorine production are shown in Figs. 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, 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 min compared to the 105 min 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 12 V applied are similar, as the linear killing rate has a steeper slope with the increased area; 100% inactivation is achieved in 35 min with the single area and in 20 min with the triple area [Fig. 4(a)]. The amount of chlorine generated with the single area and 12 V applied was in the region of 0.7 mg/L while 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

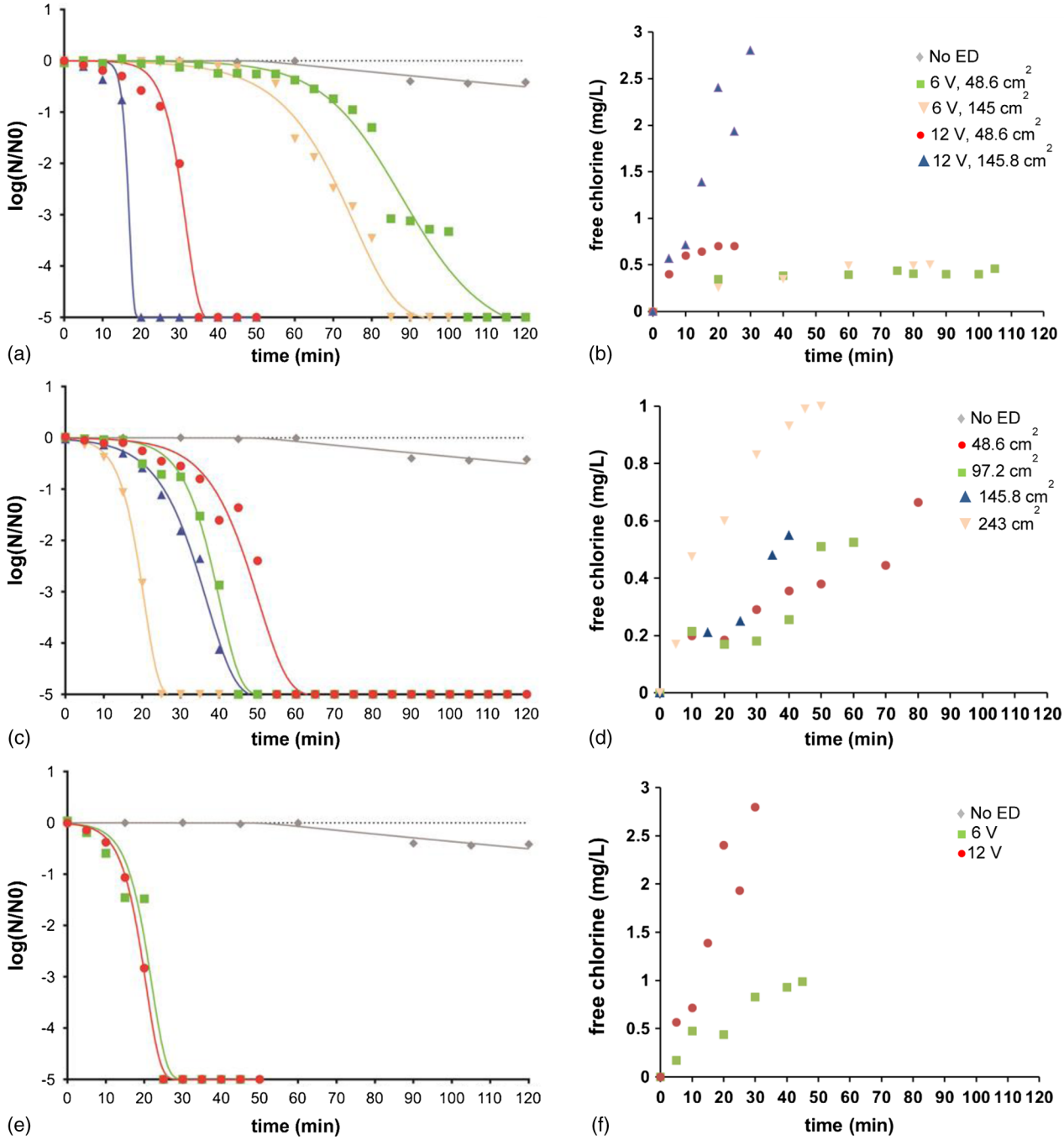



**Fig. 4.** (a) Inactivation rate of *E.coli* in dependence on electrode area; (b) free chlorine production rate in dependence on electrode area in tap water at 30°C with 5-mm electrode spacing; (c) inactivation rate of *E.coli* in dependence on electrode area; (d) free chlorine production rate in dependence on electrode area in water at 30°C with 6 V and 2-mm electrode spacing; (e) comparison of the inactivation curves when applying 1 A; (f) free chlorine production rate when applying 1 A

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 Figs. 4(c and d). Successive increase in the area of the electrode resulted in a stepwise 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.

Notably, the 25 min killing time achieved at 12 V with the 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 [Fig. 4(e)]. Indeed, with a current density of 4 mA/cm$^2$ at 6 V generating free chlorine levels of 1 mg/L, the performance of the electrolytic cell is analogous to that when using 12 V, with current density of 11 mA/cm$^2$ producing 3 mg/L free chlorine [Fig. 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.

### 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 upstream. Therefore, the experimental procedure was changed slightly to simulate what happens in a recirculation system. The experimental flask was spiked with bacteria, either prior to, and 5 or 10 min after turning on the electrodes. Bacteria were inactivated rapidly once introduced into the water that had previously undergone ED, as expected (Fig. 5). Measurements taken 5 min 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 min after electrolysis, and 15% when spiking occurred 10 min after electrolysis, confirming that the water had accumulated more oxidants over time, which where necessary for bacterial elimination.

### 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 interelectrode spacing of 2 mm. As depicted in Fig. 6(a), a >5-log inactivation was achieved in 15 min with 2.5 L, and in 25 min with 3.5 L of water, with a similar starting density of bacteria. At 10 min, only 7% of the bacteria are viable with the lower volume, compared to 42% in the larger volume. Fig. 6(b) confirms that with a lower volume, the concentration of free chlorine increases more rapidly, suggesting that the size of the cell and the water residence time are important parameters to take into consideration during the design process.

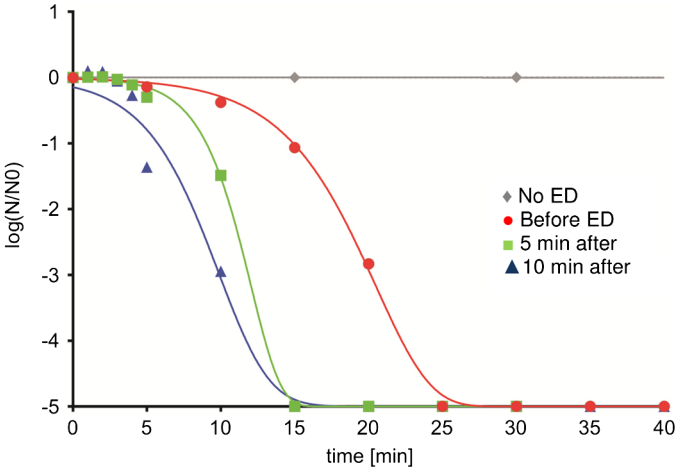



**Fig. 5.** Inactivation rate of *E.coli* in dependence on the different spiking time in tap water at 30°C with 6 V, 2-mm electrode distance, and 243 cm$^2$ area

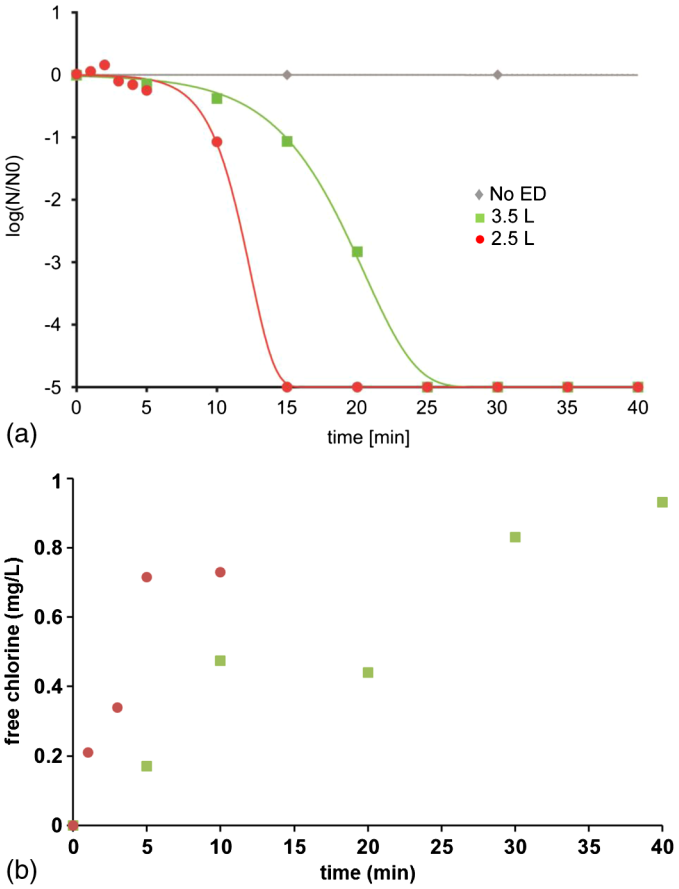



**Fig. 6.** (a) Inactivation rate of *E.coli*; (b) free chlorine production rate in dependence on the contaminated water volume in tap water at 30°C at 6 V with electrode area of 243 cm$^2$ and interelectrode spacing of 2 mm

### Enhancing Mixing

The final operational parameter assessed in this study was the effect of increasing mixing in the flask; the results are shown in Fig. 7(a) and are obtained using 6 V, electrode area of 243 cm$^2$,

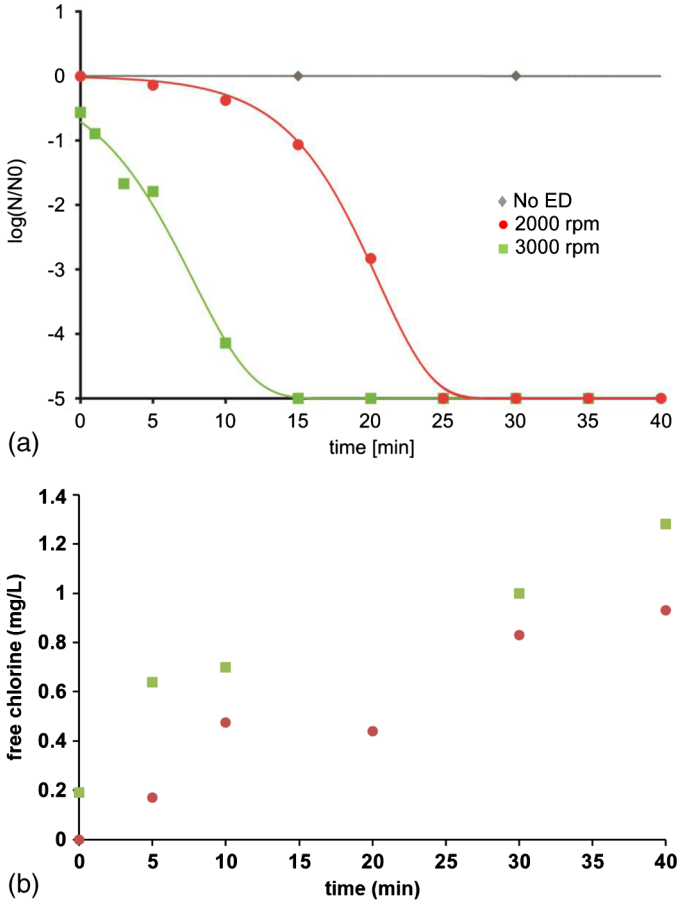



**Fig. 7.** (a) Inactivation rate of *E.coli* in dependence; (b) free chlorine production rate in dependence on the mixing speed in tap water at 30°C at 6 V with electrode area of 243 $cm^2$ and interelectrode spacing of 2 mm

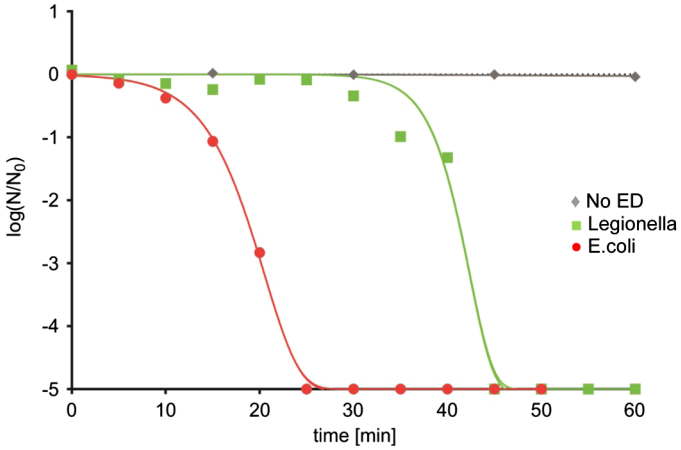



**Fig. 8.** Comparison between *Legionella* pneumophila and *E. coli* inactivation curves in tap water at 30°C at 6 V with electrode area of 243 $cm^2$, interelectrode spacing of 2 mm, and 3.5 L volume

interelectrode spacing of 2 mm, 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 revolutions per minute (rpm), with only 2% of bacteria remaining viable after 5 min. 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 helps the ED process meet the water chlorine demand more rapidly. After 5 min, the free chlorine concentration is three times higher [Fig. 7(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.

### Comparison between Legionella and E. coli Inactivation

Fig. 8 compares the inactivation curves of *E. coli* and *Legionella pneumophila* in tap water at 30°C, 6 V applied, electrode area of 243 $cm^2$, interelectrode spacing of 2 mm, 3.5 L volume, and 2,000 rpm stirring speed. A >5-log inactivation of *Legionella* was achieved after 45 min compared to 25 min for *E. coli*, demonstrating that *Legionella* is more resilient. A 25 min time lag can be observed in the *Legionella* inactivation curve despite the chlorine demand of the water having been fulfilled. This result indicates that *Legionella* requires higher levels of disinfectants for inactivation to occur, as reported previously (Kuchta et al. 1983). In the present experiment the level of free chlorine after 5 min was 0.6 mg/L while at 30 min (when the levels of *Legionella* start to diminish) the free chlorine was 1 mg/L, suggesting that these are the values necessary to trigger 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 *Legionella* and other more resilient bacteria.

### Building Monitoring

The sampling locations and the samples types taken at each outlet are shown in Table 4. Also shown are the TVCs, *Pseudomonas* species and *Pseudomonas aeruginosa* results of the samples taken at the four location points prior to turning on the ED device in May 2013. Samples labeled with not tested (NT), were not processed due to administration errors at the laboratory. Although *Legionella* bacteria were not found, high concentrations of *Pseudomonas* species were recorded in all samples, and *Pseudomonas aeruginosa* was detected in the kitchen postflush sample. TVCs were also found to be relatively high. The analysis revealed *Pseudomonas* in both the postflush samples taken from Tap A and the kitchen, suggesting contamination in the recirculation loop, whilst the high preflush results in Tap B suggested a local outlet problem. High bacteria counts were unanticipated due to the fact that the building was relatively new, temperatures were maintained across the whole hot water system, and a flushing regime of underused outlets was carried out regularly.

Figs. 9 and 10 present the average preflush and postflush sample results for TVCs and *Pseudomonas* species, respectively. The survival ratio is calculated by dividing the monthly mean results by the mean results in May 2013. On average, there was a marked 77–94% (preflush) and 95–99% (postflush) reduction in the number of counts measured in water samples once ED has started. There was a declining trend in the amount of bacteria measured in both the preflush and postflush samples over time, with commencement of ED. The total bacteria counts in the same tap were up to 60-fold higher in preflush samples than in postflush 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 premixed with cold water to achieve 45°C. Bacteria levels in the recirculation loop have been

**Table 4.** Results from the First Sampling Routine (May 2013) Prior to Electrochemical Disinfection

| Sample locations | Sample type | TVCs 22°C 72 h CFU/mL | TVCs 37°C 48 h CFU/mL | *Pseudomonas* spp. 30°C CFU/100 mL | *Pseudomonas aeruginosa* CFU/100 mL |
|---|---|---|---|---|---|
| Tap A, toilets | Preflush | NT | NT | NT | NT |
| Tap A, toilets | Postflush | 8,400 | 8,400 | 11,000 | 0 |
| Tap B, toilets | Preflush | 15,000 | 9,400 | 13,000 | 0 |
| Kitchen | Postflush | 8,400 | 7,400 | 11,000 | 11 |

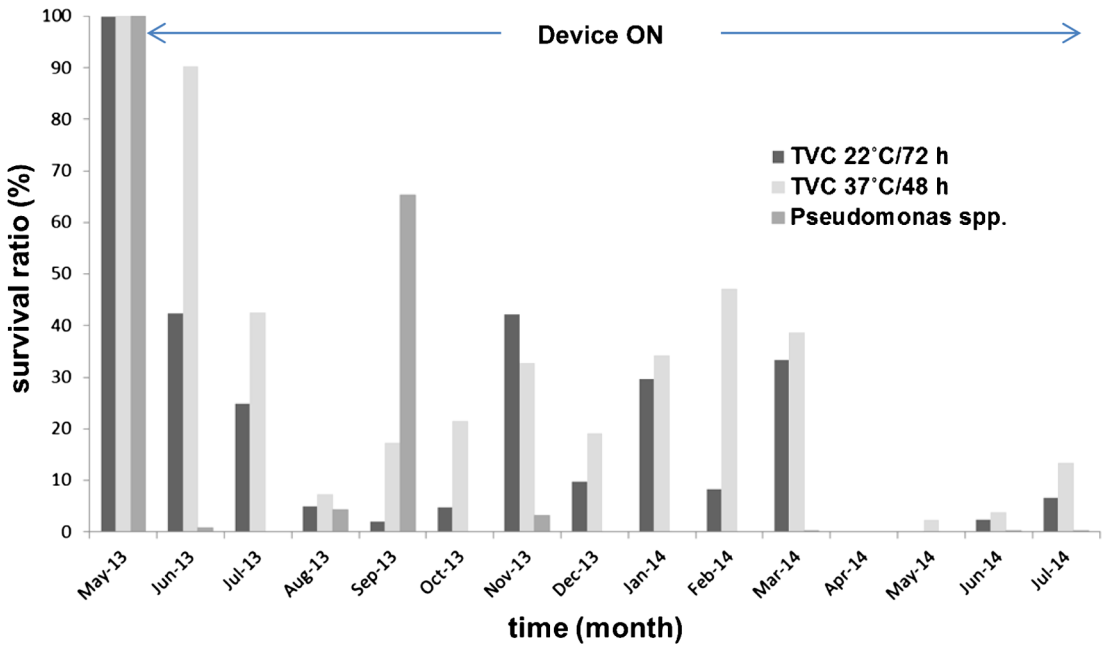



**Fig. 9.** Average of preflush results for *Pseudomonas* species grown at 30°C, TVCs grown at 22°C for 72 h, and TVCs grown at 37°C for 48 h

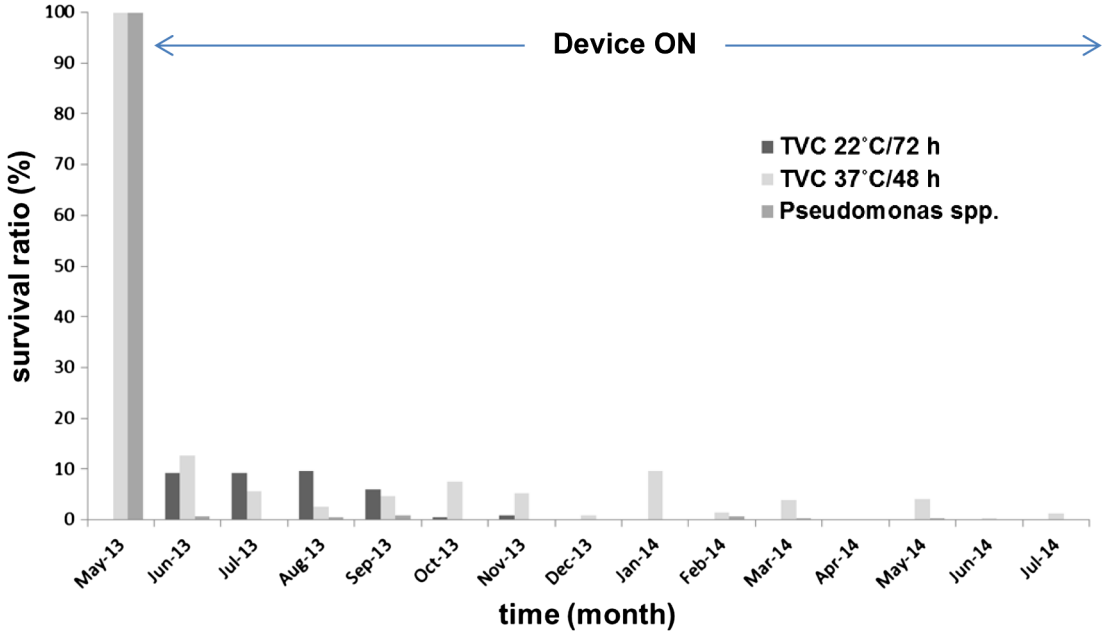



**Fig. 10.** Average of post-flush sample results for *Pseudomonas* species grown at 30°C, TVCs grown at 22°C for 72 h, and TVCs grown at 37°C for 48 h

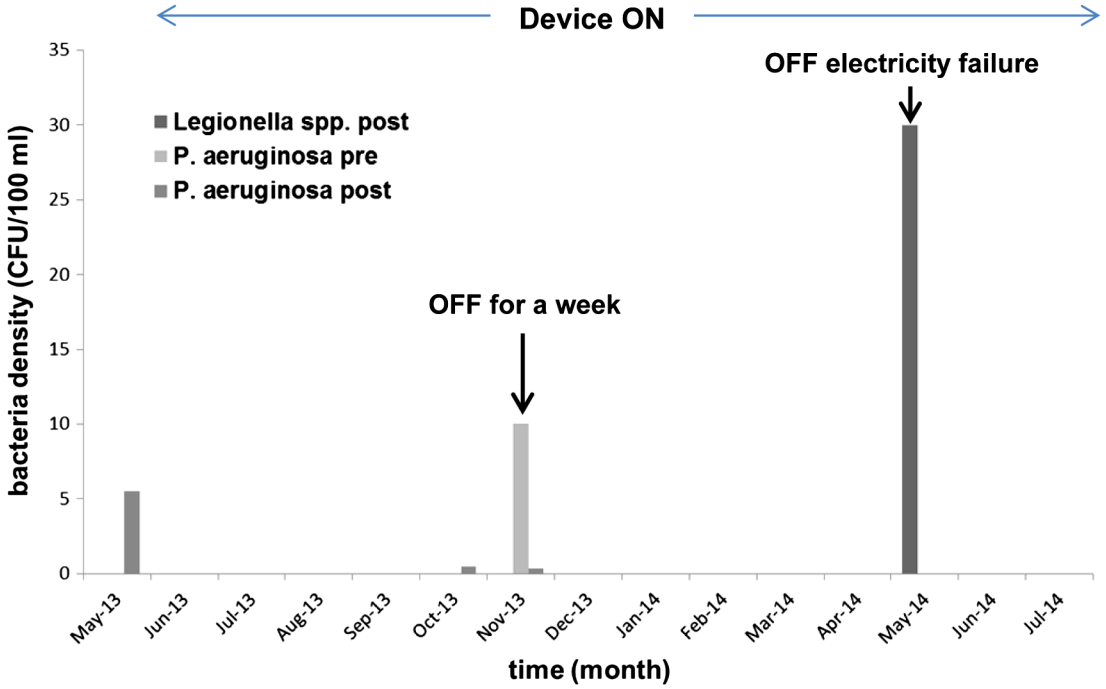



**Fig. 11.** Pathogens detected during the monitoring period including *Legionella* species and *Pseudomonas aeruginosa* (CFU/100 mL)

reduced significantly by ED and these levels are maintained throughout the monitoring period (Fig. 10).

Despite almost continuous operation from May 2013–June 2014, the device was purposefully 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 an 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 preflush and postflush samples after the ED was turned off, pathogenic bacteria (*Legionella* species and *Pseudomonas aeruginosa*) were detected above control limits at these times (Fig. 11).

One possible weakness of this study is that monitoring of the device performance was carried out in one building only. Therefore, the results cannot be generalized to all buildings given the diversity of hot water distribution systems. However, the laboratory results provide proof of the efficacy of ED in vitro and the tools to adapt the device to the building conditions, while the field monitoring provides confimation of the efficacy of the device in an individual building. The results of this initial evaluation of ED should be followed by controlled long-term studies in other buildings and by confirmatory reports from different buildings as suggested by Stout and Yu (2003).

## Conclusions

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

The small-scale laboratory experiment results contributed to the understanding of how geometrical and operational parameters influence the inactivation rate of *E.coli*, the common indicator of water contamination.

These findings were then applied in the design of the electrolytic prototype cell to be installed in the hot water distribution system of a medical center, making this study the first reported long-term monitoring of an ED device for pathogen control in the hot water recirculation system of a commercial building.

This investigation indicates that ED has potential for use in the disinfection of hot water systems and, given these successful results, the authors recommend the long-term monitoring of ED devices in other buildings. It is also advisable to measure chlorine levels at the outlet of the device, monitor flow rate, investigate DBPs formation, and monitor the performance of the device at the reduced temperature of 45°C with the due precautions in place. In a previous study, the authors estimated that reducing the hot water temperature from 60°C to 45°C in commercial buildings could generate savings of £62 million (Cossali et al. 2012). Further reductions in $CO_2$ emissions can also be achieved by heating the water with green technologies that become more efficient if one demands water at a lower temperature. The implementation of ED in the hot water recirculation of buildings may also result in waste water with lower levels of bacteria and lower levels of chemicals, enabling the reuse of the greywater for flushing toilets and landscape irrigation, further reducing energy and the use of chemicals.

The results obtained from this study also point toward the potential use of ED in other low-temperature water systems, including food production (e.g., the disinfection of quenching water for food processing and disinfection of the mains water used into fresh-cut washing systems) and society at large (e.g., long-term maintenance of water quality in hot water tubs and birthing pools).

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## References


Affinity Water. (2014). "Water quality report for Uxbridge/Heathrow, period 01-Jan-2014 to 31-Dec-2014." ⟨https://www.affinitywater.co.uk/my-water.aspx⟩ (Mar. 15, 2016).

Bergmann, H., Iourtchouk, T., and Hartmann, J. (2014). "On the THM formation during inline electrolysis—First systematic laboratory-scale studies." *Chem. Eng. Trans.*, 41, 127–132.

Bergmann, H., Iourtchouk, T., Schops, K., and Bouzek, K. (2002). "UV irradiation and direct electrolysis—Promising methods for water disinfection." *Chem. Eng. J.*, 85(2), 111–117.

Bergmann, H., and Koparal, S. (2005). "The formation of chlorine dioxide in the electrochemical treatment of drinking water for disinfection." *Electrochim. Acta*, 50(25), 5218–5228.

Bergmann, H., Tansu Koparal, A., Savas Koparal, A., and Ehrig, F. (2008). "The influence of products and by-products obtained by drinking water electrolysis on microorganisms." *Microchem. J.*, 89(2), 98–107.

BSI (British Standards Institute). (1998). "Water quality. Microbiological methods: Detection and enumeration of Legionella." *BS 6068-4.12: 1998/ISO 11731:1998*, London.

BSI (British Standards Institute). (2008). "Sampling for Legionella bacteria in water systems-code of practice." *BS 7592:2008*, London.

Chen, Q., Ai, S., Li, S., Xu, J., Yin, H., and Ma, Q. (2009). "Facile preparation of $PbO_2$ electrode for the electrochemical inactivation of microorganisms." *Electrochem. Commun.*, 11(11), 2233–2236.

Cossali, G., Ratcliffe, M. S., Routledge, E. J., Karayiannis, T. G., and Fielder, J. (2012). "The cost of Legionellosis and technical ways forward." *CIBSE Technical Symp.*, Chartered Institution of Building Services Engineers (CIBSE), London.

Delaedt, Y., et al. (2008). "The impact of electrochemical disinfection on Escherichia coli and Legionella Pneumophila in tap water." *Microbiol. Res.*, 163(2), 192–199.

Diao, H. F., Li, X. Y., Gu, J. D., Shi, H. C., and Xie, Z. M. (2004). "Electron microscopic investigation of the bactericidal action of electrochemical disinfection in comparison with chlorination, ozonation and Fenton reaction." *Process Biochem.*, 39(11), 1421–1426.

Drees, K. P., Abbaszadegan, M., and Maier, R. M. (2003). "Comparative electrochemical inactivation of bacteria and bacteriophage." *Water Res.*, 37(10), 2291–2300.

ECDC (European Centre for Disease Prevention and Control). (2011). "Legionnaires' disease in Europe 2010." Stockholm, Sweden.

Fang, Q., Shang, C., and Chen, G. (2006). "MS2 inactivation by chloride-assisted electrochemical disinfection." *J. Environ. Eng.*, 10.1061/(ASCE)0733-9372(2006)132:1(13), 13–22.

Furuta, T., Tanaka, H., Nishiki, Y., Pupunat, L., Haenni, W., and Rychen, Ph. (2004). "Legionella inactivation with diamond electrodes." *Diamond Relat. Mater.*, 13(11), 2016–2019.

Ghernaout, D., and Ghernaout, B. (2010). "From chemical disinfection to electrodisinfection: The obligatory itinerary?" *Desal. Water Treat.*, 16(1–3), 156–175.

Gomez-Lopez, V. M., Gobet, J., Selma, M. V., Gil, M. I., and Allende, A. (2013). "Operating conditions for the electrolytic disinfection of process wash water from the fresh-cut industry contaminated with E. coli O157:H7." *Food Control*, 29(1), 42–48.

Goodburn, C., and Wallace, C. A. (2013). "The microbiological efficacy of decontamination methodologies for fresh produce: A review." *Food Control*, 32(2), 418–427.

HSE (Health and Safety Executive). (2012). "What is the issue over copper-ionisation in legionella control?" ⟨http://www.hse.gov.uk/legionnaires/faqs.htm#silver-copper-systems⟩ (Jun. 13, 2013).

HSE (Health and Safety Executive). (2013). "The approved code of practice for the control of legionella bacteria in water systems (L8)." ⟨http://www.hse.gov.uk/pubns/books/l8.htm⟩ (Jun. 16, 2013).

HSL (Health and Safety Laboratory). (2012). "Legionella outbreaks and HSE investigations: An analysis of contributory factors." Buxton, U.K.

Jeong, J., Kim, C., and Yoon, J. (2009). "The effect of electrode material on the generation of oxidants and microbial inactivation in the electrochemical disinfection processes." *Water Res.*, 43(4), 895–901.

Jeong, J., Kim, J. Y., Cho, M., Choi, W., and Yoon, J. (2007). "Inactivation of Escherichia coli in the electrochemical disinfection process using a Pt anode." *Chemosphere*, 67(4), 652–659.

Kerwick, M. I., Reddy, S. M., Chamberlain, A. H. L., and Holt, D. M. (2005). "Electrochemical disinfection, an environmentally acceptable method of drinking water disinfection?" *Electrochim. Acta*, 50(25), 5270–5277.

Kraft, A., et al. (1999a). "Electrochemical water disinfection. Part I: Hypochlorite production from very dilute chloride solutions." *J. App. Electrochem.*, 29(7), 859–866.

Kraft, A., Blaschke, M., Kreysig, D., Sandt, B., Schroder, F., and Rennau, J. (1999b). "Electrochemical water disinfection. Part II: Hypochlorite production from potable water, chlorine consumption and the problem of calcareous deposits." *J. App. Electrochem.*, 29(8), 895–902.

Kuchta, J. M., States, S. J., McNamara, A. M., Wadowsky, R. M., and Yee, R. B. (1983). "Susceptibility of Legionella pneumophila to chlorine in tap water." *App. Environ. Microbiol.*, 46(5), 1134–1139.

Li, X. Y., Diao, H. F., Fan, F. X. J., Gu, J. D., Ding, F., and Tong, A. S. F. (2004). "Electrochemical wastewater disinfection: Identification of its principal germicidal actions." *J. Environ. Eng.*, 10.1061/(ASCE)0733-9372(2004)130:10(1217), 1217–1221.

Liang, W., Qu, J., Chen, L., Liu, H., and Lei, P. (2005). "Inactivation of Microcystis aeruginosa by continuous electrochemical cycling process in tube using Ti/RuO2 electrodes." *Environ. Sci. Tech.*, 39(12), 4633–4639.

Nakajima, N., et al. (2004). "Evaluation of disinfective potential of reactivated free chlorine in pooled tap water by electrolysis." *J. Microbiol. Methods*, 57(2), 163–173.

Panizza, M., Michaud, P. A., and Cerisola, G. (2001). "Electrochemical treatment of wastewaters containing organic pollutants on boron-doped diamonds electrodes: Prediction of specific energy consumption and required electrode area." *Electrochem. Commun.*, 3(7), 336–339.

Patermarakis, G., and Fountoukidis, E. (1990). "Disinfection of water by electrochemical treatment." *Water Res.*, 24(12), 1491–1496.

Polcaro, A. M., Vacca, A., Mascia, M., Palmas, S., Pompei, R., and Laconi, S. (2007). "Characterization of a stirred tank electrochemical cell for water disinfection processes." *Electrochim. Acta*, 52(7), 2595–2602.

*Prism 6.04* [Computer software]. GraphPad Software, San Diego.

Saidan, M., Rawajfeh, K., and Fayyad, M. (2013). "Investigation of factors affecting THMs formation in drinking water." *Am. J. Envir. Eng.*, 3(5), 207–212.

Sapers, G. M. (2001). "Efficacy of washing and sanitizing methods for disinfection of fresh fruit and vegetable products." *Food Technol. Biotech.*, 39(4), 305–311.

Sarkka, H., Vepsalainen, M., Pulliainen, M., and Sillanpaa, M. (2008). "Electrochemical inactivation of paper mill bacteria with mixed metal oxide electrode." *J. Hazard. Mater.*, 156(1), 208–213.

Stout, J. E., and Yu, V. L. (2003). "Experiences of the first 16 hospitals using copper-silver ionization for Legionella control: Implications for the evaluation of other disinfection modalities." *Infect. Control Hospital Epidemiol.*, 24(8), 563–568.

Thames Water. (2013). "Thames water utilities water quality report-2013 data." ⟨https://secure.thameswater.co.uk/dynamic/cps/rde/xchg/corp/hs.xsl/899_5787.htm⟩ (Mar. 15, 2016).

U.K. Environment Agency. (2010). "The microbiology of drinking water. Part 8: The isolation and enumeration of Aeromonas and Pseudomonas aeruginosa by membrane filtration." Environment Agency, Almondsbury, Bristol, U.K.

U.K. Environment Agency. (2012). "The microbiology of drinking water. Part 7: Methods for the enumeration of heterotropic bacteria." Environment Agency, Almondsbury, Bristol, U.K.

Xiong, R., Xie, G., Edmondson, A. E., and Sheard, M. A. (1999). "A mathematical model for bacterial inactivation." *Int. J. Food Microbiol.*, 46(1), 45–55.