USE OF CHLORINE DIOXIDE FOR *LEGIONELLA* CONTROL IN HOSPITAL WATER SYSTEMS

by

Zhe Zhang

B.E. Civil Engineering, Wuhan University of Technology, 1999

M.S., Environmental Science, Wuhan University, 2002

Submitted to the Graduate Faculty of

School of Engineering in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2007

UNIVERSITY OF PITTSBURGH

SCHOOL OF ENGINEERING

This dissertation was presented

by

Zhe Zhang

It was defended on

April 3, 2007

and approved by

Leonard W. Casson, Associate Professor, Department of Civil & Environmental Engineering

Robert Ries, Assistant Professor, Department of Civil & Environmental Engineering

Janet E. Stout, Research Professor, Department of Infectious Disease

Stanley States, Pittsburgh Water and Sewer Authority

Dissertation Director: Radisav D.Vidic, Professor, Department of Civil & Environmental Engineering

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Zhe Zhang, PhD

University of Pittsburgh, 2007

The safety and efficacy of chlorine dioxide (ClO_2) for *Legionella* control were evaluated in a controlled prospective study conducted in two hospitals. The results showed that a significant reduction of *Legionella* positivity in the hot water was achieved by ClO_2 treatment in these two hospitals. ClO_2 application was safe based on the EPA MRDL for ClO_2 and MCL for chlorite (ClO_2^-).

The impacts of pH, temperature and total organic carbon on ClO_2 decay were investigated in a batch reactor to investigate the causes of the low ClO_2 residual in hot water observed in the field. Temperature and TOC are both important factors governing ClO_2 demand in hot water systems. The effect of pipe corrosion scale on ClO_2 efficiency was investigated to predict the ClO_2 loss in water distribution systems. Goethite (α-FeOOH) and magnetite (Fe_3O_4) were identified as the main component phases of iron corrosion scale. Cuprite (Cu_2O) was the major component of copper corrosion scale. The first order reaction rate constants for ClO_2 reaction with iron corrosion scales and magnetite ranged from 0.0251-0.0829 min^{-1}. The first order reaction rate constants for ClO_2 reaction with cuprite ranged from 0.0052-0.0062 min^{-1}. Cuprite and magnetite were the main compounds in the scales that caused ClO_2 loss in this study. The loss of ClO_2 in the corroded iron pipe was dominated by reactions between ClO_2 and these ferrous compounds in the iron pipe corrosion scales.

Possible synergy between ClO_2 and free chlorine to provide more effective control of *Legionella* in a hospital water system was investigated in a model plumbing system. Combination of ClO_2 and chlorine did not show significant synergistic effect on inactivation of *Legionella* in the model plumbing system. However, maintaining of 0.2 mg/L of ClO_2 residual led to a significant reduction of *Legionella* at 40 degree Celsius in the model plumbing system.

Based on the results of this study, it can be concluded that ClO_2 represents a viable alternative approach for controlling *Legionella* in institutional distribution systems provided that the initial demand due to the presence of corrosion scales is satisfied and that sufficient residual at distal outlets is achieved.

Keywords: *Legionella*, chlorine dioxide, hospital water system, disinfection byproducts

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ACKNOWLEDEGEMENTS

I would like to express my sincere gratitude to my advisor, Dr. Radisav D. Vidic for offering me the great opportunity to work on this project. My thanks are extended for his guidance, patience and great advice throughout my study.

No words can exactly express my cordial gratitude to Drs. Victor L. Yu and Janet E. Stout for their strong support and great advice both academically and financially. Without their extensive knowledge and experience in Legionnaires' diseases and *Legionella* control, this work would not have been accomplished. I also like to thank Drs. Leonard W. Casson, Robert Ries and Stanley States for their serving on my doctoral committee and their insightful suggestions and great efforts.

I deeply thank all the staff at the Special Pathogen Laboratory, VA Pittsburgh Healthcare Systems, especially Sara Vaccarello, Sue Mietzner, Laura Morris, Asia Obman, Pat Sheffer and Jaclynn L. Shannon and John Rihs. It is a great experience to work with this big family.

Finally I would like to express my deepest appreciation to my wife Liu Qi, my father and my brother for their continuous support. This dissertation is dedicated to my late mother.

1.0 INTRODUCTION

1.1 APPLICATION OF CHLORINE DIOXIDE FOR *LEGIONELLA* CONTROL IN HOSPITAL WATER SYSTEMS AND MONITORING THE FATE OF DISINFECTION BYPRODUCTS

Chlorine dioxide (ClO_2) has recently been used in the U.S. for disinfection of hospital water systems and to prevent hospital-acquired Legionnaires' disease [1, 2]. The Environmental Protection Agency determined the Maximum Residual Disinfectant Level (MRDL) for chlorine dioxide at 0.8 mg/L [3]. The disinfection byproducts of chlorine dioxide are chlorite (ClO_2^-) and chlorate (ClO_3^-) ions. These disinfection byproducts may also pose high health risks for consumers and the Maximum Contaminant Level (MCL) for chlorite is set at 1.0 mg/L [3].

Although chlorine dioxide and its disinfection byproducts persistence in water treatment plant and large distribution systems has been studied since it became increasingly popular for drinking water treatment [4, 5], its efficacy and safety as a disinfection approach in secondary distribution systems, such as hospital water systems, has not been studied extensively. The fate and levels of chlorite and chlorate generated during continuous chlorine dioxide disinfection of hospital water system are not known. The objective of this study was to evaluate the efficacy of chlorine dioxide to control *Legionella* in hospital water systems and to verify that the levels of ClO_2, $ClO_2^-$and ClO_3^- did not exceed EPA limits.

1.2 THE EFFECT OF WATER QUALITY PARAMETERS (PH, TEMPERATURE AND TOC) ON CHLORINE DIOXIDE DECAY IN DRINKING WATER

Previous studies with chlorine dioxide for controlling *Legionella* in a hospital system showed that an extended time (>20 months) was needed to achieve significant reduction in *Legionella* positivity in hot water system [1, 2]. Such behavior was attributed to the fact that the chlorine dioxide residual in the hot water was significantly lower than that in the cold water. Accordingly, *Legionella* positivity of the hot water samples was much higher than in the cold water samples. The low chlorine dioxide residual in the hot water may be due to chlorine dioxide volatilization at high temperature, faster reactions rate of chlorine dioxide reaction with organic matters at higher temperatures, or the effect of high organic load in the hot water. The increased reaction rate in hot water is an important consideration that may be overlooked by typical batch studies that are typically conducted at room temperature. And *Legionella* are thermophilic bacteria and proliferate in hot water of 45-55 °C. If a sufficient disinfectant residual is not maintained in a hot water distribution system, complete eradication or suppression of *Legionella* may not be possible.

In this study, chlorine dioxide decay kinetics in drinking water with different total organic carbon concentration was investigated in room temperature (25 ± 2 °C) and at 45 °C and compared to the reaction kinetics with humic substance at those temperatures. The formation of disinfection by-products (chlorite and chlorate) was also analyzed to establish a mass balance equation to evaluate the chlorine dioxide reaction mechanism:

Chlorine dioxide dose = chlorine dioxide residual +

chlorite concentration +

chlorate concentration +

chloride produced

1.3 THE EFFECT OF PIPE CORROSION SCALE ON CHLORINE DIOXIDE CONSUMPTION IN DRINKING WATER

New electrochemical generation system uses electrochemical cassettes and membrane technology to generate a stock solution of approximately 500 mg/L ClO_2 from 25% sodium chlorite solution. The equipment is easy to install and safe to operate in the institutional plumbing system. Several studies have been conducted to evaluate the efficacy and safety of ClO_2 generated by this electrochemical process for controlling water borne pathogens in hospital water systems [1, 2].

However, the loss of ClO_2 due to corrosion scales has not been studied in detail. And chlorine dioxide is a strong oxidant and will oxidize ferrous ions released from the iron corrosion scale. The reactions of ClO_2 with corrosion scales will lead to undesirable losses in the disinfectant residual. In this study, the corrosion scales from a galvanized iron pipe and a copper pipe that have been in service for more than 10 years were characterized by energy dispersive spectroscopy (EDS) and X-ray diffraction (XRD). The impact of corrosion scale materials on ClO_2 decay was investigated in DI water at 25°C and 45°C in a batch reactor to simulate the application of chlorine dioxide in hospital hot and cold water systems. In addition, ClO_2 decay

was also investigated in a specially designed reactor made from the iron and copper pipes to obtain more realistic reaction rate data.

1.4 SYNERGISTIC EFFECT OF CHLORINE DIOXIDE AND FREE CHLORINE FOR CONTROLLING *LEGIONELLA* IN A MODEL PLUMBING SYSTEM

A field study showed that *Legionella* positivity in one hospital water system was reduced in a much shorter period of time (6 -10 months) compared to the other two hospitals included in the study. It was also found that the incoming drinking water received from the city's supply system contained high levels of free chlorine because the hospital was very close to the water treatment plant and was the first big consumer in the distribution system. It can be hypothesized that the coincident reaction of two disinfectants (chlorine and chlorine dioxide) might provide additional synergistic effects for controlling *Legionella* in the water distribution system. Another possibility is that disinfection with chlorine followed by chlorine dioxide may be an effective approach to treat *Legionella*. Advantages of combining these disinfectants are that chlorine is still used as the primary disinfectant in most water treatment plants. When chlorine is used as the primary disinfectant, it can meet some oxidant demand in the distribution system so that the chlorine dioxide injected in the health care facility can be maintained at a stable residual concentration. In addition, the reaction of chlorine and chlorite may reduce the formation of chlorine dioxide disinfection byproducts.

The synergistic effects of combined disinfectant residuals (free chlorine and chlorine dioxide) for controlling *Legionella* were investigated in a model plumbing system. And the effect

of temperature on the efficacy of chlorine and chlorine dioxide for controlling *Legionella* was also investigated.

1.5 OBJECTIVES AND SCOPE

The objectives of this study were to evaluate the safety and efficacy of chlorine dioxide for *Legionella* control in controlled prospective study in individual hospitals as the step 3 of a 4-step evaluation process. The factors that would impact the efficiency of chlorine dioxide in hospital water systems were investigated in laboratory experiments. More specifically:

To evaluate the efficacy of chlorine dioxide for *Legionella* control in individual hospitals;

To monitor the fate of chlorine dioxide disinfection byproducts in hospital water systems;

To investigate the impacts of pH, temperature and total organic carbon on chlorine dioxide decay in drinking water in a batch reactor with floating glass cover to optimize the application of chlorine dioxide in the field;

To investigate the effect of pipe corrosion scale on chlorine dioxide efficiency to predict the chlorine dioxide loss in water distribution systems;

To investigate the synergistic effect combined disinfectant residuals (free chlorine and chlorine dioxide) for controlling *Legionella* in a model plumbing system.

2.0 LITERATURE REVIEW

2.1 CHLORINE DIOXIDE

2.1.1 Physical chemical characteristic of chlorine dioxide

Pure chlorine dioxide is a green-yellow gas [6, 7]. Chlorine dioxide gas is readily soluble in water, in which it forms a green yellow solution. In contrast to the hydrolysis of chlorine gas in water, chlorine dioxide in water does not hydrolyze to any appreciable extent but remains in solution as a dissolved gas [8]. Chlorine dioxide exists almost entirely as monomeric free radicals with an unpaired electron in nature and is five times more soluble than chlorine [6, 7]. General methods of preparing chlorine dioxide include the reactions between acid and sodium chlorite, or chlorine gas and sodium chlorite. Chlorine dioxide is light sensitive and will decompose readily when exposed to sunlight or fluorescent lights. Zika [9] showed that chlorine dioxide has a lifetime of less than 0.5 minute in water exposed to bright sunlight.

Chlorine dioxide is effective in removing iron and manganese [6, 7] and controlling taste and odor [10], especially if caused by phenolics, chlorophenolics, and algal by-products. Chlorine dioxide and its byproducts, chlorite and chlorate, do not react with humic or fulvic acids to form THMs, as does chlorine [11, 12]. Reactions of chlorine dioxide with organic compounds, such as phenol [13-16] and humic substances [17], have been widely studied. In general, chlorine dioxide

reacts primarily by one electron oxidation mechanism [14, 15, 18]. In contrast, chlorine reacts not only via oxidation but also by electrophilic substitution, resulting in a variety of chlorinated organic products, among them the THMs [18]. Chlorine dioxide also appears to be more selective as evidenced by somewhat lower demand for chlorine dioxide as compared to chlorine. The use of chlorine dioxide has been proven to reduce THMs formation in many drinking water supplies [11, 12, 19-21]. Chlorine dioxide does not react with amines to form chloramines and does not react with bromide to form brominated byproducts [7].

2.1.2 Chlorine dioxide as a disinfectant

The efficacy of chlorine dioxide as a bactericide was demonstrated in the 1940s[22, 23]. 1-5 mg/L of chlorine dioxide was shown to be effective against *Escherichia coli and Bacillus* anthracoides [22, 23]. Chlorine dioxide was found to be more effective than chlorine in sewage effluent [24], and the rate of chlorine dioxide inactivation was extremely rapid compared with chlorine [25]. Other studies showed that biocidal efficiency of chlorine dioxide is equal or superior to chlorine [26-32]. Chlorine dioxide effectively inactivates *Legionella* [1, 2], Cryptosporidium parvum oocysts[33-36], viruses [37, 38] and other water borne pathogens [32, 39]. Laboratory studies showed chlorine dioxide to be effective in controlling biofilms that form on the surfaces of distribution pipes [40]. Chlorine dioxide has also been applied extensively in the paper pulp process as alternative bleaching chemical to chlorine.

The mode of inactivation of viruses or bacteria by chlorine dioxide is still under debate. Alvarez concluded that chlorine dioxide inactivated poliovirus by reacting with the viral ribonucleic acid (RNA) and impairing its synthesis [36]. Other studies showed chlorine dioxide to react readily with the amino acids (e.g., cysteine, tryptophan and tyrosine), but not with RNA. It

was concluded that virus inactivation was due to altering viral capsid proteins by chlorine dioxide [37, 41]. Chlorine dioxide disruption of the outer membrane permeability was reported as a third possible inactivation mechanism [25].

Since chlorine dioxide has so many advantages, it has been used as an alternative disinfectant to chlorine not only in food industry[42-48], but also in drinking water and wastewater treatment [26, 49-51].

Chlorine dioxide (ClO_2) has recently been used in the U.S. for disinfection of hospital water systems and to prevent hospital-acquired Legionnaires' disease [1, 2]. Environmental Protection Agency set the Maximum Residual Disinfectant Level (MRDL) for chlorine dioxide at 0.8 mg/L [3]. The disinfection byproducts of chlorine dioxide are chlorite (ClO_2^-) and chlorate (ClO_3^-) ions. These disinfection byproducts may also pose high health risks for consumers and the Maximum Contaminant Level (MCL) for chlorite is set at 1.0 mg/L [3].

Chlorine dioxide and its disinfection byproducts persistence in water treatment plant and large distribution systems has been studied since it became increasingly popular for drinking water treatment [4, 5], however its efficacy and safety as a disinfection approach in secondary distribution systems, such as hospital water systems, has not been studied extensively. The fate and levels of chlorite and chlorate generated during continuous chlorine dioxide disinfection of hospital water system are not known. The objective of this study was to evaluate the efficacy of chlorine dioxide to control *Legionella* in hospital water systems and to verify the levels of ClO_2, ClO_2^- and ClO_3^- that will occur throughout the distribution system.

2.2 *LEGIONELLA*

2.2.1 *Legionella* and Legionnaires' diseases

Legionnaires' disease is a pneumonia caused by *Legionella pneumophila. Legionella*e bacteria are ubiquitous in natural and manmade aquatic environments, such as rivers, ground waters [52], streams and thermally polluted waters [53]. *Legionella* proliferate within manmade water systems, especially water distribution systems that provide favorable water temperatures (about 45-50°C), physical protection, nutrients (sediments and biofilms), and commensal microorganisms [53-56]. Experiments also showed that *Legionella* can colonize on the surface of various plumbing materials, such as polyvinyl chloride and stainless steel [53-55, 57]. Even rubber fittings in showers and taps were experimentally shown to support the growth of *Legionella* [58, 59].

The first known epidemic of nosocomial *Legionella*-induced pneumonia occurred in July 1965 in St. Elizabeth's hospital in Washington, D.C [54]. Since then, *Legionella* was isolated from potable water distribution systems of numerous hospitals experiencing outbreaks of Legionnaire's disease [60-62].

2.2.2 *Legionella* in hospital water systems

Legionella is an opportunistic pathogen and its presence in water systems poses a high risk for individuals with compromised immune systems, especially in hospitals where a large number of patients with compromised immunity are likely to be exposed to water contaminated with *Legionella*. The mortality of health care-acquired Legionnaires' disease is estimated to be

approximately 40% [63]. And hospital hot water systems have been demonstrated to be colonized by *Legionella* [56].

Aerosolization was considered as the primary mode of transmission since outbreaks of Legionnaires' disease were widely linked to aerosol-generating systems, such as cooling towers [64]. But evidence also implicated aspiration as a mode of transmission [64] and water distribution systems contaminated with *Legionella* were documented as sources of nosocomial and community-acquired Legionnaires' disease [53-55]. Disinfecting water distribution systems is the key for suppressing the growth of *Legionella* in a hospital water system and preventing hospital-acquired Legionnaires' disease [57].

2.3 DISINFECTION METHODS FOR *LEGIONELLA* CONTROL

Superheat and flush, hyperchlorination, ozonation, ultraviolet (UV) light irradiation and copper-silver ionization have been used to control *Legionella* in hospital water systems [65]. Each of these methods has its advantages and drawbacks. Hence, chlorine dioxide has been considered as an alternative for disinfection of potable water systems and control of *Legionella* proliferation.

2.3.1 Superheat and flush

Legionella can be eradicated by elevating hot water tank temperature to 70°C followed by flushing all water outlets, faucets and showers for 30 minutes [65-68]. The main advantage of superheat and flush is that no special equipment is required. However, the method is time-

consuming and recolonization of the system with *Legionella* will occur shortly after the superheat and flush event [65-68].

2.3.2 Hyperchlorination

Hyperchlorination refers to the addition of free chlorine concentrations as high as 2 to 6 mg/liter through the addition of calcium hypochlorite or sodium hypochlorite solution [65-68]. Hyperchlorination is often combined with superheat and flush to control *Legionella* in water distribution systems. The notable drawback of hyperchlorination is its inability to completely eradicate *Legionella* from the water system and its corrosive impact on distribution pipes over time [65-68]. In addition, high concentration of free chlorine enhances the production of trihalomethanes and other disinfection byproducts, which are carcinogenic or mutagenic [65-68].

2.3.3 Ultraviolet light irradiation

U.V. irradiation kills bacterial cells by producing thymine dimers in DNA, which subsequently hampers DNA replication [69]. Continuous UV light treatment with filtration can control *Legionella* from colonizing water fixtures that were near the point of use [69-72]. UV light systems are easy to install and will not form any disinfection byproducts in the treated water. However, UV light systems provide no residual protection at distal sites and are not suitable as the sole disinfection measure [71].

2.3.4 Copper silver ionization

Copper and silver ionization systems have been applied in more than 30 hospitals in the United States to control *Legionella* in their water systems [73]. Copper and silver systems can be easily installed and maintained and provide lasting residual protection [74]. However, scale accumulation on the electrodes and high pH will reduce its efficacy [53]. Long-term treatment might result in the development of *Legionella* resistance to these ions [75].

2.4 CHLORINE DIOXIDE FOR *LEGIONELLA* CONTROL

Laboratory and field studies have shown that chlorine dioxide is an effective disinfectant for *Legionella* removal in hot and cold drinking water distribution systems [37, 76-81]. Chlorine dioxide has been demonstrated to be effective against *Legionella* in potable water distribution systems of several hospitals in Europe [37, 76-81]. Walker *et al.* [53] used a shock dose of 50-80 ppm chlorine dioxide to treat a hospital water system for 1 hour. Although the biofilm was not completely removed by this procedure, no *Legionella* was recovered from both cold and hot water system after the treatment. Continuous injection of low levels (0.3-0.5mg/L) of chlorine dioxide was found to be effective in controlling *Legionella* in both cold and hot water systems [77]. Hood *et al*[77] showed that continuous dosing of chlorine dioxide (up to 0.5 mg/L) in a hospital cold water system over a 6-year period was extremely effective in controlling planktonic *Legionella pneumophila*. Chlorine dioxide was injected into the cold water system at 0.25-0.5 mg/L in a newly opened ward block in a regional cardiothoracic unit and the level of chlorine dioxide in

the hot water system was raised to 3-5mg/L in controlled phases until no *Legionella* were detected in the systems [77].

The application of chlorine dioxide in U.S. hospital water systems was not popular until recently and there are very few studies that investigated the efficacy of chlorine dioxide to control *Legionella* in U.S. hospitals [77].

2.4.1 Disinfection byproducts of chlorine dioxide

Despite its numerous advantages, chlorine dioxide still represents a potential source of health risk for consumers due to its inorganic disinfection byproducts. Chlorite and chlorate are the main disinfection byproducts of chlorine dioxide. At low levels, chlorite may cause congenital cardiac defects and hemolytic anemia through oxidative damage to the red blood cell membrane [11], while higher levels can result in an increase in methemoglobin [82]. Chlorite also affects the nervous system in infants and young children [82]. Persistence of chlorine dioxide and its disinfection byproducts in water treatment plants and large distribution systems has already been studied [4, 5]. However, the level of chlorite and chlorate generated during the continuous chlorine dioxide disinfection of hospital water systems has not been well documented. Since chlorite may cause congenital cardiac defects and hemolytic anemia for patients undergoing hemodialysis therapy, measures must be taken to remove not only chlorite but also chlorine dioxide that could affect the patients. A case study showed that granular activated carbon and reverse osmosis were effective in removing chlorite and other oxidants at a hemodialysis chronic care clinic [83]. Ferrous salts have also been shown effective in removing chlorite and chlorate in a laboratory study[84].

2.4.2 Effect of temperature on chlorine dioxide efficacy

Ruffell *et al.* revealed that the decrease in chlorine dioxide obeyed a first order rate law, $C(t)=C_o\exp(-kt)$ at 20°C [33]. They also assumed that the first-order decay in chlorine dioxide concentration was valid at temperatures other than 20°C. Olivieri found that the loss of residual chlorine dioxide follows first-order rate law at 22°C and that the half-life for chlorine dioxide in the static distribution system test was 93 min at 22°C [37].

The inactivation rates of *C.parvum oocyst* or *Bacillus subtilis*[36] with chlorine dioxide were investigated in a temperature range of 4-30 °C [33, 39]. The results showed that the inactivation rate was lower at the lower water temperature, thereby increasing Ct requirements with decreasing temperature. Water temperature was a critical factor for chlorine dioxide inactivation of *C.parvum oocyst* [34]. For every 10 °C temperature increase, the reaction constant increased by a factor of 2.3.

The decomposition of chlorine dioxide in hot water (>30°C) was not investigated extensively in the past. The inactivation rate of *Legionella* with chlorine dioxide in hot water (>30°C) had not been studied. Therefore, a model distribution system was used to investigate the fate of chlorine dioxide in both the cold and hot water.

2.4.3 The effect of pH on chlorine dioxide efficacy

It has been shown that chlorine dioxide is stable in the pH range of 4-10 [6, 7] and significant dispropotionation of chlorine dioxide only occurs at pH above 10 [6, 7]. The impact of pH on the efficacy of chlorine dioxide in controlling *Legionella* needs to be investigated due to its potential impact on the rate of inactivation as well.

2.4.4 The effect of dissolved organic carbon on chlorine dioxide efficacy

Humic substances are present in most natural water bodies at the range of 0.1-200mg/L [53]. Chlorine dioxide will not react with these humic substances to form THM compounds[12, 17, 19-21]. However, the bactericidal effect of chlorine dioxide will be affected by higher organic carbon in water due to the reaction of chlorine dioxide with organic compounds. Chlorine dioxide will react with available natural organic matters in drinking water, which will reduce the chlorine dioxide residual in the distribution system and its bactericidal efficiency.

In a previous study [1, 2], and in a current field study, the chlorine dioxide residual in the hot water was significantly lower than that in the cold water. Accordingly, *Legionella* positivity of the hot water samples was much higher than in the cold water samples. The observed low chlorine dioxide residual in the hot water may be due to several factors, including chlorine dioxide gassing off at high temperature, faster reactions of chlorine dioxide at higher temperatures, or the presence of high organic load in the hot water.

2.4.5 The effect of corrosion scale on chlorine dioxide decay

The compounds usually found in iron corrosion scales include goethite (α-FeOOH), lepiodcrocite (γ-FeOOH), magnetite (Fe_3O_4), siderite ($FeCO_3$), ferrous hydroxide ($Fe(OH)_2$), ferric hydroxide ($Fe(OH)_3$), ferrihydrite ($5Fe_2O_3 \cdot 9H_2O$), green rusts ($Fe^{II}_4Fe_2^{III}(OH)_{12}(CO_3)$) and calcium carbonate [85, 86]. Literature showed that high concentration of readily soluble Fe (II) content was present inside the scale and that iron was released to bulk water primarily in the ferrous form [85-89]. Free chlorine consumption induced by iron corrosion in a drinking water

system has been investigated [85-89], but the chlorine dioxide consumption by pipe corrosion scale has not yet been reported.

2.4.6 The effect of chlorine residual on the efficacy of chlorine dioxide for *Legionella* control

Reactions (1) and (2) represent possible reactions between chlorine (as molecular chlorine or hypochlorous acid) and chlorite that have been widely studied with respect to chlorine dioxide generation [90-96]. Reaction 1 predominates at pH 5 and high concentration of reactants. Reaction 2 predominates in high pH region, but negligible chlorine dioxide was formed at pH above 7.

$$HOCl + 2ClO_2^- + H^+ \rightarrow 2\,ClO_2 + Cl^- + H_2O \quad (1)$$

$$OCl^- + 2ClO_2^- \rightarrow ClO_3^- + Cl^- \quad (2)$$

For drinking water conditions at neutral pH and milligram-per-liter concentrations of reactants, very little information on the reaction rates is available [17, 96]. The reaction between chlorite and hypochlorite ion was very slow, requiring several hours at high concentrations of reactants to produce measurable concentrations of chlorate as the only product [90-96]. Werdehoff investigated the reaction of 2.1 mg/L of Cl_2 and 1.1 mg/L of chlorite at pH 7 [84]. No detectable chlorine dioxide was measured during the period of 7 days and the chlorate was thought to be the main products.

Katz *et.al* applied the equal dose of chlorine dioxide and chlorine to disinfect the effluent from a municipal sewage treatment plant [84]. The results showed that the combination of two disinfectants produced relatively stable high residual of both disinfectants, reduced the

concentration of the undesirable disinfection byproduct (i.e. chlorite ion), while increasing the concentration of chlorine dioxide. They explained such behavior due to Reaction 2. Several studies showed that mechanically mixed oxidants achieved considerable disinfection efficiency for selected microorganisms [97]. However, the level of the enhanced disinfection efficiency remains unclear and the synergistic effect of the mixed oxidants also needs to be confirmed. One study showed that sequential disinfection with chlorine dioxide followed by free chlorine is an effective approach to treating *Cryptosporidium parvum* [98]. The synergistic effect of sequential treatment may be caused by the unique activity of each disinfection agent reacting with specific chemical groups of the cell wall. The combination of chlorine dioxide and chlorine for *Legionella* control has not yet been investigated.

3.0 MATERIALS AND METHODS

3.1 FIELD STUDY

3.1.1 Hospital A

Healthcare-acquired legionellosis due to *Legionella pneumophila* was diagnosed in an immunocompromised patient in hospital A with 364 patient beds and 74 skilled nursing beds. Following the initial case, steps were taken to control *Legionella* in the water distribution system and ClO_2 was chosen to treat the hospital water system. Hospital A is comprised of two buildings: Building 1 (referred to as B1) and Building 2 (referred to as B2). Both buildings have 8 floors (Table 1). 83% of pipe in the hospital water system is copper; the other 17% is brass. The hospital water is supplied by the city water department. *Legionella* has been detected in the hot water systems of both buildings of this hospital since October 2002. The extent of *Legionella* colonization is expressed as percent *Legionella* positivity, which is the percentage of all sample sites that tested positive for *Legionella*. The risk of legionnaires' disease in hospital patients has been shown to be better predicted by the percentage of water-system sites testing positive for *Legionella* than by the concentration of *Legionella* bacteria in individual samples [99]. The percentage of *Legionella* positive hot water outlets was 67% (6/9) before the installation of the ClO_2 system. ClO_2 generating system was online and operational in January 2003.

Table 1. Comparison of study parameters of three hospitals using chlorine dioxide for *Legionella* control

Parameter	Study No.1 (Geisinger)	Study No. 2 (Hospital A)	Study No. 3 (Hospital B)
Size	437 beds 23 buildings	401 beds 2 buildings	687 beds 1 building
ClO_2 injection point	520,000 gal. reservoir	Cold water main	Cold water main
Sample frequency & duration	Monthly for 22 months	Bi-monthly for 20 months	Bi-monthly for 14 months & ongoing
Chlorite Monitoring	No	Yes	Yes

3.1.1.1 ClO_2 generation system

One ClO_2 generating unit (Halox Inc. Bridgeport, CT) was installed in each building by Environmental Hygiene Services (Nalco Co., Naperville, IL). The modular electrochemical cassettes generate a solution with approximately 500 mg/L ClO_2 using a 25% sodium chlorite solution. The ClO_2 was injected into the incoming cold water main pipe at the target ClO_2 concentration of 0.5-0.7 mg/L based on the flow rate of the incoming cold water.

3.1.1.2 Sample collection

Sampling locations for *Legionella* were selected throughout the distribution system in hospitals A. 13 sampling locations in B1 and 7 sampling locations in B2 were located on the second, fourth, fifth, sixth and eighth floors of hospital A. Hot and cold water samples were collected from distal outlets (sink and shower) at each sampling location. The hot water storage tank was also sampled. Sampling in hospital A was performed every two months from June 2004 to August 2005 and extended to June 2006.

120 ml water samples were collected for *Legionella* culture immediately after the outlet tap was turned on. *Legionella* testing was performed in the VA Special Pathogens Laboratory, as previously described[1]. The distal outlets were then flushed for 1 minute to collect representative water samples for ClO_2 analysis. Temperature measurements were taken directly from the flow stream after the flush. A 10-ml sample was taken for ClO_2 analysis at the time of collection and 100g/L of glycine was used to eliminate free chlorine interference. Levels of ClO_2 were analyzed in both hot and cold water samples using the Hach Method 10101 –DPD Method for ClO_2 (0.00 to 5.00 mg/L) utilizing a glycine reagent and Hach DPD Free Chlorine Reagent (Hach Company, Loveland, CO). The colorimetric measurements were performed using the Hach DR/2010 Spectrophotometer (Hach Company, Loveland, CO).

Hospital personnel also monitored ClO_2 residual in cold water throughout the distribution system every month in hospital A. ClO_2 residual measurements by the hospital personnel were performed using a pocket colorimeter following the DPD method with the same reagents as for the study samples.

Samples for ClO_2^- and ClO_3^- analysis were chosen to represent various distances from the ClO_2 injection point (closest, midpoint and farthest sites). A total of 7 hot water samples and 5

cold water samples were collected for ClO_2^- and ClO_3^- analysis every two months from 5 locations in B1 and 2 locations in B2. Samples for ClO_2^- and ClO_3^- analysis were sparged with nitrogen gas for 10 minutes immediately following the collection to remove ClO_2 residual. 30 ml of the sample was filtered through a 0.2 micron filter followed by the addition of 50 mg/L of ethylenediamine to each sample. ClO_2^- and ClO_3^- were measured by ion chromatography (DX-500, Dionex Inc, Sunnyvale, CA) with suppressor and conductivity detector according to USEPA method 300.1 [100]. Two samples were also sent to a reference laboratory each time as a quality control measure to ensure accuracy of ClO_2^- and ClO_3^- analysis (Novachem Laboratories Inc., Oxford, OH).

Water quality of the municipal water supply collected in the hospital was evaluated in October 2003 and June 2004 by the Pittsburgh Water and Sewer Authority, Pittsburgh, PA. Water samples from the city water supply were collected and stored at 4°C before transfer to the Pittsburgh Water and Sewer Authority for analysis using standard laboratory procedures.

3.1.2 Hospital B

From March 2001 to January 2002, three cases of hospital-acquired *Legionella pneumophila* pneumonia occurred in hospital B. Cultures from the water distribution system reflected the presence of *Legionella pneumophila* serogroup 5 and were identical to *Legionella* cultured from one of the identified patients. Following the initial cases, steps were taken to eliminate *Legionella* in the water distribution system. This included superheating and flushing the hot water system and replacement of a water tank found to be colonized with *Legionella*. Despite these measures, another case of hospital-acquired *Legionella pneumophila* pneumonia was identified in January 2002. The optimal method for long-term disinfection has not yet been

identified and no recommendations exist at this time for long-term treatment of water distribution systems [101]. Chlorine dioxide was the disinfection method chosen to treat the hospital water system and was installed in April 2004.

In hospital B, the 12-floor building that is treated with ClO_2 has 672 operating patient beds. Pre-disinfection baseline cultures were collected from 2002-2004. There were 17 sampling locations in the hospital B building that were located from the third to the twelfth floor. Hot and cold water samples were collected from distal outlets (sink and shower) at each sampling location. The hot water storage tank was also sampled. Sampling was performed every two months from June 2004 through 2005 and extended to 2006.

120 ml water samples were collected for *Legionella* culture immediately after the outlet tap was turned on. *Legionella* testing was performed in the VA Special Pathogens Laboratory, as previously described[1]. The distal outlets were then flushed for 1 minute to collect representative water samples for ClO_2 analysis. Temperature measurements were taken directly from the flow stream after the flush. A 10-ml sample was taken for ClO_2 analysis at the time of collection and 0.1 ml of 100g/L of glycine was used to eliminate free chlorine interference. Levels of ClO_2 were analyzed in both hot and cold water samples using the Hach Method 10101 –DPD Method for ClO_2 (0.00 to 5.00 mg/L) utilizing a glycine reagent and Hach DPD Free Chlorine Reagent (Hach Company, Loveland, CO). The colorimetric measurements were performed using the Hach DR/2010 Spectrophotometer (Hach Company, Loveland, CO).

Hospital personnel also monitored ClO_2 residual in cold water throughout the distribution system every month in hospital A. ClO_2 residual measurements by the hospital personnel were performed using a pocket colorimeter following the DPD method with the same reagents as for the study samples.

Samples for ClO_2^- and ClO_3^- analysis were chosen to represent various distances from the ClO_2 injection point (closest, midpoint and farthest sites). A total of 7 hot water samples and 5 cold water samples were collected for ClO_2^- and ClO_3^- analysis every two months from 5 locations in B1 and 2 locations in B2. Samples for ClO_2^- and ClO_3^- analysis were sparged with nitrogen gas for 10 minutes immediately following the collection to remove ClO_2 residual. 30 ml of the sample was filtered through a 0.2 micron filter followed by the addition of 50 mg/L of ethylenediamine to each sample. ClO_2^- and ClO_3^- were measured by ion chromatography (DX-500, Dionex Inc, Sunnyvale, CA) with suppressor and conductivity detector according to USEPA method 300.1 [100]. Two samples were also sent to a reference laboratory each time as a quality control measure to ensure accuracy of ClO_2^- and ClO_3^- analysis (Novachem Laboratories Inc., Oxford, OH).

Water quality of the municipal water supply collected from the hospital was evaluated in June 2004 by the Pittsburgh Water and Sewer Authority, Pittsburgh, PA. Water samples from the city water supply were collected and stored at 4°C before transfer to the Pittsburgh Water and Sewer Authority for analysis using standard laboratory procedures.

3.1.3 Statistical analysis

Statistic software Stata 9.0 (StataCorp**,** College Station, Texas) was used for statistical analysis. The significant differences were evaluated by t-tests; analysis of variance (ANOVA), Chi-square test and regression analysis under 95% confidence intervals. Significant differences among samples were defined when the p value obtained from the statistical analysis was less than 0.05. The null hypothesis of t-test, h_0: $\mu_a = \mu_b$ (μ_a and μ_b were the mean values of two sets of samples), would be rejected if the p value is less than 0.05.

3.2 BATCH REACTOR EXPERIMENTS

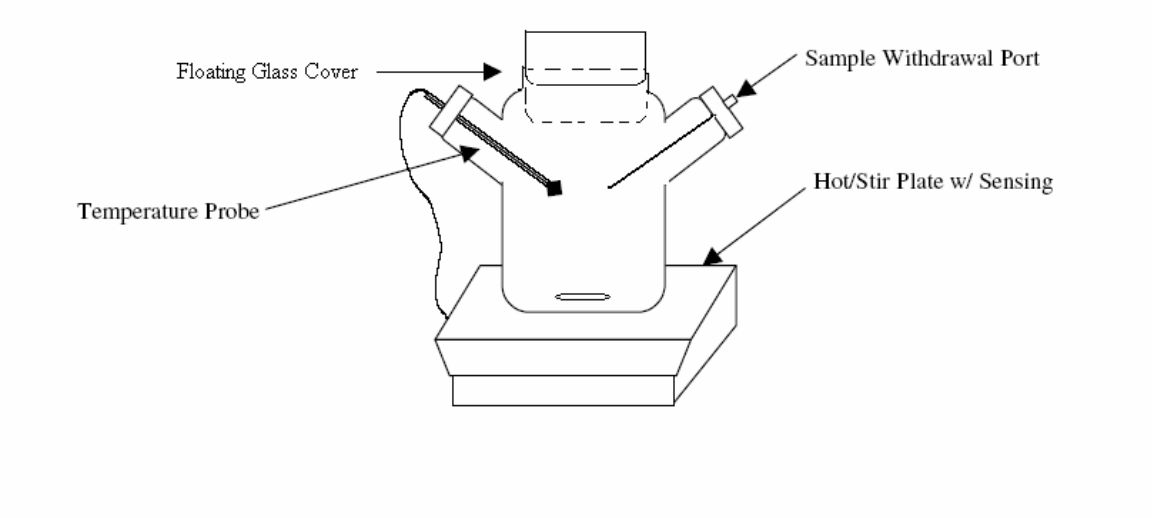


Figure 1. The batch reactor with the floating glass cover

A cell culture flask (Wheaton Science Products, Millville, NJ) capable of holding 2,500-ml of liquid was used as the batch reactor. A floating glass cover was used to prevent exchange of gases between the headspace and room air and minimize the volatility of ClO_2. Single port 45-mm red rubber stoppers with a 0.25" hole (Wheaton Science Products, Millville, NJ) were used on the side arms as a temperature monitoring port and a sample withdrawal port. The stoppers were sealed gas tight using a 45-mm inlet cap (Wheaton Science Products, Millville, NJ). The reactor and all parts were autoclaved prior to each experiment. The reactor was soaked overnight in 50 mg/L ClO_2 solution to satisfy disinfectant demand of the reactor material and rinsed with DI water before use.

The experiments were carried out at room temperature which varied in a very narrow range of 25±2°C. Hot water temperatures were maintained by heating the flask in a water bath on the hot plate with a temperature probe feedback. Temperature monitoring was performed using the temperature probe of the hot plate (PMC Industries Inc. San Diego, CA).

A ClO_2 generator (Diox, Klenzoid Inc.) provided a concentrated ClO_2 stock solution. The effluent solution was wasted until the generator achieved ClO_2 concentration of approximately 500 mg/L. The ClO_2 concentration of the concentrated stock solution (fresh stock solution was prepared for each experiment) was monitored using the Hach Method 8138 (0-700mg/L). The appropriate amount of the ClO_2 stock solution was pipetted into the batch reactor to achieve a target ClO_2 concentration of 1.0 mg/L. The decay of ClO_2 was monitored after the addition of the corrosion scales at various times. ClO_2 concentration during batch experiments was monitored using the Hach Method 10101 –DPD Method for ClO_2 (0.00 to 5.00 mg/L) utilizing a glycine reagent and Hach DPD Free Chlorine Reagent (Hach Company, Loveland, CO). The colorimetric measurements were made using the Hach DR/2010 Spectrophotometer (Hach Company, Loveland, CO).

Chlorite, chlorate and chloride samples were collected for the analysis at the beginning and the end of the experiment. Chlorite, chlorate and chloride were measured by ion chromatography (DX-500, Dionex Inc, Sunnyvale, CA) with suppressor and conductivity detector according to USEPA method 300.1. Chlorite, chlorate and chloride concentrations produced through ClO_2 reaction with scales were determined by the difference between the final and initial concentration to eliminate the interference of chlorite, chlorate and chloride present in the stock solution. pH of the solution was buffered with 0.1M phosphate buffer and it was adjusted by the addition of 0.1M NaOH.

Cold potable water samples were collected from the incoming drinking water main of the studied hospitals. Hot water samples were collected in 10-L polyethylene carboys from the building that was not treated with chlorine dioxide and stored at 4°C. The pH of the potable water samples was not adjusted. All water samples were sterilized prior to use by autoclaving the waters at 120 °C for 30 minutes. TOC concentration of the potable water samples was measured using the high temperature combustion method on a Model 1555B TOC Analyzer (Ionics, Incorporated Instrument Division, Watertown, MA).

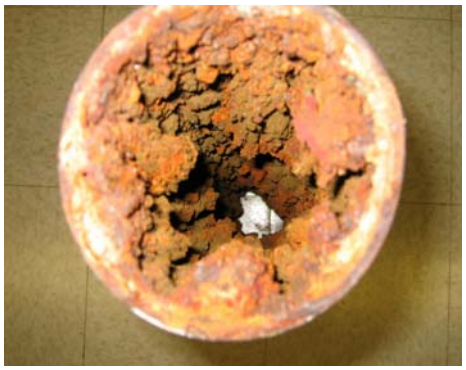
The TOC stock solution was prepared by dissolving the humic substance extracted from the Suwannee River (International Humic Substances Society, St. Paul, MN) and Aldrich humic acid as sodium salt (Aldrich, Milwaukee, WI) in a 0.001 M KOH solution and diluting to 1 liter with deionized water. The stock solution was stored at 4 °C.

UV-VIS spectra of humic acid before and after the reaction with chlorine dioxide were analyzed in the range of 200-600 nm on a Cary 5000 UV-VIS spectrophotometer (Varian Instruments, Walnut Creek, CA) in 1 cm path length quartz cells to detect the change of the absorbance spectrum of humic acid.

The corrosion scale material for the batch experiment was sampled from the corroded iron pipe and ground to powder without sieving before adding to the batch reactor. Commercial cuprite and magnetite powder (Aldrich, PA) were also used in the batch reactor experiment. The appropriate amount of the ClO_2 stock solution was pipetted into the batch reactor to achieve a target ClO_2 concentration of 1.0 mg/L. The decay of ClO_2 was monitored after the addition of the corrosion scales at various times.

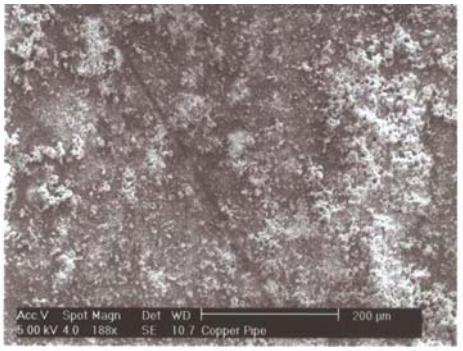
3.3 PIPE REACTOR

A 30 inches long, 4 inches diameter galvanized iron pipe and a 15 inches long, 2 inches diameter copper pipe were obtained from a local hospital water system and used for this study. The iron pipe was covered with deposits of corrosion products and heavily tuberculated as shown in Figure 2(a). The copper pipe was comparatively clean (Figure 2. (b)), only a thin film of corrosion scale formed on the copper pipe wall as shown by scanning electron microscopy (SEM) image in Figure 2 (c).



a)

(b)



(c)

Figure 2. (a) The inner surface of the galvanized iron pipe, (b) the inner surface of the copper pipe from a local hospital water system and c) SEM image of the copper pipe wall

3.3.1 Characterization of corrosion scale on the iron and copper pipe

The scales were scraped from the top, middle and bottom layer of the tubercles close to the end of the iron pipe and grounded into powder. The scales from the copper pipe were scraped from the copper pipe wall from both ends of the pipe. The elemental composition of the scales was analyzed by energy dispersive spectroscopy (EDS). Philips XL Series 30 scanning electron microscope and X-ray energy dispersive spectrometer (Philip Analytical Inc, Natick, MA) was used for EDS studies. The X-ray diffraction patterns of the samples were obtained with a Philips X'PERT diffractometer (Philip Analytical Inc, Natick, MA) using a standard Ni-filtered Cu Kα radiation source operating at 40 kV and 30 mA. X-ray patterns were analyzed using pattern processing software based on the latest Joint Committee on Powder Diffraction Standards (JCPDS) files. Samples were also sent to Materials Characterization Laboratory (Pennsylvania State University, University Park, PA) for X-ray photoelectron spectroscopy.

3.3.2 Pipe reactors setup

Copper and galvanized iron pipes were used to set up the experimental system shown in Figure 3. The flow rate of ClO_2 stock solution (1.0 mg/L) was adjusted to achieve the retention time for sampling port 1, 2 and 3 of 10, 20 and 30 minutes, respectively. The pipe reactor was flushed by the tap water for 24 hours before the experiment to re-wet the pipe surface and flush out any easily dislodged tubercles. 1.0 mg/L of ClO_2 was pumped through the pipe reactor and the ClO_2 residual at each sampling port was measured during the experiment until the ClO_2 residual at each sampling port stabilized. The experiments were conducted in duplicate. The first order kinetic expression was used to evaluate the data.

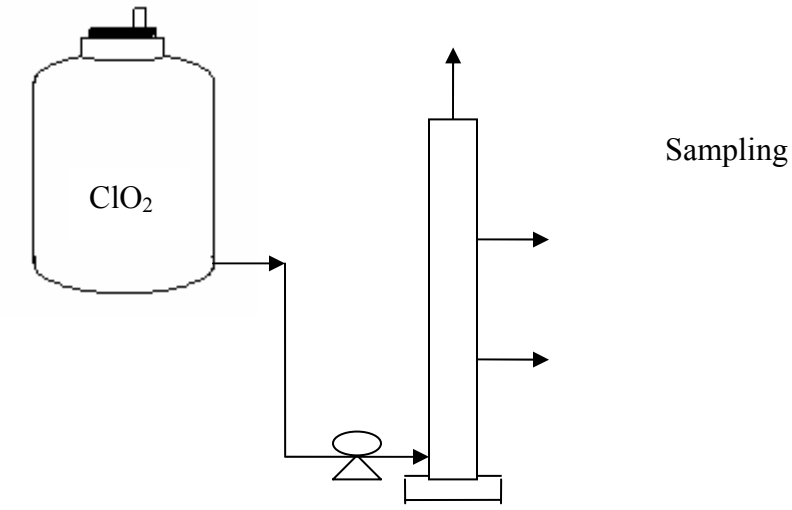


Figure 3. The pipe reactors setup

3.4 THE MODEL PLUMBING SYSTEM

3.4.1 Model plumbing system

The model plumbing system consists of three parallel transparent PVC pipes (1"×8') which can be operated in a single pipe network or as separate loops (Figure 4). One pipe is used as a control pipe and the other two receive different disinfectant doses. The flow throughout the plumbing system was turbulent, with Reynold's number (Re) between 10,000 and 11,000 at flow rate at 3.2 gpm. The pipes were wrapped with aluminum foil and the room was kept dark as much as possible to eliminate the effects of the UV light emitted by florescent lamps. Interference due to chlorine was eliminated by using dechlorinated water.

Figure 4. Model plumbing system

3.4.1.1 Stock solution The source of naturally-grown *Legionella* and HPC bacteria was sediment and associated water collected from filter cartridges in the recirculating hot water system of a VA Hospital in Pittsburgh. The presence of *Legionella pneumophila* in the harvested and collected filter/water suspension was confirmed using direct fluorescent antibody staining (DFA). The solution was maintained at room temperature in a 10-liter carboy and continuously aerated to maintain the dissolved oxygen (DO) level at 6.5 – 8.0 mg/L.

3.4.1.2 System inoculation and biofilm formation The inoculum solution consisted of 1.5 liter of the *Legionella* stock solution, 8 liters of dechlorinated tap water, and 0.5 L of nutrient supplement solution containing 0.25 gram of ferric pyrophosphate, 1.0 g of alpha-ketogluterate and 0.4 g cysteine. The inoculum solution was incubated for 14 days at 37°C prior to use.

After 14-day incubation at 37°C, the inoculum solution was added to the model system with an additional 20 liters of dechlorinated tap water and recirculated intermittently for 14 days at 3.2 gpm to establish a consistent biofilm population.

3.4.1.3 Disinfection The first loop was used to investigate the synergistic efficacy of continuous addition of 0.2 mg/L chlorine dioxide and 0.5 mg/L free chlorine for controlling *Legionella* at room temperature by using syringe pumps (Cole-Parmer, Vernon Hills, Illinois) , compared with the result of the second loop with continuous addition of 0.2 mg/L of chlorine dioxide alone for controlling *Legionella*. The third loop was used as control loop.

In the second run of the test, the first loop was used to evaluate the continuous addition of 0.2 mg/L chlorine dioxide for controlling *Legionella* at room temperature. The second loop was used to investigate the effect of temperature on the efficacy of mixed disinfectant (0.2 mg/L of chlorine dioxide and 0.5 mg/L free chlorine) for controlling *Legionella*. The temperature of the

second loop was maintained at ~40°C with the heating tape. The third loop was used as the control loop. The impact of temperature on 0.2 mg/L of chlorine dioxide for *Legionella* control was also investigated in the model plumbing system. And the experiments were conducted in duplicate.

Chlorine dioxide residual in all experiments was monitored at every hour for the first 24 hours by the method described in previous section. The *Legionella* and HPC bacteria concentrations in planktonic phase and in the biofilm were monitored at 0, 1, 3, 6, 24 and 48 hours.

3.4.1.4 Planktonic and biofilm sampling The details of the procedure was similar to that used by Gao 54. A 5-ml planktonic water sample was collected from each sampling loop at the mixing tank with addition of 0.05ml of 15% sodium thiosulfate to neutralize the disinfectant residual. 0.1 ml of the solution was plated in duplicate directly and after dilution onto Buffered Charcoal Yeast Extract (BCYE), buffered charcoal yeast extract with Dyes, Glycine, Vancomycin and Polymyxin (DGVP) and R2A media plates. 1 ml of the solution was mixed with 1 ml of filter sterilized HCl-KCl solution for 3 minutes and 0.1 ml of this acid treated solution was plated in duplicate onto BCYE and DGVP media plates to culture *Legionella*. The samples were also diluted and plated if necessary. The mean value of the results from all these dilutions was used as data points for data analysis.

Biofilm samples were taken by swabbing the inner surface of a predetermined section of the pipe starting from both ends of each sampling pipe. The swab was first vortexed for 1 minute in 5 ml of sterilized water. 0.1 ml of the solution was plated in duplicate directly and after dilution onto BCYE, DGVP and R2A media plates. 1 ml of the solution was mixed with 1 ml of filter sterilized HCl-KCl solution for 3 minutes and 0.1 ml of this acid treated solution was plated

in duplicate onto BCYE and DGVP media plates to culture *Legionella*. The mean value of the results from all these dilution was used as data points for data analysis.

3.4.1.5 pH and temperature determination pH was measured using a Fisher Scientific, Accumet, pH meter, model No.25. pH of the water was 7.0 throughout the experiment. Room temperature was 25±2 °C. The hot water temperature was maintained at 40 °C by wrapping a heating cable around the loop. Water temperature was measured using a portable thermometer.

4.0 RESULTS AND DISCUSSION

4.1 APPLICATION OF CHLORINE DIOXIDE FOR *LEGIONELLA* CONTROL IN HOSPITAL WATER SYSTEMS AND MONITORING THE FATE OF THE DISINFECTION BYPRODUCTS

4.1.1 Hospital A

4.1.1.1 Water quality parameters The water quality of the municipal water supply was monitored in October 2003 and June 2004. The mean values of water quality parameters were as follows: hardness was 127 mg/L as calcium carbonate, alkalinity was 83 mg/L as calcium carbonate, pH was 7.70, total iron was 0.03 mg/L, total manganese was 0.01 mg/L, total organic carbon (TOC) was 1.96 mg/L, and turbidity was 0.40 NTU. The ClO_2 demand of the drinking water was determined to be 0.20 mg/L after 6-hour contact time at 24 °C and pH 7.9 using Method 2350C of the Standard Methods for the Examination of Water and Wastewater.

4.1.1.2 *Legionella* positivity In these studies the extent of *Legionella* colonization was expressed as *Legionella* percent positivity, which is the number of sample sites positive for *Legionella* divided by the total number of sites tested. The risk of legionnaires' disease in hospital patients has been shown to be better predicted by the percentage of water-system sites testing positive for *Legionella* than by the measured concentration of *Legionella* bacteria [73, 99]

Healthcare facilities are increasingly faced with the decision of choosing a *Legionella* disinfection method. It has been recommended that such systems undergo a 4-step evaluation process to ensure safety and efficacy [73]. This study represents step 3 of the process for chlorine dioxide; controlled prospective study in an individual hospital.

Legionella positivity in hot water was reduced from 60% (12/20) in August 2003 to 10% (2/20) in February 2006 (Figure 5). Regression analysis showed that a significant decline in *Legionella* positivity in the hot water was observed after 18 months due to low ClO_2 residual ($p<0.05$, Figure 5). We believe that this decline can be attributed to a significant increase in ClO_2 residual in the hot water. ClO_2 residual in the hot water increased significantly from 0.04 mg/L in August 2003 to 0.11 mg/L in February 2006 ($p<0.05$, Figure 5). The decline in *Legionella* positivity in the hot water can not be attributed to the variation of the hot water temperatures because hot water temperatures below 60 °C did not affect *Legionella* colonization [67, 68]. The mean distal site hot water temperature was 44 °C during the study (range from 27 to 52°C). The mean distal site cold water temperature was 18 °C (range from 4 to 31°C). *Legionella* positivity in cold water samples was below 20% with 0.3 -0.5 mg/L of ClO_2 residual (Figure 6). The increase in ClO_2 with time in the cold water was not significant ($p>0.05$).

Although the overall distal site positivity declined during the study, we did not observe a significant decrease in the concentration of *Legionella* (mean CFU/ml) in positive samples (decreased from 265 CFU/ml to 30 CFU/ml, $p>0.05$). No cases of hospital-acquired Legionnaires' disease have been detected at the hospital since the ClO_2 system was installed in January 2003.

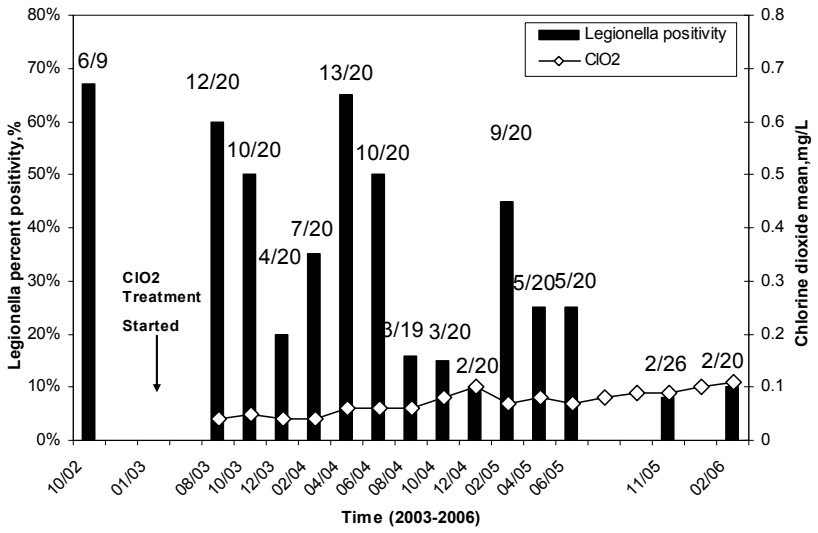


Figure 5. A significant reduction in *Legionella* postivity was observed after the ClO_2 treatment ($p<0.05$). Figure depicts *Legionella* positivity and ClO_2 in hot water samples.

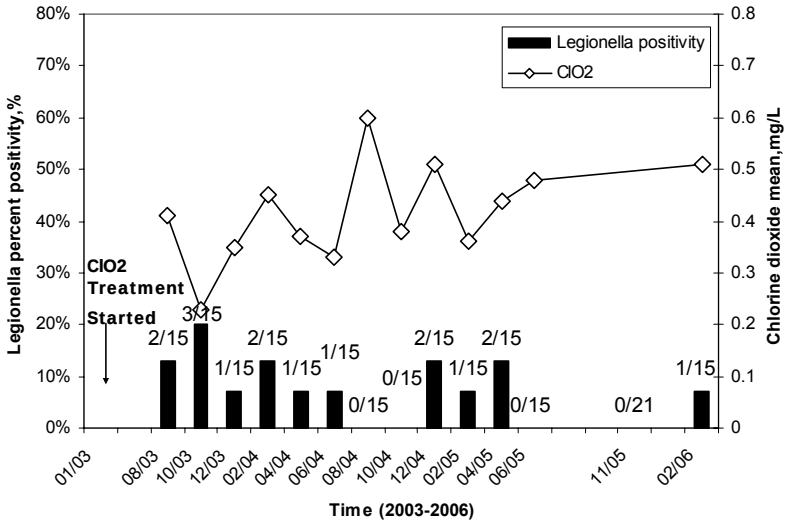


Figure 6. *Legionella* positivity was below 20% in the cold water with a 0.3-0.5 mg/L ClO_2 residual. Figure depicts *Legionella* positivity and ClO_2 in cold water samples.

In February 2005, *Legionella* positivity of hot water samples unexpectedly increased from 10% (December 2004) to 45%. No malfunction of the ClO_2 generator was reported prior to

this increase and mean ClO_2 residual remained at 0.36 mg/L in the cold water on the sampling day. The reason for the increase in *Legionella* positivity remains unclear. The feed concentration of ClO_2 was increased to 0.58 mg/L in B1 in April 2005. Samples collected in April and June 2005 showed that *Legionella* positivity returned to 25% (Figure 5).

The feed pump of the ClO_2 generation system was changed on June 8th, 2004 to allow for additional feed capacity. As shown in Figure 9, the mean monthly ClO_2 residual in the cold water increased from 0.41 mg/L to 0.54 mg/L after the pump replacement. The changes and variability in mean monthly ClO_2 residual are attributed to operational adjustments and maintenance

After the ClO_2 system was installed, a significant decrease in *Legionella* percent positivity was observed in the hot water system. However, an extended period of time (18 months) was needed to achieve a significant reduction of *Legionella* positivity in the hot water system of this hospital. This observation is consistent with the study by Sidari et al. in which the time to complete elimination of *Legionella* positivity was 1.75 years [1]. Sidari speculated that the injection of ClO_2 into the 520,000 gal reservoir and distribution over a large campus may have contributed to the prolonged lag period. In the present study, ClO_2 was injected into the incoming cold water main of a comparatively smaller secondary distribution system. The delay in *Legionella* reduction is more likely due to the low concentration of ClO_2 in the hot water. This is significant because *Legionella* species proliferate in hot water [57]. It is clear that maintaining sufficient ClO_2 residual in the hot water system is quite challenging. Elevated water temperature hastens the conversion of ClO_2 to ClO_2^- the reactions with organic compounds in the water distribution system [102]. This is consistent with the observation that ClO_2 was consumed and

converted to ClO_2^- in hot water and the mean ClO_2^- concentration in hot water was higher than in cold water (Figure 7).

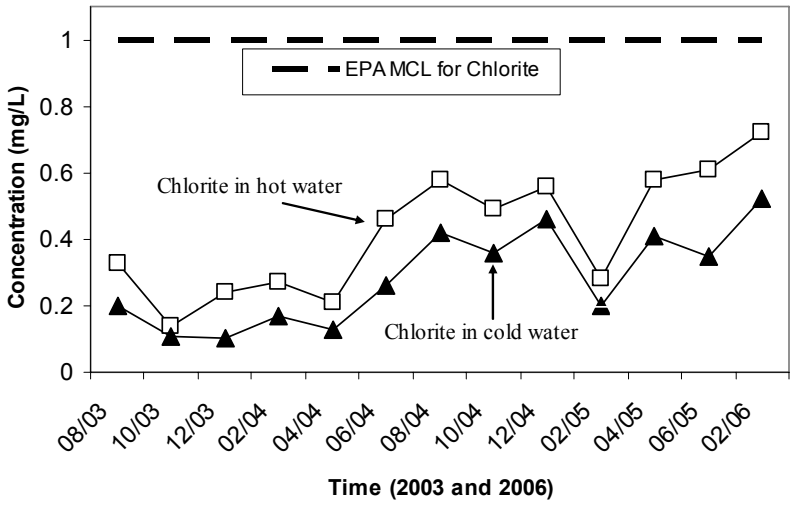


Figure 7. Mean chlorite level in the hot and cold water of hospital A

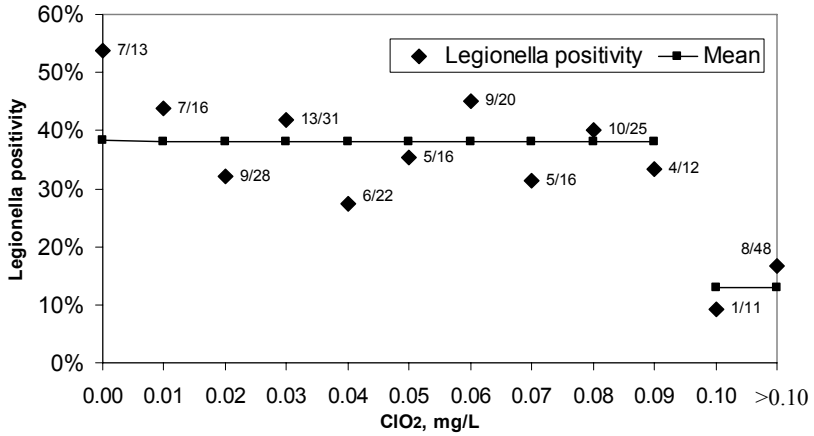


Figure 8. Effect of ClO_2 concentration on *Legionella* positivity in the hot water. The percentage of samples positive for *Legionella* decreased significantly after ClO_2 residual in the hot water increased to ≥ 0.1 mg/L (t-test, $p<0.05$)

Percent *Legionella* positivity in the hot water samples decreased as ClO_2 residual in the hot water increased to ≥ 0.1 mg/L (Figure 8). Since p value from t-test was less than 0.05, the null hypothesis that the mean *Legionella* positivity was the same between samples with chlorine dioxide residual below or above 0.1 mg/L was rejected. The decline in *Legionella* positivity in the hot water with increasing ClO_2 residual ≥ 0.1 mg/L was significant ($p<0.05$) (Figure 8). In this hospital distribution system, mean ClO_2 residual in the hot water seldom reached 0.10 mg/L, which may explain the extended time needed to accomplish significant reduction of *Legionella* positivity. Increasing ClO_2 residual up to 0.1 mg/L at distal outlets in hot water system might be helpful to the application of ClO_2 for controlling *Legionella*.

Previous field studies on the efficacy of ClO_2 for controlling *Legionella* in European hospitals showed that at least six months of continuous injection of ClO_2 was required for complete eradication of *Legionella* or to achieve a significant reduction of *Legionella* positivity [76, 103]. A field study on the efficacy of ClO_2 in European hospitals also reported that *Legionella* persisted in significant numbers (up to 20,000 CFU/L) and with little reduction in the number of positive sites with ClO_2 treatment for 2 years with the injection of 0.5 mg/L ClO_2 in both hot and cold water [104]. Our results show that *Legionella* can be suppressed in the cold water with the ClO_2 residual of 0.30-0.50 mg/L. This is consistent with results of other studies [73]. However, an extended period of time (18 months) was still needed to achieve a significant reduction of *Legionella* positivity in the hot water system since ClO_2 residual in the hot water seldom exceeded 0.10 mg/L.

It may be possible to reduce this lag period by performing shock ClO_2 treatment [105]. Makin reported that the successful application of ClO_2 in hot water system for controlling *Legionella* required increasing the ClO_2 level to 3-5 mg/L in hot water system [81]. Alternatively,

daily flushing of sinks and showers in patient rooms may also be effective [105]. However, both of these measures need to be evaluated in a controlled study.

Another possible approach to achieving higher ClO_2 residual in hot water includes adding a ClO_2 injection point to the line after the hot water tanks. This may shorten the time needed to achieve measurable ClO_2 residual at distal outlets. The impact of injecting ClO_2 directly into the hot water for controlling *Legionella* has yet to be evaluated.

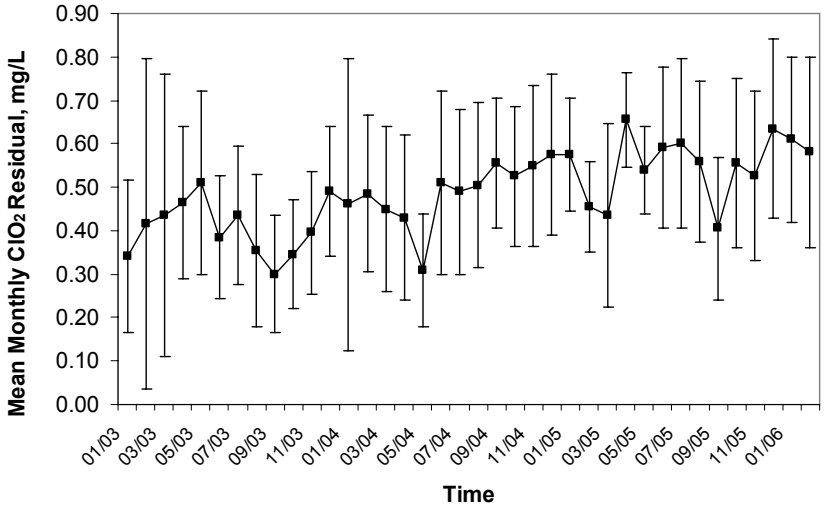


Figure 9. Mean monthly ClO_2 residual in cold water samples. The changes and variability in mean monthly residual are attributed to operational adjustments and maintenance.

4.1.1.3 Mechanical modification of the ClO_2 feed system The feed pump of the ClO_2 generation system was changed on June 8th, 2004 to allow for additional feed capacity. As shown in Figure 3, the mean monthly ClO_2 residual in the cold water increased from 0.41 mg/L

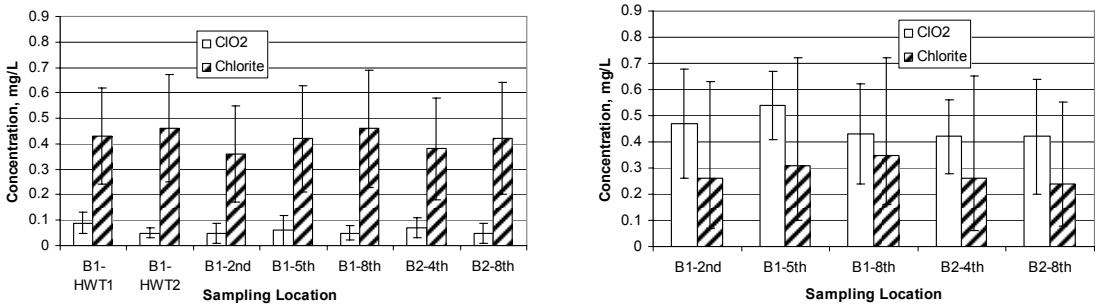
to 0.54 mg/L after the pump replacement. The changes and variability in mean monthly residual are attributed to operational adjustments and maintenance (Figure 9).

4.1.1.4 ClO_2 and its disinfection by-products ClO_2, ClO_2^- and ClO_3^- levels were monitored throughout the buildings during the study. The mean ClO_2 residual in the hot water was 0.07 mg/L. The mean ClO_2 residual in the cold water was 0.42 mg/L. The difference in mean ClO_2 concentrations between the cold water distal outlets and hot water distal outlets was significant ($p<0.05$). The mean ClO_2 residual in the hot water seldom exceeded 0.10 mg/L.

A total of 91 hot water samples and 65 cold water samples were analyzed for ClO_2^- and ClO_3^-. The average ClO_2^- concentrations in the hot and cold water were 0.42 and 0.28 mg/L, respectively. The ClO_2^- concentrations measured in this study were in agreement with those obtained by the reference lab (NovaChem Laboratories Inc., Oxford, OH). The mean difference was only 8 % ($p>0.05$).The mean ClO_2^- concentrations in cold and hot water were below the EPA MCL of 1.0 mg/L. The mean ClO_3^- concentrations in hot and cold water were below 0.10 mg/L.

The mean ClO_2 and ClO_2^- concentrations between the different sampling locations in hospital A over 2 years are presented in Figure 10 a and b. Chlorite concentration ranged from 0 to 0.82 mg/L. Chlorine dioxide concentration ranged from 0 to 0.70 mg/L. There were no significant differences ($p>0.05$) in mean ClO_2 and ClO_2^- concentrations between the sampling locations that represent various distances from the injection point in the hot water (Figure. 10a) and in the cold water (Figure 10b). F statistics obtained by ANOVA ranged from 0.49 to 1.16, whose p-values were greater than 0.05. The null hypothesis that mean chlorite and chlorine dioxide levels between different sampling locations were same was not rejected.

The operation of the ClO_2 system for controlling *Legionella* in the hospital water system was found to be safe based on the MRDL for ClO_2 and MCL for ClO_2^-. The ClO_2 residual at distal outlets was below the MRDL. The mean concentrations of ClO_2^- in the hot and cold water throughout the buildings were below the MCL levels. ClO_3^- is currently not regulated but its levels in the hot and cold water never exceeded 0.20 mg/L. Users of chlorine dioxide systems must comply with current regulations for municipal water systems. This involves daily monitoring of ClO_2 and monthly monitoring of ClO_2^-. ClO_2^- monitoring can be reduced to quarterly monitoring after monthly monitoring results show that the ClO_2^- level in the distribution system has not exceeded the MCL of 1.0 mg/L for one year [3]. Our data suggest that less frequent monitoring of ClO_2^- levels in a hospital with a small secondary water system would be sufficient to satisfy safety concerns.



a) Hot water b) Cold water

Figure 10. Distance from the ClO_2 point-of-injection did not significantly affect mean concentrations of ClO_2 and ClO_2^- in the hot and cold water (ANOVA, $p>0.05$).

Srinivasan compared the ClO_2 and ClO_2^- levels at different sampling locations between two time points in a hospital water system [2]. The ClO_2 and ClO_2^- levels in the lower floor were higher than the ClO_2 and ClO_2^- levels in the higher floor one month after the start of the

treatment. The differences disappeared after 17 months. One explanation for this phenomenon was that the ClO_2 demand of the system had been met after 17 months. In our study, the mean ClO_2 and ClO_2^- levels at different sampling locations were compared during the study. There was no significant difference in mean ClO_2 and ClO_2^- concentration in the hot water between sampling locations that represented various distances from the ClO_2 injection point in B1 and B2 (Figure 10a). There was also no significant difference in ClO_2 and ClO_2^- levels with increasing distance from the point of injection in the cold water in B1 and B2 (Figure 10b). The change of ClO_2 and ClO_2^- levels with time could be due to the mechanical modifications to the ClO_2 feed system and operational adjustments. Given that this study began six months after the installation and operation of the ClO_2 unit, the initial demand may have been met and system equilibrium reached within the first six months of continuous treatment of ClO_2.

A significant reduction of *Legionella* positivity in the hot water was achieved by ClO_2 treatment. An extended time (18 months) was needed to achieve the significant reduction of *Legionella* positivity due to low ClO_2 residual in the hot water. ClO_2 did not completely eliminate *Legionella* in the hospital hot and cold water system when the target feed concentration was 0.5-0.7 mg/L in the cold water. However, this and other studies have demonstrated that zero positivity is not necessary to prevent hospital acquired Legionnaires' disease [73].

Until the optimal operating parameters for ClO_2 are delineated, we recommend 1) regular environmental monitoring for *Legionella* must be performed, 2) all patients with hospital acquired pneumonia must be screened for Legionnaires' disease and treated empirically for Legionnaires' disease if the etiology is unknown, 3) other methods for *Legionella* control (periodic heat flush procedures or point-of-use filters for high risk areas) should be instituted in

the first months of operation (possible 6 -12 months) or until a sustained low level of positivity (<30%) can be consistently achieved.

4.1.2 Hospital B

4.1.2.1 Water quality parameters The water quality of the municipal water supply was monitored in June 2004. The values of water quality parameters were as follow: hardness was 124 mg/L as calcium carbonate, alkalinity was 69 mg/L as calcium carbonate, pH was 8.57, total iron was 0.04 mg/L, total manganese was 0.01 mg/L, TOC (total organic carbon) was 2.25 mg/L, and turbidity was 0.38 NTU. The ClO_2 demand of the drinking water was determined to be 0.20 mg/L at 23 °C and pH 7.8 using Method 2350C of the Standard Methods for the Examination of Water and Wastewater.

4.1.2.2 *Legonella* positivity Mean percent positivity of distal outlets for *Legionella* was 60 % before the ClO_2 treatment (range from 35 to 88 %, n = 72). After the ClO_2 treatment, mean percent positivity of distal outlets for *Legionella* decreased from 60 % to 8 % (range from 0 to 24%, n = 165, $p<0.05$) (Figure 11). *Legionella* positivity in the hot and cold water was reduced to 0% in 6 months and remained at 0% for three consecutive sampling events since August 2005 (Figure 11). *Legionella* positivity increased to 19% in June 2006 when the ClO_2 generator malfunctioned on the sampling date; the ClO_2 residual in hot and cold was 0.09 and 0.29, respectively, which were lower than the average values. No cases of healthcare-acquired legionellosis have been identified in the post-disinfection period.

The mean concentration of *Legionella* in positive hot water samples decreased from 166 CFU/ml (range from 10 to 520 CFU/ml) to 43 CFU/ml (range from 10 to 100 CFU/ml). The

mean concentration of *Legionella* in positive cold water samples decreased from 20 CFU/ml (range from 10 to 20 CFU/ml) to 0 CFU/ml. The decrease in mean concentration of *Legionella* in positive samples was not significant, but the overall distal site positivity decreased significantly.

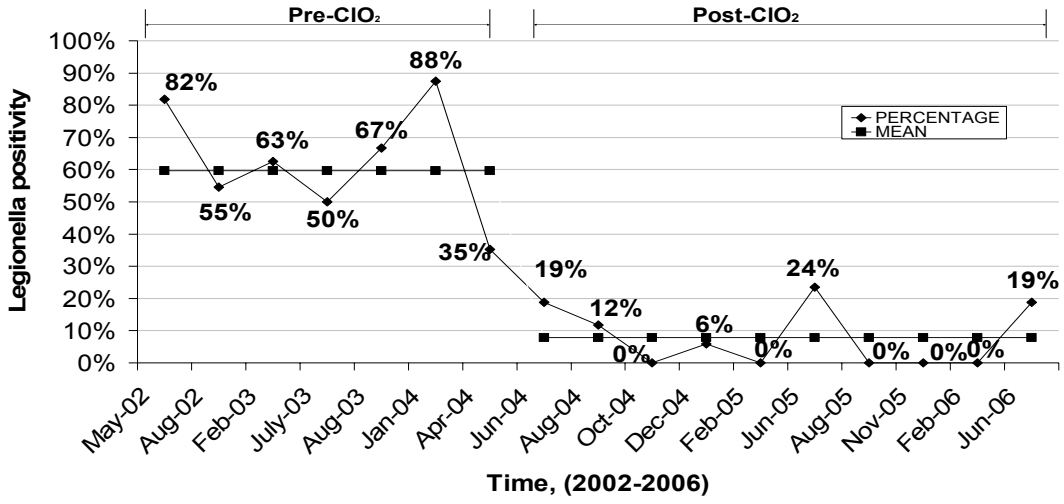


Figure 11. Mean percentage of distal outlets positive for *Legionella* was reduced from 60 % to 8 % after the ClO_2 treatment (t-test, $p<0.05$).

Legionella positivity in this hospital water system was reduced to 0% in a much shorter period of time (6 -10 months). This observation was not the same as our previous study in hospital A [1, 106]. In hospital B, the mean ClO_2 residual in hot water reached above 0.10 m/L in a much shorter time. And mean ClO_2 residual in hot water of this hospital was significantly higher than hospital A (Table 2). Statistical analysis showed that the percentage of samples positive for *Legionella* in the hot water samples decreased as ClO_2 residual increased to ≥ 0.1 mg/L (Figure 12). The null hypothesis of Chi-square test was that possibility of success for treatment with ClO_2 residual less than 0.1 mg/L or greater than 0.1 mg/L was same. Since p value obtained by Chi-square test was greater than 0.05, the null hypothesis was not rejected. The decease was not significant ($p>0.05$) since the sample size of positive samples was small. It is hypothesized that

the less time it takes to reach to ≥0.10 mg/L ClO_2 residual in hot water, the faster reduction of *Legionella* positivity can be achieved.

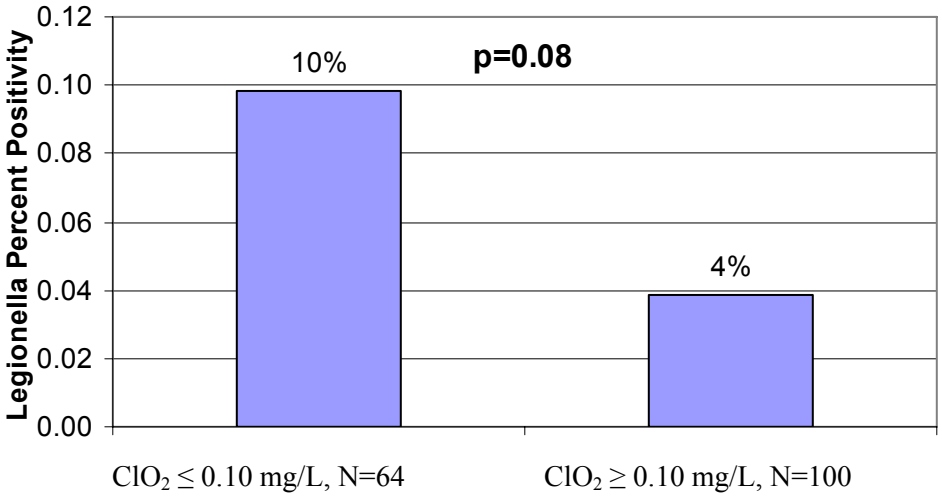


Figure 12. *Legionella* percent positivity in the hot water decreased as ClO_2 residual increased to ≥ 0.1 mg/L (Chi-square, p>0.05)

It was also found that the incoming drinking water received from the city's supply system contained high levels of free chlorine (range from 0.75 to 1.02 mg/L) because the hospital was very close to the water treatment plant (driving distance less than 5 miles). The mean chlorine in the incoming water of hospital B was higher than hospital A (Table 2). It can be hypothesized that the coincident reaction of two disinfectants (chlorine and ClO_2) provided synergistic effects for controlling *Legionella* in the water distribution system. It is also possible that the high levels of free chlorine in the drinking water can meet some oxidant demand so that ClO_2 injected in this health care facility can be maintained at a stable residual concentration in both hot and cold water systems. Katz *et.al* applied the equal dose of chlorine dioxide and chlorine to disinfect the effluent from a municipal sewage treatment plant [97, 107]. The results showed that the combination

of two disinfectants produced relatively stable high residual of both disinfectants, reduced the concentration of the undesirable disinfection byproduct (i.e. chlorite ion), while increasing the concentration of chlorine dioxide. Other studies showed that mechanically mixed oxidants achieved considerable disinfection efficiency for selected microorganisms [97]. And sequential disinfection with chlorine dioxide followed by free chlorine is an effective approach used to treat *Cryptosporidium parvum* [98]. The synergistic effect of sequential treatment may be caused by the unique activity of each disinfection agent reacting with specific chemical groups of the cell wall. However, the level of the enhanced disinfection efficiency remains unclear. The synergistic effects of ClO_2 and chlorine for *Legionella* control will be investigated in a future study to confirm these hypotheses.

Table 2. Study parameters of two hospitals using chlorine dioxide for *Legionella* control

Parameter	Hospital A	Hospital B	
Size	438 beds 2 buildings	672 beds 1 building	
ClO_2 injection point	Cold water main	Cold water main	
Mean ClO_2 in hot water (mg/L)	0.07	0.12	$p<0.05$
Mean ClO_2 in cold water (mg/L)	0.42	0.36	$p>0.05$
Mean Cl_2 in cold water (mg/L)	0.55	0.91	$p<0.05$
Months to achieve 0% *Legionella* positivity	>24 months	6-10 months	

4.1.2.3 HPC bacteria and *Pseudomonas* The efficacy of ClO_2 for controlling HPC bacteria in this hospital hot water system was also evaluated. The result in Figure 13 shows that the HPC bacteria average concentration in the hot water was reduced from 15,427 CFU/ml to 2,927 CFU/ml ($p<0.05$) after ClO_2 treatment.

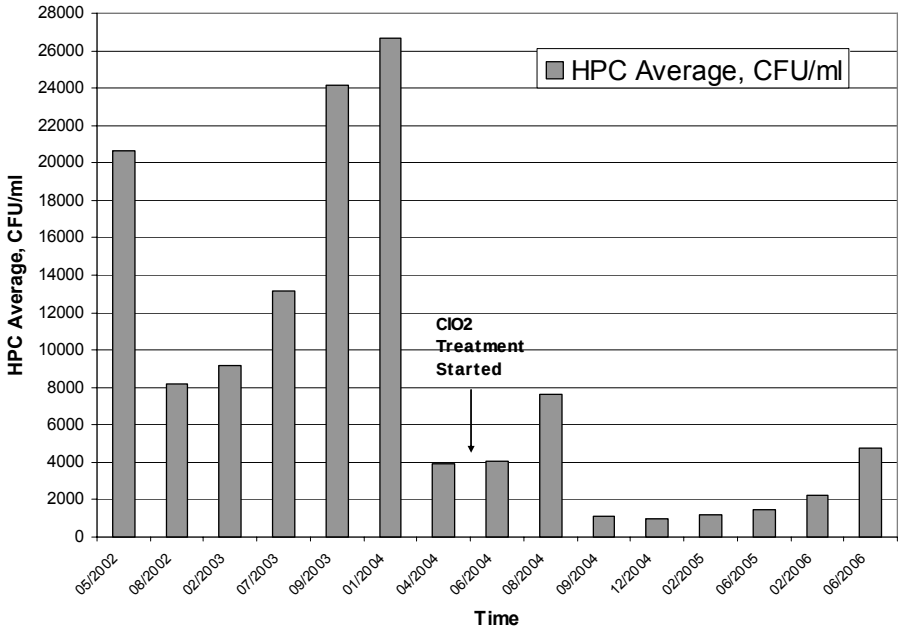


Figure 13. HPC bacteria concentration in hot water samples decreased significantly after ClO_2 treatment (t-test, $p<0.05$).

Although most of the heterotrophic bacteria in drinking water are not human pathogens, HPC bacteria in drinking water may include isolates from the genera that may be pathogenic for immunocompromised patients in hospitals, such as Pseudomonas spp., and different fungi [108]. The efficacy of ClO_2 for controlling HPC bacteria in this hospital was also evaluated to assess the risk of infection from opportunistic pathogens. The HPC bacteria average concentration was reduced from 15,427 CFU/ml to 2,927 CFU/ml after ClO_2 treatment.

In September 2006, a new chlorine dioxide unit was installed in another building housing Burn Units of hospital B. The Burn Units were heavily colonized by *Pseudomonas aeruginosa*. After 5 months of chlorine dioxide treatment, Pseudomonas positive percentage decreased from 100% (5/5) to 20% (1/5). The concentration decreased from 84,000 to 973 CFU/ml. The HPC bacteria average concentration was also reduced from 104, 031 to 1,600 CFU/ml in hot water, from 5273 to 950 CFU/ml in cold water. These results show that ClO_2 is an effective disinfectant not only for controlling *Legionella* but also for other pathogenic bacteria in drinking water.

4.1.2.4 ClO_2 and its disinfection by-products The mean ClO_2 residuals in the hot and cold water samples were 0.11 and 0.36 mg/L, respectively (Table 2.). The difference in mean ClO_2 concentrations between the cold water distal outlets and hot water distal outlets was significant ($p<0.05$). A total of 54 hot water samples and 36 cold water samples were analyzed for ClO_2^- and ClO_3^-. The mean ClO_2^- concentrations in cold and hot water were 0.42 and 0.38 mg/L, respectively. The mean ClO_3^- concentrations in hot and cold water were less than 0.10 mg/L. The ClO_2^- concentrations measured in this study were in agreement with those obtained by the reference lab (NovaChem Laboratories Inc., Oxford, OH). The mean difference was 8 % ($p>0.05$).

Figure 14 and Figure 15 present the mean ClO_2 and ClO_2^- concentration at different locations in the hot and cold water system. ANOVA analysis showed that there was no significant difference of ClO_2 and ClO_2^- levels between the different sampling locations that represent various distance from the injection point in the hot water and cold water ($p>0.05$). F statistics obtained by ANOVA ranged from 0.11 to 0.94, whose p-values were greater than 0.05. The null hypothesis that mean chlorite and chlorine dioxide levels between different sampling locations were same was not rejected.

The mean distal site water temperatures for the hot and cold water during the study were 43 °C (range from 34 to 52 °C) and 17 °C (range from 4 to 25 °C), respectively.

Our data suggest that the malfunction of the generator would directly cause the increase of *Legionella* positivity in the hospital plumbing system. So monitoring of the operational efficiency of the ClO_2 generator is also a key factor for the successful application of ClO_2 for *Legionella* control.

The operation of the ClO_2 system for controlling *Legionella* in hospital water system was found to be safe based on the MRDL for ClO_2 and MCL for ClO_2^-. The ClO_2 residual at distal outlets was below the MRDL. The mean concentrations of ClO_2^- in hot and cold water throughout the building were below the MCL levels. ClO_3^- is currently not regulated but its levels in hot and cold water never exceeded 0.30 mg/L. Users of chlorine dioxide systems must comply with current regulations for municipal water systems. This involves daily monitoring of ClO_2 and monthly monitoring of ClO_2^-. ClO_2^- monitoring can be reduced to quarterly monitoring after monthly monitoring results show that the ClO_2^- level in the distribution system has not exceeded the MCL of 1.0 mg/L for one year [3]. Our data suggest that chlorite level in hot and cold water of an open water distribution system is unlikely to exceed the EPA MCL when 0.5-0.7 mg/L of ClO_2 is injected in the incoming cold water. Less frequent monitoring of the disinfection byproducts would satisfy the safety concerns in hospital water systems.

The comparison of the mean ClO_2 residual level at different sampling locations showed that there were no significant differences of mean ClO_2 and ClO_2^- concentrations between the sampling locations that represent various distance from the injection point in hot and cold water. One possible reason that the overcome of the hospital water system demand reported by the other study [2] was not observed in the present study was that high level of free chlorine in the incoming

cold water meets some of the oxidant demand and help ClO_2 residual maintained at a relative stable level. And chlorite in hot water of hospital B was slightly higher than in cold water (Figure 16).

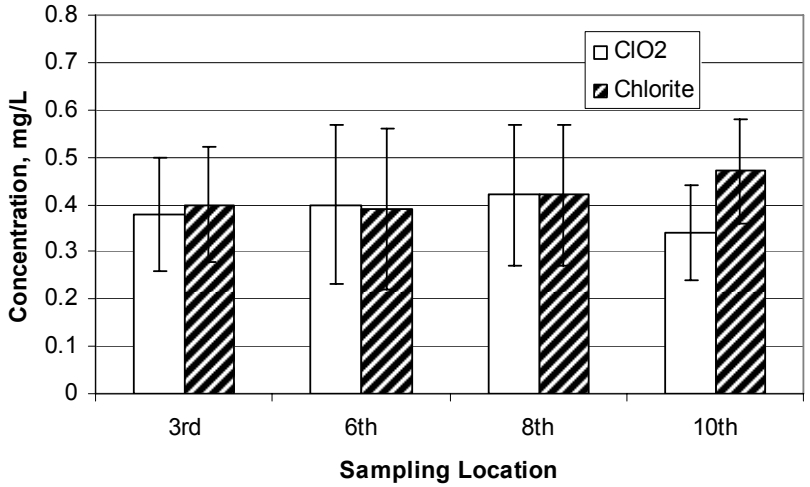


Figure 14. Distance from the ClO_2 point-of-injection did not significantly affect mean concentrations of ClO_2 and ClO_2^- in the cold water (ANOVA, $p>0.05$).

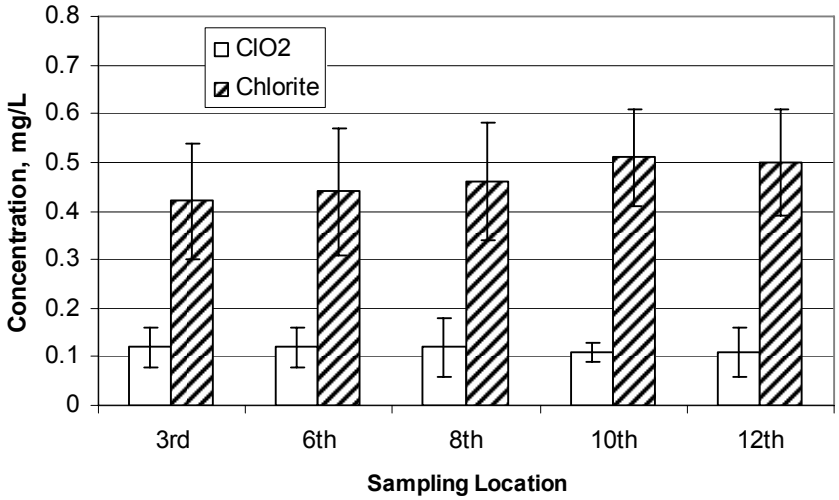


Figure 15. Distance from the ClO_2 point-of-injection did not significantly affect mean concentrations of ClO_2 and ClO_2^- in the hot water (ANOVA, $p>0.05$).

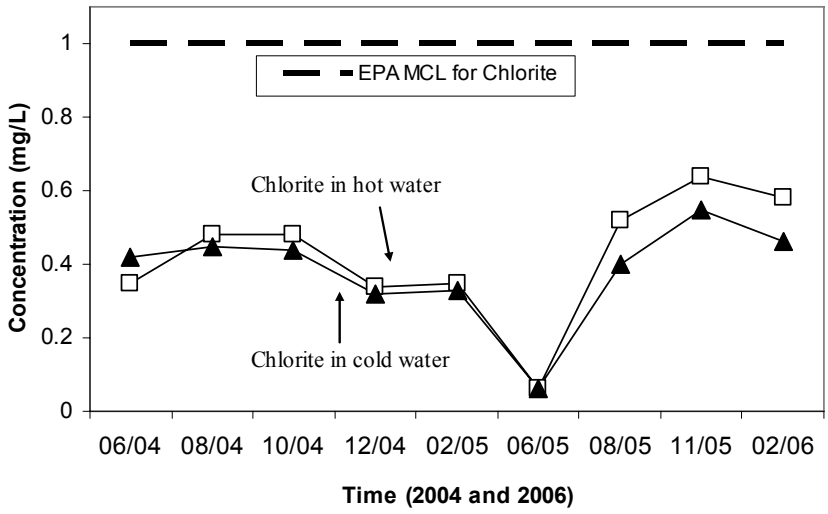


Figure 16. Mean chlorite level in the hot and cold water of the hospital B

4.1.2.5 Cost of *Legionella* control *Legionella* remediation efforts are not inexpensive. Cost estimates have been reported to be in the range of $70,000 - $80,000 for continuous hyperchlorination, and $60,000 - $100,000 for copper-silver ionization systems [57]. One hospital in Wales estimated the cost for engineering measures with chlorine dioxide at approximately $50,000 per annum [104]. The annual cost for operation and maintenance of two chlorine dioxide units at this 438-bed New York hospital was approximately $16,800 per unit per year. This amount did not include installation costs because the hospital leased the chlorine dioxide units. Hospital personnel installed the required flowmeters, filters, injection points, and piping. The annual cost for monitoring the chlorine dioxide residual and chlorite level in the hospital water system ranged from $3,000 to $5,000, depending on the frequency of monitoring. The total annual cost was approximately $39,000.

Legionella was successfully controlled by chlorine dioxide in these two hospitals. And chlorine dioxide disinfection byproducts were below EPA limits. Specifically in this study, when

ClO_2 was injected into the cold water main at 0.5-0.7 mg/L, mean concentrations of ClO_2 and chlorite (ClO_2^-) in cold and hot water samples did not exceed the EPA MRDL of 0.8 mg/L for ClO_2 and MCL of 1.0 mg/L for ClO_2^-, respectively. Distance from the point of ClO_2 injection did not significantly affect mean concentrations of ClO_2 and ClO_2^- in both hot and cold water systems. In addition to *Legionella*, ClO_2 is also a promising disinfectant for controlling other bacteria in drinking water.

4.2 THE EFFECT OF WATER QUALITY PARAMETERS (PH, TEMPERATURE AND TOC ON CHLORINE DIOXIDE DECAY IN DRINKING WATER

4.2.1 Chlorine dioxide decay in DI water

Chlorine dioxide decay in deionized water was investigated at pH 7.5 and 8.5 and at temperature of 25±2 °C and 45°C. The first order kinetics expression was fitted to experimental data and the results are shown in Figure 7. The reaction rate constant (k) can be determined by fitting an exponential curve to the data set according to equation:

$$C/C_0 = e^{-kt}$$

where,

C = concentration of chlorine dioxide at any time t

C_o= initial concentration of chlorine dioxide at time t=0

k = reaction rate constant (/minute),

t = time (minutes)

The reaction rate did not change with pH, but temperature increased the reaction rate constant from 0.0002 to 0.0004 min^{-1} (Figure 17). R^2 ranged from 0.92 to 0.98. Chlorite and chlorate analysis showed that chlorine dioxide was mainly converted to chlorite at 25°±2C (Table 3). Chlorate concentration observed at 45°C was higher than at 25°±2C because higher temperature increased the disproportionation rate of chlorine dioxide. The mass balance verified that the floating cover (Figure 1) virtually eliminated the chlorine dioxide loss due to volatility (Table 3). Therefore, the chlorine dioxide loss due to volatility was not considered in future batch experiments.

Table 3. Mass balance on ClO_2, chlorite, chlorate and chloride during ClO_2 decay in DI water after 6 hours (mg/L) (All concentrations are expressed as chlorine)

Test parameters	ΔClO_2	ΔClO_2^-	ΔClO_3^-	ΔCl^-	ClO_2 loss due to volatilization[b]
26 ºC , pH7.5	0.07	0.04[a]	0.02	0.02	-0.01
45 ºC , pH7.5	0.13	0.06	0.04	0.01	0.02
27 ºC , pH8.5	0.06	0.04	0.01	0.01	0
45 ºC , pH8.5	0.12	0.06	0.05	0.01	0

[a] Cl mass = (atomic Cl mass /MW_{ClO_2})* ΔClO_2, MW-Molecular weight

[b] ClO_2 loss due to volatilization = $\Delta ClO_2 - \Delta ClO_2^- - \Delta ClO_3^- - \Delta Cl^-$

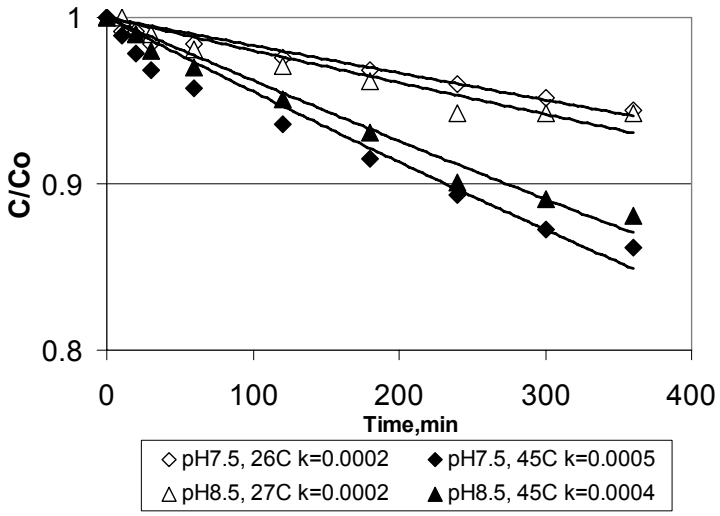


Figure 17. Chlorine dioxide decay kinetics in the deionized water at pH7.5 and pH8.5, at 25±2ºC and 45ºC

4.2.2 Chlorine dioxide decay in drinking water

The chlorine dioxide decay kinetic was also investigated in potable waters with different TOC concentrations at 25 °C and 45°C. A first-order kinetic expression does not represent the entire data set collected at 25 °C very well and the data can be divided into two phases: the initial, rapid chlorine dioxide demand (0-60min) and slower, long-term chlorine dioxide demand (120-360min) (Figure 18 and Table 4). Similar decay patterns have also been observed for chlorine dioxide [79, 109] and chlorine [110, 111]. The first order reaction rate constant for the first phase of chlorine dioxide decay in drinking water at 25 °C ranged from 0.0019 to 0.0029 min^{-1}, while the first order reaction rate constant for the second phase varied in a narrow range from 0.0006 to 0.0008 min^{-1}. The first phase of chlorine dioxide decay probably corresponds to reactions with easily oxidizable compounds, while the second phase corresponds to slower reactions with less reactive compounds. On the other hand, a first-order kinetic expression provides a good

prediction of the chlorine dioxide concentration over the entire reaction period at 45 °C (Figure 18). The estimated first order reaction rate constant for chlorine dioxide at 45 °C ranged from 0.0016 to 0.0044 min^{-1} (Table 4). Figure 19 reveals that the chlorine dioxide reaction rate constant for the first phase at 25 °C increased with the increase in TOC, while the reaction rate constant for second phase experienced no significant change. It could mean that the proportion of easily oxidizable organic carbon increased with the increasing total organic carbon concentration. With increasing the temperature, the first order reaction rate of chlorine dioxide in drinking water was increased (Figure 19). And with increasing the total organic carbon concentration, the chlorine dioxide decay rate increased at 45°C. Overall, Increase in TOC did not have as pronounced impact on the chlorine dioxide decay rate at 25 °C. The higher organic carbon concentration in hot water could be due to biomass carbon of higher thermophilic bacteria concentration at higher temperature.

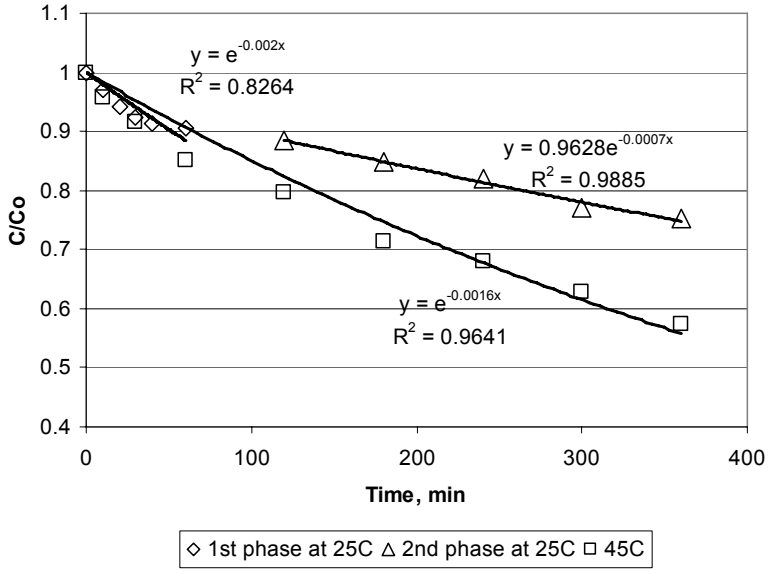


Figure 18. The two-phase decay pattern of chlorine dioxide in drinking water at 25 °C

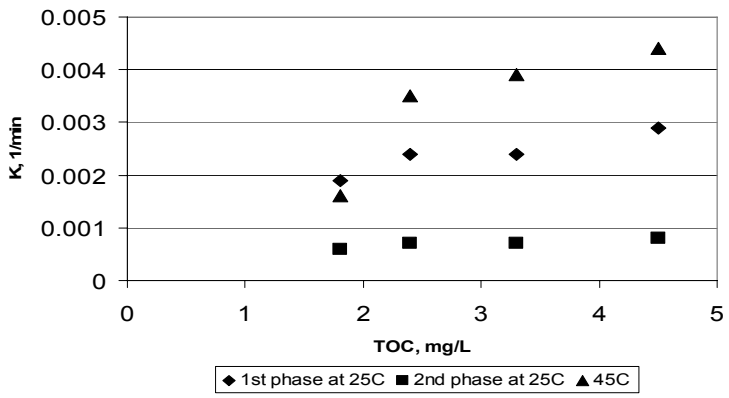


Figure 19. Impact of TOC on the first order reaction rate constant at 25 and 45 ºC

Table 4. Impact of temperature on first-order reaction rate constants for chlorine dioxide consumption in different potable water sources

Temperature		Cold Water A TOC=1.8mg/L	Hot Water A TOC=2.4 mg/L	Cold Water B TOC=3.3 mg/L	Hot Water B TOC=4.5 mg/L
25 °C	1st phase	K= 0.0019 R^2=0.85	K= 0.0024 R^2=0.9772	K= 0.0023 R^2=0.9651	K= 0.0029 R^2=0.9780
	2nd phase	K= 0.0006 R^2=0.9886	K= 0.0007 R^2=0.9974	K= 0.0007 R^2=0.9876	K= 0.0008 R^2=0.9935
45 °C	Whole data	K= 0.0016 R^2=0.97	K= 0.0035 R^2=0.9647	K= 0.0039 R^2=0.9644	K= 0.0044 R^2=0.9751

34% -76% of chlorine dioxide consumed in potable water was converted to chlorite at room temperature and 45°C (Table 5). Chlorate accounts for only 9-19% of chlorine dioxide consumed. The mass balance for chlorine dioxide, chlorite and chlorate can not be set up

because chlorine dioxide is not always converted to chlorite at 1:1 ratio. For example, 25% of chlorine dioxide was converted to chlorite for the reaction of chlorine dioxide with indene, 50% for the reaction with phenol and 100% for the reaction with hydroquinone [14-16, 18]. Other authors reported that 70% percent of chlorine dioxide consumed in drinking water is typically converted to chlorite [17, 90]. Slight difference between the results of this study and literature values could be due to the difference in organic and inorganic composition of portable water sources used for experimental investigation.

The chloride concentrations were not shown in the Table 5 because they were outside the calibration curve and the background chloride concentrations in the potable water sources were too high to show any significant changes associated with chlorine dioxide decay.

Table 5. Change in chlorine dioxide and chlorite, chlorate concentrations in potable water after 6 hours (mg/L)

Water Source	Temperature (^{o}C)	ΔClO_2	ΔClO_2^-	ΔClO_3^-	$\Delta ClO_2^-/\Delta ClO_2$
Cold Water A	25	0.26	0.09	0.05	34%
TOC 1.8 mg/L	45	0.40	0.21	0.06	51%
Hot Water A	25	0.33	0.22	0.05	66%
TOC 2.4 mg/L	45	0.70	0.54	0.05	76%
Cold Water B	25	0.28	0.16	0.03	57%
TOC 3.3 mg/L	45	0.77	0.56	0.06	73%
Hot Water B	25	0.34	0.24	0.03	72%
TOC 4.5 mg/L	45	0.81	0.47	0.07	58%

4.2.3 Reactions of chlorine dioxide with natural organic matter

Controlled experiments with Suwannee River Humic Acid (SRHA) showed that 1.0 mg/L of chlorine dioxide was rapidly consumed in the presence of 1.0 or 2.0 mg/L of this humic substance within 60 minutes. A first-order kinetic expression does not represent the entire data set at 25 or 45 °C very well. Temperature accelerated the consumption of chlorine dioxide in the presence of 1.0 and 0.5 mg/L of SRHA (Figure 20). The reaction of chlorine dioxide with SRHA showed that 63-73% of chlorine dioxide consumed was converted to chlorite (Table 6). Other authors have also reported that natural organic matter reacts with chlorine dioxide very fast in the first few hours followed by a slow reaction [14, 92].

Aldrich humic acid (AHA) was used to investigate the impact of temperature on the reaction mechanism of chlorine dioxide with representative organic matters. 1 g/L of AHA was reacted with approximately 0.5 g/L of chlorine dioxide at room temperature and 45 °C and samples were diluted 100 times for UV-VIS spectra measurement. UV-Vis spectra of Aldrich humic acid is broad, featureless and monotonously decreases with increasing wavelength (Figure 21) [112]. After 3 hours of reaction with chlorine dioxide at 45 °C, substantial decreases in specific absorbance was observed (55% decrease in absorbance at 254 nm and 78% decrease in absorbance at 465nm). It took much longer reaction time (more than 6 hrs) to achieve similar decrease in absorbance at 25 °C (46% decrease in absorbance at 254 nm and 71% decrease in absorbance at 465nm). The decrease of absorbance at 254nm and 465nm suggested that chlorine dioxide radical attack proceeded mainly on the aromatic moieties and color forming moieties of the molecule. And humic acid molecule was degraded by chlorine dioxide to less UV-Vis absorbing compounds. The removal efficiencies of UV absorbing centers could be ascribed relatively slower compared to color forming moieties. It is postulated that the reaction

mechanism between chlorine dioxide and humic acid was the same at different temperatures; higher temperature only increased the reaction rate of chlorine dioxide and humic acid. UV-Raman spectroscopy, C^{13}NMR and GC-MS have also been used to identify the change of functional groups or the organic products of Aldrich humic acid after the reaction with chlorine dioxide at 25 and 45 °C. No meaningful results were obtained by these analytic methods, which is most probably due to the low concentration of reaction products generated and the complexity of humic acid molecules.

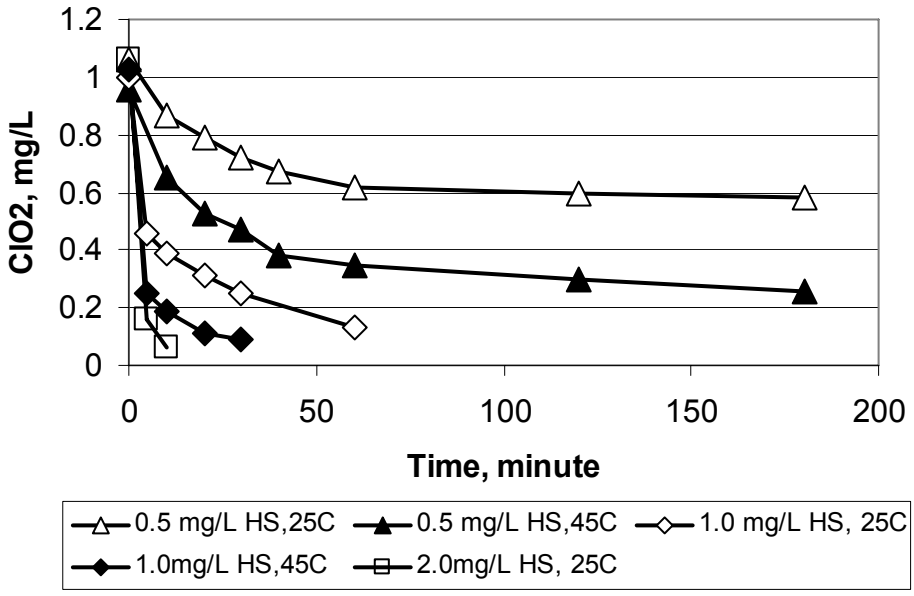


Figure 20. The effect of temperature on chlorine dioxide reaction with humic substance

Hoigné reported that chlorine dioxide is a highly selective oxidant with respect to specific functional groups of organic compounds like phenolic group and tertiary amino groups [13]. Naturally occurring humic substance is a complex organic material containing a variety of

structures and functional groups, such as polyphenolic and carboxylic groups. These functional groups exhibit different reactivity and reaction mechanisms with chlorine dioxide [17, 92]. Temperature increase may have caused sufficient increase in the total energy in the system to overcome the activation energy for the reaction with additional functionalities, which lead to greater consumption of chlorine dioxide at higher temperature. Since natural humic material is a complex substance containing a variety of functional groups, there is no clear theoretical explanation about such high chlorite yield [17, 90].

Another reason that chlorite residual only accounts for 70% of chlorine dioxide is that chlorite is also an effective oxidizing agent that is converted to chloride at a much slower rate than chlorine dioxide conversion to chlorite [113].

Table 6. Change in chlorine dioxide, chlorite, chlorate and chloride generated during the chlorine dioxide reaction with SRHS substance (mg/L)

SRHS Concentration (mg/L)	Temperature (^{o}C)	ΔClO_2	ΔClO_2^-	ΔClO_3^-	$\Delta ClO_2^-/\Delta ClO_2$
2.0	25	0.97	0.71	0.04	73%
1.0	25	0.90	0.63	0.05	70%
	45	0.93	0.68	0.04	73%
0.5	25	0.48	0.30	0.03	63%
	45	0.63	0.41	0.02	65%

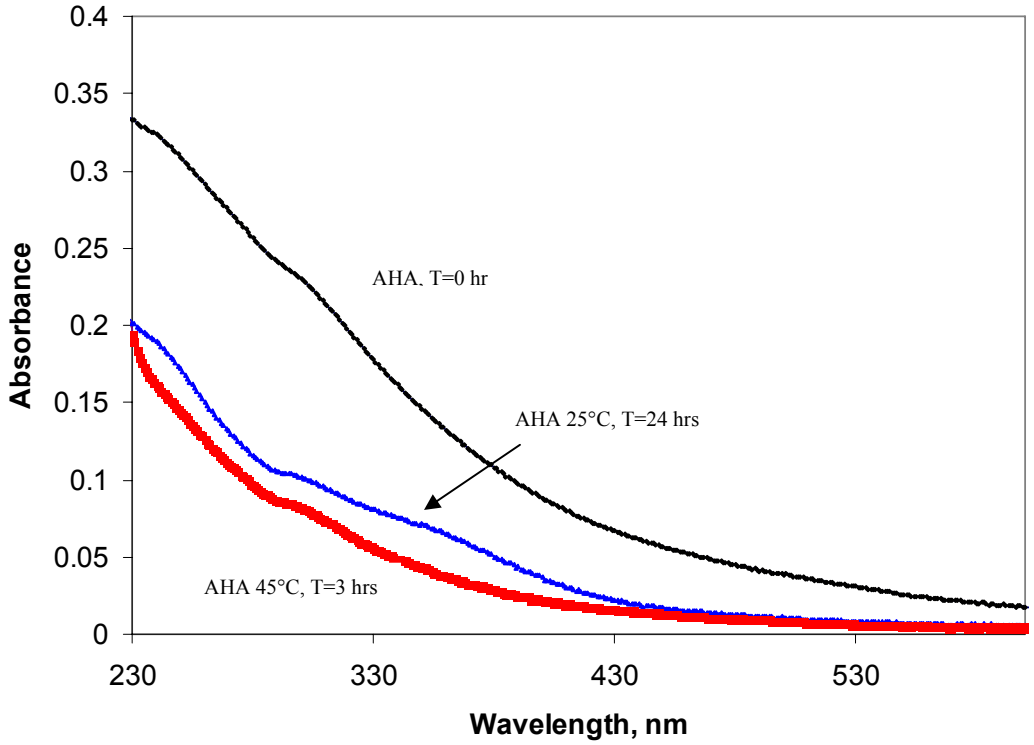


Figure 21. UV-Vis spectra of Aldrich humic acid after the reaction with chlorine dioxide at 25 and 45 °C

TOC concentration in the hot water was higher than in the cold water of the two studied hospitals. The estimated first order reaction rate constant for chlorine dioxide at 45 °C ranged from 0.0016 to 0.0044 min^{-1} and increased with an increase in drinking water TOC. Higher chlorite concentrations observed at 45°C also confirmed the faster reaction rate between chlorine dioxide and organic matter at higher temperature. Increase in TOC did not have as pronounced impact on the chlorine dioxide decay rate at 25 °C. This study suggests that temperature and TOC are both the key factors governing chlorine dioxide demand in hot water systems. The increased consumption of chlorine dioxide due to faster reaction rate and high organic load in hot

water requires an increased initial dose of chlorine dioxide or directly injection of chlorine dioxide into the hot water system to maintain the same residual as in a cold water system.

4.3 THE EFFECT OF PIPE CORROSION SCALE ON CHLORINE DIOXIDE CONSUMPTION IN THE DRINKING WATER

4.3.1 Characterization of corrosion scales

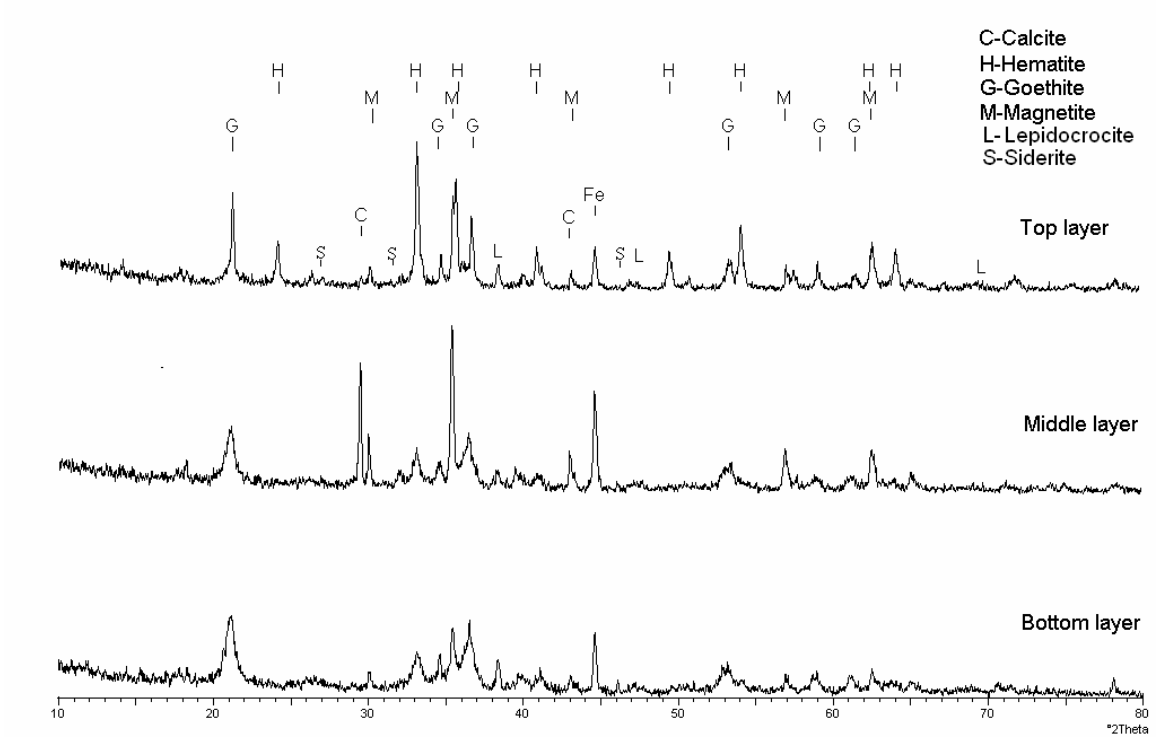


Figure 22. XRD patterns of the corrosion scale on different layers of the corroded iron pipe

The EDS measurements on powder samples identified that iron was the major component element of the scale. XPS analysis showed that iron was present at 28% (atomic percent), carbon at 18% and oxygen at 48%. The top layer, middle layer and bottom layer scale samples of the iron pipe corrosion scale were analyzed by XRD for the changes in compositions in Figure 22. Goethite (α-FeOOH), magnetite (Fe_3O_4), hematite (α-Fe_2O_3), calcium carbonate and elemental Fe were identified as the main components. All layers of the scales had a very similar set of peaks, but a few differences were discernible. Comparison of the peak heights of hematite suggests that hematite is mainly present in the top layer of the scale. Comparing the heights of magnetite peaks and elemental Fe peaks, it can be inferred that the content of magnetite and elemental Fe increased from the top layer to the middle layer. The scale structure proposed by Sarin [85] also indicated that magnetite was the dominate component in the shell-like layer of iron pipe corrosion scale. Based on the XRD results, it can be concluded that ferrous compounds are mainly associated with magnetite. The lower peak intensity of the bottom samples indicated that relative quantities of magnetite was small and goethite was the dominate component of the bottom lay of the scale. Siderite was likely present in minor amount since siderite is not stable when exposed to air. These results were similar to the results by other researchers [85, 86, 114, 115]. Since the composition of the iron scales depends on water quality, water flow patterns, seasonal temperature fluctuation and other factors, the composition of the scales obtained from different sources would be different. Green rust was not detected in the iron scale since green rust is not stable and can further be oxidized in contact with air to a more stable phase like goethite or magnetite.

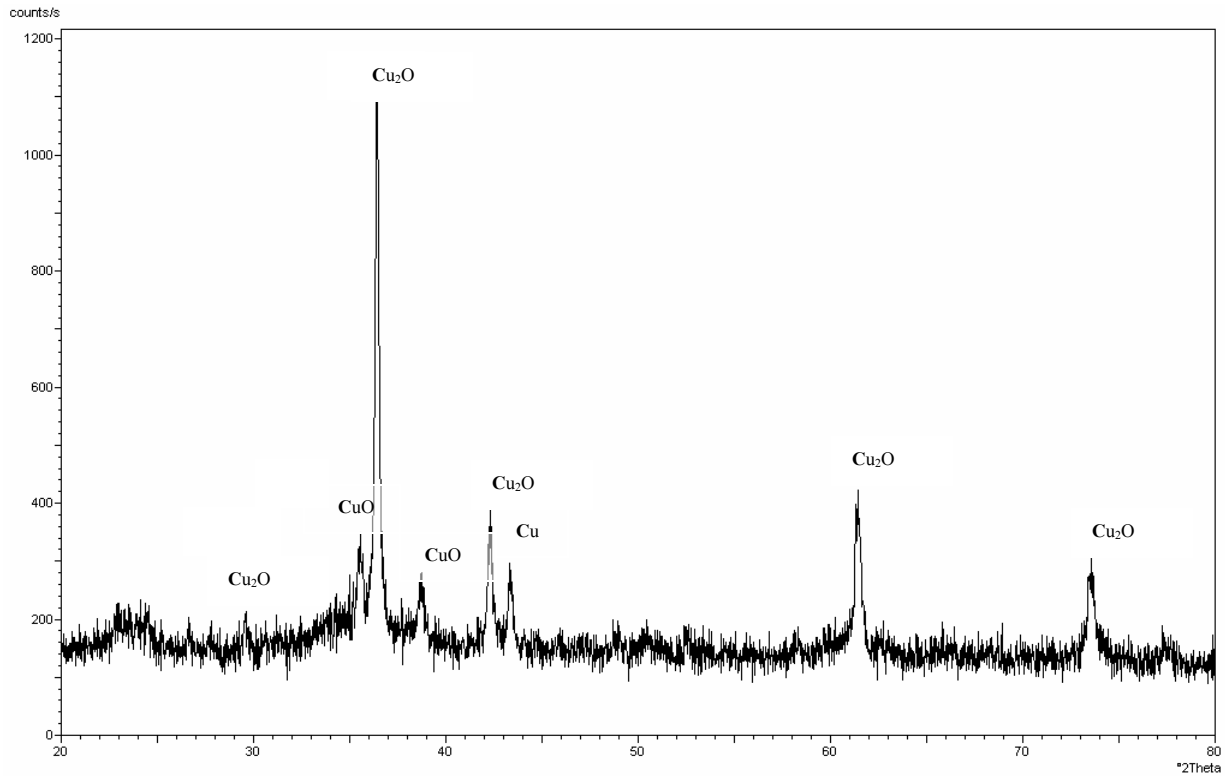


Figure 23. XRD patterns of the corrosion scale on the copper piper

The EDS measurements on powder samples of the copper pipe scale identified copper as the major component element of the scales. XPS analysis confirmed that copper was present at 10% (atomic percent), carbon at 19% and oxygen at 55%. XRD results showed that the copper corrosion scale primarily consisted of cuprite (Cu_2O), copper oxide (CuO) and metallic copper (Figure 23). And cuprite has been identified as a major corrosion product in drinking water corrosion [116]. The change of light brown cuprite film to light green malachite fibers by slow oxidation process has been reported [116]. But malachite was not found in the XRD examination of green film samples this study. It is possible that the malachite concentration in the corrosion scale was too low to be detected by XRD in this study (Figure 23).

4.3.2 Reactions of ClO_2 with iron and copper corrosion scales

The results of ClO_2 interaction with 1.0 g/L of corrosion scale from iron pipe at 25°C and 45°C are shown in Figure 24.

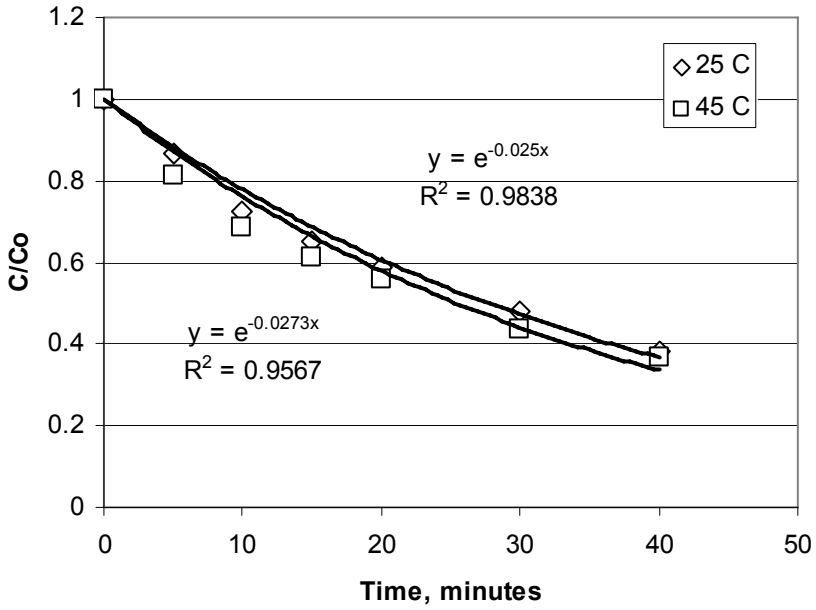


Figure 24. ClO_2 decay in DI water due to reaction with 1.0 g/L of corrosion materials from iron pipe at pH 7.5, 25 °C and 45 °C

Similar tests were conducted with 2.0 and 5.0 g/L of corrosion scales and the results are summarized in Table 7. A first-order kinetic expression provides a good fit of ClO_2 decay in DI water containing 1.0 g/L, 2.0 g/L and 5.0 g/L of pipe corrosion scale over the entire time period (Figure 22. and Table 7) at 25 °C and 45 °C. The first order reaction rate constant for ClO_2 consumption ranged from 0.025-0.083 min^{-1}. Temperature did not significantly affect the ClO_2 reaction rate with iron scales since the reaction of ClO_2 with reduced iron species is very fast.

Table 7. The mean first order rate constant for the reaction of ClO_2 with the iron corrosion scale, magnetite and cuprite

Scale concentration (g/L)	Temperature (°C)	Reaction Rate Constant (min^{-1})	R^2
1.0	25	0.0251	0.9783
	45	0.0273	0.956
2.0	25	0.0368	0.9913
	45	0.0408	0.977
5.0	25	0.0803	0.9746
	45	0.0829	0.9712
1.0 g/L of Fe_3O_4	25	0.070	0.9557
	45	0.076	0.9635
2.0 g/L of Cu_2O	25	0.0052	0.9781
	45	0.0062	0.9745

However, there was a clear relationship between the amount of corrosion scale present in the reactor and the first order reaction rate constant. Fig. 7 shows that the rate constant increased with increasing scale concentration in the reactor. Such behavior indicates that the availability of corrosion scale in the batch reactor represents the rate limiting step. The reaction order with respect to scale concentration was investigated using data collected at initial ClO_2 concentration of 1.0 mg/L and scale concentration of 1.0, 2.0, 5.0 and 10.0 g/L. The rate of ClO_2 decay is described by the pseudo first order expression:

$$-d\frac{[ClO_2]}{dt} = k[ClO_2]$$

Where, k = k'[Scale]$^{\alpha}$. It is possible to identify the effect of scale concentration on the reaction rate by linearizing the expression for k:

ln k = lnk' + αln[Scale]

The reaction order with respect to scale concentration was determined by plotting lnk vs ln[Scale] (Figure 25). The slope of 0.83-0.85 indicates that the reaction is not exactly the first order in scale concentration. Based on these results with powder scale from iron pipe, it can be concluded that the rate expression for ClO_2 consumption due to the reaction with the scale has the form:

$$-d\frac{[ClO_2]}{dt} = k'[ClO_2] * [Scale]^{0.84}$$

Where, k' varies from 0.021 to 0.027 min^{-1} (g/L)$^{-0.84}$

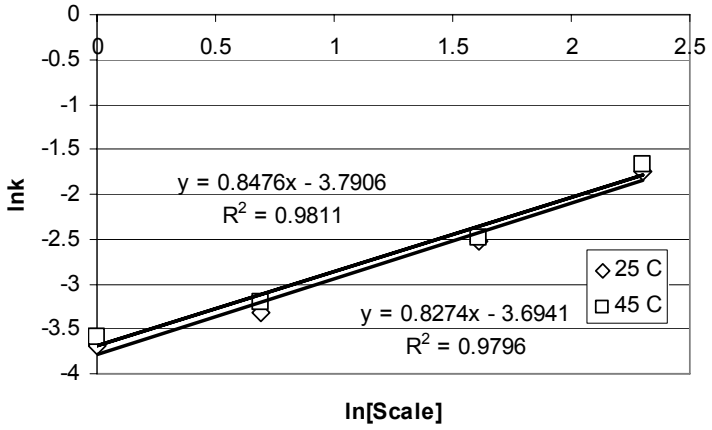


Figure 25. Determination of the reaction order with respect to scale concentration at 25 and 45 ºC

The reaction of chlorine dioxide with iron scale follows the first order reaction kinetics with respect to chlorine dioxide, but not exactly the first order reaction with respect to scale concentration (Figure 25). Such findings imply a complex reaction mechanism, which is likely due to heterogeneous reaction and the fact that the corrosion scale is a mixture of iron compounds and heterogeneous reaction between chlorine dioxide and the corrosion scale. Compared with the ClO_2 decay rate constant (0.0011-0.0044 min^{-1}) in drinking water due to reaction with organic matter [102], the rate constants of the reaction of ClO_2 with the iron pipe corrosion scales ranged from 0.0251-0.0829 min^{-1}, which was almost 20 times greater. Therefore, the major loss of ClO_2 would be caused by the corrosion scales in iron pipes in water distribution systems where heavy corrosion occurred. The decay rate of free chlorine with iron corrosion deposits was reported to range from 0.0027-0.034 h^{-1} in the batch reactor [117], which is much smaller than the decay rate of chlorine dioxide observed in this study.

The chlorite analysis showed that nearly 100% of ClO_2 was converted to chlorite (Table 8). It can be concluded that ClO_2 reacts with corrosion scale by one-electron mechanism whereby ClO_2 oxidizes the ferrous compounds in the corrosion scale to ferric compounds.

Table 8. The mean mass change of ClO_2 and chlorite during the ClO_2 reaction with the iron corrosion scale, magnetite and cuprite

Scale concentration (g/L)	Temperature (°C)	ΔClO_2	ΔClO_2^-	$\Delta ClO_2^-/\Delta ClO_2$
1.0	25	0.66	0.63	95%
	45	0.66	0.62	94%
2.0	25	0.76	0.72	95%
	45	0.78	0.74	95%
5.0	25	0.93	0.75	81%
	45	0.97	0.82	84%
1.0 g/L of Fe_3O_4	25	0.86	0.74	86%
	45	0.91	0.81	90%
2 .0g/L of Cu_2O	25	0.68	0.53	78%
	45	0.76	0.65	86%

Magnetite and cuprite were identified as the major components of the corrosion scales on the iron pipe and copper pipe, respectively. These compounds are the possible compounds that will cause ClO_2 loss because magnetite has one Fe(II) in each molecule and ClO_2 can oxidize Fe(II) to Fe(III). ClO_2 can also oxidize cuprous compound to cupric compound. The reactions of ClO_2 with cuprite and magnetite were confirmed using commercial powder cuprite and magnetite. The reaction rate between 1.0 g/L of commercial magnetite and chlorine dioxide was very close to the chlorine dioxide decay rate in the iron pipe (Figure 26) and the reaction rate of

chlorine dioxide with 5 g/L of corrosion scale in a batch reactor (Table 7). The reaction rate constant of ClO_2 reaction with 1.0 g/L magnetite was 0.070 min^{-1} at 25ºC. The reaction rate constant slightly increased to 0.076 min^{-1} as the temperature increased from 25 ºC to 45 ºC. Therefore, the reactions between ClO_2 and magnetite in the corrosion scales account for most of the ClO_2 loss in the iron pipes. Although Sarin et al [86] reported that soluble iron was released to bulk water primarily in the ferrous form, the amount of soluble ferrous ion is typically very small and can not account for the significant ClO_2 loss observed in this study. Other studies showed that free chlorine loss in the distribution system is also dominated by the reactions with corrosion scale or materials deposited on the surface of the pipe [117]. Since corrosion scale on the copper pipe was limited and cuprite was identified as the main component of copper corrosion scale, 2.0 g/L of commercial cuprite was used to study reactions between chlorine dioxide and copper corrosion scale. The results in Table 3 show that the reactions of ClO_2 with cuprite followed the pseudo first order kinetic expression. The reaction rate of chlorine dioxide with cuprite ranged from 0.005-0.006 min^{-1}, which is very close to the chlorine dioxide decay rate in the copper pipe (Figure 24). The reaction rate constant slightly increased to 0.0062 min^{-1} as the temperature increased from 25 ºC to 45 ºC. This confirmed the chlorine dioxide loss in copper pipe was primarily caused by the reaction of chlorine dioxide with cuprite. Although ClO_2 loss caused by copper pipe scale was much slower in the copper pipe than in iron pipe, it may be still necessary to prevent the corrosion and unnecessary loss of disinfectant due to the corrosion scale in the copper pipe distribution system to maintain an effective disinfectant residual.

78%-96% of ClO_2 consumed by the iron corrosion scales, cuprite and magnetite was converted to chlorite (Table 8.), which suggests that ClO_2 reacts with these solids by one-electron mechanism and oxidizes the ferrous and cuprous ions to ferric and cupric ions,

respectively. Since chlorite is also a weak oxidant, chlorite can be further reduced to other chlorine species with excess ferrous ions [84, 118, 119], which may explain why the recovery of chlorite decreased as the concentration of corrosion scales increased.

4.3.3 ClO_2 consumption in the pipe reactors

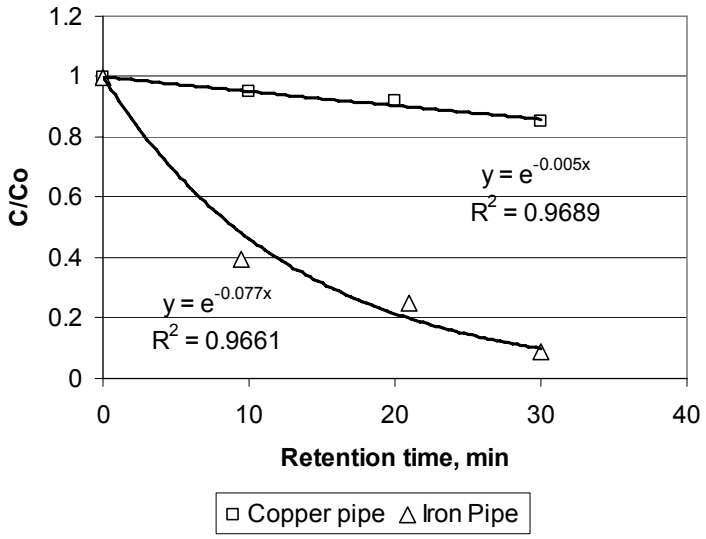


Figure 26. ClO_2 residual at each sampling port after the steady state was achieved

The results of the tests with an iron pipe reactor and a copper pipe reactor (Figure 2) are presented in Figure 26. During the first two hours of the test in the iron pipe, there was no measurable ClO_2 at any of the three sampling ports, which means that ClO_2 was completely consumed in the first 10 inches of the pipe. After approximately 6 hours, the mean ClO_2 residual at sampling ports 1, 2 and 3 was stable. Using the first order kinetic expression to fit all the data points, the mean reaction rate constant of 0.077 min^{-1} (R^2 = 0.9661) was obtained. It is clear that the reaction between ClO_2 and scale was not limited by the concentration of the scale in the pipe

reactor. Similar reaction rate constant was obtained in the batch reactor with iron scale concentration of 5.0 g/L. The decay rate of free chlorine due to reactions with the pipe wall reaction ranged from 0.07-0.26 h^{-1} [88, 89], which is much smaller than that of chlorine dioxide in this study. Such behavior is to be expected since chlorine dioxide is a free radical and very reactive to ferrous compounds [120]. The corrosion condition of the pipe and pipe material are also related to the decay rate of free chlorine and chlorine dioxide due to the wall reaction.

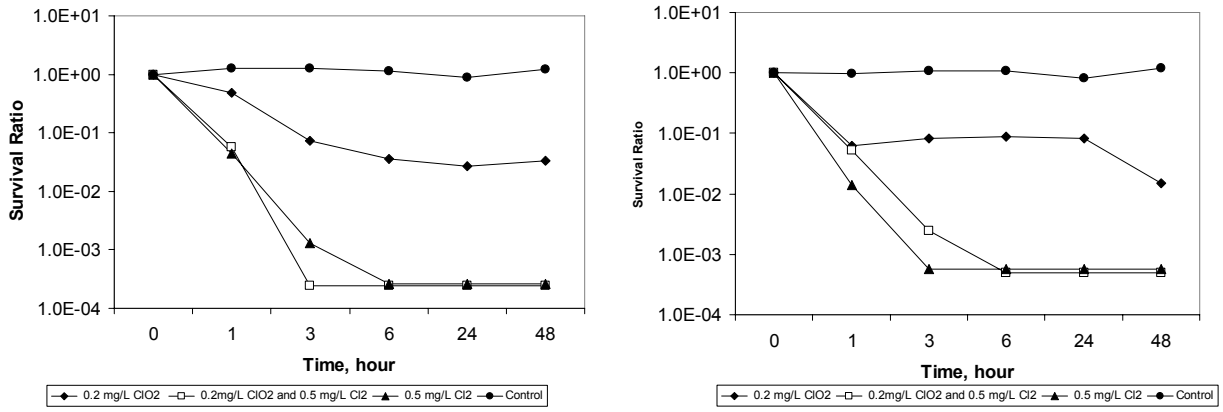
ClO_2 consumption was much slower in the copper pipe than in the iron pipe. After approximately 6 hours, the mean ClO_2 residual at sampling ports 1, 2, 3 was stable. Using first order kinetic expression to fit all the data points, the reaction rate constant of 0.005 min^{-1} (R^2 = 0.9689) was obtained. Similar reaction rate constant was obtained in the batch reactor with 2.0 g/L of cuprite.

Based on these results, it can be concluded that corrosion scales will cause much more significant ClO_2 loss in corroded iron pipes of the distribution system than the total organic carbon that may be present in finished water.

4.4 THE SYNEGISTIC EFFECT OF CHLROINE DIOXIDE AND FREE CHLORINE FOR *LEGIONELLA* CONTROL IN A MODEL PLUMBING SYSTEM

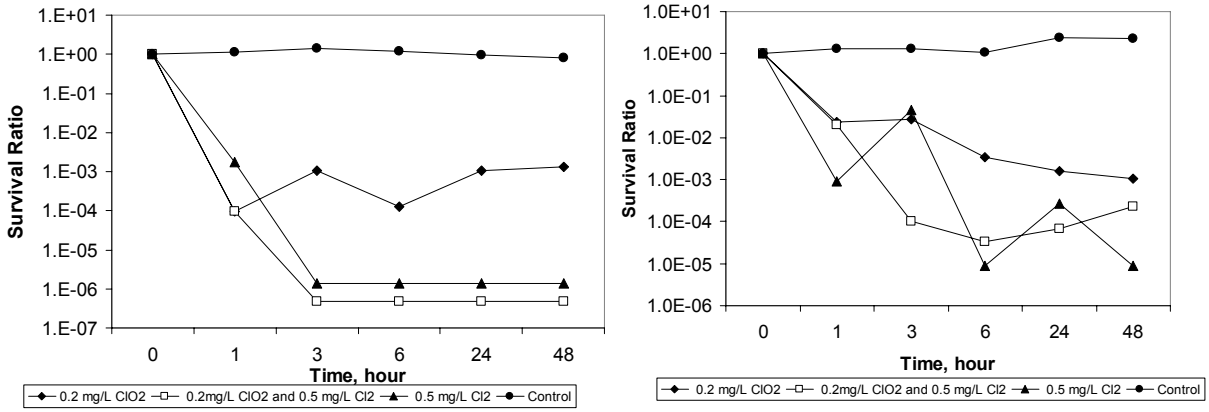
4.4.1 Comparison of free chlorine and chlorine dioxide for *Legionella* and HPC inactivation

Figure 27 and 28 depict the efficacy of chlorine, chlorine dioxide and the combination of chlorine and chlorine dioxide against *Legionella* and heterotrophic bacteria in both biofilm and planktonic phases. The duplicate experiments had similar results (Appendix, Figures 31-34).



a). Planktonic phase b). Biofilm phase

Figure 27. Inactivation of planktonic and biofilm associated *Legionella* in a model plumbing system by residual maintenance of 0.2 mg/L of chlorine dioxide, 0.5 mg/L free chlorine and 0.2 mg/L of chlorine dioxide with 0.5 mg/L free chlorine at 26 °C and pH 7.0



a). Planktonic phase b). Biofilm phase

Figure 28. Inactivation of planktonic and biofilm associated HPC bacteriain a model plumbing system by residual maintenance of 0.2 mg/L of chlorine dioxide, 0.5 mg/L free chlorine and 0.2 mg/L of chlorine dioxide with 0.5 mg/L free chlorine at 26 °C and pH 7.0

More than 99.9 % of biofilm associated and planktonic *Legionella* were inactivated in 3 hours by maintaining 0.5 mg/L of free chlorine residual and combined residuals of 0.2 mg/L of chlorine dioxide and 0.5 mg/L of free chlorine (Figure 27). Only 98% of biofilm associated and planktonic *Legionella* were inactivated in 48 hours by 0.2 mg/L of chlorine dioxide residual maintenance. Free chlorine was shown to be more effective in eradicating *Legionella* in both planktonic and biofilm phases at the conditions used in this study. Although this result is consistent with the previous study [54], the result still demonstrated the discrepancy between the laboratory study and the field study since the efficacy of free chlorine is significantly impacted by pH, TOC and other water quality parameters of drinking water in the distribution systems [54].

Figure 28 shows the efficacy of chlorine and/or chlorine dioxide against heterotrophic bacteria. More than 99.9% of biofilm associated and planktonic HPC bacteria were inactivated in 6 hours by maintaining 0.5 mg/L of free chlorine residual and combined residuals of 0.2 mg/L of chlorine dioxide and 0.5 mg/L of free chlorine. It took 48 hours for 0.2 mg/L of chlorine dioxide residual to inactivate 99.9% of biofilm associated and planktonic HPC bacteria in the model plumbing system.

Since free chlorine itself demonstrated high efficacy for inactivation of biofilm associated and planktonic *Legionella* and HPC bacteria in the model plumbing system, the synergistic effect of combined disinfectant residuals of chlorine and chlorine dioxide could not be determined in this study. However, the results clearly support the hypothesis that high level of free chlorine in the drinking water improves the efficacy of chlorine dioxide for *Legionella* control in hospital water systems.

4.4.2 Impact of temperature on chlorine dioxide inactivation of *Leigonella* and HPC bacteria

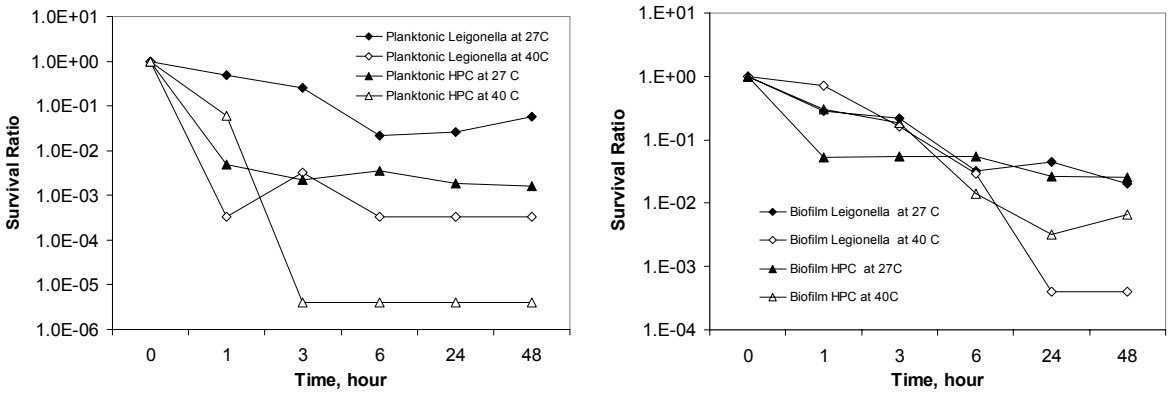


Figure 29. Temperature improved the efficacy of 0.2 mg/L chlorine dioxide residual inactivation of *Legionella* and HPC bacteria at pH 7.0 in both biofilm and planktonic phases

Figure 29 shows the impact of temperature on 0.2 mg/L chlorine dioxide residual inactivation of *Legionella* and HPC bacteria in both biofilm and planktonic phases. As the temperature increased from 27 to 40 °C, more than 99.9% of *Legionella* and HPC bacteria were inactivated in 6 hours by maintaining 0.2 mg/L of chlorine dioxide residual in both biofim and planktonic phases. The efficacy of chlorine dioxide inactivation of *C.parvum oocyst* has been demonstrated to be affected by water temperature [34]. For every 10 °C temperature increase, the reaction constant increased by a factor of 2.3.

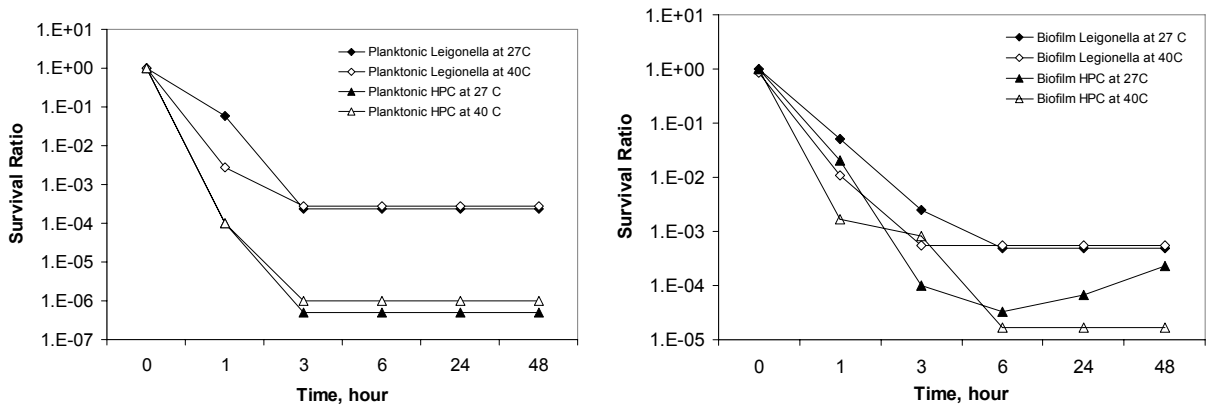


Figure 30. Temperature did not impact the efficacy of combined disinfectant residuals of 0.2 mg/L chlorine dioxide and 0.5 mg/L chlorine inactivation of *Legionella* and HPC bacteria at pH 7.0 in both biofilm and planktonic phases

Since more than 99.9% of *Legionella* and HPC bacteria were inactivated in 3 hours by combined disinfectant residuals of 0.2 mg/L chlorine dioxide and 0.5 mg/L chlorine at 27 °C, the efficacy of combined disinfectant residuals was not significantly improved as the temperature increased to 40 °C (Figure 30).

To maintain the same level of chlorine dioxide residual in the hot water in the model plumbing system, more chlorine dioxide stock solution (approximately 1.5×) needed to be injected. The reasons could be chlorine dioxide gassing off at higher temperature since the model plumbing system is not a completely closed system. High temperature also increased the reaction rate of chlorine dioxide with organic load in the system. It is important to monitor the level of the disinfection byproduct of chlorine dioxide if chlorine dioxide will be injected directly into the hot water system in future to improve its efficacy.

5.0 SUMMARY AND CONCLUSIONS

A significant reduction in *Legionella* colonization in two hospital water systems was achieved using ClO_2. Further cases of health-care-associated Legionellosis have not been identified in the studied hospitals after the ClO_2 treatment.

When ClO_2 was injected into the cold water main at 0.5-0.7 mg/L, the mean concentrations of ClO_2 and chlorite (ClO_2^-) in cold and hot water samples did not exceed the Environmental Protection Agency maximum residual disinfectant level (EPA MRDL) of 0.8 mg/L and maximum contaminant level (MCL) of 1.0 mg/L, respectively. Distance from the ClO_2 point-of-injection did not significantly affect mean concentrations of ClO_2 and ClO_2^- in the hospital hot and cold water systems. ClO_2 application was safe based on the EPA MRDL for ClO_2 and MCL for ClO_2^-.

ClO_2 is a promising disinfectant for controlling not only *Legionella* but also other bacteria in drinking water. Successful application of this new technology requires prospective environmental monitoring and cooperation between infection control, facilities engineers and the water treatment specialist.

TOC concentration in the hot water was higher than in the cold water of the distribution system in two studied hospitals. The chlorine dioxide decay rate at 45 °C increased with an increase of the total organic carbon concentration. Increase in TOC did not have as pronounced

impact on the chlorine dioxide decay rate at 25 °C. This study suggests that the temperature and TOC are both the key factors governing chlorine dioxide demand in hot water systems.

Goethite (α-FeOOH) and magnetite (Fe_3O_4) were identified as the main component phases of iron corrosion scale. Cuprite (Cu_2O) was identified as the major component of copper corrosion scale. The estimated first order reaction rate constants for ClO_2 reaction with iron corrosion scales and magnetite ranged from 0.0251-0.0829 min^{-1}. The estimated first order reaction rate constants for ClO_2 reaction with cuprite were much smaller, ranging from 0.0052-0.0062 min^{-1}. Cuprite and magnetite were the main compounds in the scales that caused ClO_2 loss in this study. The loss of ClO_2 in the corroded iron pipe was dominated by reactions between ClO_2 and these ferrous compounds in the iron pipe corrosion scales. Additionally, chlorine dioxide oxidizes iron corrosion scale, magnetite and cuprite by one electron transfer. The application of ClO_2 in water distribution systems using cast iron pipe will not be recommended unless measures to prevent corrosion are taken.

Temperature improved the efficacy of chlorine dioxide inactivation of *Legionella* and HPC bacteria in both planktonic and biofilm phases in the model plumbing system. So it is feasible to evaluate the direct injection of chlorine dioxide into the hot water system to improve the efficacy of chlorine dioxide for *Leigonella* control in hospital water systems.

6.0 ENGINEERING SIGNIFICANCE OF THE STUDY

This study included a field evaluation of chlorine dioxide efficacy for *Legionella* control in hospital water systems and investigation of the effects of water quality parameters and pipe conditions on chlorine dioxide efficacy in the batch study. Although chlorine dioxide has been very effective for controlling *Legionella* in laboratory studies, there are many other factors in the hospital water system that could deteriorate its efficiency in filed applications. Field studies have already shown that the efficiency of chlorine dioxide in the hot water system of hospitals was not as good as expected. The study of the impact of temperature, TOC and pipe corrosion on the decay of chlorine dioxide help to understand the behavior of chlorine dioxide under realistic process conditions. The study reveals that the most important factors that govern chlorine dioxide efficiency is corrosion scale at the conditions used in this study. Based on the results of this study, engineers can decide that removal of TOC, addition of corrosion inhibitors or temperature adjustment may be needed to ensure successful use of chlorine dioxide in a given hospital.

Currently, chlorine dioxide is usually injected into the incoming cold water main. The mean chlorine dioxide residual at the hot water tank outlet is typically bellowing 0.2 mg/L even if 0.5- 0.7 mg/L of chlorine dioxide is injected into the incoming cold water. Changing the chlorine dioxide injection point to outlet pipe of the hot water tank might shorten the time needed to reach a measurable chlorine dioxide residual at the distal outlets of the hot water system and

facilitate faster eradication of *Legionella* from the hot water system. Using the maximum chlorine dioxide decay rate constant due to reaction with TOC measured in this study (i.e., k = 0.0044 min^{-1}) and assuming a 6 hour detention time from the injection point to the farthest distal outlet, the chlorine dioxide residual at the distal outlet can reach 0.16 mg/L when 0.8 mg/L of chlorine dioxide is injected after the hot water tank. Therefore, separating chlorine dioxide injection point between the cold water and hot water systems might shorten the time that is needed to reach a measurable chlorine dioxide residual at distal outlets of the hot water system and improve the efficacy of chlorine dioxide for *Legionella* control in hot water systems.

Advantage of combining free chlorine and chlorine dioxide is that chlorine is still used as primary disinfectant in most water treatment plants. Therefore, chlorine can satisfy some oxidant demand in the institutional distribution system and facilitate maintenance of a stable chlorine dioxide residual concentration in both the hot and cold water systems. In addition, the reaction between chlorine and chlorite may reduce chlorine dioxide disinfection byproducts in the water system. The presence of two different disinfectants in the distribution system may help prevent the development of bacterial resistance to disinfection that has been observed when chlorine was used as the only disinfectant.

Our data demonstrated that a malfunction of the generator would directly cause the increase of *Legionella* positivity in the hospital plumbing system. Therefore, monitoring of the operational efficiency of the ClO_2 generator is also a key factor in the successful application of ClO_2 for *Legionella* control.

7.0 RECOMMENDATIONS FOR FUTURE WORK

This study demonstrated that temperature improves the efficacy of chlorine dioxide inactivation of *Legionella* and HPC bacteria in both planktonic and biofilm phases in a model plumbing system. Maintaining a sufficient chlorine dioxide residual in the hot water system would be a key factor to control *Legionella* in hospital water systems. Evaluating the direct injection of chlorine dioxide into the hot water system to improve the efficacy of chlorine dioxide for *Leigonella* control in hospital water system should be our next step.

Proper model system control is critical to the quality of the experimental data. To be able to perform experiments with more water quality parameters, a new model plumbing system with better design and control needs to be developed. A new model plumbing system should have a recirculation line to simulate the recirculation situation in hospital hot water systems. Automatic control valves should be installed in the inlet and outlet of the model system to simulate the usage of the water so that continuous water flow can be maintained in the model plumbing system. Automated devices for controlling and monitoring pH and temperature, automated injection system also should be considered. The new model should be designed to allow testing of different pipe materials, such as copper, iron and PVC pipes. A sampling device or loop for biofilm sampling should be considered in the new model because manually disconnecting the loop and taking biofilm samples from the pipe surface often causes plumbing leakage and errors.

The impact of pipe corrosion scale on chlorine dioxide decay in drinking water was investigated in this study. Based on the results, a corrosion inhibitor is recommended for the water distribution system where heavy corrosion occurred. But only one study showed that phosphate had little impact on disinfectant reaction with iron corrosion scale [117]. Further research of the impact on phosphate on chlorine dioxide reaction with copper corrosion scale is required since most pipes in the hospital water system are copper.

Our preliminary data showed that chlorine dioxide is also effective for controlling *Pseudomonas* in hospital water systems. Further study is required to confirm this result in the field.

APPENDIX

ADDITION TABLES AND FIGURES

Table 9 .Chlorine dioxide and its byproducts in cold and hot water samples from hospital A

	Chlorine dioxide, mg/L		Chlorite, mg/L		Chlorate, mg/L	
Sample Month	Hot water, n=27	Cold water, n=20	Hot water	Cold water	Hot water	Cold Water
08/03	0.04	0.41	0.33	0.2	<0.10*	<0.10
10/03	0.05	0.23	0.14	0.11	<0.10	<0.10
12/03	0.04	0.35	0.24	0.10	<0.10	<0.10
02/04	0.04	0.45	0.27	0.17	<0.10	<0.10
04/04	0.06	0.37	0.21	0.13	<0.10	<0.10
06/04	0.06	0.33	0.46	0.26	<0.10	<0.10
08/04	0.06	0.60	0.58	0.42	0.13	0.13
10/04	0.08	0.38	0.49	0.36	<0.10	<0.10
12/04	0.1	0.51	0.56	0.46	<0.10	<0.10
02/05	0.07	0.36	0.28	0.2	<0.10	<0.10
04/05	0.08	0.44	0.58	0.41	0.10	<0.10
06/05	0.07	0.48	0.61	0.35	<0.10	<0.10
02/06	0.11	0.51	0.72	0.52	<0.10	<0.10
Mean	0.07	0.42	0.42	0.28	<0.10	<0.10

*0.10 mg/L was the lowest calibration point for chlorite and chlorate analysis

Table 10. Chlorine dioxide and its byproducts in cold and hot water samples from hospital B

	Chlorine dioxide, mg/L		Chlorite		Chlorate	
Sample Month	Hot water	Cold water	Hot water	Cold water	Hot water	Cold water
04/04	NA	NA	NA	NA	NA	NA
06/04	0.09	0.22	0.35	0.42	NA	NA
08/04	0.06	0.35	0.48	0.45	0.15	0.13
10/04	0.16	0.35	0.48	0.44	0.10	<0.10
12/04	0.13	0.41	0.34	0.32	0.11	<0.10
02/05	0.12	0.36	0.35	0.33	<0.10*	<0.10
06/05	0.08	0.27	<0.10	<0.10	<0.10	<0.10
08/05	0.12	0.4	0.52	0.40	<0.10	<0.10
11/05	0.13	0.43	0.64	0.55	<0.10	<0.10
02/06	0.15	0.49	0.58	0.46	<0.10	<0.10

*0.10 mg/L was the lowest calibration point for chlorite and chlorate analysis

Table 11. Chlorine dioxide residual at each sampling port of the iron pipe reactor (mg/L)

The First Experiment					Time, minutes												
sampling port	0-30 min	30-60	60 -90	90-120	120-150	150-180	180-210	210-240	240-270	270-300	300-330	330-360	360-390	420-480			Avg value
0	1.02	1	1	0.98	1	1	1	1.02	1	0.98	1	1	1	1	1	1	1
1	0	0	0	0	0.05	0.05	0.11	0.12	0.1	0.11	0.11	0.21	0.36	0.38	0.36	0.41	0.38
2	0	0	0	0	0	0	0	0	0	0	0.04	0.06	0.11	0.22	0.24	0.26	0.24
3	0	0	0	0	0	0	0	0	0	0	0	0	0.04	0.07	0.04	0.09	0.08
The Second Experiment																	
sampling port	0-30 min	30-60	60 -90	90-120	120-150	150-180	180-210	210-240	240-270	270-300	300-330	330-360	360-390	420-480			Avg value
0	1.01	1	1	0.99	1	1	1	1.02	1	0.98	1	1	1	1	1	1	1
1	0	0	0	0	0.05	0.05	0.09	0.09	0.09	0.1	0.12	0.22	0.38	0.42	0.41	0.39	0.41
2	0	0	0	0	0	0	0	0	0	0	0.06	0.06	0.15	0.23	0.29	0.27	0.26
3	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0.09	0.1	0.11	0.1

Table 12. Chlorine dioxide residual at each sampling port of the copper pipe reactor (mg/L)

The First Experiment	Time, minutes																
sampling port	0-30 min	30-60	60 -90	90-120	120-150	150-180	180-210	210-240	240-270	270-300	300-330	330-360	360-390	420-480			Avg value
0	1.02	1.02	1	1	1	1	1	1	1	1.01	1.01	1	1.01	1.05	1.05	1.05	1.05
1	0.96	0.98	0.85	0.9	0.96	0.96	0.96	0.85	0.94	0.95	0.95	0.96	0.96	0.99	0.99	0.99	0.99
2	0.9	0.94	0.83	0.81	0.9	0.9	0.91	0.91	0.9	0.91	0.91	0.92	0.91	0.97	0.97	0.97	0.97
3	0.88	0.92	0.72	0.75	0.85	0.84	0.87	0.86	0.83	0.84	0.85	0.85	0.83	0.9	0.9	0.9	0.9
The Second Experiment																	
sampling port	0-30 min	30-60	60 -90	90-120	120-150	150-180	180-210	210-240	240-270	270-300	300-330	330-360	360-390	420-480			Avg value
0	1.02	1.02	1	1	1	1	1	1	1	1.01	1.01	1	1.01	1	1	1	1.02
1	0.96	0.98	0.85	0.9	0.96	0.96	0.96	0.85	0.94	0.95	0.95	0.96	0.96	0.96	0.96	0.96	0.96
2	0.9	0.94	0.83	0.81	0.9	0.9	0.91	0.91	0.9	0.91	0.91	0.92	0.91	0.92	0.92	0.92	0.92
3	0.88	0.92	0.72	0.75	0.85	0.84	0.87	0.86	0.83	0.84	0.85	0.85	0.83	0.85	0.84	0.84	0.84

Table 13. Results of 48-hr disinfection on chlorine dioxide and chlorine against *Legionella* and HPC bacteria at pH 7.0 in a model plumbing system

		Control			0.2 mg/L ClO_2 at 27 °C			0.2mg/L ClO_2 and 0.5 mg/L Cl_2 at 27 °C		
Sample Type	Time (hour)	CFU[1]/ml	log CFU/ml	Kill Ratio	CFU/ml	log CFU/ml	Kill Ratio	CFU/ml	log CFU/ml	Kill Ratio
Planktonic *Legionella*	0	4250	3.6284	0	3900	3.5911	0	3500	3.5441	0
	1	1450	3.1614	0.658824	1870	3.2718	0.520513	736	2.8669	0.789714
	3	10600	4.0253	-1.49412	976	2.9894	0.749744	90	1.9542	0.974286
	6	7050	3.8482	-0.65882	86	1.9345	0.977949	10	1.0000	0.997143
	24	2200	3.3424	0.482353	100	2.0000	0.974359	1[2]	0.0000	0.999714
	48	4600	3.6628	-0.08235	220	2.3424	0.94359	1	0.0000	0.999714
Planktonic HPC	0	1680000	6.2253	0	1595000	6.2028	0	1600000	6.2041	0
	1	1840000	6.2648	-0.09524	7750	3.8893	0.995141	6800	3.8325	0.99575
	3	1110000	6.0453	0.339286	3610	3.5575	0.997737	3120	3.4942	0.99805
	6	1450000	6.1614	0.136905	5640	3.7513	0.996464	1250	3.0969	0.999219
	24	720000	5.8573	0.571429	2980	3.4742	0.998132	1	0.0000	0.999999
	48	1280000	6.1072	0.238095	2580	3.4116	0.998382	1	0.0000	0.999999
Sample Type		CFU/cm^2	log CFU/cm^2	Kill Ratio	CFU/cm^2	log CFU/cm^2	Kill Ratio	CFU/cm^2	log CFU/cm^2	Kill Ratio
Biofilm *Legionella*	0	75500	4.8779	0	7500	3.8751	0	12750	4.1055	0
	1	51250	4.7097	0.321192	2105	3.3233	0.719333	333	2.5224	0.973882
	3	46250	4.6651	0.387417	1645	3.2162	0.780667	230	2.3617	0.981961
	6	39250	4.5938	0.480132	238	2.3766	0.968267	163	2.2122	0.987216
	24	115000	5.0607	-0.52318	330	2.5185	0.956	1	0.0000	0.999922
	48	87000	4.9395	-0.15232	150	2.1761	0.98	1	0.0000	0.999922
Biofilm HPC	0	1030000	6.0128	0	65000	4.8129	0	40000	4.6021	0
	1	450000	5.6532	0.563107	3375	3.5283	0.948077	1280	3.1072	0.968
	3	1405000	6.1477	-0.36408	3600	3.5563	0.944615	2600	3.4150	0.935
	6	1270000	6.1038	-0.23301	3600	3.5563	0.944615	2625	3.4191	0.934375
	24	625000	5.7959	0.393204	1740	3.2405	0.973231	1400	3.1461	0.965
	48	1140000	6.0569	-0.1068	1655	3.2188	0.974538	50	1.6990	0.99875

[1] CFU: Colony Forming Unit

[2] 1: Under detection limit

Table 14. Results of 48-hr disinfection on chlorine dioxide and chlorine against *Legionella* and HPC bacteria at pH 7.0 and 40 °C in a model plumbing system

		Control			0.5 mg/L Cl_2 at 26 °C			0.2mg/L ClO_2 and 0.5 mg/L Cl_2 at 40 °C		
Sample Type	Time (hour)	CFU[1]/ml	log CFU/ml	Kill Ratio	CFU/ml	log CFU/ml	Kill Ratio	CFU/ml	log CFU/ml	Kill Ratio
Planktonic *Legionella*	0	3700	3.5682	0	3800	3.5798	0	3550	3.5502	0
	1	4650	3.6675	-0.25676	170	2.2304	0.955263	10	1.0000	0.997183
	3	4700	3.6721	-0.27027	5	0.6990	0.998684	1	0.0000	0.999718
	6	4200	3.6232	-0.13514	1[2]	0.0000	0.999737	1	0.0000	0.999718
	24	3300	3.5185	0.108108	1	0.0000	0.999737	1	0.0000	0.999718
	48	4500	3.6532	-0.21622	1	0.0000	0.999737	1	0.0000	0.999718
Planktonic HPC	0	1195000	6.0774	0	715000	5.8543	0	1000000	6.0000	0
	1	1325000	6.1222	-0.10879	1250	3.0969	0.998252	100	2.0000	0.9999
	3	1680000	6.2253	-0.40586	100	2.0000	0.99986	1	0.0000	0.999999
	6	1410000	6.1492	-0.17992	1	0.0000	0.999999	1	0.0000	0.999999
	24	1175000	6.0700	0.016736	1	0.0000	0.999999	1	0.0000	0.999999
	48	955000	5.9800	0.200837	1	0.0000	0.999999	1	0.0000	0.999999
Sample Type		CFU/cm^2	log CFU/cm^2	Kill Ratio	CFU/cm^2	log CFU/cm^2	Kill Ratio	CFU/cm^2	log CFU/cm^2	Kill Ratio
Biofilm *Legionella*	0	1725	3.2368	0	1763	3.2463	0	1550	3.1903	0
	1	1700	3.2304	0.014493	25	1.3979	0.98582	20	1.3010	0.987097
	3	1875	3.2730	-0.08696	1	0.0000	0.999433	1	0.0000	0.999355
	6	1875	3.2730	-0.08696	1	0.0000	0.999433	1	0.0000	0.999355
	24	1415	3.1508	0.17971	1	0.0000	0.999433	1	0.0000	0.999355
	48	2100	3.3222	-0.21739	1	0.0000	0.999433	1	0.0000	0.999355
Biofilm HPC	0	150000	5.1761	0	110000	5.0414	0	60000	4.7782	0
	1	72500	4.8603	0.516667	100	2.0000	0.999091	100	2.0000	0.998333
	3	75000	4.8751	0.5	5000	3.6990	0.954545	50	1.6990	0.999167
	6	70000	4.8451	0.533333	1	0.0000	0.999991	1	0.0000	0.999983
	24	65000	4.8129	0.566667	30	1.4771	0.999727	1	0.0000	0.999983
	48	57500	4.7597	0.616667	1	0.0000	0.999991	1	0.0000	0.999983

[1] CFU: Colony Forming Unit

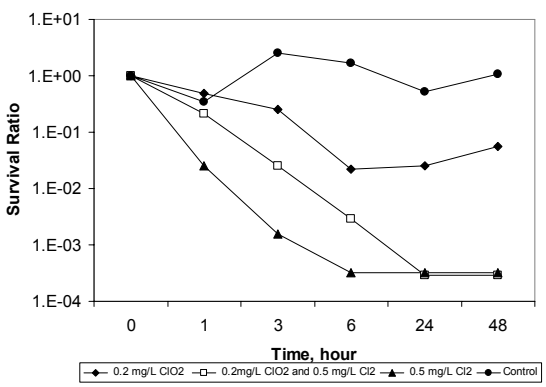
[2] 1: Under detection limit

Table 15. Temperature improved the disinfection efficacy of 0.2 mg/L chlorine dioxide residual against *Legionella* and HPC bacteria at pH7.0 in a model plumbing system

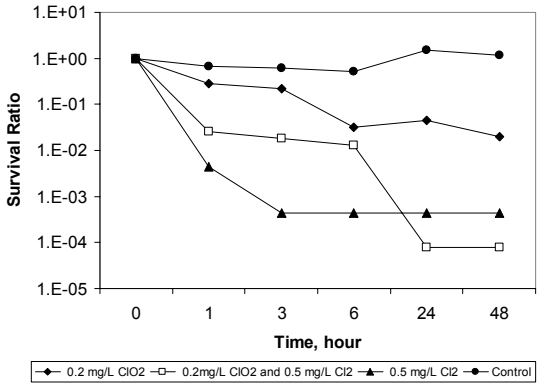
		Control			0.2 mg/L ClO_2 at 27 °C			0.2mg/L ClO_2 at 40 °C		
Sample Type	Time (hour)	CFU[1]/ml	log CFU/ml	Kill Ratio	CFU/ml	log CFU/ml	Kill Ratio	CFU/ml	log CFU/ml	Kill Ratio
Planktonic *Legionella*	0	4250	3.6284	0	3900	3.5911	0	3075	3.4878	0
	1	1450	3.1614	0.658824	1870	3.2718	0.520513	1[2]	0.0000	0.999675
	3	10600	4.0253	-1.49412	976	2.9894	0.749744	10	1.0000	0.996748
	6	7050	3.8482	-0.65882	86	1.9345	0.977949	1	0.0000	0.999675
	24	2200	3.3424	0.482353	100	2.0000	0.974359	1	0.0000	0.999675
	48	4600	3.6628	-0.08235	220	2.3424	0.94359	1	0.0000	0.999675
Planktonic HPC	0	1680000	6.2253	0	1595000	6.2028	0	250000	5.3979	0
	1	1840000	6.2648	-0.09524	7750	3.8893	0.995141	15000	4.1761	0.94
	3	1110000	6.0453	0.339286	3610	3.5575	0.997737	1	0.0000	0.999996
	6	1450000	6.1614	0.136905	5640	3.7513	0.996464	1	0.0000	0.999996
	24	720000	5.8573	0.571429	2980	3.4742	0.998132	1	0.0000	0.999996
	48	1280000	6.1072	0.238095	2580	3.4116	0.998382	1	0.0000	0.999996
Sample Type		CFU/cm^2	log CFU/cm^2	Kill Ratio	CFU/cm^2	log CFU/cm^2	Kill Ratio	CFU/cm^2	log CFU/cm^2	Kill Ratio
Biofilm *Legionella*	0	75500	4.8779	0	7500	3.8751	0	2500	3.3979	0
	1	51250	4.7097	0.321192	2105	3.3233	0.719333	1805	3.2565	0.278
	3	46250	4.6651	0.387417	1645	3.2162	0.780667	407	2.6096	0.8372
	6	39250	4.5938	0.480132	238	2.3766	0.968267	73	1.8633	0.9708
	24	115000	5.0607	-0.52318	330	2.5185	0.956	1	0.0000	0.9996
	48	87000	4.9395	-0.15232	150	2.1761	0.98	1	0.0000	0.9996
Biofilm HPC	0	1030000	6.0128	0	65000	4.8129	0	50000	4.6990	0
	1	450000	5.6532	0.563107	3375	3.5283	0.948077	14900	4.1732	0.702
	3	1405000	6.1477	-0.36408	3600	3.5563	0.944615	9150	3.9614	0.817
	6	1270000	6.1038	-0.23301	3600	3.5563	0.944615	715	2.8543	0.9857
	24	625000	5.7959	0.393204	1740	3.2405	0.973231	160	2.2041	0.9968
	48	1140000	6.0569	-0.1068	1655	3.2188	0.974538	325	2.5119	0.9935

[1.] CFU: Colony Forming Unit

[2.] 1: Under detection limit

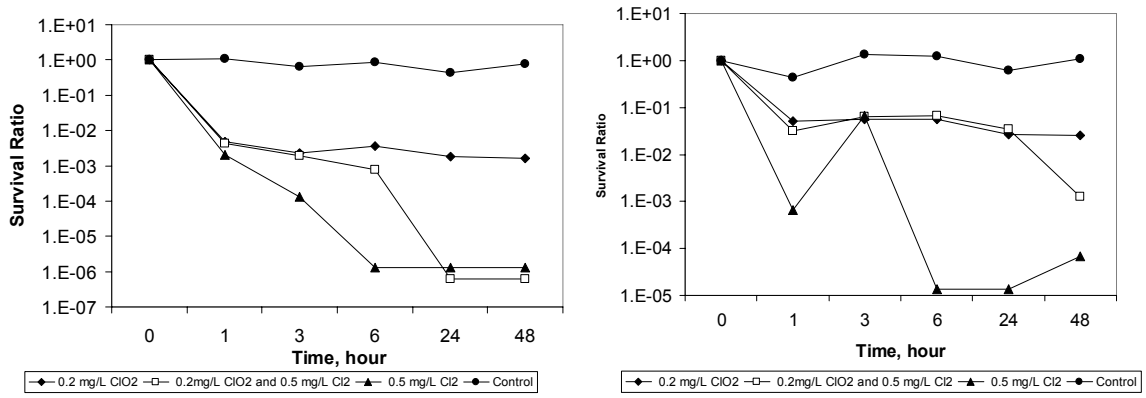


a). Planktonic phase



b). Biofilm phase

Figure 31. The results of the second test of inactivation of planktonic and biofilm associated *Legionella* in a model plumbing system by residual maintenance of 0.2 mg/L of chlorine dioxide, 0.5 mg/L free chlorine and 0.2 mg/L of chlorine dioxide with 0.5 mg/L free chlorine at 27 °C and pH 7.0



a). Planktonic phase b). Biofilm phase

Figure 32. The results of the second test of inactivation of planktonic and biofilm associated HPC bacteriain a model plumbing system by residual maintenance of 0.2 mg/L of chlorine dioxide, 0.5 mg/L free chlorine and 0.2 mg/L of chlorine dioxide with 0.5 mg/L free chlorine at 27 °C and pH 7.0

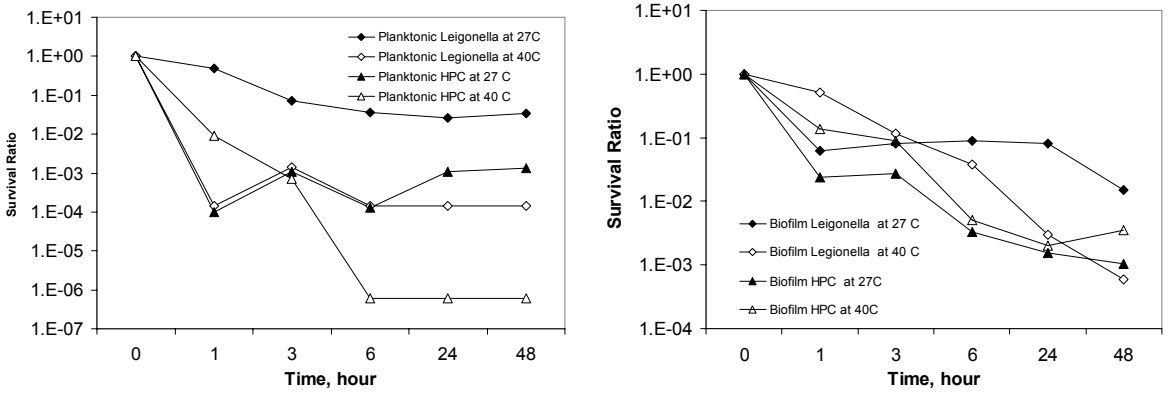


Figure 33. The results of the second test of effect of temperature on the efficacy of 0.2 mg/L chlorine dioxide residual inactivation of *Legionella* and HPC bacteria at pH 7.0 in both biofilm and planktonic phases

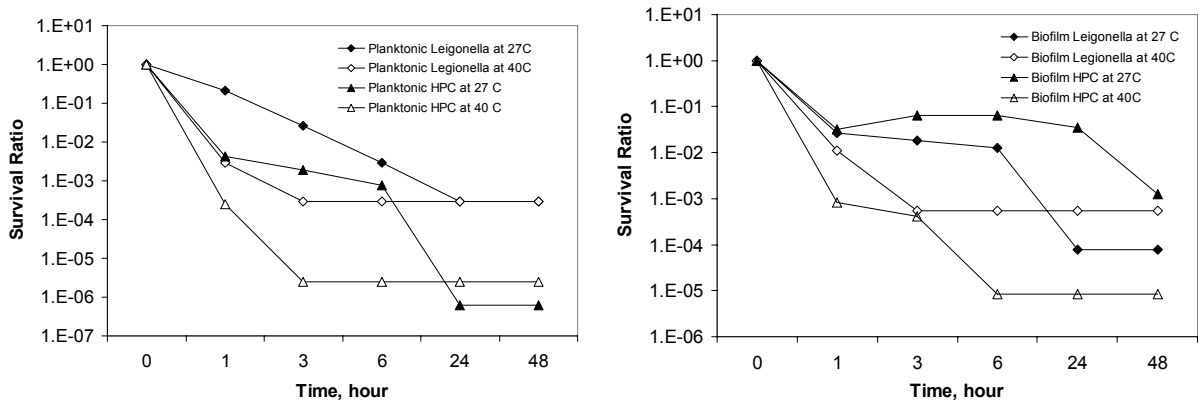


Figure 34. The results of the second test of effect of temperature on the efficacy of combined disinfectant residuals of 0.2 mg/L chlorine dioxide and 0.5 mg/L chlorine inactivation of *Legionella* and HPC bacteria at pH 7.0 in both biofilm and planktonic phases

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