Effect of disinfectants on microbial ecology in model distribution systems

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ABSTRACT

This research was conducted to assess the impact of various disinfectants on bacterial water quality within model distribution systems (i.e. annular reactors). After colonization with non-disinfected water, annular reactors were treated with relatively low doses of chlorine (0.4 mg/l), chlorine dioxide (0.15 mg/l), or chloramines (0.9 mg/l). Under the tested conditions, bacterial inactivation varied as a function of disinfectant type (ranking by efficiency per mg of oxidant: ClO_2 > Cl_2 > $ClNH_2$) and sample type (bulk water vs. biofilm). Depending on the disinfectant, the log inactivation of suspended and attached bacteria were 0.7–1.2 and 0.5–1.0, respectively. The characterization of microbial communities in drinking water can be performed using biochemical and/or molecular methods. In this study, biochemical tests were used, showing that pseudomonad and pseudomonad-like bacteria, as in other studies, were the most predominant micro-organisms (e.g. *Pseudomonas fluorescens*, *Brevundimonas vescularis*). The ratio Gram-positive to Gram-negative organisms was 1 to 3. No drastic differences were observed between the non-treated and disinfected pipes. Based on the bacteriological data presented in these experiments, chlorine dioxide represents an alternative to chlorine for certain distribution systems.

Key words | disinfectants, distribution systems, microbial ecology, water quality

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INTRODUCTION

During the distribution of drinking water, bacterial regrowth may lead to a deterioration of bacterial water quality, amplification of corrosion, generation of bad tastes and odors, and proliferation of macroinvertebrates (Levy *et al.* 1986; Geldreich 1996). Suspended bacteria present in the water column come mostly from biofilms formed on the surface of distribution pipes, suspended particles, or sediments accumulated in storage tanks (Van der Wende *et al.* 1989; Geldreich 1996). Microbial accumulation in biofilms is governed by the three processes of adhesion, growth, and detachment (Bryers and Characklis 1982; Van der Wende *et al.* 1989; Block *et al.* 1993). Bacteria adhering to the pipe surface may come from the source water through the treatment plant, regrowth of biofilm cells existing already within the distribution system, and introduction during main repair or cross connection. Growth of bacteria fixed on pipes is a combination of cell multiplication and mortality. Biofilm levels at steady state in distribution systems generally range between 10^5–10^7 bacteria/cm^2 (Geldreich 1996). The detachment of biofilm bacteria into the water column can be induced by fluid shear stress at the biofilm surface or other water quality changes, such as a change in disinfectant type or concentration. Consequently biofilm control has become recognized as an important part in the operation of drinking water plants and distribution systems.

The practice of primary disinfection and the maintenance of a disinfectant residual level within the distribution system are necessary to control microbial contaminants and restrain bacterial regrowth. Chemical disinfection must be kept in balance because of the potential to form disinfection

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by-products (DBPs) which, in turn, may have potential adverse health effects (Dodds *et al.* 1999). Free chlorine has been applied to drinking water since the early 1900s. However, it is well established that free chlorine can react with natural organic matter to form harmful halogenated DBPs (Rooks 1977). Consequently, the water industry has actively sought alternative disinfection strategies. Under certain conditions, monochloramine can be a better biocide for controlling biofilm growth in distribution systems (Norton and LeChevallier 1997). However, chloramination can also lead to nitrification in the distribution systems (Wilczak *et al.* 1996). Chlorine dioxide, a strong disinfectant, has different reaction pathways from chlorine in the formation of DBPs. Chlorine dioxide, however, forms inorganic by-products such as chlorite and chlorate (Faust 1999).

Characterization of bacterial water quality can be performed quantitatively or qualitatively. The determination of bacterial density is one way to characterize bacterial populations. Culture methods can be used to enumerate aquatic bacteria (APHA 1998). However, culture methods generally recover no more than 10% of the total viable bacteria in drinking water. Microscopic counts with acridine orange or DAPI (4′-6-diamino 2-phenyl indole) reveal the total amount of cells (dead and living), while staining using CTC (5-cyano-2, 3 ditolyl tetrazolium chloride) reveals respiring bacteria. The composition of communities can be determined using culture and/or molecular method. After isolation, bacteria can be identified depending on substrate usage (e.g., API identification systems or BIOLOG systems). One convenient method of characterizing bacteria is based on the analysis of cellular fatty acids: Fatty Acid Methyl Esterase method (Briganti & Wacker 1995). Molecular methods for identification of microorganisms include DNA analysis, in situ hybridization, polymerase chain reaction, ribotyping, or antibody labelling (Characklis & Marshall 1990).

It is critical to assess both qualitative and quantitative change in bacterial population. The primary objective of this project was to evaluate the effect of disinfection on bacterial water quality in drinking water distribution systems. The research was conducted at pilot scale using annular reactors. This study investigated (i) the effectiveness of chlorine dioxide, monochloramine and free chlorine for limiting bacterial regrowth, and (ii) the predominant type of bacteria present in water.

MATERIALS AND METHODS

Annular reactor description

Annular reactors (ARs) (Model LJ, BioSurface Technologies Corporation, Bozeman, MT) were used as model distribution systems, and consist of a stationary outer glass cylinder and a rotating inner drum that simulate shear stress. A biofilm accumulates on exposed surfaces and it can be sampled from 20 removable coupons, which are flush mounted to the surface of the inner drum. Three ARs were operated independently with low levels of chlorine (0.4 mg/l), monochloramine (0.90 mg/l), and chlorine dioxide (0.15 mg/l). A fourth AR was operated with no disinfectant (control AR). The disinfectant residuals were selected to simulate the end of a system. The application of disinfectants was initiated after a four-week acclimation period was completed and a steady-state biofilm was achieved, as indicated by a relatively constant number of biofilm heterotrophic plate counts (HPCs). The retention time for each AR was one hour to ensure that the growth of suspended cells was negligible in comparison to the growth rate of biofilm cells and biofilm detachment rate.

Water preparation

Tap water supplied by the Halifax Regional Water Commission (HRWC) was used as the primary process water for the ARs. On average, the treated surface water has an alkalinity of 35 mg/l as $CaCO_3$, pH of 8.5, a total organic carbon (TOC) concentration of approximately 1.5 mg/l, and a free chlorine residual of approximately 0.6 mg/l. Tap water was passed up flow into a granular activated carbon (GAC) filter to remove any free chlorine present and then passed into a biologically activated carbon (BAC) filter to reduce the background organic carbon concentration and supply the distribution system with an inoculum of microorganisms. The resulting water was pumped into each of the four annular reactors which were augmented with a cocktail of carbon, nitrogen ($NaNO_3$), phosphorous (K_2HPO_4 and

KH_2PO_4), as well as a disinfectant. The cocktail solution or biodegradable organic material was composed of ethyl alcohol, propionaldehyde, oxalate, pyruvate, and acetate (Camper 1996), which is equivalent to an organic carbon concentration of 100 ug C/l. The cocktails were pumped into the ARs to obtain a molar C:N:P ratio of 100: 20: 5. Experiments were conducted at a temperature of 20 ± 1°C.

Disinfectant generation and analysis

After a four week colonization period with non-disinfected water, the annular reactors received disinfected water for 2 months. Chlorine dioxide was produced according to the method outlined in the Standard method (APHA 1998). The method uses sodium chlorite addition to a strong acid to generate chlorine dioxide. The system generates a stock solution of approximately 2.0 g/l and is essentially free of all other chlorine species other than chlorine dioxide. Free chlorine stock was prepared by diluting reagent grade sodium hypochlorite in phosphate buffered saline (pH 7.4). Monochloramine was prepared by combining stoichiometrically equal amounts of ammonium chloride and sodium hypochchlorite in phosphate buffered saline (pH 9.5) and diluting and adjusting to a final pH of 7.4.

Bacterial enumeration

On a weekly basis, AR influent and effluent water samples were collected in sterile containers containing sodium thiosulfate. Biofilm samples for fixed microbial analysis were also collected on a routine basis. Polycarbonate coupons were removed aseptically from the annular reactor and transferred into 150-ml sterile test tube containing 25 ml phosphate buffered saline (PBS) and 25 μl of 10% (w/v) sterile sodium thiosulfate. The attached cells were immediately removed as described by Gagnon & Slawson (1998). The coupons and saline solution were transferred to a sterile stomacher bag (177 × 304 mm) and placed into the stomacher (Stomacher 400 Lab Blender, Seward, London, UK). The stomacher was operated at 230 rpm for 2 minutes to ensure adequate removal. The resulting bulk liquid was then transferred aseptically into sterile 15 ml disposable plastic tubes (Corning Inc., Acton, MA). The removed coupons were cleaned, sterilized and reinserted into the ARs to maintain constant volume and hydraulic conditions. Heterotrophic plate count (HPC) enumeration was conducted on R2A agar (Becton, Dickinson and Company, Franklin Lakes, NJ). Plates were incubated at room temperature (20 ± 1°C) for 7 days. Acridine orange direct counts (AODC) were performed by thoroughly homogenizing and vortexing the samples. Then, serial dilutions were prepared from each sample. A 1 ml sample from each appropriate dilution was withdrawn and mixed with 1 ml of a 0.1% (w/v) solution of acridine orange (ICN Biochemicals, Costa Mesa, CA) for five minutes at 20°C. The samples were then filtered on black cellulose nitrate filters, and washed once with 95% ethanol. The filters were observed by epifluorescence microscopy with a blue (450–480 nm) excitation filter used in combination with a 520 nm barrier filter. (Olympus BX-60 microscope, Melleville, NY). Fluorescent (orange to green) bacterial cells were counted in 10 different randomly-chosen fields. The counting was automated using an image analysis system (Esprit™ 1.0, Olympus) and a digital CCD camera. Some coupons were also directly stained at 21°C by immersion in a solution of 0.01% (w/v) acridine orange for 5 minutes. After staining, the coupons were gently rinsed in sterile deionized water and dried. The coupons were observed by epifluorescence microscopy using an Olympus BX-60 microscope. Bacterial removal (log_{10} inactivation) was also calculated after disinfection and corresponded to the log transformed ratio of the steady state HPC levels during chemical disinfection and prior to disinfection.

Bacterial identification

To determine the most predominant organisms, colonies from the highest dilution R2A plates from the annular reactors were identified by Gram-staining, the oxidase test, and API identification systems (API 20E and API 20NE from BioMérieux, Hazelwood, MO). These two systems are specific for Gram-negative bacteria. Pipe influent bacteria were not identified to the genus level. Gram-positive cocci and aerobic Gram-positive rods were not identified to the species level.

Most of the data sets were not normally distributed, hence the geometric means were calculated. Non-parametric tests were performed to determine whether two data sets were

statistically different (comparison of medians). Statistical tests were conducted at a 5% level of significance (α).

RESULTS AND DISCUSSION

Quantitative effect of disinfection

This section describes the effects of disinfectants on bacterial water quality within model distribution systems fed with low levels of chlorine, chloramines, chlorine dioxide or no oxidant (control). At first, all the annular reactors were colonized with undisinfected water to achieve an identical steady state in all reactors prior to implementing chemical disinfection. HPC and AODC levels in the bulk water were 2.8×10^4 to 5.5×10^4 CFU/ml, and 1.1×10^6 to 1.4×10^6 cells/ml, respectively. In comparison the HPC and AODC levels in the biofilm were 3.2×10^6 to 5.7×10^6 CFU/cm^2, and 7.0×10^6 to 8.6×10^6 cells/cm^2. There was no statistical difference among reactors in the number of HPCs and AODCs for either the bulk water or biofilm phases.

Following the initial colonization period, the annular reactors (except the control) received an oxidant. The effect of disinfectants on heterotrophic bacteria was clearly quantifiable in the annular reactors, while other factors affecting bacterial regrowth (temperature, nutrient levels, hydraulic conditions and pipe material) were controlled and maintained constant. An initial microbial inactivation period was quickly observed after the application of disinfectant. This period lasted several days (data not shown). Then bacteria levels stabilized to a steady state. Mean steady state bacterial concentrations are shown in Figure 1 for the control (no disinfectant) and disinfected systems. A significant decrease in bulk water HPC levels was observed ($p < 0.002$). Depending on disinfectant conditions, the log-inactivation of bulk water and biofilm HPC were 0.7 to 1.2 (average of 1 log-reduction), and 0.5 to 1.0 (average of 0.7 log-reduction), respectively. Disinfection was more effective in bulk water than on biofilm bacteria ($p < 0.05$). Adhesion was described as a factor of protection of attached bacteria (Camper 1997; LeChevallier *et al.* 1988). Biofilm bacteria grown on different surfaces were 150 to more than 3000 times more resistant to hypochlorous acid than were unattached cells (LeChevallier *et al.* 1988). The partial penetration of biofilm by disinfectants can explain the reduced efficacy of such agents against biofilm bacteria.

The proportion of cultivable bacteria decreased drastically in the bulk water following disinfection (Table 1). In the absence of disinfectant, the HPC/AODC ratio stabilized at 1.8% while it was 0.1–0.3% after disinfection. With regards to biofilm data, the HPC/AODC ratio was 3.5% without disinfection, compared to 0.6–2.1% with disinfection. As a comparison, the proportion of suspended viable bacteria stabilized at 0.2% of the total bacteria population after chlorine dioxide disinfection (Toulouse system, France, Gatel *et al.*, 1995). The HPC/AODC ratio was lower in a Canadian system (Volk *et al.*, 2002) in which heterotrophic bacteria represented 0.01–0.05% of the total bacteria concentration. In another study, Block (1997) found that 1% of the bacteria from a pilot distribution system with free chlorine could be cultured on R2A agar.

The present study also showed that disinfection efficiencies were the following: $ClO_2 > Cl_2 > ClNH_2$. When the log inactivation of HPC was reported per unit of

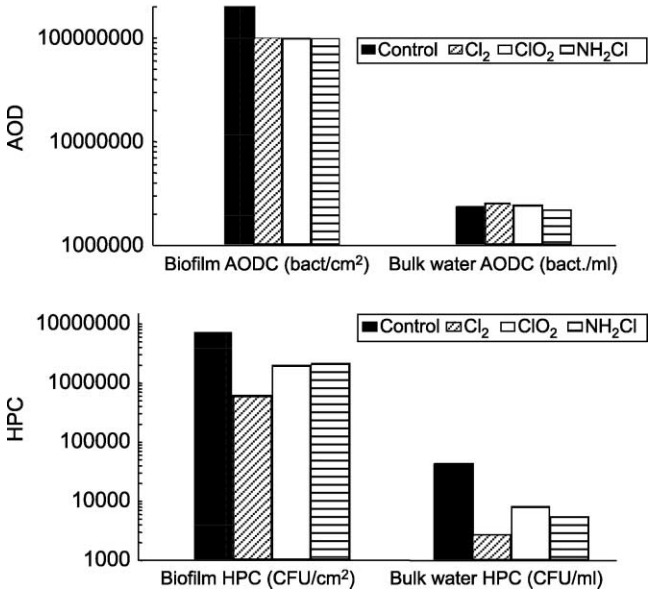

Figure 1 | Steady state biofilm and bulk water bacteria levels (HPC and AODC) in absence of disinfectant or in presence of 0.4 mg/l of free chlorine, 0.15 mg/l of chlorine dioxide, or 0.9 mg/l of chloramines (counts represent geometric means of 10 samples collected over the experiment).

Table 1 | Bulk water and biofilm bacteria HPC to AODC ratio (%) for the different treatment conditions

Treatment conditions	Biofilm bacteria (% HPC/AODC)	Bulk water bacteria (% HPC/AODC)
Control (no disinfection)	3.5	1.8
Chlorine	0.6	0.1
Chlorine dioxide	1.9	0.3
Chloramines	2.1	0.3

disinfectant, chlorine dioxide was 1.6 fold and 3 fold more effective on suspended bacteria than chlorine or chloramines, respectively (data not shown). The same trend was observed for another set of experiments, when chlorine and chlorine dioxide were compared from annular reactors operating with concentration x time (*Ct*) values of 30 mg.min./l. ClO_2 provided bulk water heterotrophic bacteria inactivation of 4 and 2.8 log-reduction in polycarbonate and cast iron coupon reactors, whereas free chlorine provided inactivation of 2.2 and 1.4 log for the same materials (Gagnon *et al.* 2003). The comparison of free chlorine and chloramines at a *Ct* of 60 mg.min./l showed that chlorine outperformed chloramines. Biofilm removal showed the same trend materials (Gagnon *et al.* 2003). Although chlorine dioxide is known to be a stronger disinfectant than free chlorine or chloramines, relatively few studies have examined its ability to control bacterial regrowth in distribution systems. Limoni and Teltsch (1985) reported low suspended heterotrophic plate counts in a distribution system treated with a chlorine dioxide residual concentration of approximately 0.2 mg/L. A Canadian system (Laval distribution system), which has used ClO_2 disinfection since 1984, did not experience bacteriological problems (Lafrance *et al.* 1992). Between 1987 and 1991, the HPC levels (on R2A, 20°C, 7 day incubation) were less than 10 CFU/100 ml.

In general, there are conflicting results on the ability of disinfectants such as chlorine or chloramines to limit bacteria proliferation (Camper 1997). The results were found to be system specific and showed the complexity of a biofilm response to an interrelated set of parameters. Pipe conditions (such as corrosion, pitting and tuberculation) are fundamental to the presence of biofilms, their metabolic activity, and the release of bacteria into the water column. Pipe conditions also indirectly impact biofilm bacteria by affecting disinfection efficiency, especially with free chlorine. Rompré *et al.* (1997) reported that free chlorine produced a rapid decrease in biofilm density (>1 log) in polycarbonate reactors, whereas chlorine did not affect the biofilm in grey iron reactors. The benefit of chloramines compared to that of chlorine has been debated for many years and conflicting results have been reported, depending on the study conditions (LeChevallier *et al.* 1990; Block 1992). Pilot studies showed that the density of biofilm bacteria on concrete pipes was ten times lower in the presence of chlorine than in the presence of 1 mg/l of chloramines. In addition, in the presence of 0.4 mg/l of chlorine, concrete pipes showed a lower biofilm accumulation (up to 100 times lower) than on PVC and polyethylene pipes. However, when chlorine was replaced by chloramines, the three materials gave similar results (Block 1992). Other experiments (LeChevallier *et al.* 1990) showed that low levels of chlorine and chloramines (1 mg/l) could reduce heterotrophic bacteria levels more than a hundredfold (2 log) for biofilm grown on galvanized and plastic pipes. However, chlorine residuals of 3–4 mg/l were ineffective on iron pipes, while biofilm treated with 2 mg/l of chloramines exhibited a more than 3 log die-off. It has been suggested that monochloramine is better able to penetrate the biofilm, while free chlorine reacts with various constituents (corrosion products) and is consumed prior to penetrating the biofilm (LeChevallier *et al.* 1990; Camper *et al.* 1997).

A marginal decrease in biofilm AODC levels was observed after disinfection (a 0.3 log removal or 50% reduction) (Figure 1). The decrease was not statistically significant ($p = 0.10$). The bulk water AODC levels were not affected by disinfection ($p > 0.3$) (Figure 1). AODC measurement yields total cell counts (dead and living microorganisms), but it cannot be used alone to assess the effects of disinfection, unless there is complete cell destruction after treatment. Experiments on biofilms established on stainless steel surfaces treated with 2 mg/l of chloramines showed relatively little biofilm removal as evidenced by total cell direct counts, while plate counts indicated an

average 1.3 log decrease (Huang *et al.* 1995). Reactor coupons were also analysed microscopically to investigate the structure of the biofilm in the control reactor and after a 2 month exposure to the three secondary disinfectants investigated (Figure 2). Visual analysis of the coupons also showed a slight difference (due to disinfection), in the structure of the biofilm and the number of total bacteria present as determined from the acridine orange stained used (Figure 2). The structure of the biofilm in the control reactor appeared very dense in comparison to the disinfected pipes. It has been reported that the presence of disinfectant affects substrate consumption and the regrowth of heterotrophic bacteria in the system (Butterfield *et al.* 1997; 1999). In the presence of chlorine, biofilm showed greater carbon removal, higher specific biofilm growth rate, but lower biofilm yield, and lower biofilm densities than unchlorinated biofilm (Butterfield *et al.* 1997; 1999). These authors suggested that there is a protective mechanism for bacteria cells that may require more substrate in the presence of disinfectant. Bacteria may produce larger amounts of extra cellular polymeric substances (EPS) to react with and neutralize chlorine (explaining the higher substrate uptake and carbon requirement). Acting as a sacrificial barrier, EPS are critical in limiting chlorine penetration into the biofilm (Butterfield *et al.* 1999)

Qualitative effect of disinfection

Bacterial identification

Overall, 107 heterotrophic bacteria were isolated from the annular reactors and tested during this study. The identified bacteria were collected from the highest dilution plates, to characterize the most representative microorganisms present in the water. Bacterial identification to the species level was performed only on Gram-negative bacilli from the control annular reactor and the reactors with chlorine and chlorine dioxide. Among the 107 isolates, Gram-negative and positive bacteria were detected at a percentage of 68.2% and 31.8%, respectively (Table 2). The most predominant bacteria were *Pseudomonas fluorescens* (23.3% of isolates), and *Brevundimonas vescularis* (10.3%) a pseudomonad-like bacterium previously classified as a *Pseudomonas* (Table 2). More specifically, *P. fluorescens* is a ubiquitous bacterium that is commonly associated with soil and water. It is not considered to be pathogenic to humans, but can

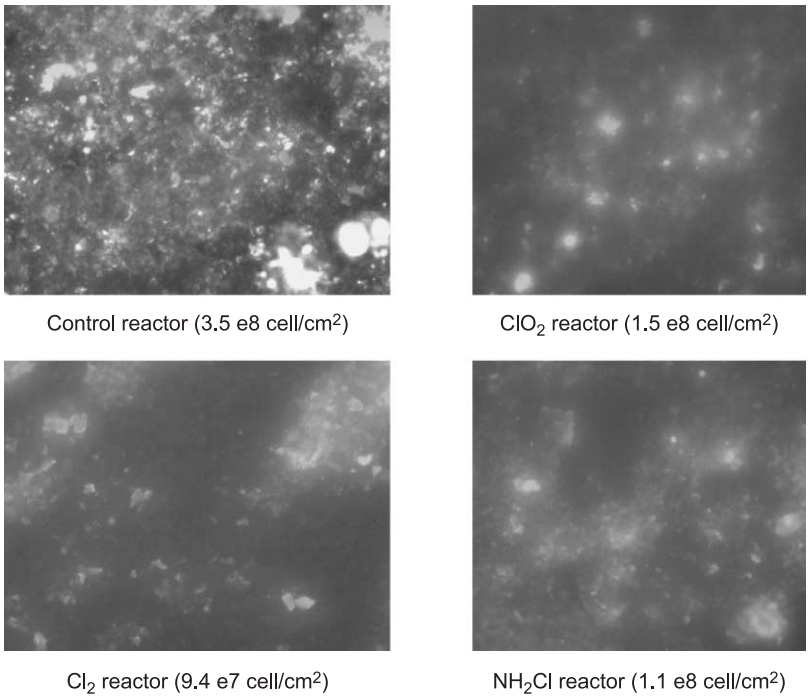

Figure 2 | Microscopic observation of annular reactor coupons stained with acridine orange (400X magnification) after 56 days of chemical disinfection.

commonly form biofilms in distribution systems (Oliveira *et al.* 1994; Vieira and Melo 1995). *Brevundimonas* is an opportunistic pathogen, capable of causing septicemia. It is very common in soil, water, and wastewater. In one recent study, it was found to be one of the most dominant bacterial species identified from an activated sludge (Juang and Morgan 2001).

The Gram-negative genus *Aeromonas* was also identified; it is a fish and opportunistic human pathogen commonly occurring in surface waters supplying municipal drinking water systems (Huys *et al.* 1997; Kühn *et al.* 1997). *A. hydrophila* was recognized as an emerging waterborne pathogen (Embrey *et al.* 2003), causing hemorrhagic septicemia or red sore disease, but its occurrence is rare in adequately disinfected systems (Chauret *et al.* 2001). The species *Aeromonas salmonicida* is better known as the causal agent of furunculosis in salmonid fishes, a debilitating and lethal disease encountered in aquaculture. Although *A. salmonicida* is not pathogenic to humans, it is closely related to many human and animal pathogens, and its presence suggests the possibility that other strains of aeromonads can be present. Several studies documented the presence of various heterotrophic bacteria in distribution systems, including *Flavobacterium*, *Acinetobacter*, *Pseudomonas*, *Bacillus*, *Moraxella*, *Staphylococcus* (Bourbigot *et al.* 1984; LeChevallier *et al.* 1987; Payment *et al.* 1988; Geldreich 1996) and sometimes coliforms (*Klebsiella*, *Escherichia coli*, *Enterobacter*, etc.) (LeChevallier 1990; Camper *et al.* 1991; Geldreich 1996). The major types of bacteria isolated from various systems depended on the distribution system conditions, type and concentration of disinfection, nutrient levels, retention time, and water temperature. For instance, genera of *Pseudomonas*, *Azotobacter* and *Actinobacter* were present each at a level of 30% in dead end sections of a distribution system in Zurich, Switzerland (Jaeggi & Schmidt-Lorenz 1990). In another study, samples collected from a water tank in Sao Polo, Brazil (Penna *et al.* 2002) showed that the three following bacteria genera were identified: *Pseudomonas*, *Flavobacterium*, and *Acinetobacter*, with a prevalence for *P. aeruginosa* (32%).

Table 2 | Type of bacterial isolates identified from the annular reactors and frequency of isolation

Bacterial types or species[a]	Frequency of Isolation (%)
Gram negative bacteria	68.2
Gram positive bacteria	31.8
Total	100.0
Pseudomonas fluorescens	23.3
Gram positive cocci	16.8
Gram positive spore forming rods	12.1
Brevundimonas vescularis	10.3
Gram positive rods (no spores)	2.8
Moraxella spp.	1.9
Agrobacterium radiobacter	1.9
Aeromonas salmonicida subs. *achromogenes*	1.0
Other bacterial isolates	23.4
Total	100.0

[a]Only identifications with at least a 80% confidence level are reported in this table (based on the bioMérieux database).

In addition, Gram-positive cocci and aerobic Gram-positive spore-forming rods made up 16.8% and 12.1% of the isolates, respectively. The common detection of spore-forming bacteria is not surprising because of their relative resistance to disinfection. Full-scale studies in Canada assessed the impact of temperature, nutrient levels, disinfectant level and types on bacterial population (LeChevallier *et al.* 1998). These researchers found a preponderance of Gram-positive organisms near the plant in cold waters. The preponderance decreased with the retention time. In warm water, the trend was reversed with most of the identified isolates being Gram-negative organisms. In a distribution system with high nutrient level, bacteria of the genera *Micrococcus* and *Bacillus* constituted most of the Gram-positive bacteria; however, these genera were present in lower numbers in a low nutrient system. A more diversified population was found in the nutrient poor system. In general, populations were more diversified in warm water than in cold waters, and the diversity increased with the

residence time (related to the absence of disinfectant) (LeChevallier *et al.* 1998).

Regarding disinfection, analysis of bacterial distribution systems based on Gram reaction showed that prechlorination resulted in a rapid shift from predominantly Gram-negative bacteria in the raw water to mostly Gram-positive organisms in the chlorinated water (LeChevallier *et al.* 1998). Means *et al.* (1986) also found that full-scale chloramination resulted in an increase in the percentage of Gram-positive bacteria isolated from the distribution system. It was suggested that the nature of the thick Gram-positive bacterial cell wall and the presence of waxy lipid membranes could account for the resistance to chlorine disinfection. In another study, it was reported that the Gram-positive spore forming bacilli and actinomycetes were among the most resistant bacteria to chlorine. The most sensitive bacteria were *Corynebacterium*, *Arthrobacter*, *Klebsiella*, *Pseudomonas alcaligenes*, *Flavobacterium*, *Moraxella*, *Acinetobacter* and most Gram–positive micrococci (Ridgway and Olson 1982).

Impact of disinfectants

Figure 3 shows bacterial characterization performed at the inlet and outlet of the reactors. Annular reactor influent contained 67% of Gram-negative and 33% of Gram-positive bacilli. These bacteria came from the granular activated carbon filters (HPC = 1.2 10^3 CFU/ml) used to remove the disinfectant and organic matter in tap water. They could also originate from the pre-colonized biologically active carbon (collected from biological filters) and the distribution system tap water used as source water for the experiment. Comparison of the pipe inlet and outlet shows that the proportion of cocci shaped bacteria increased in the annular reactor. Cocci shaped bacteria were not found in the feed water, but they represented 18.5% to 24% of the bacteria in the reactors.

Based on Gram staining, no drastic differences could be observed among the treated and untreated pipes. Gram-negative bacilli represented 67% of the bacteria isolated in the control reactor while they represented 68% and 56% of the bacteria in the Cl_2 and ClO_2 reactors, respectively (slightly less gram-negative bacteria with ClO_2, the difference was not significant at a level of 5%). Gram-positive rods, cocci shaped bacteria, and especially Gram-positive spore formers, were found from each type of sample. Of note, one isolate of *Aeromonas salmonicida* was isolated from a disinfected (Cl_2) annular reactor. This observation suggests that attachment to surfaces provided adequate protection to allow survival of organisms otherwise susceptible to disinfection such as Gram-negative bacteria. LeChevallier *et al.* (1998) conducted a study on pilot distribution systems with iron or plastic pipes fed with conventionally or biologically treated water and subjected to disinfection with free chlorine or chloramine. In general, the ratio of Gram-positive to Gram-negative organisms was approximately 30% to 70%. However, the chloraminated plastic network biofilms contained 83% of Gram-positive bacteria. In another distribution system, Actinomycetes and *Aeromonas* species were the two most common groups of HPC bacteria in a chlorinated distribution system, while *Aeromonas* spp. and *Enterrobacter agglomerans* were the two most common bacteria in raw water (LeChevallier *et al.* 1980). A study conducted in a full-scale system showed that, when comparing distribution systems fed with chlorine or chlorine dioxide water, the proportion of Gram-negative bacteria in cold water was higher near the plant using BAC and chlorine dioxide than in another system using chlorine. It appeared that Gram-negative bacteria dominated the speciation when ClO_2 was used as a post-disinfectant (LeChevallier *et al.* 1998).

The results presented in this study could be related to the presence of oxidants as well as factors other than the disinfectants.Caution should be taken when conducting bacterial identification in distribution systems. The identification of cultivable bacteria does not detect all bacteria present in the distribution system. At first, the identification of bacteria in distribution system depends on the culturability of bacteria affected by various parameters. The media used could affect the results since standard media typically recover less than more sensitive (low nutrient) media such as R2A. For instance, bacteria of the genera *Aeromonas*, *Shewanella*, *Pseudomonas*, and enterobacteriaceae were detected in distributed waters (using the German standard plate count agar) (Wernicke *et al.* 1990). However, additional bacteria were detected when using lower nutrient media and longer incubation times,

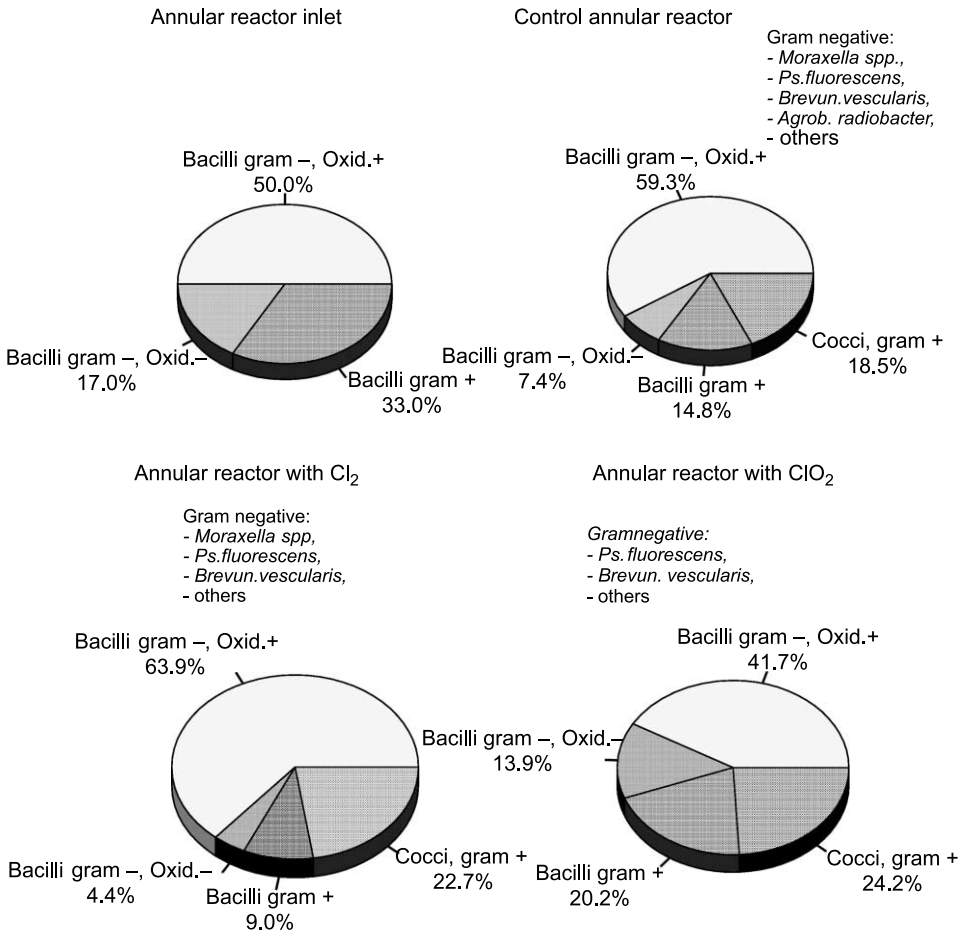

Figure 3 | Type of bacteria identified at the inlet and outlet of the annular reactors subjected to various treatment conditions.

and were found to belong to the genera *Pseudomonas*, *Alcaligenes*, *Acinetobacter*, and *Flavobacteriu*m. Moreover, injured bacteria are not detected on most cultivation media. Secondly, different types of disinfectants (free chlorine vs. chlorine dioxide) may also impact the cultivability of bacteria sampled. It is possible that the shifts in population observed reflect changes in the ability of bacteria to be cultured rather than drastic changes in populations (LeChevallier *et al.* 1998). In the present study, the bacteria types were similar in the control and disinfected reactors, which is likely to be a result of the initial colonization period that was identically-prepared with non-disinfected water. Disinfectants might not have a strong selection effect on a pre-established biofilm. Finally, the annular reactors were operated for a limited period of time; a longer period of time may be necessary to allow a well-diversified biofilm to be established. Banks and Bryers (1991) found that biofilms were quickly dominated by faster-growing organisms. Slow growing organisms were not eliminated from the biofilm, rather they increase in number over time.

CONCLUSIONS

This study reported on the disinfection capabilities of low levels of chlorine, chlorine dioxide and chloramines in simulated drinking water distribution systems. Depending on the disinfectant conditions, bulk water and biofilm heterotrophic bacteria showed a 0.7–1.2 and 0.5–1.0 log inactivation, respectively. In an absolute sense, when reported per mg of oxidant, the inactivation of heterotrophic bacteria with

chlorine dioxide was superior to free chlorine and chloramines. Based on Gram characterization, the bacteria isolated from the annular reactors were $\frac{2}{3}$ Gram-negative and $\frac{1}{3}$ Gram-positive. The most common bacteria in all samples were pseudomonads and pseudomonad-like bacteria.

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