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Disinfectant efficacy of chlorite and chlorine dioxide in drinking water biofilms

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Abstract

The drinking water industry is closely examining options to maintain disinfection in distribution systems. In particular this research compared the relative efficiency of the chlorite ion (ClO_2^-) to chlorine dioxide (ClO_2) for biofilm control. Chlorite levels were selected for monitoring since they are typically observed in the distribution system as a by-product whenever chlorine dioxide is applied for primary or secondary disinfection. Previous research has reported the chlorite ion to be effective in mitigating nitrification in distribution systems. Annular reactors (ARs) containing polycarbonate and cast iron coupons were used to simulate water quality conditions in a distribution system. Following a 4 week acclimation period, individual ARs operated in parallel were dosed with high (0.25 mg/l) and low (0.1 mg/l) chlorite concentrations and with high (0.5 mg/l) and low (0.25 mg/l) chlorine dioxide concentrations, as measured in the effluent of the AR. Another set of ARs that contained cast iron and polycarbonate coupons served as controls and did not receive any disinfection. The data presented herein show that the presence of chlorite at low concentration levels was not effective at reducing heterotrophic bacteria. Log reductions of attached heterotrophic bacteria for low and high chlorite ranged between 0.20 and 0.34. Chlorine dioxide had greater log reductions for attached heterotrophic bacteria ranging from 0.52 to 1.36 at the higher dose. The greatest log reduction in suspended heterotrophic bacteria was for high dose of ClO_2 on either cast iron or polycarbonate coupons (1.77 and 1.55). These data indicate that it would be necessary to maintain a chlorine dioxide residual concentration in distribution systems for control of microbiological regrowth.
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Keywords: Distribution system; Chlorine dioxide; Chlorite; Biofilm; Disinfection; Heterotrophic bacteria

1. Introduction

The practice of primary disinfection and the maintenance of a disinfectant residual within the distribution system are widely used strategies for controlling microbial contaminants and bacterial re-growth. Chlorine dioxide (ClO_2) is a strong disinfectant and oxidant that

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has demonstrated promise as a secondary disinfectant in full-scale distribution systems (Volk et al., 2002). The formation of organohalogens (e.g., trihalomethanes and haloacetic acids) with ClO_2 is typically much lower when compared to the use of free chlorine (Cl_2) (Hofmann et al., 1999; Werdehoff and Singer, 1987). This is primarily attributed to the difference in oxidation reaction mechanisms, where ClO_2 reacts via free radical electrophilic abstraction versus oxidative substitution and addition for Cl_2 (Baribeau et al., 2002). Lafrance et al. (1993) showed that when chlorine dioxide was implemented at two water treatment plants in Laval, Quebec, trihalomethanes were less than the reported detection limit of 2 μg/l in most samples. The changeover to ClO_2 also led to 85% reduction in trihalomethanes and 60% in haloacetic acids after replacing chlorine in a full-scale distribution system (Volk et al., 2002). Other benefits regarding the use of ClO_2 are that it is effective as a disinfectant over a much broader range of pH than chlorine, and is capable of inactivating chlorine resistant parasitic pathogens such as *Cryptosporidium parvum* (Chauret et al., 2001; Finch and Belosevic, 1997; Liyanage et al., 1997; LeChevallier et al., 1996). In addition, ClO_2 does not react with ammonia or primary and secondary amines (Thompson, 1993), which may increase chlorine demand in distribution systems.

Concerns related to the use of ClO_2 in drinking water treatment have arisen as the result of toxicological studies, including the observation of hematologic alterations at various exposures in laboratory rats (Abdel-Rahman et al., 1984). However other studies conducted in both controlled laboratory experiments and full-scale systems have failed to link the ingestion of water treated with ClO_2 to any adverse health effects in humans (Lubbers et al., 1981, 1982, 1984; Michael et al., 1981). In addition, studies attempting to link adverse birth outcomes to ClO_2 in drinking water have either been inconclusive or have also failed to prove a connection (Tuthill et al., 1982; Kanitz et al., 1996).

Chlorite (ClO_2^-) is a known by-product of ClO_2 (Baribeau et al., 2002; Gordon, 2001). When applied to drinking water, a portion of the ClO_2 will form ClO_2^- upon reaction with natural organic matter (NOM). Widely varying concentrations of ClO_2^- formed from ClO_2 have been observed and reported in the literature, with proportions ranging between 30% and 70% (Gordon, 1992). Chlorite can be removed during treatment by the addition of iron salts, softening or contact with granular-activated carbon (GAC) (Iaturu and Knocke, 1992; Simpson, 1995).

Eisnor and Gagnon (2004) found that ClO_2^- had very low corrosion rates in a tuberculated cast-iron pipe loop. Specifically, it was reported that corrosion rates and iron release were lowest in pipe loops that were disinfected with ClO_2^- when compared to ClO_2, free chlorine, chloramines, or a control system that had no disinfectant added (Eisnor and Gagnon, 2004). Furthermore, McGuire et al. (1999) concluded that ClO_2^- has potential benefits as a biocide for inactivating ammonia-oxidizing bacteria (AOB), which are known to cause nitrification in distribution systems. In particular, McGuire et al. (1999) reported that the occurrence of nitrification in full-scale systems could be acutely mitigated by switching from chloramines to chlorite. In that study, ClO_2^- was found to have a strong potential to inactivate suspended AOB, although no information was provided concerning its long-term affect on suppressing heterotrophic microorganisms. Because ClO_2^- is a by-product of ClO_2 the data presented in the literature has not been clear as to which chemical provides long-term benefit as a secondary disinfectant. Thus the primary objective of this project was to determine the extent of biocidal control on heterotrophic biofilms provided by ClO_2^-, relative to ClO_2, under controlled laboratory experiments.

2. Materials and methods

2.1. Description of model distribution system

Annular Reactors (ARs) were used to represent model distribution systems. The AR model used for this experiment was the 1120 LS (BioSurface Technologies Corporation, BioSurface Technologies, Bozeman, MT). Each AR consists of an outer glass cylinder that encompasses an inner rotating drum. A variable speed motor located above the reactor controls the rotation of the drum, which in turn controls the shear stress on the outer cylinder wall. Influent water flows through an annular gap and is mixed by the rotating drum, which contains draft tubes to ensure sufficient vertical and horizontal distribution. The hydraulic retention time of 2 h was controlled by the volumetric flow rate of the influent entering an individual AR. The total working volume in the annular gap was approximately 950 ml. Each AR was maintained at a temperature of 20 ± 1 °C fixed rotational speed of 50 rpm, which results in a shear stress of 0.25 N/m^2 at the outer wall. This shear stress approximately corresponds to a flow of 0.30 m/s in a 100 mm diameter smooth pipe and is similar to the shear conditions of other pilot- and bench-scale investigations (Sharp et al., 2001; Camper, 1996). All non-opaque exposed surfaces were covered to reduce the potential of phototrophic growth in the bench-scale system.

In the reactor, water flows through the annulus that is created between the inner rotating cylinder, the glass cylinder and the support plates. The AR accommodates twenty removable coupons that are flush-mounted on the side of the inner rotating cylinder and which support biofilm growth. Of the eight ARs running in parallel,

four were equipped with polycarbonate coupons and four had cast iron coupons.

Tap water from Halifax, Nova Scotia was used as the primary process water for the ARs. Prior to use the tap water was passed through a filter containing fresh GAC media to remove free chlorine present in the tap water. A second GAC filter operating biologically was used to remove biodegradable organic matter (BOM) in the tap water. Sterile influent cocktails of organic carbon were also pumped into each AR. The cocktail solution of biodegradable organic compounds is composed of ethyl alcohol, propionaldehyde, oxalate, pyruvate, and acetate. These compounds were chosen because they represent major classes of organic compounds found in drinking water that are relatively non-reactive with disinfectants (Camper, 1996). Each compound was added to the BOM cocktail in equimolar concentrations on the basis of oxygen demand. An organic carbon concentration of 100 μg C/L was used for all experimental trials.

To ensure a carbon-limiting system, ARs were also dosed with a separate cocktail of nitrogen ($NaNO_3$) and phosphorous (K_2HPO_4 and KH_2PO_4). The solutions were pumped into the ARs such that a C:N:P molar ratio of 100:20:5 could be obtained. The influent mixture remained constant throughout all experimental trials and the added substrate was monitored to compare utilization rates with biofilm growth.

2.2. Experimental design

One of the ARs with polycarbonate coupons and one of the cast iron ARs were not dosed with any disinfectant and served as controls. Chlorite and chlorine dioxide were used as secondary disinfectants. ClO_2^- was dosed from a stock solution of sodium chlorite ($NaClO_2$), and applied using post GAC-filtered water as influent to the ARs. ClO_2^- was applied to achieve an effluent ClO_2^- residual concentration of 0.1 and 0.25 mg/l. The remaining ARs, also receiving post GAC-filtered water, were dosed with ClO_2. ClO_2 was produced according to a method similar to one outlined in *Standard Methods for the Examination of Water and Wastewater* (APHA et al., 1998). The method includes the addition of sodium chlorite to a strong acid to generate chlorine dioxide. A gas-washing bottle filled with Milli-Q® water and placed in an ice bath for collecting the chlorine dioxide. This system generates a stock solution with concentrations ranging from 2.0 to 5.0 g/l, and is in effect free of all other chlorine species other than chlorine dioxide. ClO_2 was dosed into the ARs to achieve an effluent residual concentration of 0.25 and 0.5 mg/l. Following an acclimation period of 4 weeks for the biofilm to reach steady-state in all ARs was followed by application of disinfectant (ClO_2^- and ClO_2) for 12 weeks.

2.3. Analytical methods

Sampling and testing protocols were carried out as described in the *Standard Methods for the Examination of Water and Wastewater* (APHA et al., 1998). Microbiological parameters were monitored on a weekly basis, and disinfectant concentration, flow, pH and temperature was measured 2–3 times per week. Chlorine dioxide and chlorite residuals were measured using a spectrophotometric method employing lissamine green with a detection limit of 0.05 mg/l (Hofmann et al., 1998).

Heterotrophic plate counts (HPC) were performed using a spread-plate technique on R2A agar (BD, Franklin Lakes, NJ), where the R2A plates were incubated at 20 °C and counts were carryout out after 7 days. Acridine orange direct counts (AODC) were used to enumerate all microbial cells. One millilitre of sample was mixed with 1 ml of a 0.1% (w/v) solution of acridine orange for 5 min. The mixture was filtered on black cellulose nitrate filters (Millipore, Bedford, MA, USA). The filters were observed by epifluorescence microscopy (Olympus model BX-60, Melville, NY, USA), and enumerated using an image analysis system (Esprit™, Olympus).

Polycarbonate coupons were removed and replaced with sterile coupons that were not subsequently sampled aseptically from the ARs on a weekly basis. They were then transferred into 150 ml test tubes containing 25 ml autoclaved phosphate-buffered saline (PBS) and 0.1% w/v sodium thiosulfate. The attached cells were immediately removed using a stomacher removal method (Gagnon and Slawson, 1999) that, in comparison to scraping, was found to have a 3-fold increase in HPC recoverability. The coupons along with the saline solution were then deposited into a sterile stomacher bag ($177 \times 304\,mm^2$) and placed into a stomacher (Stomacher 400 Lab Blender, Seward, London, UK). To ensure adequate removal, the stomacher was operated for 2 min at 230 rpm. The resulting bulk liquid was then transferred aseptically into sterile 15 ml disposable plastic tubes (Corning Inc., Acton, MA) and plated on agar to be analyzed for HPCs. The cast iron coupons were scraped (front and back of coupons) to remove biological material, as the stomacher could not be used to process the metal coupon. Biofilm scraped from the cast-iron coupon was homogenized with a stomacher for 2 min at 230 rpm. AODC could not be performed from the material obtained from the cast iron coupons because the corrosion-related particulate material would fluoresce along with the bacteria.

The CTC (5-cyano-2,3-ditolyl tetrazolium)-reduction was used to measure respiring cells. CTC-reducing (respiring) cells were estimated by thoroughly vortexing the contents of one coupon with 10 ml of deionized water containing 0.01% Tween 20 (w/v). Serial dilutions

were prepared from each sample. A 1 ml sample from each appropriate dilution was withdrawn and mixed with 1 ml of a solution of CTC in phosphate buffered saline (PBS, pH 7.4). The final concentration of CTC was 5 mM. This suspension was incubated for 2 h at 20 °C. The samples were then filtered on black cellulose nitrate filters and washed once with PBS. The filters were observed by epifluorescence microscopy (Olympus BX-60). Fluorescent red bacterial (respiring) cells were counted in 25 different randomly chosen fields. The counting was automated using an image analysis system and a digital CCD camera.

2.4. Data analysis

Analysis of variance (ANOVA) tests at a significance level of 95% were used to compare the impact of the various combinations of disinfectant dose, and disinfectant type on biofilm growth. Differences between the average influent and effluent values for the water quality parameters measured.

3. Results

3.1. Acclimation/pre-disinfection period data

The ARs were operated for a period of four weeks prior to any application of disinfection to establish steady-state biofilm conditions. Pre-disinfection conditions were determined for ARs that would eventually receive no disinfectant, ClO_2, and ClO_2^-.

The overall mean number of suspended heterotrophic bacteria in water from all ARs was $8.98 \times 10^4 \pm 4.18 \times 10^4$ CFU/ml. The overall mean number of total cell counts (AODC) in the bulk water was $8.8 \times 10^5 \pm 3.78 \times 10^5$ CFU/ml during the acclimation period. The cast iron surface appeared to have lower suspended HPC counts than polycarbonate, which were $7.57 \times 10^4 \pm 3.65 \times 10^4$ and $1.04 \times 10^5 \pm 4.59 \times 10^4$ CFU/ml, respectively, though, no statistically significant difference was observed, ($p = 0.314$). There was, however, a significant difference between cast iron and PC for AODC bulk water counts ($p = 0.0455$).

The mean and standard deviation of the biofilm densities for HPCs prior to disinfection were $8.59 \times 10^6 \pm 2.46 \times 10^6$ and $1.14 \times 10^6 \pm 5.0 \times 10^5$ CFU/cm^2 for cast iron and polycarbonate coupons, respectively. Cast iron coupons had a statistically higher number than polycarbonate ($p = 0.00266$) for biofilm heterotrophic bacteria. During acclimation, there was also a significant difference between bulk water HPC and biofilm HPC that had been removed from the coupon into a 25 ml sample volume ($p = 0.00633$), disregarding coupon composition. In consideration of coupon composition there was no significant difference between bulk water and biofilm HPCs in cells per given volume on polycarbonate coupons, although there was significantly more bacteria attached to the metal surface than in suspension.

In addition to heterotrophic and total cell counts, non-viable and viable cell counts were also determined as per the CTC-reduction assay. The data for viable bulk water and biofilm bacteria are provided in Table 1, which were calculated by taking the difference between the total and non-viable cell counts. The mean number of non-viable cells in bulk water was $3.17 \times 10^5 \pm 1.37 \times 10^5$ and $2.13 \times 10^6 \pm 1.54 \times 10^6$ for biofilm. The corresponding number of viable cells for the bulk water was $5.63 \times 10^5 \pm 2.63 \times 10^5$ and $2.29 \times 10^6 \pm 1.34 \times 10^6$ CFU/cm^2 for biofilm. Disregarding coupon material there was a significant difference between bulk water and biofilm non-viable and viable cells ($p = 0.10$, non-viable and $p = 0.0831$, viable).

The ratio of heterotrophic to total cells was determined in order to establish the proportion of culturable bacteria in the bulk water and the biofilm (Table 2). The mean ratio for bulk water was 0.12 ± 0.05, and for biofilm the mean was 0.29 ± 0.12, indicating higher culturability on the bacteria attached to the coupon. There was no difference between bulk water ratios and biofilm ratios ($p = 0.0673$). The mean ratio for bulk water for polycarbonate coupons was 0.10 ± 0.03 and for cast iron coupons was 0.14 ± 0.06 with no significant difference ($p = 0.30$).

3.2. Post-disinfection HPC data

During the disinfection experimental portion of Phase 3 ARs were either not disinfected (control), disinfected with ClO_2, or with ClO_2^-. Over a 50-day period HPCs for suspended bulk water and biofilm were monitored. A decreasing trend was seen in all data for both suspended bulk water and biofilm, especially in ARs treated with ClO_2 (Fig. 1). Disregarding treatment type and coupon material there is a significant difference between mean cell counts for suspended versus biofilm cells removed from the coupon into a sample volume ($p = 0.0268$). While there is no statistically significant difference between log reductions for suspended versus biofilm ($p = 0.0995$), there was a greater log reduction for HPCs in suspended bulk water (mean suspended was 0.90 ± 0.51 and mean biofilm was 0.50 ± 0.50), which was consistent with previous studies that have demonstrated higher log reductions of suspended heterotrophic bacteria during disinfection (e.g., Gagnon et al., 2004; Camper, 1996).

A significant decrease in bulk water suspended HPC levels was found for the disinfection period, when comparing pre-disinfection and post-disinfection data ($p = 1.54 \times 10^{-4}$). There was no significant difference between the suspended heterotrophic bacteria in either

Table 1
Number of viable and total cell counts during acclimation and disinfection

AR	Acclimation				Disinfection			
	Bulk viable (cells/ml)[a]	Bulk total (cells/ml)	Biofilm viable (cells/cm^2)	Biofilm total (cells/cm^2)	Bulk viable (cells/ml)	Bulk total (cells/ml)	Biofilm viable (cells/cm^2)	Biofilm total (cells/cm^2)
PC control	9.27×10^5	1.25×10^6	1.44×10^6	3.73×10^6	2.17×10^5	3.70×10^5	1.74×10^6	3.17×10^6
CI control	4.28×10^5	6.91×10^5	n.d.	n.d.	2.73×10^5	3.74×10^5	n.d.	n.d.
PC ClO_2 (Low dose)[b]	5.53×10^5	8.49×10^5	1.95×10^6	3.81×10^6	1.11×10^5	2.59×10^5	5.38×10^5	1.41×10^6
CI ClO_2 (Low dose)	5.47×10^5	7.25×10^5	n.d.	n.d.	9.40×10^4	2.29×10^5	n.d.	n.d.
PC ClO_2 (High dose)	2.79×10^5	5.42×10^5	4.31×10^5	6.44×10^6	4.80×10^4	1.83×10^5	8.50×10^5	2.00×10^6
CI ClO_2 (High dose)	4.17×10^5	8.71×10^5	n.d.	n.d.	5.25×10^4	2.27×10^5	n.d.	n.d.
PC Chlorite (Low dose)[c]	6.97×10^5	1.04×10^6	1.11×10^6	5.11×10^6	1.99×10^5	4.63×10^5	3.25×10^6	5.39×10^6
CI Chlorite (Low dose)	2.77×10^5	5.08×10^5	n.d.	n.d.	2.46×10^5	5.57×10^5	n.d.	n.d.
PC Chlorite (High dose)	9.58×10^5	1.59×10^6	2.54×10^6	4.52×10^6	2.55×10^5	4.08×10^5	3.16×10^6	5.09×10^6
CI Chlorite (High dose)	4.28×10^5	6.98×10^5	n.d.	n.d.	1.65×10^5	4.24×10^5	n.d.	n.d.

n.d., Not determined, as cast iron material would interfere with detection of cells by epifluorescence microscopy.
[a]Cell counts represented an average of 5 replicated samples.
[b]ClO_2 was dosed into the ARs to achieve an effluent residual concentration of 0.25 mg/l (low dose) and 0.5 mg/l (high dose).
[c]ClO_2^- was applied to achieve an effluent ClO_2^- residual concentration of 0.1 mg/l (low dose) and 0.25 mg/l (high dose).

Table 2
Ratio of the number of HPCs to AODCs during acclimation and disinfection

AR	Acclimation ratio of culturable to total cells		Post-disinfection ratio of culturable to total cells	
	Bulk cells[a]	Biofilm cells	Bulk cells	Biofilm cells
PC control	1.02×10^{-1}	3.27×10^{-1}	1.17×10^{-1}	3.01×10^{-1}
CI control	9.97×10^{-2}	n.d.	8.50×10^{-2}	n.d.
PC ClO_2 (Low dose)[b]	8.89×10^{-2}	1.85×10^{-1}	4.21×10^{-4}	6.67×10^{-2}
CI ClO_2 (Low dose)	1.18×10^{-1}	n.d.	6.59×10^{-3}	n.d.
PC ClO_2 (High dose)	1.49×10^{-1}	1.29×10^{-1}	8.20×10^{-5}	3.50×10^{-3}
CI ClO_2 (High dose)	4.25×10^{-2}	n.d.	1.80×10^{-4}	n.d.
PC Chlorite (Low dose)[c]	1.36×10^{-1}	1.19×10^{-1}	1.90×10^{-2}	7.14×10^{-2}
CI Chlorite (Low dose)	2.07×10^{-1}	n.d.	1.01×10^{-1}	n.d.
PC Chlorite (High dose)	8.99×10^{-2}	3.81×10^{-1}	3.60×10^{-2}	2.08×10^{-1}
CI High Chlorite (High dose)	1.73×10^{-1}	n.d.	7.88×10^{-2}	n.d.

n.d., Not determined, as cast iron material would interfere with detection of cells by epifluorescence microscopy.
[a]Cell counts represented an average of 5 replicated samples.
[b]ClO_2 was dosed into the ARs to achieve an effluent residual concentration of 0.25 mg/l (low dose) and 0.5 mg/l (high dose).
[c]ClO_2^- was applied to achieve an effluent ClO_2^- residual concentration of 0.1 mg/l (low dose) and 0.25 mg/l (high dose).

of the polycarbonate or cast iron ARs, ignoring treatment ($p = 0.391$, mean cast iron $= 2.59 \times 10^4 \pm 2.2 \times 10^4$, mean polycarbonate $= 1.46 \times 10^6 \pm 1.7 \times 10^4$). For bulk water samples initial HPC counts on Day 0 were at about 1×10^5 CFU/ml. Control counts after the 50 day disinfection period decreased to 4.32×10^4 and 3.18×10^4 CFU/ml for polycarbonate and cast iron ARs, respectively, which corresponds to log reductions of 0.47 and 0.34. ARs that were disinfected had levels reduced ranging from 6.18×10^2 to 5.62×10^4 CFU/ml, with log reductions ranging from 0.27 to 1.77, disregarding coupon material and treatment type.

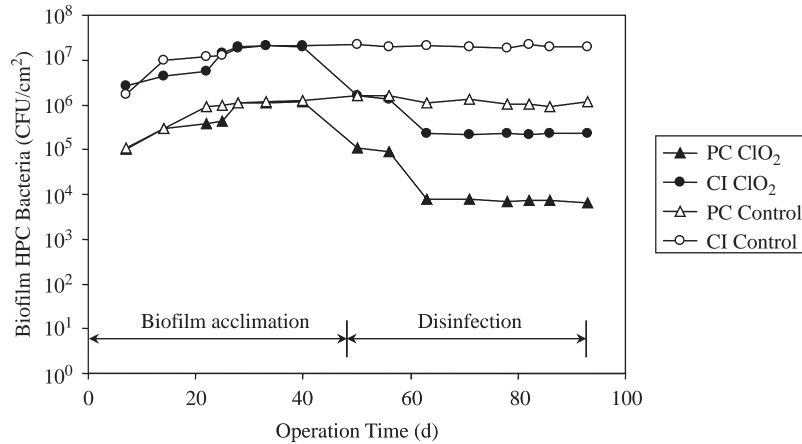


Fig. 1. Heterotrophic biofilm bacteria for chlorine dioxide ARs (high dose) and control ARs.

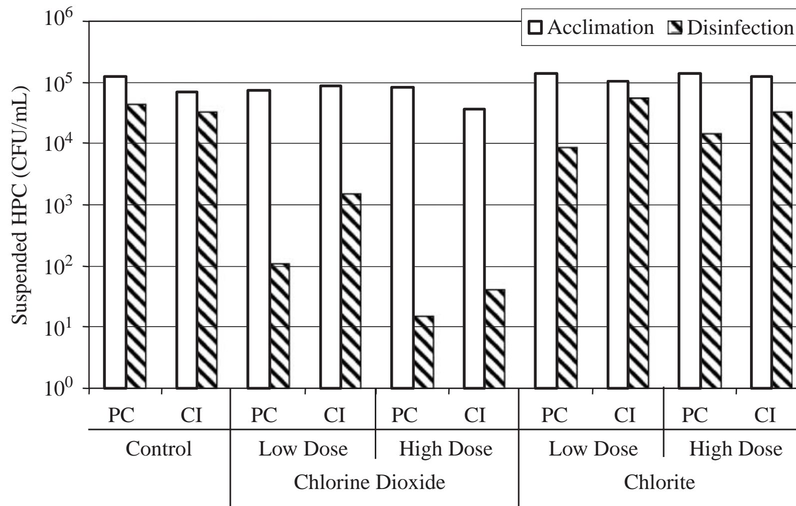


Fig. 2. Comparison of chlorite and chlorine dioxide disinfection of suspended HPCs on cast iron (CI) and polycarbonate (PC) surfaces.

No significant decrease was observed in the number of biofilm heterotrophic bacteria during the disinfection period ($p = 0.106$) for all ARs excluding those dosed with ClO_2 having a residual of 0.5 mg/l. There was no significant difference in biofilm HPC between polycarbonate and cast iron coupons, disregarding treatment ($p = 0.645$). HPC levels on Day 0 ranged from 1×10^6 to 1.5×10^7 CFU/ml. For the control ARs there was a decrease in HPCs to 9.55×10^5 and 7.90×10^6 CFU/ml for polycarbonate and cast iron ARs, respectively, which corresponds to log reductions of 0.11 and −0.07. A similar decreasing trend was observed for ARs receiving treatment with HPC levels reduced from 9.4×10^4 to 4.52×10^6 CFU/ml, with log reductions ranging from 0.20 to 0.73. Both cast iron and polycarbonate ARs receiving high ClO_2 doses had greater and comparable log reductions at 1.36 and 1.33, respectively (Figs. 2, 3).

3.3. Post-disinfection total cell counts data

After the 50-day disinfection period, total cell counts, as measured by the AODC method, were determined for each treatment type and coupon material with a mean count of $3.85 \times 10^5 \pm 8.94 \times 10^4$ for bulk water and $3.62 \times 10^6 \pm 1.64 \times 10^6$ for biofilm. The mean log reduction for bulk water was greater than the mean log reduction for biofilm (0.33 ± 0.19 for bulk water and 0.12 ± 0.25 for biofilms). There was a significant difference between mean concentrations of bulk water and biofilm ($p = 2.03 \times 10^{-3}$); however, there was no

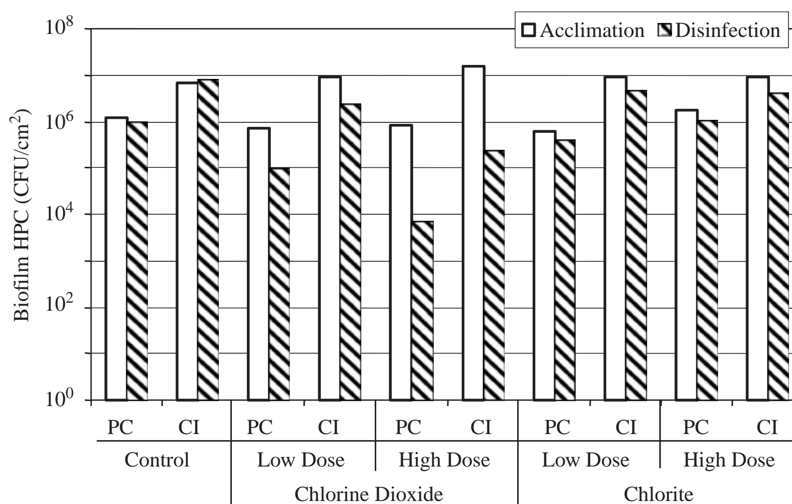


Fig. 3. Comparison of chlorite and chlorine dioxide disinfection of biofilm HPCs on cast iron (CI) and polycarbonate (PC) surfaces.

significant difference between the final AODC log reduction ($p = 0.138$) when ignoring treatment and coupon material.

There was a significant decrease in bulk water total cell counts from pre-disinfection to post-disinfection ($p = 0.000966$). In comparison, the polycarbonate and cast iron ARs, disregarding treatment type, did not display a significant difference for total cell counts ($p = 0.485$). Control levels after the 50-day disinfection period were 3.7×10^5 and 3.74×10^5 CFU/ml for polycarbonate and cast iron, respectively, which corresponds to log reductions of 0.53 and 0.27. During disinfection, reduced levels in total cell counts ranged from 2.64×10^5 to 5.57×10^5 CFU/ml with log reductions ranging from -0.04 to 0.59, disregarding coupon material and treatment type.

No significant decrease was seen for total cell counts in the biofilm after disinfection ($p = 0.388$). The control ARs had total biofilm cell counts of 3.17×10^6 CFU/cm^2 for the polycarbonate AR. This level corresponded to a log reduction in AODC of 0.07. The ARs that were disinfected had total cell counts ranging from 1.14×10^6 to 5.39×10^6 CFU/cm^2 with log reductions ranging from -0.02 to 0.49, depending on the type of disinfectant and dose combination.

In general the viable suspended cells were decreased by treatment with ClO_2 (Table 1). Overall for both disinfectants, there was a significant decrease in the number of suspended non-viable and viable cells ($p = 1.65 \times 10^{-5}$ for mean $2.02 \times 10^5 \pm 6.6 \times 10^4$ non-viable cells; $p = 0.000419$ for mean $1.83 \times 10^5 \pm 6.3 \times 10^4$ viable cells). However, for biofilm there was no significant decrease in the number of non-viable and viable cells ($p = 0.638$ for mean $1.75 \times 10^6 \pm 7.6 \times 10^5$ non-viable cells; $p = 0.658$ for mean $1.88 \times 10^6 \pm 1.3 \times 10^6$ viable cells). There was no significant difference between polycarbonate and cast iron coupons for bulk water in non-viable or viable cells ($p = 0.395$ non-viable and $p = 0.913$ viable).

3.4. Post-disinfection ratio of heterotrophic to total cells

No significant decrease was seen for total cell counts in the biofilm after disinfection ($p = 0.388$), which is consistent with Gagnon et al. (2004) findings. The ratios of heterotrophic to total cells (HPC/AODC ratio) were also calculated for post-disinfection data (Table 2). The HPC/AODC ratio decreased significantly in bulk water from a mean of 0.12 ± 0.05 pre-disinfection to 0.05 ± 0.04 post-disinfection. This finding corresponds to results presented by Gagnon et al. (2004) and Volk et al. (2002), where HPC/AODC ratios decreased after treatment with chlorine dioxide. In addition to decreases in bulk water ratios, the biofilm HPC/AODC ratio also decreased from 0.29 ± 0.12 to 0.16 ± 0.12 during post-disinfection, although this decrease was not statistically significant ($p = 0.127$).

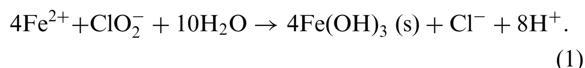
There was a significant difference between bulk water and biofilm ratios ($p = 0.018$). There was no difference between bulk water polycarbonate and cast iron coupon ratios ($p = 0.536$) and no statistical difference was found from the perspective of disinfectant type ($p = 0.147$).

4. Discussion

4.1. Comparison of coupon material

Comparing cast iron to polycarbonate coupons for biofilm heterotrophic bacteria in the pre-disinfection

stages, the cast iron coupons had statistically higher counts, which is consistent with previous drinking water biofilm studies (e.g., Gagnon et al., 2004; Camper, 1996). In most cases the greatest reduction in heterotrophic bacteria was observed for the polycarbonate coupons. However, the high ClO_2 level resulted in statistically significant reduction for both polycarbonate and cast iron ARs. Lower log reductions were observed for the polycarbonate ARs at low and high chlorite concentrations. For similar chemical dosages, chlorite was less effective than chlorine dioxide regardless of coupon material. However, chlorite was particularly ineffective in the ARs containing cast iron coupons. Iaturu and Knocke (1992) found that ClO_2^- can oxidize Fe^{2+} into ferric iron (Fe^{3+}) and follows the oxidation–reduction reaction.



$$4Fe^{2+} + ClO_2^- + 10H_2O \rightarrow 4Fe(OH)_3\,(s) + Cl^- + 8H^+. \quad (1)$$

Although the effluent residual concentration was maintained at either 0.1 or 0.25 mg/l, it is plausible that in the cast iron ARs the chlorite oxidized iron corrosion deposits rather than with disinfecting heterotrophic bacteria. Consequently, chlorite appeared to be less effective in the cast iron ARs.

4.2. *Comparison of disinfectants*

The greatest log reduction in suspended heterotrophic bacteria was observed for the high dose of ClO_2 on both cast iron and polycarbonate coupons (1.77 and 1.55). As anticipated there was virtually no HPC reduction for the two control ARs (0.27–0.56) which implies that the chemical agents were providing the disinfection and not any other external factors (e.g., changes in water matrix over the duration of the experiment.

Log reduction for chlorite in polycarbonate ARs was about 1 log, and in the cast-iron ARs, chlorite was even less effective with an average log reduction of 0.5. From these HPC log reductions it does not appear that a chlorite concentration of either 0.1 or 0.25 mg/l will influence suspended heterotrophic growth in distribution systems. This is comparable to laboratory and field results presented by McGuire et al. (1999). In that study McGuire et al. (1999) found that chlorite was not toxic to heterotrophic bacteria, but AOB were more readily disinfected by chlorite. McGuire et al. (1999) hypothesized that localized concentrations of chlorine dioxide would be produced in the cell. These localized concentrations of chlorine dioxide were a result of ClO_2^- diffusing across the cell membrane of an AOB that was subsequently reacted with H^+ that was produced during ammonia oxidation. According to that hypothesis chlorine dioxide would become the active agent for disinfection of AOB. Because heterotrophic bacteria do not oxidize ammonia and would be less likely to have localized acidic conditions at the cell membrane, chlorine dioxide would not be produced by ClO_2^- in these organisms. Practically, these data in combination with the data and microbial mechanisms presented by McGuire et al. (1999) suggest that chlorite has a greater microbial specificity than conventional secondary disinfectants and would be more effectively used in combination with other disinfectants (e.g., chloramines).

Similar to the suspended heterotrophic bacteria, ClO_2 had a greater reduction on biofilm heterotrophic bacteria than chlorite. In particular polycarbonate ARs receiving high ClO_2 doses had a 2.0 log reduction, which was greatest reduction in biofilm bacteria. Cast iron and polycarbonate ARs with low dose ClO_2 had lower log reductions (0.52 and 0.73, respectively) than the high dose of ClO_2. Nevertheless the log reduction from the low dose with ClO_2, was greater than any disinfectant dose with chlorite.

For bulk water total cell counts ClO_2 provided about 1 log disinfection, whereas chlorite provided very little disinfection for the total cell numbers. However, while there was a significant change in AODC counts as a result of disinfection there was no significant difference between the types of disinfectant applied.

5. Conclusions

Although previous studies have reported that chlorite concentrations in the range of 0.1–0.3 mg/l was capable to mitigating nitrification in distribution systems (McGuire et al., 1999), this study demonstrated that chlorite was essentially ineffective in inactivating heterotrophic bacteria (with residual concentrations of 0.1–0.25 mg/l). In particular, chlorite was ineffective for both suspended and biofilm bacteria, regardless of the coupon material. Chlorine dioxide was able to achieve a log-inactivation of 1.6–1.8 for suspended cells and just under 1 log inactivation of biofilm heterotrophic bacteria at a low concentration of 0.25 mg/l. From a practical perspective these data suggest that it is critical to maintain a chlorine dioxide residual and not to rely on the disinfection capacity of chlorite for mitigating heterotrophic growth in drinking water distribution systems.

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References

APHA, AWWA, WEF (American Public Health Association, American Water Works Association, Water Environment Federation), 1995. Standard Methods for the Examination of Water and Wastewater, 19th ed. APHA, Washington DC.

Abdel-Rahman, M.S., Couri, D., Bull, R.J., 1984. The kinetics of chlorite and chlorate in the rat. J. Am. Coll. Toxicol. 3, 277–284.

Baribeau, H., Prévost, M., Desjardins, R., Lafrance, P., Gates, D.J., 2002. Chlorite and chlorate ion variability in distribution systems. J. Am. Water Works Assoc. 94 (7), 96–105.

Camper, A.K., 1996. Factors Limiting Microbial Growth in Distribution Systems: Laboratory and Pilot-Scale Experiments. AWWA Research Foundation, Denver, CO.

Chauret, C.P., Radziminski, C.Z., Lepuil, M., Creason, R., Andrews, R.C., 2001. Chlorine dioxide inactivation of Cryptosporidium parvum oocysts and bacterial spore indicators. Appl. Enviro. Microbiol. 67 (7), 2993–3001.

Eisnor, J.D., Gagnon, G.A., 2004. Impact of secondary disinfection on corrosion in a model water distribution system. J. Water Supply: Res. Technol. (Aqua) 53 (7), 441–452.

Finch, G.R., Belosevic, M., 1997. Effect of Various Disinfection Methods on the Inactivation of *Cryptosporidium* (90734). AWWA Research Foundation, Denver, CO.

Gagnon, G.A., Slawson, R.M., 1999. An efficient biofilm removal method for bacterial cells exposed to drinking water. J. Microbiol. Methods 34, 203–214.

Gagnon, G.A., O'Leary, K.C., Volk, C.J., Chauret, C., Stover, L., Andrews, R.C., 2004. Comparative analysis of chlorine dioxide, free chlorine and chloramines on bacterial water quality in model distribution systems. ASCE—J. Environ. Eng. 130 (11), 1269–1279.

Gordon, G., 1992. Water chemistry of the oxy-chlorine species. Proceedings of the Second International Symposium on Chlorine Dioxide: Drinking Water Issues, Houston, TX.

Gordon, G., 2001. Is all chlorine dioxide created equal? J. Am. Water Works Assoc. 93 (4), 163–174.

Hofmann, R., Andrews, R.C., Ye, Q., 1998. Comparison of spectrophotometric methods for measuring chlorine dioxide in drinking water. Environ. Technol. 19 (8), 761–773.

Hofmann, R., Andrews, R.C., Ye, Q., 1999. Impact of *Giardia* inactivation requirements on ClO_2 by-products. Environ. Technol. 20, 147–158.

Iaturu, A., Knocke, W.R., 1992. Removing chlorite by the addition of ferrous iron. J. Am. Water Works Assoc. 84 (11), 63–68.

Kanitz, S., Franco, Y., Patrone, V., Caltabellotta, M., Raffo, E., Riggi, C., Timitilli, D., Ravera, G., 1996. Associations between drinking water disinfection and somatic parameters at birth. Environ. Health Perspect. 104, 516–520.

Lafrance, P., Duchesne, D., Arcoutte, N., Prevost, M., 1993. The use of ClO_2: case study of Laval. Proceedings of Chlorine Dioxide: Drinking Water Issues: Second International Symposium (AWWA), Houston, TX.

LeChevallier, M.W., Shaw, N., Smith, D.B., 1996. Full-scale studies of factors related to coliform regrowth in drinking water. Appl. Environ. Microbiol. 62, 2201–2211.

Liyanage, L.R.J., Finch, G.R., Belosevic, M., 1997. Effect of aqueous chlorine and oxy-chlorine compounds on *cryptosporidium parvum* oocysts. Environ. Sci. Technol. 31 (7), 1992.

Lubbers, J.R., Chauhan, S., Bianchine, J.R., 1981. Controlled clinical evaluations of chlorine dioxide, chlorite and chlorate in man. Fund. Appl. Toxicol. 1, 334–338.

Lubbers, J.R., Chauhan, S., Bianchine, J.R., 1982. Controlled clinical evaluations of chlorine dioxide, chlorite and chlorate in man. Environ. Health Perspect. 46, 57–62.

Lubbers, J.R., Chauhan, S., Miller, J.K., 1984. The effect of chronic administration if chlorine dioxide, chlorite and chlorate to normal health adult male volunteers. J. Environ. Pathol. Toxicol. Oncol. 5, 239–242.

McGuire, M.J., Lieu, N.I., Pearthree, M.S., 1999. Using chlorite ion to control nitrification. J. Am. Water Works Assoc. 91 (10), 52–61.

Michael, G.E., Miday, R.K., Bercz, J.P., Miller, R.G., Greathouse, D.G., Kraemer, D.F., Lucas, J.B., 1981. ClO_2 water disinfection: a prospective epidemiology study. Arch. Environ. Health 36, 20–27.

Simpson, G.D., 1995. Biofilm: removal and prevention with chlorine dioxide. Proceedings of the Third International Symposium on Chlorine Dioxide, New Orleans, LA.

Sharp, R.R., Camper, A.K., Crippen, J.J., Schneider, J.D., Leggiero, S., 2001. Evaluation of drinking water biostability using biofilm methods. ASCE—J. Environ. Eng. 127 (5), 403–411.

Thompson, A.L., 1993. Chlorine dioxide process treatment data and cost. Chlorine dioxide use in water treatment. Proceedings of the Second International Symposium on Chlorine Dioxide, Houston, TX.

Tuthill, R.W., Giusti, R.A., Moore, G.S., 1982. Health effects among newborns after prenatal exposure to ClO_2-disinfected drinking water. Environ. Health Perspect. 46, 39–45.

Volk, C.J., Hofmann, R., Chauret, C., Gagnon, G.A., Rangers, G., Andrews, R.C., 2002. Implementation of chlorine dioxide disinfection: effects of the treatment change on drinking water quality in a full-scale distribution system. J. Environ. Eng. Sci. 1 (5), 323–330.

Werdehoff, K.S., Singer, P.C., 1987. Chlorine dioxide effects on THMFP, TOXFP and the formation of inorganic by-products. J. Am. Water Works Assoc. 79 (9), 107–113.