Haloacetic acid degradation by a biofilm in a simulated drinking water distribution system

ABSTRACT
Haloacetic acids (HAAs) are disinfection by-products formed as a result of the reaction between chlorine and natural organic matter found in water. HAA concentrations have been observed to decrease at distribution system extremities. This decrease is associated with microbiological degradation by pipe wall biofilm. The objective of this study was to evaluate HAA degradation in a drinking water system in the presence of a biofilm and to identify the factors that influence this degradation. Degradation of dichloracetic acid (DCAA) and trichloroacetic acid (TCAA) was observed in a simulated distribution system. The results obtained showed that different parameters came into play simultaneously in the degradation of HAAs, including retention time, water temperature, biomass, composition of organic matter, and pipe diameter. Seasonal variations had a major effect on HAA degradation and biomass quantity was lower by 1 to 2 logs in the winter and spring compared with the fall. HAA removal decreased with increasingly large pipe diameters. The specific effects of each of these factors were difficult to isolate from each other owing to interactions.

Key words | biofilm, biomass, disinfection by-products, distribution system, drinking water, haloacetic acids

ABBREVIATIONS
BAC Biologically activated carbon
BCAA Bromochloroacetic acid
BDOC Biodegradable dissolved organic carbon
CBPs Chlorination by-products
DBAA Dibromoacetic acid
DCAA Dichloroacetic acid
DOC Dissolved organic carbon
HAAs Haloacetic acids
MBAA Monobromoacetic acid
MCAA Monochloroacetic acid
NOM Natural organic matter
PCR Polymerase chain reaction
ROM Residual organic matter
TCAA Trichloroacetic acid
THMs Trihalomethanes

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INTRODUCTION

Disinfection by-products

Chlorine is the most commonly used disinfectant in the drinking water industry. In the seventies, it was established that chlorine reacts with natural organic matter (NOM) found in raw water to form various substances known as chlorination by-products (CBPs) (Bellar et al. 1974; Rook 1974). More than 500 by-products have been identified (Richardson 1998; Richardson et al. 2002). The most common are trihalomethanes (THMs) and haloacetic acids (HAAs), followed by haloacetonitriles, haloketones, aldehydes, chlorophenols and chloropicrin (Krasner et al. 1989; Chen & Weisel 1998). Some CBPs are potentially carcinogenic and may have harmful effects on human gestation and development (Health Canada 2000).

Several parameters influence CBP formation and speciation: the amount and nature of NOM (Croué et al. 1999); concentration of bromide ions (Trussell & Umphres 1978); water pH and temperature (LeBel et al. 1997; Williams et al. 1997; Chen & Weisel 1998; Krasner 1999) and the dose of disinfectant and its contact time with the water (Singer 1994). Studies on the spatio-temporal occurrence of CBPs in the distribution system show that THM concentrations increase with residence time and reach a maximum at network extremities. However, HAA levels may increase and then decrease, especially when water reaches network extremities, which corresponds to the lowest residual chlorine levels and the maximum water residence time in most distribution systems (LeBel et al. 1997; Williams et al. 1997; Chen & Weisel 1998; Rodriguez & Sérodes 2001; Rodriguez et al. 2004). Dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) are the two HAAs most commonly present in distribution networks (LeBel et al. 1997; Williams et al. 1997; Sérodes et al. 2003; Maliarou et al. 2005). As a rule, a greater reduction of DCAA is observed in the water distribution system than for TCAA (Williams et al. 1994; Chen & Weisel 1998; Rodriguez et al. 2004).

Microbiological degradation of HAAs

Although the chemical degradation of HAAs may occur under certain circumstances (Hozalski et al. 2001; Zhang & Minear 2002; Wang & Zhu 2010), low HAA concentrations found at network extremities are associated primarily with biodegradation mechanisms attributable to various types of bacteria (Williams et al. 1994, 1998; Tung & Xie 2003). Monochloroacetic acid (MCAA), DCAA and TCAA can be biologically degraded in the soil (Meusel & Rehm 1993; Matucha et al. 2003; Torz & Beschkov 2005). Dehalogenase activity from Xanthobacter autotrophicus can degrade MCAA, DCAA, monobromoacetic acid (MBAA) and dibromoacetic acid (DBAA), but not TCAA (Ploeg et al. 1991). Grigoescu & Hozalski (2010) reported that high biomass densities (>10⁵ cells/cm²) were needed for HAA removal and that this removal is possible in a distribution system with low chlorine residual levels. Zhou & Xie (2002) also specified that in the absence of residual chlorine, HAAs are degraded by bacteria. These authors demonstrated the existence of much greater degradation kinetics for bacteria collected from biologically activated carbon filters than for microorganisms from finished water. The following degradation order was noted: MCAA > DCAA > TCAA. More recently, Bayless & Andrews (2008) tested the degradation of six HAAs through biofilm in columns filled with glass beads. They established the following biodegradation order: MBAA > MCAA > bromochloroacetic acid (BCAA) > DCAA > DBAA > TCAA. TCAA was not degraded.

Biofilm in water distribution pipes

Biofilm develops over the entire surface in contact with an aqueous vehicle. Development can be divided into three phases: induction (organic adsorption, transportation and fixation of microorganisms); accumulation (microorganism growth); and a plateau phase (dynamic balance between growth and death of microorganisms, constant thickness of biofilm) (Trulear & Characklis 1982). In reactors simulating a drinking water system, biofilm can form very quickly and reach a state of equilibrium within a week (Manuel et al. 2007). Biofilm formation is influenced by many factors, including the nature of the support material (Hallam et al. 2001; Lehtola et al. 2004), temperature (Gallardo-Moreno et al. 2004), hydraulics (Blanchard et al. 1998; Melo & Vieira 1999), and organic matter and minerals found in the water (LeChevallier et al. 1991; Song & Leff 2006).
Depending on the conditions, the biofilms' bacterial density can vary from 10 to 10⁸ cells/cm² (Niquette et al. 2000; Lehtola et al. 2004; Baribeau et al. 2005; Manuel et al. 2007). Water chlorination does not prevent the formation of biofilm on new materials (Paquin et al. 1992). Resistance to chlorine by fixed bacteria may be explained by the limited penetration of chlorine into the biofilm (De Beer et al. 1994) and oxidant consumption by the biofilm surface on water pipes and by the pipe material, especially pipes made of iron (Hallam et al. 2002). Several authors have also used pipes to study biofilm in water distribution systems (Paquin et al. 1992; Lehtola et al. 2004, 2005; Lipponen et al. 2004).

To date, studies focusing on HAA biodegradation by bacteria have been conducted in reactors, with bacteria collected from drinking water distribution systems or wastewater (Zhou & Xie 2002; McRae et al. 2004; Bayless & Andrews 2008; Zhang et al. 2009). A few studies have evaluated HAA biodegradation by a biofilm in a drinking water network (Baribeau et al. 2005; Grigorescu & Hozalski 2010) without focusing on the impacts of seasonal variations. The objective of the present study is to evaluate HAA degradation by a biofilm in a drinking water system and to identify the factors that explain this degradation. The study is based on an in situ test site within a full-scale distribution system and was conducted over a period exceeding one year to consider variable seasonal conditions.

MATERIALS AND METHODS

Choice of test site

The main criterion for choosing the test site was that the pipe feedwater had to have the lowest possible residual concentration of chlorine in order to obtain optimal conditions for biofilm formation. In fact, residual concentrations of total chlorine above 0.3 mg/L slow down the formation of biofilm (Paquin et al. 1992). Moreover, water had to flow continuously for several months. The test models were installed in a public building at one extremity of the municipal water distribution system. Water originated from the main drinking water production plant of Québec City, which supplies approximately 240,000 people and uses the Saint-Charles River as a source of raw water. The Saint-Charles River is supplied in the north of Quebec City by a lake in a process of natural eutrophication and whose water has high levels of color. The watershed is moderately urbanized with relatively low agricultural activities. The source water quality is highly influenced by meteorological events. In this region, seasonal variations during the year are considerable. Winters are long and very cold, whereas summers are relatively short, but generally warm. Given the large amounts of snow in the winter, there is considerable runoff of watershed waters in the spring. In the fall, fallen leaves are a major source of NOM in the water. These factors strongly influence the quality of the surface water used to produce drinking water (Rodríguez & Sérodes 2001). The treatment process involves coagulation–floculation by ballasted flocs, sedimentation, filtration (multimedia), ozonation and post-chlorination to ensure the presence of residual chlorine in the distribution system. As northern climatic conditions have a strong impact on the temperature and quality of raw water, experimental work was conducted over a period of more than one year in order to study the effects of seasonal variations on HAA degradation.

Experimental systems

Four experimental systems were implemented to simulate a drinking water distribution system. They were designed based on the characteristics specified in Table 1. They consisted of HDPE (high-density polyethylene) pipes referred to as A, B, C and D. Plastic pipes are considered to be non-reactive with HAAs (Hallam et al. 2002). Metal pipes were avoided, as HAA levels can be reduced by elemental iron and zinc (Hozalski et al. 2001; Wang & Zhu 2010). Pipes with two different internal diameters (12.7 mm for pipes A and B; 25.4 mm for pipes C and D) were used to study the influence of biofilm versus the volume of water circulating in the pipe (Table 1). Sampling points were installed along the pipes to sample water at different residence times (0, 15, 30, 45 and 60 min at a flow rate of 60 mL/min for A and B, and a flow rate of 120 mL/min for C and D) to establish the kinetics of HAA degradation. The pipes were supplied continuously with drinking water. In order to maintain a constant flow, the water was pumped using a
peristaltic pump (Masterflex, Cole-Palmer, Vernon Hills, IL, USA) from a reservoir supplied continuously by distribution system water and equipped with an overflow. Although the test site was selected for its low concentration of residual chlorine, the water was dechlorinated using a solution of sodium thiosulfate (Na₂S₂O₃) so that it would not be subjected to variations in residual chlorine concentrations during the experiments. This solution was injected continuously using a multi-head peristaltic pump (Masterflex, Cole-Palmer, Vernon Hills, Illinois, USA) at a rate of 1 mL/min (A and B) or 2 mL/min (C and D). The Na₂S₂O₃ concentration was adjusted according to the chlorine residual in the network water (0–250 mg/L of Na₂S₂O₃).

This same pump also served to continuously inject a solution spiked with DCAA and TCAA at a rate of 1 mL/min (pipes A and B) or 2 mL/min (pipes C and D) (Table 1). In order to take samples of biofilm, a device filled with HDPE coupons was installed at the beginning and end of the experimental systems. HDPE coupons (same material as the pipes) were installed to run parallel to the water flow and sampled at different times to characterize the biofilm. A schematic diagram of one of the four experimental systems is shown in Figure 1.

Prior to their operation, the pipes were supplied for 48 h with a de-ionized solution of water containing 100 mg Cl₂/L and then supplied with de-ionized water for four days. Thereafter, the pipes were supplied with distribution system water for 3 months to allow the biofilm time to generate and reach a state of equilibrium. In existing studies, the period required to obtain biofilm at equilibrium varies from 1 week (Manuel et al. 2007) to 3 months (Zhang & Huck 1996). During the experiments, the pipes were supplied for 4 weeks with dechlorinated water from the distribution system and an HAA solution, whereas during the periods between experiments, only dechlorinated water was supplied to the pipes. HPDE bottles referred to as T1 and T2 were used as controls to ensure that the reduction in HAA concentrations was not due to chemical degradation, volatilization or extraction used for HAA analyses, but definitely due to degradation by the biofilm. As there may be a loss of HAAs during extraction, controls help to determine whether the observed decrease is not due to extraction. Bottles T1 and T2 were filled with dechlorinated water and the retention times were the same as the times used in the pipes. Targeted HAA concentrations during the experiments are presented in Table 1. They were chosen to correspond to a concentration range that could exist in an actual distribution system (Dion-Fortier et al. 2009). Different DCAA and TCAA concentrations and ratios were tested to determine

Table 1  | Characteristics of the experimental system

<table>
<thead>
<tr>
<th>Characteristics of pipes</th>
<th>Targeted HAA concentrations</th>
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<tbody>
<tr>
<td></td>
<td>Week 1</td>
</tr>
<tr>
<td></td>
<td>DCAA (μg/L)</td>
</tr>
<tr>
<td>A 30 12.7 60</td>
<td>30 30</td>
</tr>
<tr>
<td>B 30 12.7 60</td>
<td>60 60</td>
</tr>
<tr>
<td>C 15 25.4 120</td>
<td>30 30</td>
</tr>
<tr>
<td>D 15 25.4 120</td>
<td>60 60</td>
</tr>
<tr>
<td>T1 / / /</td>
<td>60 60</td>
</tr>
<tr>
<td>T2 / / /</td>
<td>30 30</td>
</tr>
</tbody>
</table>

Figure 1  | Experimental system.
whether the proportion had an impact on HAA degradation. In the first week, the DCAA/TCAA ratio was equal to 1. However, as TCAA seems more difficult to biodegrade than DCAA in equal concentrations, DCAA may be consumed before TCAA. Therefore, during the following weeks, a DCAA/TCAA ratio <1 was applied (Table 1).

Sampling took place on days 0, 1, 7, 8, 14, 15, 21 and 22 (day 0 corresponded to the beginning of the first day of every sampling campaign). Five campaigns were carried out: one for each season in 2009 and one at the beginning of the summer of 2010. During each campaign, the test process presented in the Sampling and analyses section was repeated. The characteristics for drinking water quality during the study period (February 9, 2009, to July 6, 2010) are presented in Table 2.

An additional campaign was conducted in 2011 (June 28 to October 12, 2011). The purpose of this campaign was to confirm and expand the results. In this campaign, only experimental system A and bottle T1 were used. The retention times and sampling days were modified. The water was recirculated in a closed circuit to obtain retention times of 1, 2, 4, 6, 8 and 24. Sampling took place every 2 weeks. During experimentation days, the pipes were supplied with dechlorinated water from the distribution system and a HAA solution. The targeted concentration was 40 μg/L for both DCAA and TCAA. Between experiments, the pipes were supplied only with dechlorinated tap water.

**Sampling and analyses**

Table 3 presents the analyses carried out during each experiment. Water samples for measuring pH, conductivity, turbidity, UV-254 nm absorption and the dissolved organic carbon (DOC) were collected in 250 mL Nalgene bottles and analyzed in the laboratory within a 24 h period. DOC samples were filtered with 0.45 μm filter carbonized at 550 °C and transferred in glass vials specially cleaned for carbon analyses. Free available residual chlorine and temperature were measured onsite. Water samples for measuring HAAs were filtered using sterile syringe filters with 0.2 μm porosity and collected in 40 mL vials containing ammonium chloride. The filtration step is necessary to prevent the presence of microorganisms that may lead to HAA degradation in the vial between the time of sampling and analysis. Checks were made to ensure that the filter used did not affect HAA concentrations in the sample water (results not shown).

Temperatures were measured using a mercury thermometer. Free available residual chlorine was measured using a colorimeter (HACH DR/890) based on the colorimetric dosing method using DPD (standard 4500-Cl-F

### Table 2: Physico-chemical characteristics of drinking water for the distribution system under study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Winter (February) 2009 Mean</th>
<th>Spring (April) 2009 Mean</th>
<th>Summer end (Mid-August-early September) 2009 Mean</th>
<th>Fall (October) 2009 Mean</th>
<th>Early summer (June end-early July) 2010 Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>3.6</td>
<td>3.8</td>
<td>18.1</td>
<td>13.5</td>
<td>13.9</td>
</tr>
<tr>
<td>Residual chlorine (mg/L)</td>
<td>0.50</td>
<td>0.48</td>
<td>0.20</td>
<td>0.04</td>
<td>0.38</td>
</tr>
<tr>
<td>pH</td>
<td>7.6</td>
<td>7.4</td>
<td>7.6</td>
<td>7.7</td>
<td>7.5</td>
</tr>
<tr>
<td>Conductivity (mS/cm)</td>
<td>259</td>
<td>181</td>
<td>205</td>
<td>182</td>
<td>201</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>0.501</td>
<td>0.586</td>
<td>0.536</td>
<td>0.764</td>
<td>0.508</td>
</tr>
<tr>
<td>UV-254 nm (cm⁻¹)</td>
<td>0.027</td>
<td>0.028</td>
<td>0.023</td>
<td>0.032</td>
<td>0.027</td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>1.73</td>
<td>1.78</td>
<td>1.99</td>
<td>2.15</td>
<td>1.79</td>
</tr>
<tr>
<td>SUVA (L/mg m)</td>
<td>1.54</td>
<td>1.58</td>
<td>1.19</td>
<td>1.51</td>
<td>1.51</td>
</tr>
<tr>
<td>DCAA (μg/L)</td>
<td>6</td>
<td>11</td>
<td>4</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>TCAA (μg/L)</td>
<td>7</td>
<td>12</td>
<td>22</td>
<td>15</td>
<td>19</td>
</tr>
</tbody>
</table>

SUVA = (UV-254 nm/DOC) × 100.
method). Conductivity was measured using a conductivimeter (HACH DR/890). DOC was analyzed using a carbon analyzer (Shimadzu ASI 5000 model) following the standard 5310B method. UV absorbance was measured using a UV-visible 254 nanometer spectrophotometer (Pharmacia, model 80–2097–62) with 50 mm path length quartz cells. pH was measured with a pHmeter AP15 from Denver Instrument. Turbidity was measured using a turbidimeter (Hach model 2100N).

**HAAs** were measured using the EPA 552.2 method (USEPA 1998) with a gas-phase chromatograph equipped with an electron capture detector (GC-ECD). The detection limits were 0.9 μg/L for DCAA and 1.2 μg/L for TCAA.

**Quantification of total bacteria**

In this study, microorganism quantification was carried out using molecular biological techniques. This approach provides a value of the total biomass found in the biofilm, which quantifies both cultivable and non-cultivable bacteria compared with classic microbial cultures (Zhang & Fang 2006). HDPE coupons were installed in support devices at the end of each pipe (A, B, C, D) and at the entrance of pipes A and B (Figure 1). The coupons were sampled in sterile Falcon tubes containing 15 mL of dechlorinated network water. The biofilm was removed from the coupons with a rubber policeman. This method proved to be more effective for the retrieval of biofilm than sonication (results not shown). DNA extractions were carried out using a QIAamp DNA Mini Kit (Qiagen, Mississauga, ON, Canada). The bacterial biomass was evaluated by a 5′-nuclease real-time polymerase chain reaction (PCR). This technique involves measuring the amount of polymerized DNA at each cycle through the liberation of a fluorescent marker (Holland et al. 1991). Universal primers EUBf and EUBr (Table 4) specifically amplified bacterial 16S rRNA genes (Bach et al. 2002).

Duplicate samples were placed in a 96-well tray. Each reaction occurred in a total volume of 25 μL containing the following reactants: 1X IQ Supermix (Bio-Rad, Mississauga, ON, Canada), 1 μmol L⁻¹ of each primer (EUBf, EUBr), 0.5 μmol L⁻¹ of Taqman probe (EUBp), as well as 2 μL of DNA (extracted from the sample or coming from the standard). The calibration curve (or standard) was generated by a dilution series (10¹ to 10⁷ copies) of a plasmid containing the coding gene sequence for *Escherichia coli* 16S rRNA (ATCC 25922) obtained using the TOPO-TA cloning vector kit (Invitrogen, Carlsbad, CA, USA). A threshold cycle (Ct) was obtained for each dilution and its value was inversely proportional to the log of the number of copies initially found in the sample. The amount of bacteria was calculated from the value of Ct obtained for each sample. The following program was used: 3 min at 94°C followed by 45 cycles of 15 s at 94°C, 2 min at 62°C, tray reading. The primers and probe were synthesized by International DNA Technology (Coralville, IA, USA). The analyses were carried out using Opticon Monitor software (version 2.02.24; Bio-Rad), whereas PCR amplifications were performed on the DNA Engine Opticon 2 (Bio-Rad).

**Mathematical and statistical processing**

SPSS 15.0 (SPSS Inc., Chicago, IL, USA) software was used for the statistical processing of data. Analyses of variance were carried out to determine whether the DCAA removal obtained was statistically different from the controls.

**Table 3** | Measured parameters according to the type of sample

<table>
<thead>
<tr>
<th>Samples</th>
<th>Analytical parameters</th>
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</thead>
<tbody>
<tr>
<td>Water of distribution system</td>
<td>pH, Conductivity, Turbidity, UV254 nm absorbance, DOC</td>
</tr>
<tr>
<td>Water of the distribution system + experimental system samples</td>
<td>Temperature, Free residual chlorine, HAAs</td>
</tr>
<tr>
<td>Biofilm</td>
<td>Bacteria quantification</td>
</tr>
</tbody>
</table>

**Table 4** | Primers used for quantitative PCR

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUBp</td>
<td>5′-FAM-TKC GCG TTG CDT CGA ATT AAW CCA C-3′</td>
<td>Bach et al. (2002)</td>
</tr>
<tr>
<td>EUBf</td>
<td>5′-GTT AGT CYA YGC MST AAA CG-3′</td>
<td>Bach et al. (2002)</td>
</tr>
<tr>
<td>EUBr</td>
<td>5′-GAC ARC CAT GCA SCA CCT G-3′</td>
<td>Bach et al. (2002)</td>
</tr>
</tbody>
</table>

FAM: 6-carboxyfluorescein. 
IBTMFQ: Iowa Black Fret Quencher.
RESULTS

Seasonal variations of the physical-chemical characteristics of the drinking water supply

Table 2 presents the physico-chemical water quality in the distribution network supplying the experimental systems. Specific absorbance (SUVA) is an indicator of the aromaticity of organic matter present in the water. The higher the SUVA, the greater the proportion of humic substances in the organic matter. Humic substances (fulvic acids and humic acids) have a high molecular weight and aromatic rings in their structure. They are considered more resistant to biodegradation than other more labile compounds such as amino acids and some carbohydrates. According to earlier investigations, their biodegradability varies from 0 to 30% (Agbekodo & Legube 1995; Volk et al. 1997; Gremm & Kaplan 1998). The results obtained show that fall is the period of the year when turbidity and DOC values are at their highest and when the residual chlorine concentration in the water from the distribution system is at its lowest. Higher levels of turbidity and DOC in the fall could be related to runoff and soil leaching as a rapid decay of vegetation, which increases organic matter level in raw water (Maurice et al. 2002). The residual chlorine concentration is at its highest in winter, and then drops over the seasons, reaching minimum levels in the fall. The SUVA level is lower at the end of the summer compared with the rest of the year when it is relatively stable. The temperature of drinking water supply is at its highest at summer end and at its lowest in winter and spring. Temperature of water in the pipes was about 10°C in winter and spring, about 22°C in summer, about 14°C in fall and about 18°C in early summer. Temperatures were higher in pipes because the experimental systems were located in a room at ambient temperature, which warmed up the water.

HAA degradation

Figure 2 illustrates the results obtained for HAA degradation. At the end of the summer of 2009, there was statistically significant degradation (p < 0.05) of DCAA, but not TCAA. During the winter and spring of 2009, neither of the two HAAs had degraded. The observed negative percentages and positive percentages are not statistically significant (p > 0.1). In fact, the recovery of HAAs may vary during the extraction; this is why small variability in HAA measures can be observed. During the fall, there was degradation of DCAA (statistically significant p < 0.05) in pipes A and C but not in pipes B and D. In the experiment conducted at the beginning of the summer of 2010, there was very slight degradation (statistically significant p < 0.05) of DCAA in Pipe A and C (B and D were not studied). In all cases where DCAA was reduced, it can safely be stated that biodegradation and not chemical degradation or absorption occurred on the pipe walls as there was no reduction in HAAs in the control group. In order to illustrate the effect of HAA concentration and DCAA/HAA ratio, the results of the 2009 summer campaign were used, as DCAA degradation was observed in all four pipes. When comparing the data obtained for A vs B and C vs D, there was no total HAA concentration effect. An analysis of variance was conducted to investigate the effect of the initial concentration on the DCAA degradation. Hypotheses for homogeneity and normality were verified. The DCAA removal was not statistically different between B (60 μg/L DCAA + 60 μg/L TCAA) (69%) and A (50 μg/L DCAA + 50 μg/L TCAA) (61%) (p > 0.1), but statistically different between C (30 μg/L DCAA + 30 μg/L TCAA) (57%) and D (60 μg/L DCAA + 60 μg/L TCAA) (22%) (0.05 < p < 0.1). Also, DCAA removal was greater in B (20 μg/L DCAA + 80 μg/L TCAA) (75%) than in A (20 μg/L DCAA + 40 μg/L TCAA) (56%) (difference statistically significant p < 0.1), but not statistically different between C (20 μg/L DCAA + 40 μg/L TCAA) (33%) and D (20 μg/L DCAA + 80 μg/L TCAA) (34%) (p > 0.1). When comparing DCAA removal obtained for each pipe with a different DCAA/HAA ratio, DCAA removal was not statistically different in pipe A between ratio = 0.5 and ratio = 0.33 (p > 0.1). In pipes B and C, the DCAA/HAA ratio (p > 0.1) had no impact on DCAA removal. However, in pipe D the degradation rate was higher with the DCAA/HAA ratio = 0.2 than with the DCAA/HAA ratio = 0.5 (p < 0.1). The results obtained in this study did not allow the establishment of a conclusion regarding the effect of the DCAA/HAA ratio on DCAA removal. As there was no degradation of TCAA observed in these
campaigns, it was not possible to say whether the DCAA/HAA ratio had an impact on its degradation.

Results from the first series of campaigns in 2009 and 2010 showed that a residence time of 1 h is not sufficient to observe TCAA degradation. It is possible that degradation might occur at higher residence times. Moreover, in the early summer and fall, a retention time of 1 h led to a slight degradation of DCAA. Retention times of up to 24 h were used in a new round of sampling conducted between June 28 and October 12, 2011. This campaign was carried out to confirm and further explore certain issues raised by the 2010 results, namely: Is the degradation of DCAA actually higher in the fall than early summer? Could the TCAA be degraded using longer retention times?

Table 5 illustrates the results obtained for HAA degradation during the 2011 campaign. In the early summer (June 28), no degradation of either DCAA or TCAA was
observed. DCAA degradation became visible on July 12, but was weak. From August 11 thereon, a significant degradation of DCAA (for a period of retention times of less than 8 h) was detected and continued in September and October. These results confirmed the results obtained in 2009 and 2010. Indeed, in 2011, the degradation of DCAA was the highest in late summer (August 31) and higher in the fall (late September-early October) than in early summer (June–July). Degradation of TCAA was perceptible on August 31 with a retention time of 4 h (22%) (Table 5). On the same date, and for the same retention time, the degradation of DCAA was 71%. TCAA degradation began in late summer (August 31), but was optimal in the fall (September 27-October 12) (Table 5). We also noted that on August 11, TCAA increased quite sharply instead of decreasing. As the water was dechlorinated, this increase was not due to the reaction of chlorine with organic matter. Hydrolysis of other byproducts, such as haloacetonitriles, is a hypothesis that might explain this increase (Glezer et al. 1999).

### Seasonal variations of biofilm biomass

Sampling devices were installed at the end of each pipe (A, B, C, D) to check biomass development during water supply. Similar devices were also set up at the beginning of pipes A and B to see whether the number of bacteria/cm² varied between the two extremities of the experimental systems. The results obtained in 2009–2010 showed that the location of the device had no effect on the number of bacteria/cm² (data not shown); there were just as many

<table>
<thead>
<tr>
<th>Table 5</th>
<th>DCAA and TCAA removal for campaign 2011</th>
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#### DCAA (Pipe A)

<table>
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<tr>
<th></th>
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<tr>
<td>0 h</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>1 h</td>
<td>–4% (ns)</td>
<td>5% (ns)</td>
<td>0%</td>
<td>4% (ns)</td>
<td>58%</td>
<td>13%</td>
<td>27%</td>
<td>24%</td>
</tr>
<tr>
<td>2 h</td>
<td>–3% (ns)</td>
<td>4% (ns)</td>
<td>1% (ns)</td>
<td>8% (ns)</td>
<td>62%</td>
<td>26%</td>
<td>53%</td>
<td>48%</td>
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<tr>
<td>4 h</td>
<td>0%</td>
<td>8% (ns)</td>
<td>7% (ns)</td>
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<td>71%</td>
<td>56%</td>
<td>67%</td>
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<td>6% (ns)</td>
<td>10%</td>
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<td>88%</td>
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<td>67%</td>
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<tr>
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<td>8%</td>
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<td>89%</td>
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<td>80%</td>
<td>96%</td>
</tr>
<tr>
<td>24 h</td>
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<td>76%</td>
<td>80%</td>
<td>90%</td>
<td>92%</td>
<td>99%</td>
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#### TCAA (Pipe A)

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<tbody>
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<td>0%</td>
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<td>0%</td>
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<tr>
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<td>–2% (ns)</td>
<td>–3% (ns)</td>
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<td>–1% (ns)</td>
<td>–15%</td>
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<td>9% (ns)</td>
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<td>–20%</td>
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<td>57%</td>
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</tr>
<tr>
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<td>5% (ns)</td>
<td>–3% (ns)</td>
<td>–28%</td>
<td>81%</td>
<td>79%</td>
<td>79%</td>
<td>97%</td>
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<tr>
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<td>–6% (ns)</td>
<td>–17%</td>
<td>–71%</td>
<td>94%</td>
<td>93%</td>
<td>98%</td>
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#### Temperature (°C)

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<tbody>
<tr>
<td>Temperature</td>
<td>21.2</td>
<td>22.8</td>
<td>22.4</td>
<td>21.9</td>
<td>20.0</td>
<td>18.2</td>
<td>19.7</td>
<td>17.0</td>
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#### Bacteria/cm²

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</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>1.19×10⁴</td>
<td>2.24×10⁴</td>
<td>2.35×10⁶</td>
<td>1.61×10⁵</td>
<td>1.36×10⁶</td>
<td>1.31×10⁶</td>
<td>1.81×10⁶</td>
<td>3.69×10⁶</td>
</tr>
</tbody>
</table>

ns: not statistically significant.
bacteria in the biofilm at the beginning of the pipe as there were at the end.

The period of year played a major role in the biomass found in the biofilm (Figure 3). The number of bacteria was lower by 1 to 2 log units during the winter and spring campaigns, compared with summer and fall. This variation may be due to the thermal regime of waters in the Québec region. In the winter, raw water sources are covered and protected by a layer of ice. As water temperatures are very low, the number of bacteria brought to the watershed is lower, whereas during the ice-free seasons, warmer temperatures and watershed supply promote bacterial growth and potentially affect the composition of the biofilm.

Unlike the corresponding campaign conducted early in the summer of 2010 (June–July) where the number of bacteria/cm² was about 10⁶ (June–July), the number of bacteria/cm² was about 10⁴ for June 28 and July 12, 2011 (Table 5). Subsequently, the number of bacteria/cm² increased by two orders of magnitude to stabilize at some 10⁶ bacteria/cm² for the rest of the 2011 sampling season ended October 12. This lag in time of the amount of biomass present in the biofilm system may be explained by an early spring in 2010 compared with 2011 (Environment Canada 2010).

Effect of pipe diameter on HAA degradation

The effect of the diameter of the ducts is related to the ratio of the internal surface of the pipe (i.e., biofilm) to the volume of water circulating inside. This ratio ($S/V$) depends strictly on pipe diameter ($S/V = 4$/diameter). In test pipes, the greater the $S/V$ ratio, the more likely the total water mass will come into contact with the biofilm. During this study, the $S/V$ ratio for pipes A and B was two times higher than that for pipes C and D. In order to illustrate the effect of the $S/V$ ratio, the results of the 2009 summer campaign were used, as DCAA degradation was observed in all four pipes (Figure 4).

When comparing pipe A to C (supplied with the same HAA concentrations) and B to D (supplied with the same HAA concentrations), it was found that DCAA removal was significantly lower ($p < 0.001$) in the larger diameter than the smaller diameter pipes (Figure 4). Therefore, HAA degradation increased when the $S/V$ ratio increased.

According to Grigorescu & Hozalski (2010), increasing the water flow velocity decreased HAA removal. In this study, the water flow velocity was two times faster in pipes A and B ($7.9 \times 10^{-3}$ m.s⁻¹) than in pipes C and D ($3.9 \times 10^{-3}$ m.s⁻¹), but in both cases very low to have an impact on HAA biodegradation.

DISCUSSION

Baribeau et al. (2005) and Bayless & Andrews (2008), observed DCAA degradation and no TCAA degradation, but Zhou & Xie (2002), Wu & Xie (2005) and Zhang et al.
observed degradation of both HAAs. In this study, with a residence time of 1 h, results obtained for the campaigns of 2009 and 2010 showed DCAA degradation and no TCAA degradation. As TCAA is less biodegradable than DCAA (Zhou & Xie 2005; Wu & Xie 2005), it is possible than degradation might occur at higher residence times. Higher residence times were used in campaign of 2011 and TCAA degradation was observed. The wide variability in the results obtained between the studies may be explained by the variable test conditions used by the researchers. Bacterial species present in the various systems can also have an impact on biodegradation results.

The statistical analysis showed that pipe diameter has a strong impact on the degradation of DCAA. In fact, when the diameter decreases, the S/V ratio increases and DCAA removal increases. The S/V ratio is higher in BAC columns, glass bead columns and GAC filters than in pipes. Retention times of 5 min are sufficient to observe a degradation of HAAs in BAC columns and glass bead columns (Wu & Xie 2005; Bayless & Andrews 2008); however, when experiments are conducted in batch conditions (Zhou & Xie 2002) or in pipes with relatively large diameters (Baribeau et al. 2005), several hours or days are required to observe the degradation of HAAs. The diameters of the pipes used in our study were smaller than those used in municipal distribution networks (5 cm to 2.4 m) (Grigorescu & Hozalski 2010), but they correspond to the diameters of the plumbing pipes inside homes. DCAA degradation is strongly influenced by the season. Seasons affect water temperatures, the amount and type of NOM, and microorganism survival and activity. All these parameters may come into play in DCAA degradation. Temperature is certainly a major parameter. In the study of Baribeau et al. (2005), dihalogenated HAAs were degraded in warm water (17–22 °C) but not in cold water (12–14 °C). Wu & Xie (2005) also observed that water temperature had a strong impact on the HAA removal in BAC columns; after an empty bed contact time (EBCT) of 5 min, the removal of DCAA was 27% at 4 °C, 56% at 10 °C, 96% at 20 °C and 98% at 30 °C and the removal of TCAA was 7% at 4 °C, 23% at 10 °C, 56% at 20 °C and 88% at 30 °C. In our study, DCAA degradation was highest at high water temperatures in the distribution system (about 22 °C during summer 2009) and was not observed at all at low water temperatures (during winter and spring 2009, about 10 °C). However, results obtained in 2011 showed that DCAA degradation was higher in the fall (September and October) when the water temperature was about 18.0 °C than at the beginning of summer (June and July) when the water temperature was about 18.0 °C than at the beginning of summer (June and July) when the water temperature was about 22.0 °C. Regarding TCAA, its optimal period of degradation is in the fall, not summer (campaign of 2011). Other factors could come into play, such as the bacteria biomass amount and the presence of NOM. However, when analyzing the results, water temperature and
the number and amount of organic matter are parameters that are difficult to isolate from each other when using water from a real drinking water system to perform experiments. However, the impact of these parameters could be considered in an experimental system where each one could be independently controlled.

In a natural setting, the causes underlying reductions in water temperature also influence the amount of biomass, an important parameter. According to Grigorescu & Hozalski (2010), an amount of biomass above 10^5 bacteria/cm^2 is necessary to obtain a significant degradation of HAAs. When comparing the end of the summer of 2009 with the fall of 2009, the amount of biomass is of the same order. However, DCAA removal was slower in the fall due to a drop in bacterial activity in water at lower temperatures. It was also noted that at the beginning of the summer of 2009, the amount of biomass was relatively important (around 10^6 bacteria/cm^2) and the water temperature warm (18°C). Yet DCAA degradation was very low. At the beginning of the summer, temperatures are warm, but the amount of biomass in the pipes is emerging from a ‘cold’ period and may have to adapt to new environmental conditions. Of course, the warm-up period does not occur over a few days; it is a gradual process spanning weeks, even months. At the end of the summer, the water temperature is warm and the amount of biomass high, creating favorable conditions for DCAA degradation. Indeed, DCAA degradation is at its maximum during this period. In the fall, although the biomass faces drops in temperature, it is still active and plentiful. It is like a ‘summer effect’ that may affect water quality still remains during this period of the year. In addition, during the fall there is an additional supply of NOM in the raw water source due to vegetation decomposition in the watershed (Maurice et al. 2002; Beaulieu et al. 2009).

Organic matter is also a major parameter that could determine the amount of biomass. A larger quantity of organic matter fosters bacterial growth and activity. Thus, a higher concentration of DOC and a low SUVA could promote HAA degradation. The amount of organic matter is not the only factor influencing HAA degradation; the composition of organic matter might also play an important role. As Beaulieu (2010) pointed out, the hydrophobic and hydrophilic proportions of NOM found in raw water and in residual organic matter (ROM) in the distribution system are not the same, as water treatment affects the constituents of NOM. The treatment process tends to increase the hydrophilic character of ROM, increase the incorporation of halogen atoms and oxygen into its molecular structure, reduce the average molecular mass and promote its biodegradability (Nissinen et al. 2003). Moreover, the properties of NOM and ROM vary according to the seasons. In the summer, the ROM contains a greater proportion of hydrophilic compounds. These compounds have a lower molecular mass, are easier to biodegrade and can thus stimulate biofilm regrowth (Beaulieu et al. 2009). At the beginning of the summer and in the fall, SUVA values are comparable, but TOC is higher in the fall. Therefore, the amount of organic matter available for bacterial biomass is higher in the fall than at the beginning of summer. Another element that might influence HAA degradation is the nature of the bacteria. Various bacterial species are found in the biofilm and some of them may develop over the seasons.

Residual chlorine concentrations may also play a role. Although the drinking water that supplies the experimental systems is dechlorinated, it should be noted that prior to arriving at the test site, the bacteria travel through the water treatment process and end up in the distribution system pipes in contact with free chlorine for several hours. A drop in residual chlorine concentrations may foster bacterial growth at the test sites and, in turn, DCAA degradation. This puts colder seasons at a ‘disadvantage’, as free chlorine concentrations are higher during this period than in the summer when higher temperatures promote chlorine consumption by NOM and pipes (Kiéné et al. 1996).

**CONCLUSIONS**

The results obtained allowed the following conclusions to be drawn:

- DCAA and TCAA are degraded by a biofilm in a drinking water system but TCAA needs a longer retention time than DCAA to be eventually degraded. The diameter of the pipes has a substantial impact on DCAA degradation; DCAA removal drops when diameter increases.
- Seasonal changes have a major impact on HAA degradation. DCAA removal is higher in the late summer,
lower in the fall, very low at the beginning of the summer and non-existent in the winter and spring. TCAA removal is higher in fall, lower in the late summer and non-existent at the beginning of summer.

- Seasonal changes have a major impact on the amount of biomass in the biofilm. The number of bacteria is lower – between 1 and 2 log units – in the winter and spring than in the summer and fall.
- The impact of the seasons on the composition of ROM also affects biomass and HAA degradation.

In light of these results, it is safe to state that water temperature, biomass and organic matter are major factors affecting HAA degradation, but they are difficult to isolate from each other, as they are closely related to seasonal change and they interact. This study has some limitations. Indeed, to clarify the effects of temperature on bioactivity, it might be better to measure bioactivity rather than cell numbers. Also, quantifying assimilable organic carbon or biodegradable DOC would allow the investigation of changes in organic matter composition. With regard to biomass, it would be interesting to characterize the biodiversity found in the biofilm throughout the year and then determine which species are responsible for HAA degradation. Another major aspect is that bacteria must overcome many obstacles during treatment at the potable water plant before ending up in the ducts. It would be interesting to track these different microorganisms during the various treatment steps and ascertain the impact they have on their specific degradation effect on halogenated material.

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