

## Biodegradation of six haloacetic acids in drinking water

Walt Bayless and Robert C. Andrews

### ABSTRACT

Haloacetic acids (HAAs) are produced by the reaction of chlorine with natural organic matter and are regulated disinfection by-products of health concern. Biofilms in drinking water distribution systems and in filter beds have been associated with the removal of some HAAs, however the removal of all six routinely monitored species ( $\text{HAA}_6$ ) has not been previously reported. In this study, bench-scale glass bead columns were used to investigate the ability of a drinking water biofilm to degrade  $\text{HAA}_6$ . Monochloroacetic acid (MCAA) and monobromoacetic acid (MBAA) were the most readily degraded of the halogenated acetic acids. Trichloroacetic acid (TCAA) was not removed biologically when examined at a 90% confidence level. In general, di-halogenated species were removed to a lesser extent than the mono-halogenated compounds. The order of biodegradability by the biofilm was found to be monobromo > monochloro > bromochloro > dichloro > dibromo > trichloroacetic acid.

**Key words** | biodegradation, biofilm, disinfection, disinfection by-product, haloacetic acid

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### INTRODUCTION

The need to minimize the concentration of trihalomethanes (THMs) and haloacetic acids (HAAs) in drinking water stems from the associated toxicological impact of these disinfection by-products (DBPs), which has led to the United States Environmental Protection Agency (USEPA) limiting the levels of THMs and HAAs in drinking water to 80  $\mu\text{g/L}$  and 60  $\mu\text{g/L}$ , respectively (USEPA 1999). In order to minimize the formation of DBPs while maximizing microbial inactivation by chlorination, kinetic models which predict the chemical formation of DBPs have been developed (Amy *et al.* 1987). However, predictive models for HAAs have been found to typically over-predict HAA concentrations. This overestimation has been attributed to the biological degradation of DBPs which is not currently incorporated into empirical models. While previous studies have reported that several of the HAAs can be biodegraded during filtration and in distribution systems, they do not provide a clear indication of which compounds can be degraded by biofilms, nor the removal mechanism.

HAA concentrations have been observed to either increase (i.e. form through the reaction of chlorine and organic precursors) or decrease (i.e. degrade) in chlorinated drinking

water distribution systems. Decreases in HAA concentrations have been hypothesized to be due to biodegradation (Chen & Weisel 1998). Monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), bromochloroacetic acid (BCAA), and dibromoacetic acid (DBAA) have been shown to be degraded by enriched bacterial cultures (Williams *et al.* 1995; McRae *et al.* 2004). The order of biodegradability has been reported as  $\text{MCAA} > \text{DCAA} > \text{TCAA}$ , with the corresponding brominated species being better degraded than the chlorinated species (Hashimoto *et al.* 1998; Zhou & Xie 2002). TCAA, DCAA, and MCAA have also been shown to be biodegradable in soils (Lode 1967; Lignell *et al.* 1984; Matucha *et al.* 2003).

In contrast to the biodegradation of HAA species, most trihalomethanes (THMs) are not biodegraded. Chloroform is not degraded by aerobic biofilms (Bouwer & McCarty 1984). It has also been reported that there is no degradation of bromodichloromethane (BDCM), dibromochloromethane (DBCM), tetrachloroethylene, trichloroethylene, or bromoform by biofilms under aerobic conditions (Bouwer *et al.* 1981).

The objective of this study was to determine which of the six routinely monitored HAA species (HAA<sub>6</sub>) could be biologically degraded under aerobic conditions using a bacterial culture typical of that present in a drinking water distribution system biofilm. THMs were not considered in this study, due to their well-documented lack of biodegradability.

## METHODS

### Glass bead column design

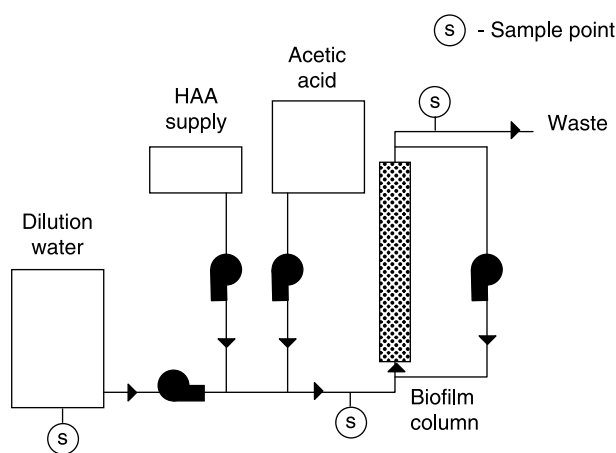
Glass bead columns have been previously used to culture biofilms and to determine kinetic parameters (Bouwer & McCarty 1984; Namkung *et al.* 1983; Zhang & Huck 1996a). However, there remains a lack of information regarding the kinetics of HAA degradation in biofilms grown from a drinking water inoculum, nor have previous studies reported the biodegradability of mono-halogenated acetic acids.

Several key factors in the design of a glass bead column include, flow, contact time, length, width, size of glass beads and whether or not a recycle loop is employed. The biofilm reactor characteristics were based on those described by Rittmann *et al.* (1986) (Table 1). A 25 cm column, 2.5 cm in diameter packed with 3 mm glass beads was used to grow the biofilm. Dilution water, HAAs, and acetate were pumped into the reactor *via* peristaltic pumps (Cole-Parmer) using Pharmed<sup>®</sup> tubing (St. Gobain Performance Plastics, New Jersey, USA) (Figure 1).

In order to biologically inoculate the columns, granular activated carbon (GAC) was obtained from the top 30 cm of the filter bed at the Mannheim Water Treatment Plant (Waterloo, ON, Canada). The column inoculum was prepared using City of Toronto tap water which was dechlorinated using a GAC filter consisting of a 40 cm long glass column (10 cm inner diameter) packed with GAC (coarse mesh, Anachemia Chemicals) and operated to achieve a 15-minute empty bed contact time (EBCT). The de-chlorinated water was then passed through a second, identical glass column packed with the biological activated carbon (BAC) obtained from the water treatment plant, such that an EBCT of 15 minutes was achieved. The inoculum was fed into the glass bead columns until no air remained, at which time the flow was shut down for a

**Table 1** | Biofilm column design characteristics

Column characteristic	Value
Length of column	215 cm
Diameter of column, $d_p$	2.5 cm
Cross-sectional area of column, $A_c$	4.91 cm <sup>2</sup>
Volume of column	105 cm <sup>3</sup>
Diameter of glass bead	0.3 cm
Area of glass bead, $A$	0.283 cm <sup>2</sup>
Specific surface area, $a$	12 cm <sup>-1</sup>
Porosity, $\varepsilon = V_v/V$	0.4
Volume of voids, $V_v$	42.2 cm <sup>3</sup>
Feed flow rate, $Q$	100 cm <sup>3</sup> /hr
Detention time, $t = V_v/Q$	25.4 min
Recycle ratio, $Q_r/Q$	5
Time of one pass through column, $V_v/(Q + Q_r)$	5.1 min
Superficial velocity of fluid, $u = (Q + Q_r)/A_c$	2.0 cm/min



**Figure 1** | Biofilm reactor and feed system, showing sampling points. Experimental apparatus was conducted in quadruple with one column acting as a control. No acetic acid was used in the control column.

period of 48-hours to allow for sorption to the glass surfaces (Namkung *et al.* 1983). Following this period the columns were switched over to the dilution water.

The columns were fed using a sterile dilution water containing a phosphate buffer and essential nutrients including, 8.5 mg/L  $\text{KH}_2\text{PO}_4$ , 21.8 mg/L  $\text{K}_2\text{HPO}_4$ , 17.7 mg/L  $\text{NaHCO}_3$ , 15.0 mg/L  $\text{KNO}_3$ , 27.5 mg/L  $\text{CaCl}_2$ , 11.0 mg/L  $\text{MgSO}_4$  and 0.15 mg/L  $\text{FeCl}_3$  (Zhang & Huck 1996b). Each nutrient solution was prepared and sterilized by filtration through a 0.2  $\mu\text{m}$  filter paper and stored in autoclaved Pyrex<sup>®</sup> media bottles. A primary substrate consisting of 3 mg/L acetate was supplied to the columns in the form of acetic acid. The pH of the dilution water was adjusted to 7.5 in the batch feed water by the addition of 10N NaOH. The acetic acid addition occurred a few centimeters (< 5 cm) upstream of the column influent in order to minimize growth in the tubing. The concentrated HAA solution and dilutions water resulted in a column influent pH of  $6.7 \pm 0.2$ . HAAs were fed from Teflon Tedlar<sup>®</sup> bags into the column influent. Each sample bag contained a concentrated solution of six haloacetic acids (MCAA, MBAA, DCAA, TCAA, BCAA and DBAA). The concentrated stock was prepared from pure salts or solutions purchased through Sigma-Aldrich (Milwaukee, WI) and diluted in Milli-Q<sup>®</sup> water. The dilution water nutrient supplies was stored in 20 L glass acid-washed carboys and prepared every 24–48 hours.

Prior to commencing substrate utilization tests, an appropriate period of time was allocated to allow the biofilm to grow and acclimatize in the glass column. Acclimatization periods reported in the literature range from two weeks (Rittmann *et al.* 1986) to more than three months (Zhang & Huck 1996a). Bouwer & McCarty (1981) reported that an acclimatization period between 10 and 40 days was sufficient to ensure that the substrate was being utilized by the biofilm. The system was classified as steady-state when the changes in the effluent carbon, nitrate, and phosphate concentrations were less than 5% and there was a visible brownish biofilm growth on the glass beads. The effluent acetate concentration was also monitored until there was no observed decrease in the effluent (approximately six weeks).

Once the columns reached steady-state conditions, HAA solutions were fed. For each experimental trial the concentration of HAAs in the bags was varied such that the column influent concentration ranged from 0.005 to 0.15 mg/L

for each HAA. The concentrations of HAAs were varied to observe the impact of diffusion into the biofilm. The utilization of substrates by biofilms is subject to the diffusion rates in the bulk fluid, fluid/biofilm interface and in the biofilm itself (Rittmann & McCarty 2001). If diffusion is slow it will limit substrate utilization, however if it is fast then it will not control the rate. The HAA concentrations were increased through the course of the experiment in seven independent trials to counter these diffusion effects. Effluent sampling was conducted over the course of 4–6 hours among the experiments. Following the completion of an experiment the HAA feed was removed and the columns were allowed to return to a steady-state with the acetate feed.

Biofilm consumption measurements were performed on three separate parallel columns receiving the same feed solutions. Concurrent with the three biofilm columns, a control column was operated to determine if HAA losses were occurring due to any non-biodegradation factors. This column was fed the same influent HAA concentration and nutrient cocktail. However, the column was never inoculated and no acetate (primary substrate) was supplied.

### Analytical methods

Acetate samples were collected and immediately passed through a Dionex<sup>®</sup> H-Cartridge and preserved with 1–2 drops of chloroform (Peldszus *et al.* 1996). Acetate was measured using ion chromatography (Dionex<sup>®</sup> column AS-9, Dionex Canada Ltd., Oakville, ON). HAAs were measured using MTBE extraction under acid conditions according to Standard Methods 6251 B – Micro Liquid-Liquid Extraction Gas Chromatographic method (APHA 1998) using an HP 5890 series II GC/ECD (Hewlett Packard Canada, Mississauga, ON). COD measurements were conducted using Standard methods 5220 D, closed reflux colorimetric method. Prepared low range COD vials were purchased from Hach<sup>®</sup> laboratories and calibrated using KHP (Hach 2125825). pH and dissolved oxygen were measured using external probes (VWR International, Mississauga, ON, Model 8015 and Yellow Springs International, Dayton, OH, Model 52, respectively).

### Determination of biofilm and DBP parameters

Following the completion of HAA trials, the biofilm columns were disassembled and the glass beads analyzed for biofilm

density and thickness using a previously reported method (Rittmann *et al.* 1986). Ten glass beads were sampled from a column at evenly distributed points such that biofilm properties would not be biased by position in the column. A sterile inoculating loop (alcohol flamed and cooled) was used to remove the beads and transfer them to a tared aluminum weight dish, which was weighed, then dried at 105°C for a 24-hour period and re-weighed. Equation 1 was used to estimate the biofilm thickness (Rittmann *et al.* 1986):

$$L_f = \frac{W}{\rho n A (0.99)} \quad (1)$$

where  $W$  is the weight of the evaporated water,  $\rho$  is the density of water at 20°C (998.203 kg/m<sup>3</sup>),  $n$  is the number of glass beads, and  $A$  is the area of one bead (m<sup>2</sup>).

To determine the biofilm density, one hundred beads were removed and transferred to sterile culture tubes. Four millilitres of sterile (autoclaved) Milli-Q<sup>®</sup> water was added to each tube. The tubes were placed on a vortex mixer to shear the biofilm from the beads. The resulting solution (2.5 ml) was transferred to a COD vial. Equation 2 was used to estimate the biofilm density (Rittmann *et al.* 1986):

$$X_f = \frac{\text{COD of biomass} \times 0.706}{n A L_f} \quad (2)$$

where 0.706 mg biomass/mg COD assumes the biomass can be represented by C<sub>5</sub>H<sub>7</sub>O<sub>2</sub>N.

## Statistical methods

Comparisons between data sets were conducted using a  $t$ -test when the variance between the two sets is equal but unknown. Data was analyzed at a 90% confidence level. When comparing the data between the control column and an experimental column a paired  $t$ -test was employed at a 90% confidence level.

## RESULTS AND DISCUSSION

Influent and effluent HAA concentrations were compared to determine the impacts of biological processes within the columns. Comparisons were also made between each column and the control column to determine if losses occurred as a result of the experimental apparatus or sampling. For discussion, HAA species have been grouped into control, mono-halogenated, di-halogenated and tri-halogenated species. This approach was used to first isolate any variability inherent in the experimental apparatus and then to group compounds with respect to similar biodegradation rates. Finally, a discussion will be presented which compares each group of halogenated species.

There were no observed losses in HAAs observed to be associated with the experimental apparatus. A  $t$ -test was used to show that there was no statistical difference between the influent and effluent concentrations at a 90% level. Seven trials were performed for each of the three columns plus the control and sufficient data was collected

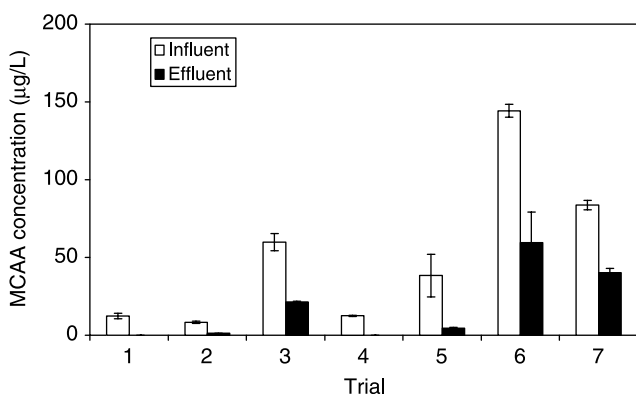
**Table 2** | Average influent HAA concentrations for each experimental trial

Trial	MCAA	MBAA	DCAA	TCAA	BCAA	DBAA
1	12.3 ± 3.3	18.6 ± 1.7	15.0 ± 2.5	12.7 ± 0.2	29.2 ± 0.4	11.3 ± 0.3
2	8.3 ± 0.7	10.4 ± 0.8	8.7 ± 2.7	5.3 ± 0.2	22.7 ± 3.0	6.2 ± 0.3
3	59.9 ± 5.2	78.1 ± 5.5	47.9 ± 4.1	38.8 ± 0.8	57.0 ± 13.1	46.8 ± 0.9
4	12.5 ± 0.3	17.2 ± 0.4	12.1 ± 0.3	10.9 ± 0.2	12.8 ± 0.3	13.8 ± 0.4
5	38.4 ± 6.2	49.8 ± 13.7	38.0 ± 1.3	32.5 ± 0.3	39.0 ± 2.5	44.0 ± 3.4
6	144.3 ± 5.5	107.8 ± 4.1	83.9 ± 2.5	92.9 ± 2.5	88.2 ± 3.0	112.8 ± 3.4
7	83.7 ± 3.1	118.6 ± 3.0	77.9 ± 0.6	100.3 ± 1.0	80.1 ± 0.4	111.3 ± 1.0

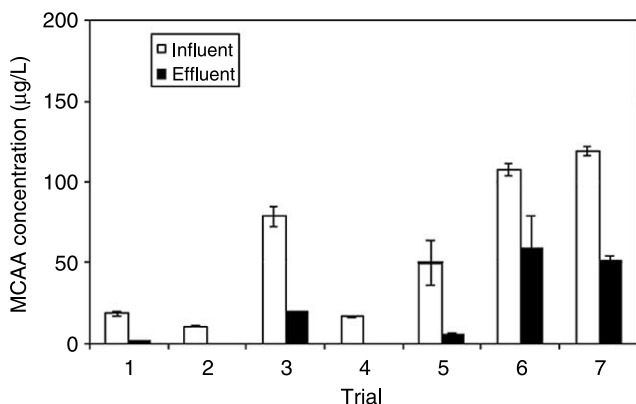
to assess the biodegradability of the compounds. The influent concentrations for each HAA in each trial are shown in Table 2.

### MCAA and MBAA

The removal of MCAA and MBAA was statistically significant (90% *t*-test) in all trials. In addition results were statistically different from the control column using these criteria. In the low concentration tests (< 15 µg/L) the MCAA and MBAA effluent concentrations were below the instrument detection limits (4 µg/L and 1 µg/L, respectively). As the trials proceeded to higher influent concentrations the effluent values became measurable (Figures 2 and 3). An alternative method of illustrating the change in effluent



**Figure 2** | Influent and effluent concentrations for monochloroacetic acid (MCAA) in column 3. Effluent concentrations were below the detection limit (4 µg/L) for three of the trials.



**Figure 3** | Influent and effluent concentrations for monobromoacetic acid (MBAA) in column 3. Effluent concentrations were below the detection limit (1 µg/L) for two of the trials.

concentration for a given influent concentration is shown in Figure 4. The data for columns 1 to 3 falls below the line of equality indicating a decrease in concentration. Data associated with the control column falls directly on the line of equality since no biodegradation was observed.

### DCAA, BCAA and DBAA

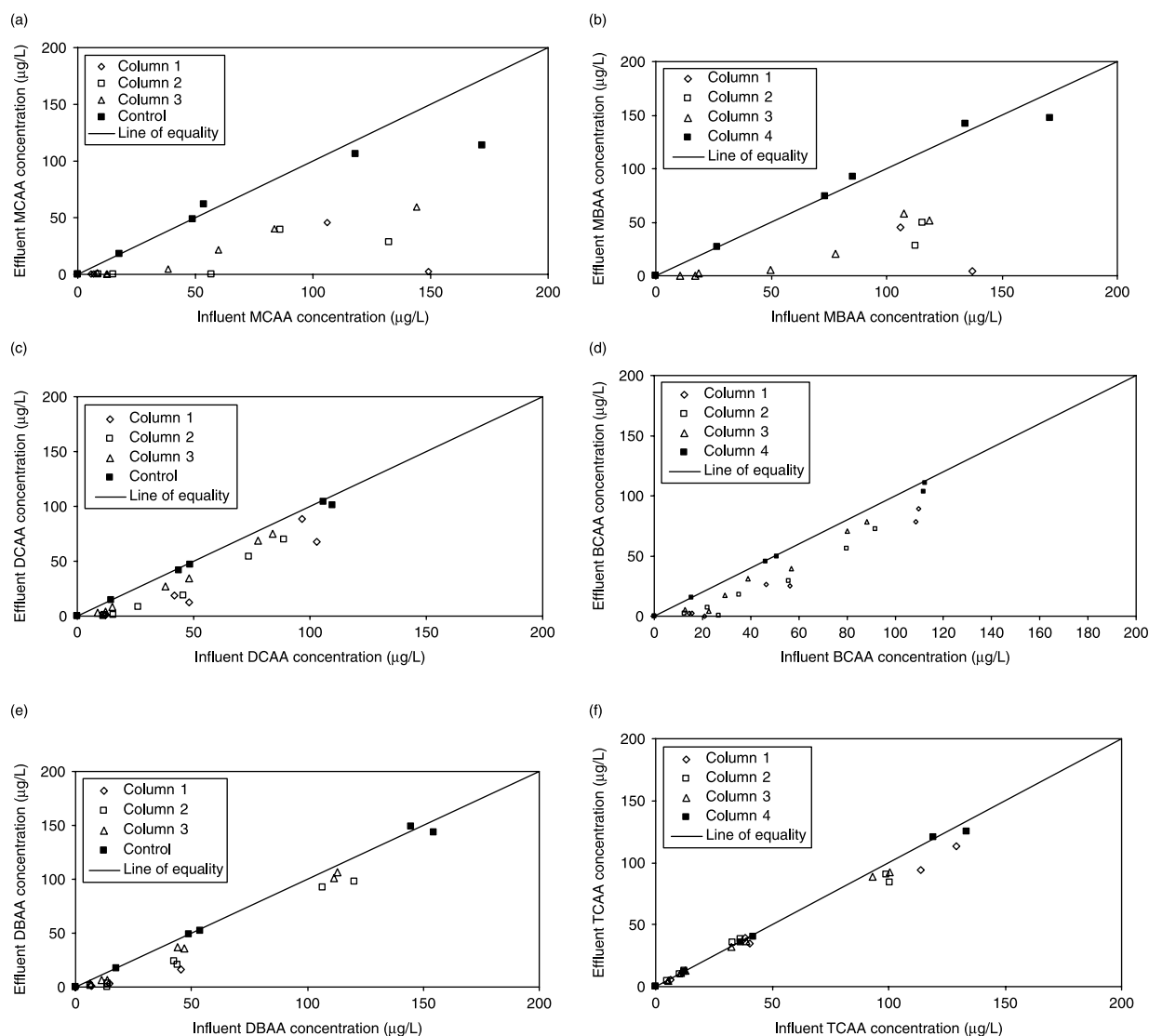
DCAA, BCAA and DBAA all showed similar degradation trends, whereby the effluent concentration for each compound was statistically lower (90% *t*-test) than the influent values. The difference in influent and effluent concentrations was also lower than the control column (90% paired *t*-test). The decrease in di-halogenated ( $X_2AA$ ) species were consistently lower when compared to the XAA compounds, possibly due to an additional step necessary for de-halogenation (Ellis *et al.* 2001). Figure 4(c), (d), (e) shows the influent and effluent concentration relationships for each of the di-halogenated compounds. When visually compared to Figure 4(a), (b) (for the mono-halogenated compounds) the difference between the line of equality and the data from the three test columns is lower (10 µg/L versus 50 µg/L difference), indicating a higher stability in the columns.

### TCAA

Trichloroacetic acid removal was not statistically significant from the columns when compared to the control at a 90% level of significance. In several experiments there was a statistically significant difference between the influent and effluent values. However, no statistical (90% paired *t*-test) differences were observed between the control column and the three test columns (Figure 4(f)). Visual interpretation of the equality plot suggests that a slight loss (maximum of 17%) of TCAA occurred for the higher concentrations, though not at a statistically significant level.

### HAA species comparison

Mono-halogenated compounds were the most degradable, followed by the di-halogenated species. The tri-halogenated species did not show any signs of biodegradation. Overall, the following order of biodegradability was observed: MBAA > MCAA > BCAA > DCAA > DBAA > TCAA (Figure 5).

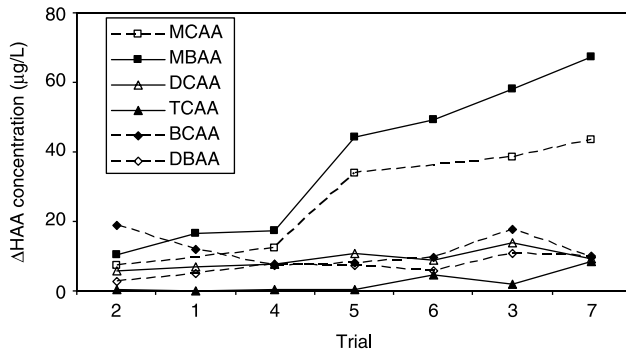


**Figure 4** | (a) Influent and effluent concentrations of monochloroacetic acid (MCAA), (b) Influent and effluent concentrations of monobromoacetic acid (MBAA), (c) Influent and effluent concentrations of dichloroacetic acid (DCAA), (d) Influent and effluent concentrations for bromochloroacetic acid (BCAA), (e) Influent and effluent concentrations of dibromoacetic acid (DBAA) (f) Influent and effluent concentrations of trichloroacetic acid (TCAA).

The biodegradation of MCAA, MBAA, DCAA, DBAA, BCAA and the stability of TCAA is consistent with previous findings (Williams *et al.* 1995). These observations contradict Chen & Weisel (1998) who reported TCAA as being biodegraded and suggested that TCAA was lost due to either biological processes or volatilization. Certain microorganisms have been reported to be able to de-halogenate TCAA (Hirsh & Alexander 1960; Lode 1967; Lignell *et al.* 1984; Hashimoto *et al.* 1998; Matucha *et al.* 2003). The cause of the variance in the reported degradability of TCAA may be due to the inoculum that was used. Literature on the degradability of

mono-chlorinated compounds in drinking water conditions is limited as concentrations are usually below detectable limits (Williams *et al.* 1995; Chen & Weisel 1998).

During the study conducted by Baribeau *et al.* (2000) the order of biodegradation appeared to be DCAA > BCAA > DBAA, which also differs from the order found in this biofilm study. Hirsh & Alexander (1960) found the order of biodegradation to be MBAA > MCAA > DCAA for a *Nocardia* 398 bacterium and DCAA > TCAA > MCAA > MBAA for *Pseudomonas* 409. The pattern for *Nocardia* 398 follows the results observed in the biofilm columns in the



**Figure 5** | Difference in influent and effluent HAA concentrations during each trial (influent concentrations were different in each trial – see Table 2). MBAA showed the highest removal and TCAA the lowest.

present study. Hashimoto *et al.* (1998) reported the order of biodegradability to be MCAA > DCAA > TCAA. Zhou & Xie (2002) and Xie & Zhou (2002) also reported this order of biodegradability by bacteria in BAC columns and McRae *et al.* (2004) similarly reported faster degradation of MCAA and MBAA than TCAA when considering bacterial enrichment cultures. The source of the bacterial culture therefore plays a role in the relative order of biodegradability of the HAAs.

### Biofilm properties

Biofilm development within the columns was readily apparent due to a brownish film which developed on the beads as previously described. Several biofilm characteristics were determined based on earlier estimations (Rittmann *et al.* 1986), including biofilm thickness and density. The biofilm thickness varied from 2 to 15 μm for each column. Rittmann *et al.* (1986) reported a value of 18 μm for an acetate fed glass bead column. Biofilm density ranged from 1,600 to 8,900 g/m<sup>3</sup> for the columns (Table 3) which were lower than previously reported values (33 000 g/m<sup>3</sup>) (Rittmann *et al.* 1986). However the recycle rates

**Table 3** | Biofilm characteristics measured following HAA experiments

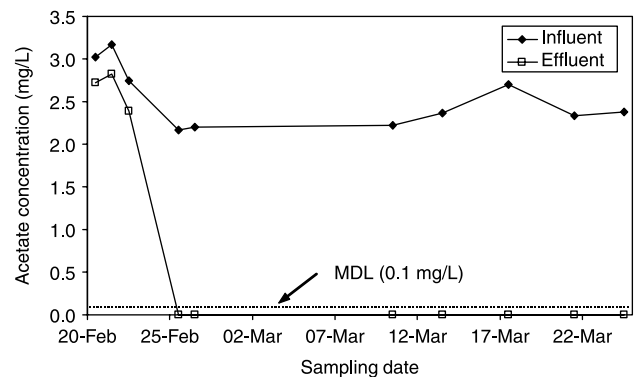
Column	L <sub>f</sub> (μm)	X <sub>f</sub> (g/m <sup>3</sup> )	Superficial fluid velocity (cm/min)
Column 1	6	2,600	2.0
Column 2	2	8,900	3.2
Column 3	15	1,600	3.3

were higher than in the current study, resulting in a higher superficial fluid velocity (8.9 cm/min). This may result in a higher density biofilm due to increased shear stresses.

Following the completion of the column experiments the bacterial culture from column 3 was viewed under a microscope (Nikon, Eclipse E600). Several microorganisms were visually identified as algae by comparison with reference images. These organisms were identified as *Chlamydomonas*, *Volvox* and *Vorticella*. This was initially unexpected as the bioreactors were protected from any light, however these organisms are capable of existing *via* photosynthesis or heterotrophically (Prescott *et al.* 1999). The organisms likely thrived in the biologically active carbon (BAC) utilized for the inoculum, which was collected from the surface of the filters. Gram staining of the samples showed a mixture of positive and negative organisms. No further characterization of the biological culture was conducted.

### Other parameters

Acetate was fed into the columns to provide a primary substrate for bacterial growth. Influent concentrations averaged 3 mg/L over the course of the trials. The effluent measurements were below the detection limit. This was expected as the S<sub>min</sub> value for acetate has been reported as 0.04 mg/L (Namkung *et al.* 1985), which was below the instrument detection limit (Figure 6). pH values were initially 7.5 in the phosphate buffered dilution water (adjusted to 7.5 with 10 N NaOH). Following the addition of acetic acid the pH was reduced to 6.7 and in the column effluents the pH was approximately 6.0 to 6.5. Dissolved oxygen measurements



**Figure 6** | Influent and effluent acetate concentrations during biofilm acclimatization period in column 3.

were found to be approximately 2 mg/L for each column, indicating that the systems were aerobic.

## SUMMARY

Monohalogenated compounds were more biodegradable by the biofilm than dihalogenated acetic acids, similar to findings from previous studies which considered bacterial enrichment cultures. The trihalogenated compounds were not observed to be biologically degraded by the biofilm under the test conditions. MBAA was more biodegradable than MCAA. Further work is needed to determine kinetic parameters which can be applied to existing drinking water biofilm models such that quantitative HAA removal estimates can be made.

## ACKNOWLEDGEMENTS

This study was funded in part by ERCO Worldwide and the Natural Sciences and Engineering Research Council of Canada (NSERC).

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First received 17 August 2006; accepted in revised form 12 December 2006. Available online November 2007.