The impact of natural organic matter on free chlorine and chlorine dioxide disinfection efficacy

Benoit Barbeau, Mélanie Rivard and Michèle Prévost

ABSTRACT

The objective of this study was to assess the effect of natural organic matter (NOM) on disinfection efficacy using two types of disinfectants: Cl_2 and ClO_2. *Bacillus subtilis* spores were used as a surrogate for monitoring disinfection efficacy. The effect of natural organic matter was tested using two series of experimental assays. Initially, assays were conducted in ultra-pure buffered waters supplemented with various NOM extracts. Second, confirmation assays were performed on three surface waters. In both natural and synthetic waters, the presence of NOM improved disinfection efficacy where chlorine dioxide was used. This phenomenon was significant, as Ct values required for 2 log inactivation of *B. subtilis* spores were reduced by a factor of 2.3 to 7.1 depending on the NOM source and concentration. However, such a phenomenon was not observed while disinfecting with free chlorine. It is proposed that (i) free radicals are generated following the reaction of chlorine dioxide and NOM and (ii) those radicals can enhance chlorine dioxide disinfection efficacy.

Key words | chlorine, chlorine dioxide, disinfection, drinking water, natural organic matter, spores

Benoit Barbeau (Corresponding author)
Mélanie Rivard
Michèle Prévost
École Polytechnique de Montréal, Chaire Industrielle-CRSNG en Eau Potable,
Department of Civil, Geological and Mining Engineering,
CP 6079, Succ. Centre-ville, Montréal,
(Québec), H3C 3A7,
Canada
Tel.: 1(514)340 4711 (2988)
Fax: 1(514)340 5918
E-mail: *benoit.barbeau@polymtl.ca*

INTRODUCTION

Despite the abundance of research related to drinking water disinfection published over the years, few studies have focused their attention on evaluating the impacts of disinfection in natural waters. The impact of natural water characteristics on disinfection efficacy was rigorously evaluated in the 1990 s (Haas *et al.* 1995; Oppenheimer *et al.* 2000). In both studies, it was concluded that water quality was a significant variable in predicting disinfection efficacy, although the exact mechanisms at play were never identified. In 2003, following an American Water Works Association Research Foundation (AWWARF) research project (Mysore *et al.* 2003), we observed that natural organic matter (NOM) could, under some conditions, increase the inactivation of *Bacillus subtilis* spores. Cho *et al.* (2003) also demonstrated an improved ozone inactivation due to NOM. Free radicals initiated by the reaction of ozone with NOM could improve inactivation of *B. subtilis* spores by 20 to 50%, depending on pH and the type of NOM. Similarly, Hijnen *et al.* (2004) observed that, for an equivalent integrated CT, the inactivation of *Clostridium perfringens* spores by ozone was higher in dissolved organic carbon (DOC)-rich waters. However, Dow *et al.* (2006) did not observe a reduced inactivation of *B. subtilis* spores in the presence of synthetic NOM-extract (from Suwannee River), although they did observe an improved ozone inactivation in natural waters (from Lake Zurich).

The fact that NOM could actually improve disinfection efficacy is counterintuitive. It is important to understand that observing this phenomenon is dependent on the Ct calculation methodology. Oxidant demand is necessarily increased by the presence of NOM in the bulk phase. Therefore, for a given dosage, disinfectant efficacy will be reduced in the presence of NOM. However, for an

doi: 10.2166/aqua.2006.025

equivalent integrated Ct (or area under the decay curve), differences have been observed in natural waters and ultra-pure waters, as described earlier.

The inactivation kinetic is dependent on the integrated Ct value, which can be described by the following relationship, where $C(t)$ represents a function describing the oxidant decay with respect to contact time:

$$Ct = \int C(t) \cdot dt \quad (1)$$

Oxidant decay is often described by a first-order kinetic, such that integrated CT values are calculated with Equation (2):

$$Integrated\ Ct = \frac{C_o}{k} \cdot (1 - \exp^{-k \cdot t}) \quad (2)$$

where:

C_o : initial disinfectant residual (after immediate demand) ($mg\,l^{-1}$)
K : first-order decay constant (min^{-1})
t : time (min)

OBJECTIVES

The purpose of this study was to assess the effect of NOM on disinfection efficacy using two types of disinfectants (Cl_2 and ClO_2). *B. subtilis* spores were used as a surrogate for monitoring disinfection efficacy. Four synthetic NOM-extracts and three natural surface waters were used to explore the potential influence of NOM characteristics.

METHODOLOGY

The effect of natural organic matter was tested using two series of experimental assays. Initially, assays were conducted in ultra-pure buffered waters supplemented with various NOM extracts. Secondly, confirmation assays were performed on several surface waters.

Synthetic waters

Four NOM extracts were purchased from the International Humic Substances Society (IHSS 2005). Two NOM extracts (SWA and Nordic) were concentrated from surface waters, while two others (Pahokee peat and Leonardite) were collected from soil. The IHSS provides the following information (IHSS 2005) on their characteristics:

- ***Suwannee (SWA) River***: The Suwannee River is located in Florida and flows southwest to the Gulf of Mexico. The Suwannee River is known for its high DOC concentrations, ranging from 25 to 75 $mg\,l^{-1}$, and pH values of less than pH 4.0.
- ***Nordic Reservoir (Nordic)***: This sample was obtained from a drinking water reservoir at Vallsjøen, Skarnes, Norway. The sample was obtained from the Sør-Odal County Waterworks intake pipe that draws water from a depth of 10 m (DOC = 10.7 $mg\,l^{-1}$).
- ***Pahokee Peat (Paho)***: The Pahokee peat is a typical agricultural peat soil from the Florida Everglades. The Pahokee series consists of very poorly drained soils that are 36 to 51 inches thick over limestone. Pahokee soils were formed in organic deposits of freshwater marshes.
- ***Leonardite (Leo)***: Leonardite is produced by the natural oxidation of exposed lignite, a low-grade coal.

NOM stock solutions were prepared in Milli-Q waters by adding 500 mg of NOM to 500 ml (1 $g\,l^{-1}$). All of the NOM had a carbon content of approximately 50%, according to the IHSS, and should therefore have produced a stock solution of approximately 500 $mg\,C\,l^{-1}$. Total organic carbon (TOC) concentrations of these suspensions were analysed using a Dohrmann DC-180 (UV-persulphate oxidation). TOC concentrations ranged from 270 $mg\,l^{-1}$ to 480 $mg\,l^{-1}$. It is possible that the TOC concentrations were underestimated due to the limited ability of UV-persulphate oxidation to entirely oxidize complex TOC materials (especially for Pahokee peat and Leonardite, both originating from soils).

Synthetic waters were prepared by adding 0, 2.5 or 5.0 $mg\,C\,l^{-1}$ (the dilution was based on a dry carbon content of 500 $mg\,C\,l^{-1}$) and *B. subtilis* spores to phosphate-buffered Milli-Q waters ($\mu = 0.05$ M, pH 6.5,

Table 1 | Water quality characteristics

NOM conditions		DOC (mg C l^{-1})	UV absorbance (cm^{-1})	SUVA (1 $mg^{-1} min^{-1}$)	Turbidity (NTU)
Buffered demand-free	0 mg l^{-1}	<0.2	<0.001	<0.5	0.24
Suwannee	5 mg l^{-1}	4.2	0.174	4.1	0.38
Leonardite	5 mg l^{-1}	0.37	0.005	1.4	2.2
Pahokee Peat	5 mg l^{-1}	0.91	0.044	4.8	0.46
Nordic Reservoir	5 mg l^{-1}	3.2	0.128	4.0	0.13
L'Assomption River	–	2.07	0.041	2.0	0.06
Mille-Iles River	–	6.09	0.192	3.2	NA
St Lawrence River	–	2.70	0.065	2.4	0.08

T = 22°C) and were left to agitate overnight at 60 rpm. Table 1 presents their characteristics prior to the start of the inactivation assays. The latter were performed in duplicate 1-l stirred batch reactors for both free chlorine and chlorine dioxide. Temperature was maintained constant using a temperature-controlled water-bath. Each microbiological observation was analysed in duplicate over two decimal dilutions. For a given disinfectant, identical dosages were used for all inactivation assays in order to minimize potential bias. Based on preliminary oxidant demand studies, applied free chlorine and chlorine dioxide dosages of 3.5 mg l^{-1} and 5.5 mg l^{-1} were employed, respectively. The higher ClO_2 dosages required reflect mostly the lower disinfectant efficacy of this disinfectant with respect to *B. subtilis* spores, compared with free chlorine. Table 2 summarizes the oxidant dosages applied during the project.

A *B. subtilis* culture was purchased from the American Type Culture Collection (#6633), and a single stock culture was prepared to use in all bench scale inactivation experiments. Spores were prepared according to the methodology described in Barbeau *et al.* (2004). For spore enumeration, biological samples were quenched in 1% (w/w) sodium thiosulphate and enumerated using a modified vacuum plate filtration method (Barbeau *et al.* 1997).

Free chlorine was measured using the DPD colorimetric method (*Standard Methods* 1998). Chlorine dioxide was measured using the ACVK method, which is based on the oxidation and resulting discoloration of acid chrome violet K (Alizarin violet 3R, colour index 6170) (Masschelein 1989). Discoloration of ACVK was measured using a Spectronic 1001 plus (Milton Roy) spectrophotometer, set at 548 nm, and 5.0 cm borosilicate glass cells. The blank was prepared by adding the reducer sodium sulphite at a final concentration of 400 mg l^{-1}.

Inactivation data were analysed using the integrated Ct concept described earlier. Spore inactivation with chlorine dioxide was described by a simple Chick-Watson kinetic:

$$Log\left[\frac{N}{N_o}\right] = -k \times Ct_{integrated} \tag{3}$$

The inactivation kinetics with free chlorine exhibited a shoulder, which was accounted for by using a delayed Chick-Watson model (Rennecker *et al.* 1999):

$$Log\left[\frac{N}{N_o}\right] = -k \times (Ct_{integrated} - Ct_{lag}) \tag{4}$$

for $Ct_{integrated} \geq Ct_{lag}$ and $Log[N/N_o] = 0$ for $CT_{integrated} < Ct_{lag}$.

The integrated CT values were calculated using Equation (2) and the first-order decay constant (k) and the initial residual oxidant (C_o) obtained by fitting a first-order decay to disinfectant demand data (Table 2).

Table 2 | Applied oxidant dosages and calculated first-order disinfectant decay constants ($k +/- s_k$) for disinfection studies using synthetic NOM

		Chlorine dioxide				Free chlorine			
NOM conditions		**Applied dosage ($mg\,l^{-1}$)**	**Immediate demand * (%)**	**K (min^{-1})**	**s_k**	**Applied dosage$mg\,l^{-1}$**	**Immediate demand * (%)**	**K (min^{-1})**	**s_k**
Buffered demand-free	$0\,mg\,l^{-1}$	6.00	3	1.35E − 03	3.54E − 04	3.50	2	1.00E − 04	1.41E − 04
Suwannee	$2.5\,mg\,l^{-1}$	7.00	39	3.20E − 03	0.00E + 00	N.D.	N.D	N.D.	N.D.
	$5\,mg\,l^{-1}$	7.00	59	8.25E − 03	4.95E − 04	3.50	27	1.09E − 02	2.76E − 03
Leonardite	$2.5\,mg\,l^{-1}$	4.00	5	2.40E − 03	4.24E − 04	N.D.	N.D	N.D.	N.D.
	$5\,mg\,l^{-1}$	4.00	14	5.45E − 03	7.07E − 05	3.50	16	8.90E − 03	2.83E − 04
Pahokee Peat	$2.5\,mg\,l^{-1}$	4.00	30	3.50E − 03	1.41E − 04	N.D.	N.D	N.D.	N.D.
	$5\,mg\,l^{-1}$	4.00	51	1.56E − 02	3.32E − 03	3.50	27	1.13E − 02	1.34E − 03
Nordic Reservoir	$2.5\,mg\,l^{-1}$	4.00	12	2.40E − 03	1.41E − 04	N.D.	N.D	N.D.	N.D.
	$5\,mg\,l^{-1}$	4.00	29	4.25E − 03	7.07E − 05	3.50	19	3.65E − 03	7.07E − 05

N.D.: Note done s_k: standard deviation on k.
*% of applied dosage consumed after 5 min.

Statistical analyses were completed using Statistica 7.0 (Statsoft, Oklahoma).

Natural waters

Three natural surface waters, exhibiting various NOM levels ($2 - 6\,mg\,C\,l^{-1}$) and aromaticity (SUVA: 1.5 – 2.9), were filtered using a 0.22 μm (cellulose acetate, Millipore) filter in order to remove particles. These waters were then buffered, seeded with spores ($10^{4.5}$ CFU ml^{-1}), and assayed under identical conditions as those previously described. Table 1 provides a summary of the natural and synthetic water characteristics used during this study.

RESULTS AND DISCUSSION

Chlorine dioxide inactivation assays

Figure 1 presents the inactivation kinetics of *B. subtilis* spores using ClO_2 for various synthetic waters. The reference condition was obtained in buffered demand-free water, while other reactors were supplemented with $5\,mg\,C\,l^{-1}$ from various NOM sources. Inactivation data from duplicate reactors were pooled together for regression analysis purposes.

Results indicate that, for all the NOMs tested, the inactivation of spores was significantly enhanced by the addition of NOM. Inactivation kinetics in NOM-rich waters were similar, except for the Nordic Reservoir NOM, which exhibited a slower inactivation.

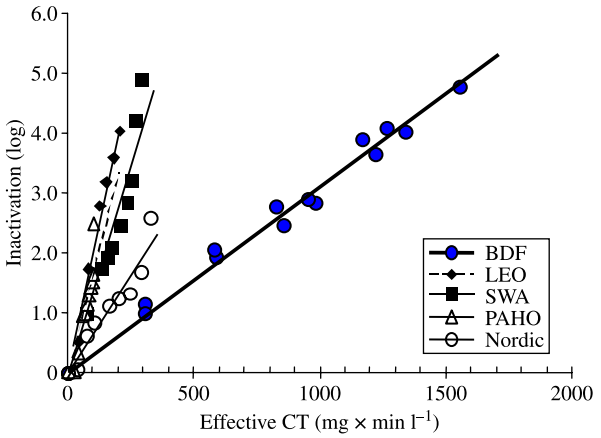

Figure 1 | Inactivation of *B. subtilis* spores using chlorine dioxide for several synthetic waters ($5\,mg\,C\,l^{-1}$). BDF (buffered demand-free) represents the reference condition. Bold lines provide the Chick-Watson kinetics. T: 22°C, pH 6.5.

As seen in Figure 3(a), a dose-effect relationship was also observed. Increasing NOM concentration from $2.5\,mg\,l^{-1}$ to $5\,mg\,l^{-1}$ always led to a statistically significant increase in disinfection efficacy, as measured by the integrated Ct required for 2 log inactivation. The lowest efficacy was observed while using ultra-pure buffered waters (without NOM). For this condition, the Ct value for 2 log inactivation was $642\,mg \times min\,l^{-1}$ (± 3%). For high-NOM conditions ($5\,mg\,C\,l^{-1}$), the Ct values for Suwannee (142 ± 6%), Leonardite (91 ± 12%), Pahokee (126 ± 11%) and Nordic Reservoir (203 ± 16%) were all significantly ($p < 0.01$) lower than the reference value of $642\,mg \times min\,l^{-1}$ obtained with buffered demand-free (BDF) water. For moderate-NOM conditions ($2.5\,mg\,C\,l^{-1}$), the favourable impact of NOM was still important and statistically significant ($p < 0.01$), although the Ct values were approximately 1.4 to 1.8 times higher than those obtained at $5\,mg\,C\,l^{-1}$.

The exact origin of this phenomenon has not yet been clearly explained. This effect was also observed in a previous study (Barbeau *et al.* 2005) using MS2 coliphages and *B. subtilis* spores. The results for *B. subtilis* spores are equivalent. During the initial study, the influence of NOM on MS2 phages inactivation was less pronounced than for *B. subtilis* spores, providing a 1.5-fold increase in the rate of inactivation (Barbeau *et al.* 2005). It is proposed that chlorine dioxide by-products may work together synergistically in the disinfection process. The efficacy of chlorite (the main chlorine dioxide disinfection by-product) was evaluated and was found to be negligible. No statistically significant reduction of spores was observed after 5 days of contact with $100\,mg\,l^{-1}$ of chlorite. The nature and concentrations of these by-products remain therefore to be identified. It is also possible that only a fraction of the NOM (hydrophilic, hydrophobic, etc.) is responsible for this reaction. According to Doré (1989), $HClO_4$· radicals can originate from the decomposition of chlorine dioxide in water. O· and ClO· radicals have also been proposed as intermediate by-products of chlorine dioxide photolysis. Joncourt *et al.* (1998) demonstrated using spin-trapping ESR spectroscopy that chlorine radicals (Cl°) were formed in conditions simulating chlorine dioxide (ClO_2) bleaching of softwood kraft pulp (in the presence of iron cations). NOM is known to act both as a promoter and inhibitor of free radicals by ozone (Siddiqui 1996). Considering that

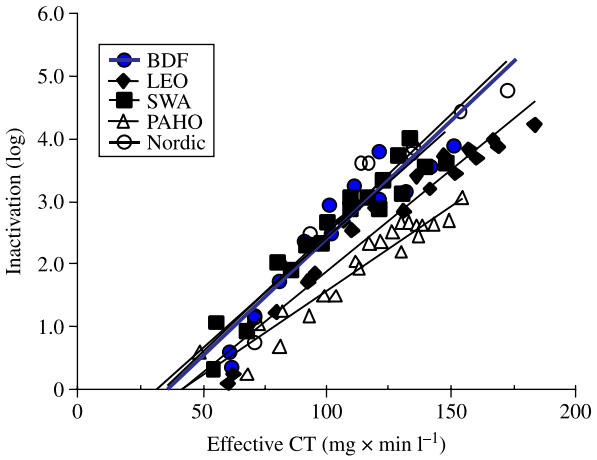

Figure 2 | Inactivation of *B. subtilis* spores using free chlorine for several synthetic waters (5 mg C l^{-1}). BDF water (buffered demand-free) represents the reference condition. Bold lines provide delayed Chick-Watson kinetics. T: 22°C, pH 6.5.

chlorine dioxide may produce free radicals (Doré 1989), a similar process may also be at play in the present case. Additional assays should also be conducted using parasites (*Giardia* or *Cryptosporidium*), as these organisms do not necessarily behave like *B. subtilis* spores.

Free chlorine inactivation assays

Figure 2 presents the inactivation kinetics of *B. subtilis* spores using free chlorine for various synthetic waters. The reference condition was obtained in buffered demand-free water, while other reactors were supplemented with 5 mg C l^{-1} of various NOM. Inactivation data from duplicate reactors were analysed with the delayed Chick-Watson model. In contrast to chlorine dioxide, the inactivation of *B. subtilis* spores with free chlorine always exhibited a significant lag phase. However, beyond the lag phase, free chlorine was much more effective than chlorine dioxide. In the absence of NOM, the Ct value for 2 log inactivation was 7.7 times higher for chlorine dioxide (642 mg × min l^{-1}) than for free chlorine (83 mg × min l^{-1}). In the presence of 5 mg C l^{-1}, this difference was lowered: the Ct values for 2 log inactivation were, on average, 1.5 times higher for chlorine dioxide, compared with free chlorine.

Figure 2 indicates that the influence of NOM was much less pronounced while disinfecting with free chlorine. In order to test this influence, the Ct 2 log values derived from the delayed Chick-Watson models presented in Figure 2 were transposed into Figure 3(b).

The 95th percentile confidence intervals confirmed that, for most synthetic waters, the presence of NOM did not significantly impact the inactivation of *B. subtilis* spores. This observation is coherent with the fact that free chlorine does not promote free radical reactions. Also, this observation was made despite the fairly high (2.1 NTU) turbidity of the Leonardite samples, due to the fact that this NOM, which originates from soil, did not completely dissolve. However, one exception was also noted. The Pahokee NOM (also from soil) produced a slightly higher

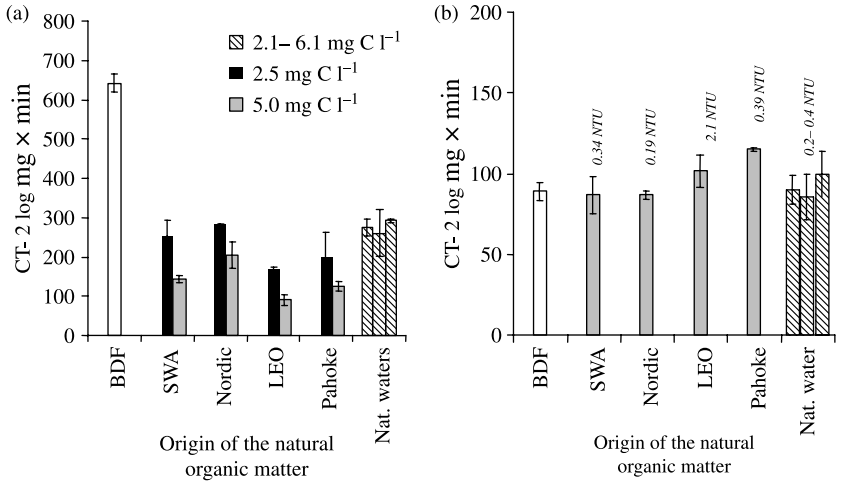

Figure 3 | Values of Ct (2 log) for inactivation of *B. subtilis* spores using chlorine dioxide (a) or free chlorine (b) for several synthetic and natural waters. BDF water (buffered demand-free) represents the reference. Error bars represent the 95th percentile confidence interval. T: 22°C, pH 6.5.

Ct value (2 log). Although statistically significant, this difference was modest (a 30% increase compared with BDF water).

Confirmation inactivation assays in natural waters

Inactivation assays were performed using three surface waters in order to confirm the trends observed with synthetic waters. These waters were filtered (0.22 μm) in order to remove suspended solids. Figure 3(a) (ClO_2) and (b) (Cl_2) provides the Ct values calculated for 2-log inactivation of *B. subtilis* spores in various synthetic and natural waters.

For the three surface waters investigated, the inactivation data (Figure 3(a)) confirmed the improved disinfection efficacy of chlorine dioxide, most likely due to the presence of NOM. However, the Ct values for 2-log inactivation were fairly similar from one surface water to another (~290 mg × min l^{-1}), even though they differed in NOM concentrations (1.9 to 6.1 mg l^{-1}) and characteristics (UVA from 0.08 to 0.27 cm^{-1}).

The chlorine inactivation assays (Figure 3(b)) also corroborated the observation that NOM had no significant impact on chlorine efficacy. Ct values (2 log) varied from 86 to 100 mg × min l^{-1} in natural waters, this variation being within the 95th percentile confidence interval for BDF water.

CONCLUSIONS

The presence of NOM hinders water treatment more than it benefits water treatment. However, it is important to understand the origins of variations in disinfection efficacy in natural waters. Such information is essential in order to adequately define our safety factors related to the application of the Ct concept.

The current research project has led to the following conclusions:

For chlorine dioxide, the occurrence of NOM improved disinfection efficacy. This phenomenon was significant, as Ct values (2 log) were reduced by a factor of 2.3 to 7.1, depending on the NOM source and concentration. The impact of NOM on chlorine dioxide appears to be concentration-dependent, as it was observed that increasing the NOM concentrations amplified this phenomenon. The favourable effect of NOM on ClO_2 efficacy was confirmed in three surface waters.

For free chlorine, the occurrence of NOM did not improve disinfection efficacy. This observation was consistent with observations in both synthetic and natural surface waters.

This work emphasizes the importance of analysing disinfection data in terms of integrated Ct values. Currently, several utilities are moving towards the use of advanced Ct calculation techniques, such as the Integrated Disinfection Design Framework. The use of such a technique requires a better understanding of the role of water quality on disinfection efficacy. In order to improve our understanding of inactivation in natural waters, this work supports the need for additional research to confirm the influence of NOM on other types of organism (especially *Giardia* and *Cryptosporidium*), and other disinfectants.

REFERENCES

Barbeau, B., Boulos, L., Desjardins, R., Coallier, J., Prevost, M. & Duchesne, D. 1997 A modified method for the enumeration of aerobic spore-forming bacteria. *Can. J. Microbiol.* **43**, 976–980.

Barbeau, B., Huffman, D., Mysore, C., Desjardins, R. & Prévost, M. 2004 Examination of discrete and confounding effects of water quality parameters during the inactivation of MS2 phages and Bacillus subtilis spores with free chlorine. *J. Environ. Eng. Sci.* **3**, 255–268.

Barbeau, B., Huffman, D., Mysore, C., Desjardins, R., Clément, B. & Prévost, M. 2005 Examination of discrete and confounding effects of water quality parameters during the inactivation of MS2 phages and Bacillus subtilis spores with chlorine dioxide. *J. Environ. Eng. Sci.* **4**, 139–151.

Cho, M., Chung, H. & Yoon, J. 2003 Disinfection of water containing natural organic matter by using ozone-initiated radical reactions. *Appl. Environ. Microbiol.* **69**(4), 2284–2291.

Doré, M. 1989 *Chimie des Oxydants et Traitement des Eaux: Technique et Documentation*, Lavoisier, Paris.

Dow, S. M., Barbeau, B., von Gunten, U., Chandrakanth, M., Amy, G. & Hernandez, M. 2006 The impact of selected water quality parameters on the inactivation of B. subtilis spores by monochloramine and ozone. *Wat. Res.* **40**, 373–382.

Haas, C. N., Joffe, J., Hornberger, J. C., Anmangandla, U., Heath, M. & Jacangelo, J. 1995 *Development and Validation of Rational Design Methods of Disinfection*, AWWA Research Foundation, Denver, Colorado.

Hijnen, W. A. M., Baars, E., Bosklopper, T. G. J., Van Der Veer, A. J., Meijers, R. T. & Medema, G. J. 2004 Influence of DOC on the inactivation efficiency of ozonation assessed with *Clostridium*

perfringens and a lab-scale continuous flow system. *Oz. Sci. Eng.* **26**(5), 465–473.

IHSS (International Humic Substances Society) 2005 www.ihss.gatech.edu/, 15 August 2005.

Joncourt, M. J., Froment, P. & Lachenal, D. 1998 Formation of radical species during ClO_2 delignification. *J. Wood Chem. Technol.* **18**(2), 159–170.

Masschelein, W. J. 1989 Determination of residual ozone or chlorine dioxide in water with ACVK: An updated version. *Oz. Sci. Eng*, **11**, 209–215.

Mysore, C., Leparc, J., Barbeau, B., Amy, G., Hernandez, M., Dow, S., Huffman, D. & Marinas, B. 2003 *Impact of Water Quality on the Inactivation of Bacterial and Viral Pathogens*, AWWA Research Foundation, Denver, Colorado.

Oppenheimer, J., Najm, I., Trussell, R. R., Aieta, E. M. & Jacangelo, J. 2000 Constructing a CT table for *Cryptosporidium* inactivation with ozone in low TOC and low turbidity waters. *Wat. Suppl.* **18**(1), 427–430.

Rennecker, J. L., Marinas, B., Owens, J. H. & Rice, E. W. 1999 Inactivation of *Cryptosporidium parvum* oocysts with ozone. *Wat. Res.* **33**(11), 2481–2488.

Siddiqui, M. 1996 Chlorine-ozone interactions: Formation of chlorate. *Wat. Res.* **30**(9), 2160–2170.

Standard Methods for the Examination of Water and Wastewater 1998 20th edition. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC.

First received 4 February 2006; accepted in revised form 20 April 2006