Inactivation of *Cryptosporidium parvum* with ozone in treated drinking water

Lijie Li and Charles N. Haas

ABSTRACT

Cryptosporidium parvum oocyst disinfection experiments were performed using ozone at bench-scale in batch reactors. The water matrix was filter effluents collected from a full-scale facility in different seasons but with similar water quality. Cell tissue culture (FDM-MPN) was used to assess the viability of *C. parvum* oocysts. The disinfection kinetics showed no significant difference in waters from different seasons. The simple Chick–Watson model was found to be the best fit model for MPN data in combination with first-order-plus-demand ozone decay kinetics. The *CT* value for 99% inactivation was determined to be 19.5 mg-min l^{-1} at 15°C when pooling data together, which was larger than values reported in other studies.

Key words | *C. parvum* oocysts, cell tissue culture, inactivation kinetics, ozone, plant filtered effluent water

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INTRODUCTION

First described by Tyzzer in 1907 (Tyzzer 1907) and first recognized as a health threat to humans in 1976 (Meisel *et al*. 1976; Nime *et al*. 1976), *Cryptosporidium parvum* is well recognized as the cause of worldwide waterborne cryptosporidiosis in humans. Cryptosporidiosis is a diarrhoeal disease with the symptoms of abdominal pain, diarrhoea, weight loss, stomach cramps, loss of appetite and a slight fever for people with an average immune system. It can be more severe or even fatal to immunocompromised patients (Petersen 1982).

Cryptosporidium lives in the intestine of infected humans or animals. It is spread by soil, food, water or surfaces that have been contaminated with the faeces from infected humans or animals. Since *Cryptosporidium* oocysts are small and highly resistant to chlorine and many other traditional disinfectants (Fayer & Ungar 1986), the removal or inactivation of *Cryptosporidium* has become a major concern in the drinking water treatment industry. To date, most *Cryptosporidium* outbreaks have been caused by contaminated drinking water, including the largest outbreak in the USA, in 1993 in Milwaukee, Wisconsin (MacKenzie *et al*. 1994).

The Interim Enhanced Surface Water Treatment Rule (USEPA 1998) set the first federal drinking water standards to control *Cryptosporidium* for public water systems that use surface water or groundwater under the direct influence of surface water (GWUDI) and serve at least 10,000 people. It established a maximum contaminant level goal (MCLG) of zero for *Cryptosporidium* and 2-log *Cryptosporidium* removal requirements for filtered systems. These requirements have been extended to small systems under the Long Term 1 Enhanced Surface Water Treatment Rule (LT1ESWTR) (USEPA 2002) along with the establishment of the Filter Backwash Recycling Rule (FBRR) (USEPA 2001). Currently EPA is proposing the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) to supplement existing regulations by requiring additional *Cryptosporidium* treatment at higher risk water suppliers (USEPA 2003).

Ozone is a strong disinfectant and has been proven to be much more effective at inactivating *C. parvum* oocysts than chlorine-based disinfectants. Earlier studies of *Cryptosporidium* inactivation using ozone showed some

disparities in required *CT* (product of disinfectant concentration, *C*, in mg/l^{-1} and contact time, *T*, in min) values. These disparities have been attributed to the various methods of assessing viability, variability of oocyst sources and different experimental designs (Rose *et al*. 2002). In previous studies, excystation and animal infectivity were most often used to detect and quantify *C. parvum* oocysts. Buffered water was used in most cases.

Cell tissue culture is a recently developed *in vitro* method used for quantifying the number of infectious *C. parvum* oocysts in water samples. Based on cell tissue culture, it combines the foci detection method (FDM) and most-probable-number method (MPN) to enumerate infectious *C. parvum* oocysts in water samples. The advantages of this method include: its ability to detect viability and infectivity of oocysts; its high sensitivity to low levels of infectious oocysts; its ability to detect oocysts in both treated and untreated waters; and time and labour savings when compared with animal infectivity studies, the current 'gold standard' for measuring *C. parvum* infectivity (Rochelle *et al*. 1997; Slifko *et al*. 1997*a*, *b*, 1998). Several studies have shown good correlation ($r>0.85$) between oocysts infectivity detection methods based on cell culture and animal infectivity analysis (Slifko *et al*. 1998; Huffman *et al*. 2000; Rochelle *et al*. 2002; Slifko *et al*. 2002), suggesting that cell culture can be used as a practical alternative for assessing oocyst infectivity and determining inactivation. Rochelle *et al*. (1997) and DiGiovanni *et al*. (1999) have also demonstrated the use of PCR and RT-PCR integrated with cell culture for the detection of infectious oocysts in water samples.

The objective of this study was to employ the FDM-MPN method in determining the inactivation of *C. parvum* oocysts by ozone. Experiments were conducted in Philadelphia Baxter Plant filtered drinking water at a temperature of 15°C and pH 7. This methodology is in contrast with the methodology used in the majority of published studies. In those studies experiments were usually conducted in phosphate buffer solution and using animal infectivity or *in vitro* excystation to assess inactivation. One of the purposes was to determine the *CT* value needed to achieve 2-log inactivation in Philadelphia Baxter Plant water.

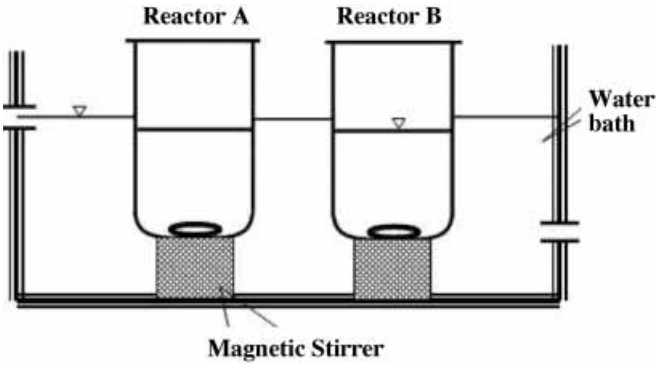

Figure 1 | Batch reactor configuration.

METHODS

General

In this study, bench-scale *C. parvum* oocyst disinfection experiments using ozone in plant filtered water were performed in batch reactors. A recently validated technique, foci detection method and most probable number method (FDM-MPN), was used to assess the viability of oocysts in all disinfection experiments.

Except for the matrix water used for disinfection experiments, all the water used for reagent preparation in this study was processed by a Milli-Q water system (Millipore Intertech., Bedford, Massachusetts). The reactor configuration is depicted in Figure 1. Two 1-l heat-resistant beakers were used as the reactors (disinfection reactor A and control reactor B). The reactors were placed on two submersible stirrers in a circulating refrigerated water bath (Model 1186, VWR Scientific, Bridgeport, New Jersey). Before the experiments, the reactors and stir bars were made ozone demand free by submerging them in ozone solution (2 mg l^{-1} or more) for 1 h or more and then drying them at 110°C for 5 h. Before each experiment, the reactors and sampling syringes were rinsed with eluting solution (pH 7.4) to minimize oocyst attachment to walls (*Standard Methods* 1995; 9711 B).

Cell line preparation and maintenance

Frozen human ileocecal adenocarcinoma cells (HCT-8 cells, ATCC# CCL-244) were employed in this study. Cell maintenance methods were used as described by Slifko *et al*. (1997*b*). Defrosted cells were maintained in maintenance medium containing 82% RPMI-1640 (Fisher Scientific, Malvern, Pennsylvania), 5% fetal bovine serum (FBS) (Atlanta Biologicals, Norcross, Georgia), 10% Opti-MEM I reduced serum medium (Life Technologies, Rockville, Maryland), 1% 200 mM L-glutamine (Fisher Scientific, Malvern, Pennsylvania), and 2% HEPES (pH 7.3, Fisher Scientific, Malvern, Pennsylvania). Growth medium was also prepared from the same recipe as maintenance medium except the FBS was increased to 10% for growing infected oocysts.

The cells were maintained in 75-cm^2 tissue culture flasks in a CO_2 incubator with an environment of 37°C, 5% CO_2 and 100% humidity. They were split/passaged by trypsinization every 2 to 3 days no more than 20 times.

A600FL antibody reagent preparation

A600FL-20 × Sporo-Glo™ is a fluorescein-labelled, purified polyclonal rat lgG antibody made for sporozoites of *C. parvum*. It was obtained from Waterborne, Inc., New Orleans, Louisiana. A 20 × working dilution was prepared and stored at 4°C for no more than 1 month.

Oocyst preparation

The *C. parvum* oocysts used for all experiments were purchased from Pleasant Hills Farm, Troy, Idaho. It is a bovine derived strain (Iowa strain) maintained in newborn calves. A suspension of 5×10^7 oocysts was shipped overnight in a chilled 15 ml centrifuge tube. Upon receipt, the oocyst stock was washed and concentrated twice by centrifugation at $4000 \times g$ for 10 min before being resuspended in 1 × phosphate buffered solution (PBS, pH 7.2). The oocysts were used for ozone disinfection experiments immediately after the cleaning procedures. The density of the oocyst stock was determined by using a hemocytometer count. The age of oocysts was recorded and the elapsed time between oocysts shedding to disinfection was in the range of 6 to 47 days.

Ozone preparation

Stock ozone solution was generated at the point-of-use by a Polymetrics Ozone Generator (Model T408, Polymetrics Inc., Colorado). Oxygen carrier gas containing approximately 5% ozone was bubbled through 400 ml of Milli-Q water in a 500 ml gas absorption flask surrounded by ice for a minimum of 30 min. The Milli-Q water was refrigerated at 4°C prior to ozone generation in order to obtain a stock ozone concentration around 25 mg l^{-1}. The generated stock ozone solution was stored in a dark bottle and kept in a 4°C refrigerator for 10 min to stabilize the ozone concentration in the stock solution. Then the concentration of the ozone stock solution was measured by the indigo colorimetric method (*Standard Methods* 1995; 4500-O_3 B) and the ozone solution was used immediately thereafter.

Source water preparation

The water used in this study was taken from the combined filter effluent of the Baxter Municipal Water Treatment Plant in Philadelphia, whose water source is the Delaware River. The filtered drinking water samples were taken in September 2000 (fall water) and January 2001 (winter water) and stored at 4°C for no longer than 3 months before use.

Experimental design and operation

Three disinfection experiments were conducted with initial ozone doses of 1.0, 2.0 and 3.0 mg l^{-1} for the water collected in the fall. These three experiments were also done with the same ozone initial doses for the water taken in the winter.

The experimental design matrix is displayed in Table 1 together with the information on source water and oocysts. All the experiments were performed at 15°C and pH 7. The temperature was controlled by a refrigerated water bath. The pH of the filtered drinking water samples was adjusted to 7.0 by 1 M KH_2PO_4 added in advance of disinfection experiments.

Table 1 | Summary of Baxter Plant water quality and experimental design

	Water quality						
Water source	pH	Turbidity (NTU)	Specific conductance (μmhos cm^{-1})	Alkalinity as $CaCO_3$ (mg l^{-1})	Ammonia as N (mg l^{-1})	Ozone dose (mg l^{-1})	Oocysts age (days)
Baxter, fall	7.4	0.07	345	55	0.05	1.0	20
Baxter, fall	7.4	0.07	345	55	0.05	2.0	47
Baxter, fall	7.4	0.07	345	55	0.05	3.0	40
Baxter, winter	7.6	0.06	374	46	0.14	1.0	26
Baxter, winter	7.6	0.06	374	46	0.14	2.0	12
Baxter, winter	7.6	0.06	374	46	0.14	3.0	6

Before each experiment, 2.5×10^7 of *C. parvum* oocysts were added into 1 l of the filtered water and mixed with the water for at least 30 min. Then the suspension was divided evenly into reactor A and reactor B (Figure 1). To start the disinfection experiment, a volume of freshly drawn ozone stock solution was added into the disinfection reactor A to achieve the desired initial ozone dose. A volume of the filtered water without oocysts equal to the ozone solution volume was also added to the control reactor B before the start of experiment to maintain equal volume and oocyst concentration in both reactors. Inactivation samples were taken by syringe at 0.5 min, 2.5 min and 7.5 min after the addition of ozone stock solution and transferred into centrifuge sampling tubes (35 ml) containing 0.5 ml of 10% (w/v) sodium thiosulfate solution to quench the remaining ozone. The inactivation samples were stored at 4°C before being analysed by the FDM-MPN method. Control samples were taken at the beginning and the end of disinfection experiments from the control reactor into centrifuge sampling tubes which were prepared in the same way mentioned above. Ozone residual samples were taken at 1 min, 5 min, 10 min and 20 min after starting disinfection. The ozone residual samples were measured immediately by the indigo colorimetric method.

HCT-8 cells were used as the hosts for *C. parvum* oocysts in reproductive stages and were maintained in 75-cm^2 tissue culture flasks in a CO_2 incubator. The HCT-8 cells suspended in maintenance medium were pipetted into 8-well covered chamber slides, with 0.7 ml of suspension in each well, and were incubated in the 37°C incubator with 5% CO_2 atmosphere for 48 h prior to infection until the monolayers in the slides became 70–90% confluent. The maintenance medium in all the wells was replaced with the growth medium before oocyst infection. The number of slides was determined by the number of samples and the number of dilutions. The basis of the most probable number (MPN) assay is a set of dilutions for determining the most probable number of oocysts in a sample. In this study, each sample required one MPN, and each MPN required six dilutions.

The oocyst samples were washed twice by PBS before being transferred into 1.5-ml microcentrifuge tubes with final volume of 900 μl. Each oocyst sample was mixed well with 100 μl (10%, v/v) of household bleach containing 5.25% sodium hypochlorite kept at 4°C and incubated for 8 min. Then the oocyst samples were centrifuged at $10{,}500 \times g$ for 4 min in a centrifuge, washed twice by PBS, and resuspended in growth medium (pre-warmed to 37°C). After sodium hypochlorite pre-treatment, six decimal dilutions were made in series in growth medium to

Table 2 | Disinfection kinetic models with first-order decay

Model	Inactivation rate	Modified survival ratio
First-order Chick-Watson	$-kCN$	$\ln\left(\frac{N}{N_0}\right) = -\frac{k}{k^*}(C_0 - x)\,[1 - \exp(k^*t)]$
Chick-Watson	$-kC^nN$	$\ln\left(\frac{N}{N_0}\right) = -\frac{k(C_0 - x)^n}{nk^*}[1 - \exp(-nk^*t)]$
Hom	$-kmNC^nt^{m-1}$	$\ln\left(\frac{N}{N_0}\right) = -\frac{mk(C_0 - x)^n}{(nk^*)^m}y(m, nk^*t)$
Power Law	$-kC^nN^x$	$\ln\left(\frac{N}{N_0}\right) = -\frac{1}{x-1}\ln\left\{1 + \frac{(x-1)k(C_0 - x)^n}{nk^*}N_0^{x-1}[1 - \exp(-nk^*t)]\right\}$
Hom-Power Law	$-nkC^nN^xt^{m-1}$	$\ln\left(\frac{N}{N_0}\right) = -\frac{1}{x-1}\ln\left\{1 + (x-1)\frac{mk(C_0 - x)^n}{(nk^*)^m}y\,(m,nk^*t)N_0^{x-1}\right\}$

Source: Haas & Finch (1999).

prepare low concentrations for each oocyst sample. 150 μl of each diluted sample was transferred to each of six of the eight wells of a slide. The other two wells of each slide were used as an uninoculated control. After infection, the slides were incubated at 37°C with 5% CO_2 for another 48 h. Then the cells were fixed with 100% methanol (Fisher Scientific, Malvern, Pennsylvania) for 10 min and labelled by A600FL antibody reagent. *C. parvum* oocysts in reproductive stages can react with the antibody and fluoresce an apple-green colour against a relatively dark background of uninfected cells under epifluorescense microscopy. Infected wells were scored as positives, while uninfected wells were scored as negatives. The number of replicates, the number of dilutions and volumes used were entered into the Information Collection Rule ICR-MPNv software program supplied by USEPA (1996) and the most probable number (MPN) with confidence intervals of infectious oocysts in the original sample was determined as MPN per ml.

Data analysis

Ozone decay in an aqueous environment can be modelled as first-order-plus-demand kinetics given as:

$$C = (C_0 - x)\exp(-k^*t) \tag{1}$$

where C is ozone residual at time t (mg l^{-1}), C_0 is ozone initial dose (mg l^{-1}), x is instantaneous ozone demand (mg l^{-1}), and k^* is the first-order ozone decay rate (min^{-1}). The values of x and k^* were determined by the method of nonlinear regression (Haas *et al*. 1995).

Five models were used to describe ozone disinfection kinetics: first-order Chick–Watson ($n = 1$) model; general Chick–Watson ($n \neq 1$) model; Hom model; Power Law model; and Hom Power Law model. Rate expressions and survival ratio expressions associated with these models are presented in Table 2 with modification to incorporate ozone initial demand and decay (Haas & Finch 1999).

Model parameters for each model were estimated via a nonlinear least squares regression method. The best-fit value of parameters for each model was estimated as the value that minimized the residual sum of squares SS.

$$SS = \sum\left[\ln\left(\frac{N}{N_0}\right)_{predicted} - \ln\left(\frac{N}{N_0}\right)_{observed}\right]^2 \tag{2}$$

where N and N_0 are the concentrations of viable microorganisms at the end and the beginning of a disinfection experiment.

Table 3 | First-order-plus-demand ozone decay parameters of ozone in fall and winter (T=15°C)

	Fall		Winter	
Ozone initial dose C_0 (mg l^{-1})	k^* (min^{-1})	x (mg l^{-1})	k^* (min^{-1})	x (mg l^{-1})
1.0	0.095	– 0.022	0.088	0.217
2.0	0.101	0.575	0.063	0.085
3.0	0.059	0.388	0.027	0.225

All fits were conducted by use of Matlab 5.2 (The MathWorks, Inc.). Best-fit models were compared using the residual sum of squares of the five models and a partial F-test. The model that significantly improved the goodness of fit with the minimum number of parameters was selected as the best-fit model (Motulsky & Ransnas 1987; Haas 1988).

RESULTS AND DISCUSSION

Ozone decay

From fall and winter experiments, the values of the initial ozone demand, x, and the first-order decay coefficient, k^*, were calculated for each experiment using first-order-plus-demand kinetics. Results are summarized in Table 3. The decay curves are plotted in Figure 2 and Figure 3. Generally, the initial ozone demand in fall water was greater than that of winter water, except at an ozone dose of 1.0 mg l^{-1}. Ozone decayed faster in fall water than in winter water.

Separate analyses of *C. parvum* oocysts fall and winter disinfection data

MPN data for fall water and winter water were separately fit to the five disinfection rate models summarized in Table 2 by the nonlinear least squares method. Since none of the other models showed significant improvements

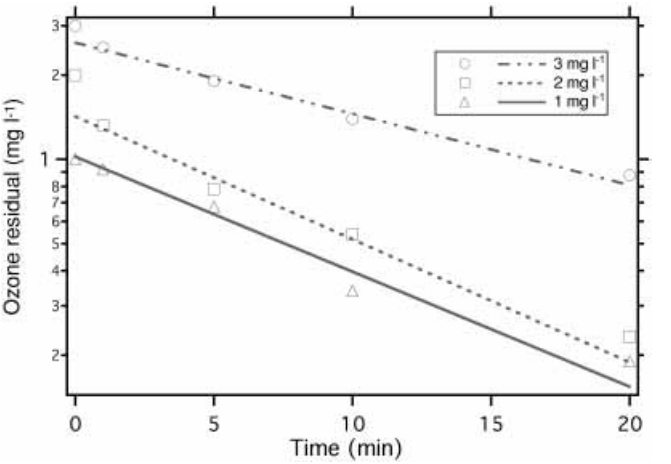

Figure 2 | First-order-plus-demand ozone decay curve in fall water (T=15°C).

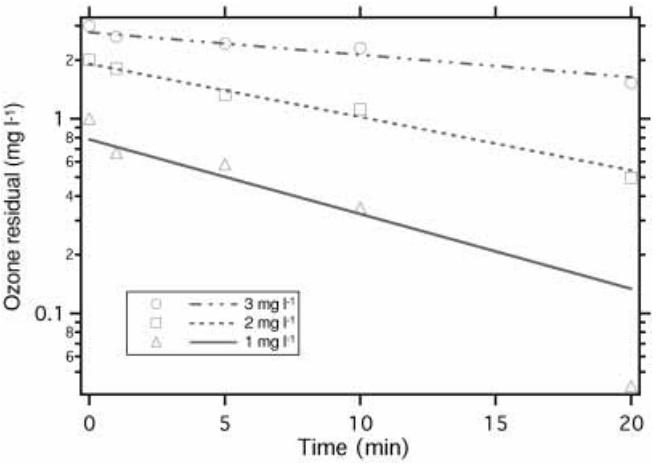

Figure 3 | First-order-plus-demand ozone decay in winter water (T=15°C).

($p>0.05$) based on the partial F-test (Table 4), the first-order Chick–Watson model was accepted as the most appropriate model to describe *C. parvum* oocysts inactivation by ozone in both fall water ($k=0.305$ min^{-1}) and winter water ($k=0.201$ min^{-1}). Survival curves generated via the first-order Chick–Watson model are displayed in Figure 4 and Figure 5.

In the first-order Chick–Watson model, k (min^{-1}) is the rate constant of the disinfection reaction. Using the first-order Chick–Watson model to compare MPN data of both seasons, the disinfection rate constant in fall water was greater than that of winter water, which means that

Table 4 | Probabilities from F-test for pairwise model comparison

Models	Probability from partial F-test (α=0.05)	
	Fall data	Winter data
Simple Chick-Watson vs. Chick-Watson	0.1007	0.0598
Chick-Watson vs. Hom	0.8950	0.1159
Chick-Watson vs. Power Law	0.0578	0.0882
Hom vs. Hom-Power Law	0.0517	0.4680
Power Law vs. Hom-Power Law	0.3604	0.6778

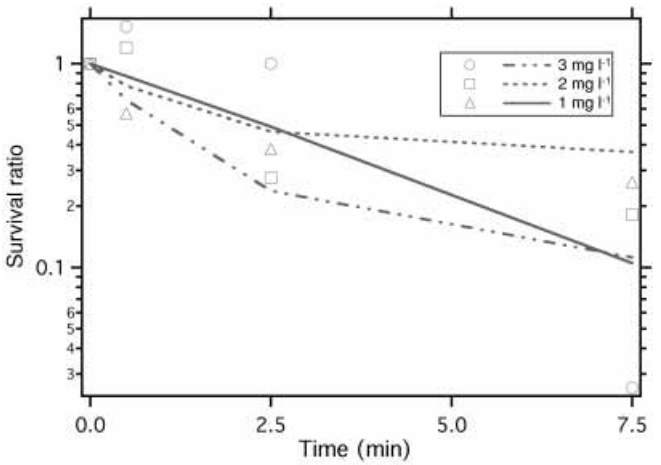

Figure 4 | The simple Chick-Watson model fitting on inactivation of *C. parvum* oocysts with ozone in fall water (T=15°C).

the ozone disinfection on *C. parvum* oocysts in fall water was more effective than in winter water. Related to the faster decay of ozone in fall water mentioned above, it seems more ozone was efficiently used for *C. parvum* inactivation in fall water.

Analyses of *C. parvum* oocysts disinfection pooled data of fall and winter

MPN data in fall and winter water were pooled together and fit by each of the five disinfection models to determine

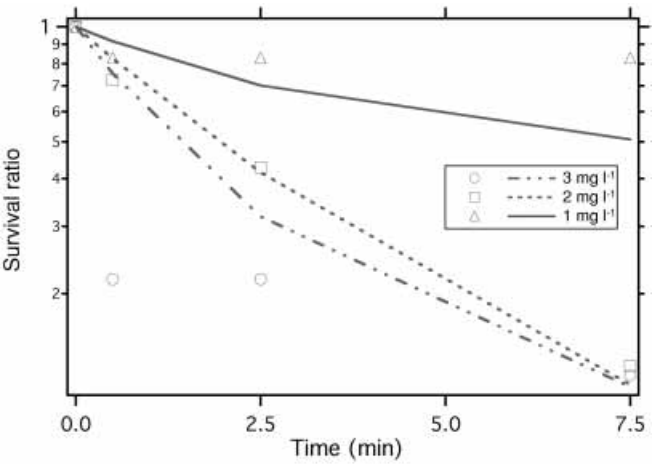

Figure 5 | The simple Chick-Watson model fitting on inactivation of *C. parvum* oocysts with ozone in winter water (T=15°C).

whether the two sets of data were significantly different. The first-order Chick–Watson model was again determined to be the most appropriate model that explained the pooled data ($k = 0.236\ \text{min}^{-1}$).

Based on the corresponding first-order Chick–Watson model, the pooled analysis was compared with the separate analysis by an F-test as described by Motulsky and Ransnas (1987). The overall sum of squares and the overall degrees of freedom from separate analysis were computed as:

$$SS_{sep} = SS_{fall} + SS_{winter} \qquad df_{sep} = df_{fall} + df_{winter} \tag{3}$$

The values of SS_{pool} and df_{pool} were obtained from pooled analysis. The significance of the improvement was determined by F value calculated from:

$$F = \frac{(SS_{pool} - SS_{sep})/(df_{pool} - df_{sep})}{SS_{sep}/df_{sep}} \tag{4}$$

Corresponding to a F value of 1.96, the separate analysis did not exhibit significant improvement over the pooled analysis at the 95% confidence level ($p = 0.18$). It indicated no significant difference exists between the disinfection kinetics derived from fall and winter data.

Comparison with other studies

Rennecker *et al.* (1999) studied ozone inactivation of *C. parvum* oocysts in 0.01 M demand-free phosphate buffer (pH = 7) and used *in vitro* excystation as the viability assay method. The experiments were performed in a semi-batch reactor. Expressing the disinfection kinetics as a delayed Chick–Watson expression, they reported the proposed minimum *CT* values (ozone residual × contact time) of 9.6 mg-min l^{-1} for 2-log inactivation on Iowa strain oocysts at 15°C. The effect of temperature on *C. parvum* oocyst ozonation was found to obey an Arrhenius law in the range of 5–30°C. The experimental values of the ozone inactivation rate constant obtained were found consistent with a later study (Rennecker *et al.* 2000) for the same oocyst strain in the temperature range of 4–20°C. The reported activation energy was demonstrated to well represent the temperature effect on the ozone inactivation kinetics using one of the lots of *C. parvum* oocysts within 1–20°C (Corona-Vasquez *et al.* 2002*b*).

Li *et al.* (2001*b*) used batch reactors containing 0.05 M demand-free phosphate buffer for *C. parvum* oocyst inactivation by ozone. Animal infectivity was used to determine the viability of oocysts. They reported the *CT* requirement of 11.8–12.4 mg-min l^{-1} for 2-log inactivation at 13 ± 0.5°C and pH 6–8 based on an incomplete gamma Hom model. They suggested incorporating a safety factor with a given level of statistical confidence. With 90% confidence intervals, the designed ozone dose is about twice the expected dose given by their IgH model.

Finch *et al.* (2001*a*) analysed two sets of the oocyst inactivation data. One was from an AWWARF (American Water Works Association Research Foundation) project directed by Finch and co-workers at the University of Alberta (UA) with demand-free buffer in a batch reactor (Finch *et al.* 2001*b*), which was the same dataset used in Li *et al.*'s study (2001*b*) but a different data processing approach was utilized. The second was from another AWWARF project conducted by Oppenheimer and co-workers at Montgomery Watson (MW) with a consortium of unfiltered and filtered water in a continuous stirred tank reactor (Oppenheimer *et al.* 2000). Both projects employed animal infectivity as the viability assay. For data at 13–15°C, the Chick model was determined to be the best-fit model for both sets of data. A *CT* value of 10.3 mg-min l^{-1} was required for 2-log inactivation from UA data and 4.6 mg-min l^{-1} *CT* value from MW data. Source waters were found to be a factor in ozone inactivation on *C. parvum* oocysts.

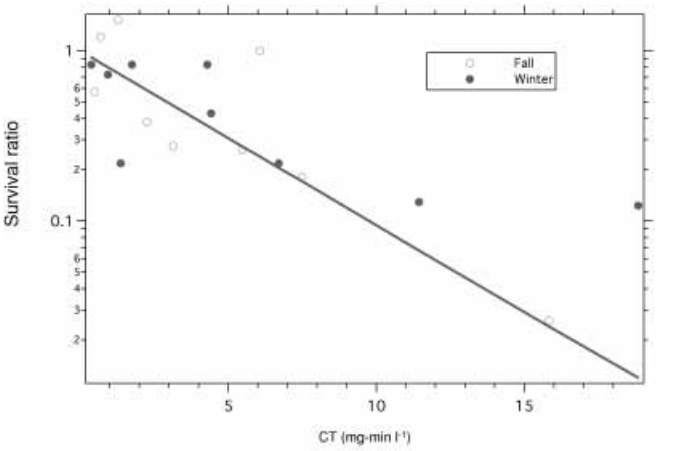

Figure 6 | Survival ratio of *C. parvum* oocysts vs. integrated *CT* values (*T* = 15°C).

In this study, plant effluent water was collected in the fall and the winter. The first-order Chick–Watson model was accepted to be the most appropriate model to describe the inactivation in both season's water at 15°C, with disinfection rate constant to be $k = 0.305$ min^{-1} for fall water and $k = 0.201$ min^{-1} for winter water. Use of a more complex model did not improve the statistical significance of the fit. The *CT* values extrapolated to 2-log inactivation at 15°C were determined to be 15.1 mg-min l^{-1} for fall data and 22.9 mg-min l^{-1} for winter data. By pooling all the data and analysing together, the first-order Chick–Watson model remained the most appropriate model to explain the pooled data with $k = 0.236$ min^{-1}, corresponding to a *CT* value of 19.5 mg-min l^{-1} for 2-log inactivation. Figure 6 displays the relation between survival ratios of *C. parvum* oocysts and integrated *CT* values from the first-order Chick–Watson model for the pooled data.

Compared with other studies with data available at 15 ± 2°C (Table 5), the data reported in this study give the most conservative *CT* values. This study used plant filtered water as the experimental water matrix and applied cell tissue culture (FDM-MPN) to assess the oocyst viability.

Table 5 | Studies used in *CT* values comparison (99% inactivation)

Study	*CT* (mg-min l^{-1})	Water type	Temperature (°C)	Viability assay	Reactor
This study	18.7	Plant effluent, pooled (fall and winter)	15	Cell tissue culture	Batch
Rennecker *et al.* (1999)	9.6	Demand-free buffer	15	*In vitro* excystation	Semi-batch
Oppenheimer *et al.* (2000)	4.644	Natural	15	Animal infectivity	CSTR
Li *et al.* (2001*b*)	11.8–12.4	Demand-free buffer	13 ± 0.5	Animal infectivity	Batch

All the other compared studies used animal infectivity or *in vitro* excystation as the assay methods. The Montgomery Watson study used filtered or unfiltered natural water as the water matrix and the others used buffered water. The source and age of oocysts has been noticed to affect oocyst infectivity and inactivation. The Iowa strain of oocysts utilized in all of the above studies was considered to be a more resistant strain than the Louisiana strain (Rennecker *et al*. 1999). Oocysts aged 30 days and older exhibited less infectivity when compared with younger oocysts (Slifko *et al*. 1999). Even though the oocyst age was not available from the studies used for direct comparison, the oocysts used in this study were aged between 6 and 47 days between shedding and testing and could be considered to have relatively high infectivity. The age of the oocysts used in the fall experiments was generally greater than that of those used in the winter experiments. This could be one of the reasons that ozone decayed faster and had higher initial demand in the fall water, especially in the case of 2 and 3 mg l^{-1} of ozone dose. Most of the other studies mentioned used the Indigo colorimetric method to determine ozone residual, except that Li *et al.* (2001*b*) used direct UV absorbance at 260 nm. Different ozone residual measurements could also cause variability in disinfection.

Sodium hypochlorite (0.0525% (v/v)) was used to pretreat oocysts in order to eliminate contamination of the tissue cultures and to trigger excystation (Rose *et al*. 2002). A synergistic effect was observed on sequential inactivation of *C. parvum* oocysts with ozone and chlorine, with ozone and monochloramine, as well as with chlorine dioxide followed by chlorine/monochloramine (Driedger *et al*. 2000, 2001; Rennecker *et al*. 2000, 2001; Li *et al*. 2001*a*; Corona-Vasquez *et al*. 2002*a*, 2002*b*). The parallel study conducted by Slifko (2001) indicated that treatment of disinfected oocysts by dilute sodium hypochlorite did not enhance *C. parvum* inactivation with UV and chlorine dioxide. Further studies are needed to determine whether the bleach pretreatment has an effect on *C. parvum* oocysts inactivation with ozone.

Clark *et al.* (2002) developed a *CT* equation based on a simple Chick–Watson model and data reported by Rennecker *et al.* (1999), Li *et al.* (2000), Oppenheimer *et al.* (2000) and Owen *et al.* (2000). A safety factor was incorporated in the equation for estimating *CT* corresponding to a 90% level of confidence. Using that equation for this study at 15°C, the *CT* value would be 18.72 mg-min l^{-1}, which is close to the predicted *CT* value for pooled data. Using a higher level of confidence of 95%, Clark's *CT* value was recalculated as 22.78 mg-min l^{-1}, which is very close to the *CT* value obtained for winter water in this study.

CONCLUSIONS

Philadelphia Baxter Plant filtered effluent water was used as the water matrix for ozone disinfection of *C. parvum* oocysts. The water quality of the fall water and the winter water was similar. Even though ozone decayed faster in the fall water than in the winter water, the disinfection kinetics of ozone inactivation of *C. parvum* oocysts was not significantly different for inactivation experiments

performed in both waters at 15°C. The first-order Chick–Watson model was accepted to be the most appropriate model to explain both the separated data (fall and winter) and the pooled data. According to the first-order Chick–Watson model, the *CT* value for 99% inactivation was determined to be 19.5 mg-min l^{-1}, which is larger than the *CT* values reported in other studies. This disparity could be caused by the source of water matrix, oocysts viability assay method, oocyst age and even ozone residual measurement.

In the disinfection experiments conducted in this study, 2-log inactivation of *C. parvum* oocysts could not be achieved using ozone at 15°C and pH 7 in the filtered drinking water.

ACKNOWLEDGEMENTS

The authors thank the Philadelphia Water Department for providing financial support for this investigation. Baxter Plant in Philadelphia provided all the filtered water used for this study. The authors also acknowledge Gary Burlingame of the Philadelphia Water Department for his good advice and Dr Joan B. Rose of Michigan State University for the technical support with the cell tissue culture method. The primary author thanks Jason Sites and Tim Bartrand for their kindly help and support.

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First received 11 June 2003; accepted in revised form 1 February 2004