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Measurement of OH radical CT for inactivating *Cryptosporidium parvum* using photo/ferrioxalate and photo/TiO_2 systems

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Abstract

Aim: This study investigates the inactivation of *Cryptosporidium parvum* using the OH radical and reports the OH radical CT (OH radical concentration × contact time) values for *C. parvum* inactivation.

Methods and Results: Although a wealth of information has demonstrated the efficacy of the microbial inactivation activity of the OH radical, no studies have performed a quantitative estimation of the OH radical for *C. parvum* inactivation. The CT value of the OH radical required for 2 log *C. parvum* inactivation was measured with two OH radical-generating systems, photo/ferrioxalate and photo/TiO_2. The OH radical was approx. 10^4–10^7-fold more effective for microbial inactivation than other popular chemical disinfectants such as ozone, chlorine dioxide and free chlorine.

Conclusions: The OH radical appears to be suitable for microbial inactivation with a calculated CT value required for 2 log *C. parvum* inactivation of $9{\cdot}3 \times 10^{-5}$ mg min l^{-1}.

Significance and Impact of the Study: This study is the first report of an investigation on the role of the OH radical in the photo/ferrioxalate and photo/TiO_2 systems and on the OH radical CT required for *C. parvum* inactivation.

Introduction

Concern has been increasing in recent years about the inactivation of pathogenic micro-organisms in water treatment systems. These pathogenic micro-organisms cause frequent microbial outbreaks in the world (Roach *et al.* 1993; Craunm *et al.* 1998; Schoenen 2002; Parshionikar *et al.* 2003; Ali 2004) and various human diseases. To inactivate these pathogens, many drinking water systems employ chlorine-based disinfectants. However, previous studies have reported that they did not effectively disinfect several chlorine-resistant micro-organisms, such as protozoan parasites (*Cryptosporidium parvum*, etc.), without excess formation of disinfection byproducts (Finch *et al.* 1993; EPA Guidance Manual 1999; Driedger *et al.* 2000; Corona-Vasquez *et al.* 2002).

Ozone, chlorine dioxide and ultraviolet (UV) irradiation have been extensively investigated as alternate disinfectants for controlling chlorine-resistant micro-organisms because of strong-oxidizing capacity or biocidal irradiation (EPA Guidance Manual 1999; Shin *et al.* 2001; Young and Setlow 2003). However, only few studies on the microbial inactivation behaviours of the OH radical have been conducted, despite the powerful oxidation potential of this radical (Wolfe *et al.* 1989; Sjogren and Sierka 1994; Wei *et al.* 1994; Kikuchi *et al.* 1997; Cho *et al.* 2005).

The OH radical, popular for recalcitrant chemical degradation, is one of the most effective oxidants (oxidation potential: OH radical 2·70 V; ozone 2·07 V; chlorine dioxide 1·91 V; free chlorine 1·36 V: Cho *et al.* 2004b). Previous studies have also demonstrated the possible activity

of the OH radical in microbial inactivation with diverse OH radical-generating systems (Watts *et al.* 1995; Wei *et al.* 1994; Guittonneau *et al.* 1996; Hislop and Bolton 1999; Elovitz *et al.* 2000; Cho *et al.* 2003, 2004b). For example, the biocidal action of the OH radical with the Peroxone (O_3/H_2O_2) system and TiO_2 photo-catalysis was reported (Wolfe *et al.* 1989; Ireland *et al.* 1993). Recently, our research group conducted quantitative studies reporting the OH radical CT (OH radical concentration × contact time) values for *Escherichia coli* inactivation, using photo/ferrioxalate reaction and photo/TiO_2 reaction (Cho *et al.* 2004a,b), and for *Bacillus subtilis* spore (*B. subtilis* spore) inactivation using high pH/ozone (Cho *et al.* 2003). However, despite the tremendous interest in *C. parvum* control in water treatment system, few studies have investigated the inactivation of *C. parvum* using the OH radical with quantitative evaluation (Curtis *et al.* 2002).

This study therefore attempted to quantitatively estimate the ability of the OH radical to inactivate *C. parvum*. The objective of this research was to investigate the effectiveness and the role of the OH radical for inactivating *C. parvum* and to determine its CT values using the disinfection kinetics model, in the application of two different OH radical-generating systems: photo/ferrioxalate/H_2O_2 at near neutral pH and photo/TiO_2. In addition, the determined OH radical CT values for inactivating *C. parvum* were compared with that for *B. subtilis* spores, which is a popular protozoa indicator micro-organism, and the results are discussed in comparison with other well-known chemical disinfectants such as ozone, free chlorine and chlorine dioxide.

Materials and methods

Reagents and experimental equipment

All glassware was washed with distilled water (Barnstead Co., lowa, USA) and autoclaved at 121°C for 15 min before use. Chemical reagents were filtered through a membrane filter (MFS, 0·22 μm, etc.) to remove any bacteria.

In the photo/ferrioxalate/H_2O_2 system, a stock solution of Fe(III) (5 mmol l^{-1}), oxalate (300 mmol l^{-1}) and H_2O_2 (35%) was prepared as previously described (Cho *et al.* 2004b). TiO_2 powder (Degussa P25), a mixture of anatase and rutile (8 : 2), was used as a photocatalyst in the photo/TiO_2 system (Cho *et al.* 2004a). Fe(III), oxalate, H_2O_2 and pCBA (*para*-chloro benzoic acid, an OH radical probe compound) are analysed as previously described (Cho *et al.* 2003, 2004a,b, 2005). In the two types of photo systems, the light source was provided by four or fewer black light blue lamps (BLB; 18 W; wavelength emission: 300–420 nm, Philips Co., Eindhoven, the Netherlands), which were placed on each side of the reactor, and the light intensity was measured by ferrioxalate actinometry (Hatchard and Parker 1956). The reaction temperature was controlled with a thermostatic chamber (20°C, Jeio Tech Co., Seoul, Korea) and the pH was adjusted using a phosphate buffer solution (KH_2PO_4 + NaOH).

Preparation and analysis of *C. parvum* and *B. subtilis* spores

Cryptosporidium parvum, purified by sucrose and percoll gradients in bovine hosts, was prepared by purchasing the commercial stock solution (viable $1{\cdot}0 \times 10^7$ oocysts in 8 ml; Waterborne Co., Louisiana, USA). The *C. parvum* stock was used within 2 months of purchase. Immediately before the experiments, the oocysts were washed twice by centrifugation with a 1·6-ml micro-centrifuge tube containing 1·0 ml of stock solution at 11 300 ***g*** for 10 min and re-suspended in 10 mmol l^{-1} phosphate buffer solution. The initial concentration of oocysts in disinfection was adjusted to $3{\cdot}1 \times 10^3$ oocysts ml^{-1} with dilution up to 1/400.

Although animal models or cell culture assays are more preferred for the measurement of *C. parvum* infectivity, the *in vitro* excystation was chosen in this study, as described by Jenkins *et al.* (1998), since in addition to limited resources, the *in vitro* excystation is one of the methods widely used for determining the *C. parvum* inactivation (Driedger *et al.* 2000; Corona-Vasquez *et al.* 2002).

The analytical procedures of *in vitro* excystation consisted of centrifugation, followed by washing the sample solution with acidified Hanks' balanced salt solution and incubating it at 37°C for 1 h in dark condition. The incubated solution was washed three times and then 0·01 ml of 1% sodium deoxycholate in Hank's minimal essential medium and 0·01 ml of 2·2% sodium bicarbonate in HBSS were added to each sample. The vortex, mixed suspension was incubated at 37°C for 3·5 h in the dark, washed and then left at room temperature for 0·5 h. After washing with 1 ml of DABCO-phosphate buffer saline (PBS), 500 oocysts were assayed at each sampling time using a microscope (80i Nikon Tokyo, Japan) containing conventional DIC and epi-fluorescence mode. Preparations were kept on ice until the counts were completed. The percentage of excysted oocysts was determined by eqn (1) (Finch *et al.* 1993; Korich *et al.* 1990).

$$\%\ \text{Excystation} = \frac{\text{No. excystated oocysts}}{\text{No. intact oocysts} + \text{No. excystated oocysts}} \times 100 \quad (1)$$

For comparison experiments, spore suspensions of *B. subtilis* (ATCC 6633) were prepared as previously

described (Nakayama *et al.* 1996; Cho *et al.* 2002, 2003). The spores were harvested by centrifuging and re-suspending in 50 ml of PBS (pH 7·0) and quantified by the spread plate method with nutrient agar grown at 37°C for 24 h (Facile *et al.* 2000; Radziminski *et al.* 2002). In several experiments using *B. subtilis* spore, heat treatment (80°C, 15 min) before the experiments was conducted to inactivate the vegetative *B. subtilis* which have not formed spore yet. The ratio of *B. subtilis* spore was over 99% and the initial concentration of *B. subtilis* spore in disinfection study was adjusted to 3×10^5 CFU ml^{-1}. In control test, no significant inactivation of *C. parvum* and *B. subtilis* spore was observed by chemicals or BLB light itself.

Experimental procedures

Table 1 presents the experimental details for the two OH radical-generating systems, photo/ferrioxalate and photo/TiO_2, and the general experimental procedures were carried out as previously described (Cho *et al.* 2004b): photo/ferrioxalate system; Cho *et al.* 2004a, 2005; photo/TiO_2). All experiments were carried out in a 60-ml Pyrex reactor (UV cut-off <300 nm) with 50 ml of solution (Lee *et al.* 2003; Cho *et al.* 2004b). In the photo/ferrioxalate system, the required solution was prepared by adding the appropriate amounts of H_2O_2, Fe^{3+}, oxalate, buffer and *C. parvum* or *B. subtilis* spores. The suspensions for photo/TiO_2 experiments were prepared by adding the presonicated TiO_2 stock (10 g l^{-1}) in 50 ml of pH-controlled reaction solution (pH 7·1), which contained *C. parvum* or *B. subtilis* spores. The photolysis experiments were started by immediately inserting the Pyrex reactor in front of the lamps. To generate the required varying amounts of OH radical formation, diverse experimental conditions were employed (Table 1). One millilitre of solution was withdrawn at each sampling time including zero time ($T = 0$) from the reactor and then microbial assay was conducted.

The OH radical was scavenged when necessary using 30 mmol l^{-1} of t-BuOH as previously described (Cho *et al.* 2004b) for the photo/ferrioxalate/H_2O_2 system. MeOH (30 mmol l^{-1}) was used to scavenge free and surface radicals (Kim and Choi 2002) in the photo/TiO_2 system.

Comparison experiments of microbial inactivation were additionally performed with ozone, free chlorine and chlorine dioxide. Preparation and measurement of the oxidants and experimental procedures were carried out as previously described (Cho *et al.* 2006). The disinfection experiments commenced when concentrated oxidant was added to the prepared solution (50 ml), containing buffer (pH 7·1, 10 mmol l^{-1}) and micro-organisms, to attain the required concentration of oxidants. Five to seven samples (1 ml) were introduced into 1·6 ml micro-tubes, each containing oxidant-quenching reagent (1 mmol l^{-1} $Na_2S_2O_3$) to stop the inactivation reaction, and the microbial assays for *C. parvum* and *B. subtilis* spores were conducted with *in vitro* excystation and spreading plate method, respectively.

A control was prepared for each disinfection experiment by injecting *C. parvum* into 10 ml of 10 mmol l^{-1} phosphate buffer for the duration of the experiment. Sampled oocysts at each sampling time were assayed by *in vitro* excystation and the percentage of excystation was calculated (% excystation$_{control}$).

Table 1 Experimental conditions and determined concentrations of the OH radical

		Exp. ID	$[H_2O_2]_0$ (mmol l^{-1})	$[Fe^{3+}]_0$ (mmol l^{-1})	Light intensity (Einstein l^{-1} s^{-1})	$[TiO_2]_0$ (g l^{-1})	Scavenger* (m mol l^{-1})	Exp. set	No. of samples	$[\cdot OH]_{ss}$ (mg l^{-1})
Photo/ferrio-xalate	*Cryptosporidium parvum*	1	2·0	0·1	$3{\cdot}4 \times 10^{-6}$	–	–	2	11	$1{\cdot}02 \times 10^{-7}$
		2	2·0	0·1	$7{\cdot}9 \times 10^{-6}$	–	–	3	24	$2{\cdot}89 \times 10^{-7}$
		3	2·0	0·5	$7{\cdot}9 \times 10^{-6}$	–	–	2	12	$4{\cdot}59 \times 10^{-7}$
		4	2·0	0·1	$7{\cdot}9 \times 10^{-6}$	–	+†	2	14	–
	Bacillus subtilis spore	5	2·0	0·1	$3{\cdot}4 \times 10^{-6}$	–	–	2	9	$1{\cdot}02 \times 10^{-7}$
		6	2·0	0·1	$7{\cdot}9 \times 10^{-6}$	–	–	3	24	$2{\cdot}89 \times 10^{-7}$
		7	2·0	0·5	$7{\cdot}9 \times 10^{-6}$	–	–	2	12	$4{\cdot}59 \times 10^{-7}$
Photo/TiO_2	*C. parvum*	8	–	–	$7{\cdot}9 \times 10^{-6}$	0·1	–	1	6	$3{\cdot}30 \times 10^{-8}$
		9	–	–	$7{\cdot}9 \times 10^{-6}$	0·5	–	1	6	$4{\cdot}62 \times 10^{-8}$
		10	–	–	$7{\cdot}9 \times 10^{-6}$	1·0	–	1	5	$7{\cdot}14 \times 10^{-8}$
		11	–	–	$7{\cdot}9 \times 10^{-6}$	1·0	+	1	6	–
	B. subtilis spore	12	–	–	$7{\cdot}9 \times 10^{-6}$	0·1	–	2	12	$3{\cdot}30 \times 10^{-8}$
		13	–	–	$7{\cdot}9 \times 10^{-6}$	0·5	–	2	10	$4{\cdot}62 \times 10^{-8}$
		14	–	–	$7{\cdot}9 \times 10^{-6}$	1·0	–	2	10	$7{\cdot}14 \times 10^{-8}$

*Photo/ferrioxalate system: 30 mmol l^{-1} of t-BuOH, photo/TiO_2 system: 30 mmol l^{-1} of MeOH.
† '+' indicates the presence of scavenger.

Measurement of steady-state OH radical concentration

The steady-state OH radical concentration was measured based on the competitive reactivity of the OH radical between the OH radical probe compound, such as pCBA in this study, and all the organic and inorganic OH radical scavengers present, ΣS_i, in each system (Cho *et al.* 2004a,b). If pCBA, as the OH radical probe compound, is present only as a trace component (1·9 μmol l^{-1} in this study) and if the ΣS_i of the photo/ferrioxalate system remains constant, the decay of pCBA in the photo-ferrioxalate system can be expressed as

$$-\frac{d[pCBA]}{dt} = k_{exp}[pCBA] \quad (2)$$

where: $k_{exp} = k_{OH,\,pCBA} \times [\text{OH radical}]_{ss}$ ($k_{OH,\,pCBA} = 5{\cdot}0 \times 10^9$ mol l^{-1}s^{-1}), if the pseudo-steady-state assumption for the OH radical concentration is valid.

Integration of eqn (2) yields:

$$-\ln\frac{[pCBA]}{[pCBA]_0} = k_{exp}\,t. \quad (3)$$

Hence, the steady-state OH radical concentration ([OH radical]$_{ss}$) can be obtained from eqn (2). The pseudo-steady-state assumption for the OH radical was confirmed by the constant degradation of pCBA under all experimental times.

Application of disinfection kinetic model

A disinfection kinetic model is usually applied to compare the results obtained from the different experimental conditions (Haas *et al.* 1995). In this study, the inactivation ratio (log scale) was calculated from the rate of excystation (Finch *et al.* 1993) and the delayed Chick–Watson model was applied to determining the CT value.

For *C. parvum*

$$\text{Inactivation ration (log unit)} = \log\left(\frac{\%E_t}{\%E_c}\right) = \begin{pmatrix} 0 & \text{if } \overline{C}T \le CT_{lag} = \frac{1}{k}\log\left(\frac{\%E_t}{\%E_c}\right) \\ -k(\overline{C}T - \overline{C}T_{lag}) & \text{if } \overline{C}T \ge CT_{lag} = \frac{1}{k}\log\left(\frac{\%E_t}{\%E_c}\right) \end{pmatrix}. \quad (4)$$

For *B. subtilis* spore

$$\text{Inactivation ration (log unit)} = \log\left(\frac{N}{N_0}\right) = \begin{pmatrix} 0 & \text{if } \overline{C}T \le CT_{lag} = \frac{1}{k}\log\left(\frac{N_t}{N_0}\right) \\ -k(\overline{C}T - \overline{C}T_{lag}) & \text{if } \overline{C}T \ge CT_{lag} = \frac{1}{k}\log\left(\frac{N_t}{N_0}\right) \end{pmatrix}, \quad (5)$$

where $\%E_t$: % excystation of disinfected *C. parvum* at time T; $\%E_c$: % excystation of control *C. parvum* at time T; N_0: initial *B. subtilis* spore population (CFU ml^{-1}); N: viable *B. subtilis* spore population at time T (CFU ml^{-1}); $\overline{C} = \int_0^t C dt/t$: time averaged disinfectant concentration (mg l^{-1}); T: reaction time (min); k: inactivation rate constant by disinfectant (l mg^{-1} min^{-1}); $\overline{C}T_{lag}$: intercept of the inactivation curve in x axis.

We have previously reported the delayed Chick–Watson model to be suitable for successfully demonstrating the inactivation kinetics, including the shoulder region (Cho *et al.* 2003, 2004a,b). The statistical estimation was achieved by using the Solver add-in function in MICROSOFT® EXCEL® XP software.

Results

The role of the OH radical

Figure 1 presents that the OH radical generated by the two photolysis systems is actively involved in inactivating *C. parvum*. In Fig. 1a, OH radical treatment by the photo/ferrioxalate system achieved a 1·8 log *C. parvum* inactivation after 300 min. This inactivation was clearly achieved solely by the OH radical as no inactivation of *C. parvum* was observed in the photo/ferrioxalate system as confirmed by the excess presence of the well-known OH radical scavenger, t-BuOH (Cho *et al.* 2004b). Figure 1b shows that the inactivation performance of *C. parvum* by the photo/TiO_2 system was much slower than that by the photo/ferrioxalate system. In addition, it was noteworthy that as the OH radical scavenger (methanol) was applied, approximately one-quarter of the inactivation was still being made, indicating that reactive oxygen species (ROS) other than the OH radical are responsible for the minor inactivation of *C. parvum*.

Effect of OH radical concentration

From using eqn (2) and $[\text{OH radical}]_{ss} = k_{exp}/k_{OH,pCBA}$, the steady-state concentration of the OH radical can be obtained in diverse OH radical-generating systems. The OH radical concentration in almost all of the experimental conditions is summarized in Table 1. Figure 2 and Table 1 show that higher OH radical concentrations increased the inactivation of the *C. parvum* in the photo/ferrioxalate system. A good square-root dependence ($R^2 = 0{\cdot}92$) was found between the time required for 0·8 log *C. parvum* inactivation (Fig. 2) and the OH radical concentration (Table 1). A similar behaviour was observed in the photo/TiO_2 system (data not shown).

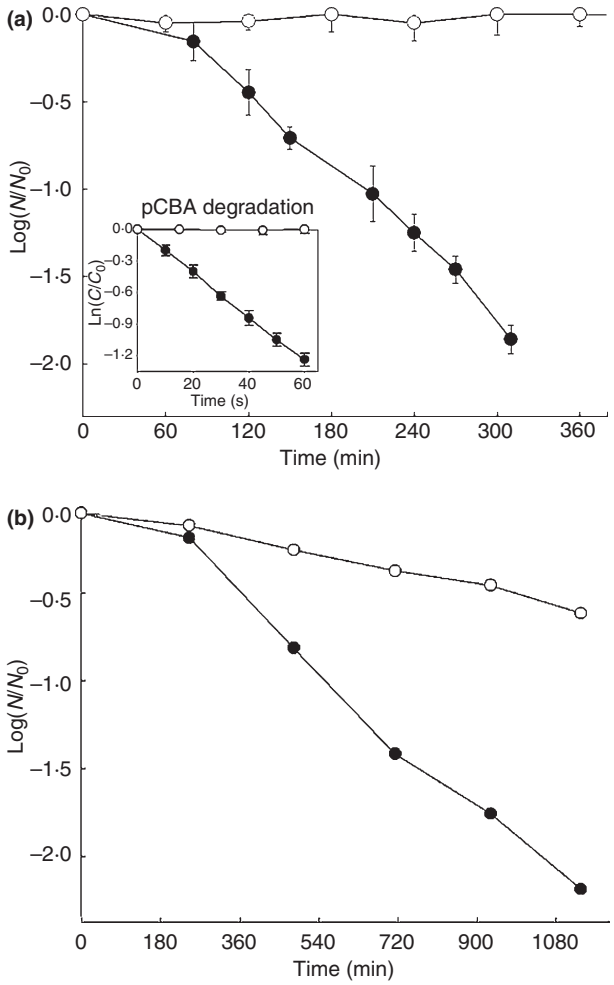

Figure 1 Role of the OH radical for inactivating *C. parvum* in two OH radical generating systems ((a) Photo/ferrioxalate system, (b) photo/TiO_2 system).

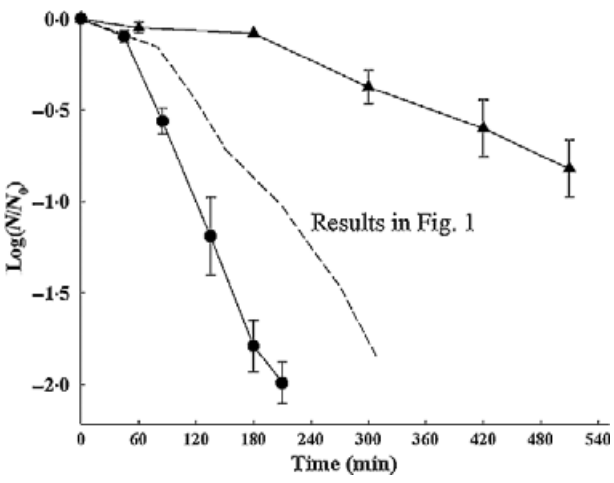

Figure 2 Inacivation of *C. parvum* in three OH radical concentrations with the photo/ferrioxalate sysem.

Determination of the OH radical CT values required for *C. parvum* inactivation

In the two experimental systems of this study, the OH radical CT was quantified by using the steady-state OH radical concentration and *C. parvum* inactivation kinetics, as previously described (Cho *et al.* 2004a,b). By utilizing the delayed Chick–Watson model [eqn (4)], the inactivation predicted by the model in these two diverse systems was observed with the measured inactivation of *C. parvum* (Fig. 3). As shown in Fig. 3, the delayed Chick–Watson model exhibited excellent applicability with high R^2 values of 0·97 in the photo/ferrioxalate system and 0·96 in the photo/TiO_2 system. From Fig. 3a, the OH radical CT values for achieving 2 log *C. parvum* inactivation were $9{\cdot}3 \times 10^{-5}$ and $7{\cdot}9 \times 10^{-5}$ mg min l^{-1} from the photo/ferrioxalate and photo/TiO_2 systems, respectively.

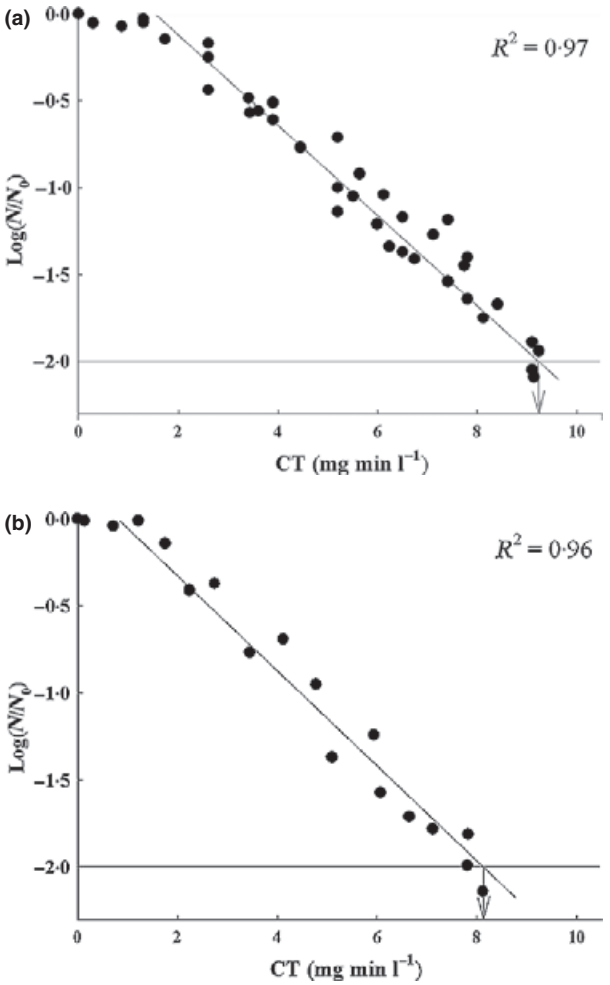

Figure 3 Determination of CT values of the OH radical for inactivating *C. parvum* from the delayed Chick-Watson model ((a) Photo/ferrioxalate system, (b) photo/TiO_2 system).

Discussion

Table 2 summarizes the OH radical CT values required for inactivating *C. parvum*. For comparison study, additional experiments were conducted for inactivating *B. subtilis* spores using the two systems (Fig. 4) and the reported OH radical CT values for *E. coli* and *B. subtilis* spore inactivation were included from our previous studies (Cho *et al.* 2003, 2004a,b).

Table 2 OH radical CT comparison between two OH radical-generating systems (for 2 log inactivation)

	CT (mg min l^{-1}) for 2 log inactivation		
System	*Cryptosporidium parvum*	*Bacillus subtilis* spore	*Escherichia coli*
Photo/ferrioxalate	$9{\cdot}3 \times 10^{-5}$	$8{\cdot}2 \times 10^{-5}$	$1{\cdot}5 \times 10^{-5}$*
Photo/TiO_2	$7{\cdot}9 \times 10^{-5}$	$7{\cdot}2 \times 10^{-5}$	$0{\cdot}8 \times 10^{-5}$†

*Cho *et al.* (2004b); †Cho *et al.* (2004a).

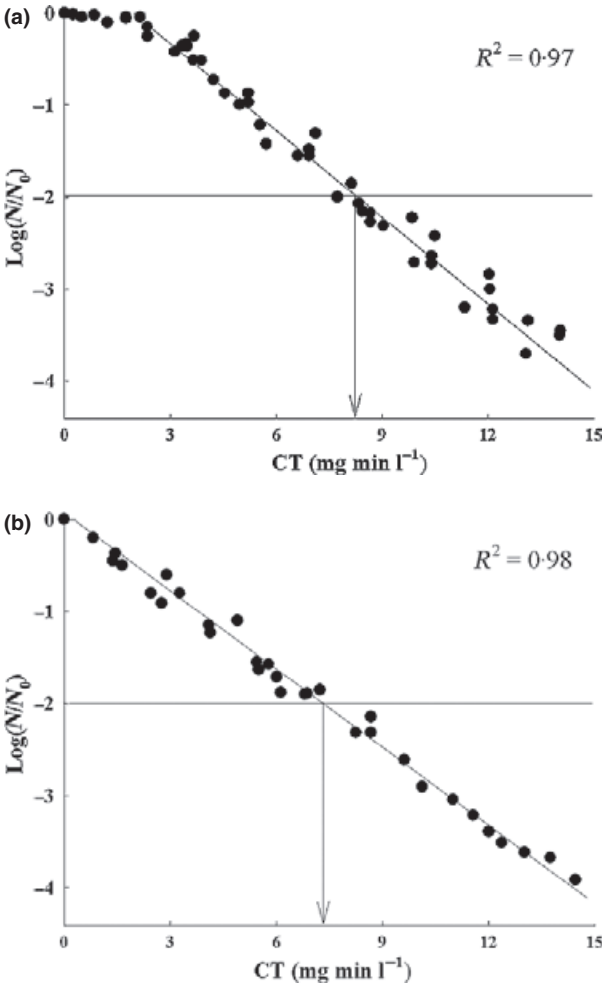

Figure 4 Determination of CT values of the OH radical for inactivating *B. subtilis* spores from the delayed Chick-Watson model ((a) Photo/ferrioxalate system, (b) photo/TiO_2 system).

As shown in Table 2, the CT value for inactivating *C. parvum* in the photo/ferrioxalate system was larger than that in the photo/TiO_2 system. This finding can be explained by the role of various ROS agents (OH radical, $O_2^{-\cdot}$ and H_2O_2) in the photo/TiO_2 system, as, as previously indicated in Fig. 1b, ROS other than the OH radical are responsible for a quarter of the *C. parvum* inactivation (24%). This tendency was found in the inactivation of *B. subtilis* spores and *E. coli* (Table 2; Cho *et al.* 2004a).

In addition, the OH radical CT values were similar between *C. parvum* and *B. subtilis* spores. For example, in the photo/ferrioxalate system, the required CT values for 2 log inactivation of *C. parvum* and *B. subtilis* spores were $9{\cdot}3 \times 10^{-5}$ mg min l^{-1} and $8{\cdot}2 \times 10^{-5}$ mg min l^{-1}, respectively. These findings support the suitability of *B. subtilis* spores as a protozoa indicator for *C. parvum*.

It should be noted that the CT values of the OH radical are based on the assumed absence of any synergistic effect between the OH radical and the other disinfectants. However, as previously mentioned in the photo/TiO_2 system, a complex interrelated influence among several oxidants, including a synergy effect and interferences, is to be expected. Such effects render difficult the exact prediction of CT values, based only on the OH radical, in various OH radical-generating systems. Unlike other systems, the OH radical was observed to play a role only for *C. parvum* inactivation in the photo/ferrioxalate system. This suggests that the results presented in Table 2 for the photo/ferrioxalate system would be the most accurate data source for determining the OH radical CT value, which was calculated to be $9{\cdot}3 \times 10^{-5}$ mg min l^{-1} for 2 log inactivation.

The CT values of several popular disinfectants that are required to achieve 2 log inactivation of *C. parvum*, *B. subtilis* spores and *E. coli* (Cho *et al.* 2003, 2004a,b) were determined in this study and are summarized in Table 3

Table 3 Comparison of the CT (mg min l^{-1}) values of four important disinfectants for the 2 log inactivation of *Cryptosporidium parvum*, *Bacillus subtilis* spores and *Escherichia coli* at pH 5·8 and 20°C

	OH radical*	Ozone	Chlorine dioxide	Free chlorine
C. parvum	$9{\cdot}3 \times 10^{-5}$	3·5	70	4200
B. subtilis spores	$8{\cdot}2 \times 10^{-5}$	4·1	50	450
E. coli	$1{\cdot}5 \times 10^{-5}$†	$6{\cdot}0 \times 10^{-3}$	$8{\cdot}0 \times 10^{-2}$†	$1{\cdot}3 \times 10^{-1}$
Oxidation potential (V)	2·70	2·07	1·91	1·36

*Results using the photo/ferrioxalate system.
†Cho *et al.* (2004a).

to present a comparison with the OH radical CT values. The CT values of the OH radical for 2 log inactivation of *C. parvum* were $3{\cdot}8 \times 10^4$, $7{\cdot}5 \times 10^5$ and $4{\cdot}5 \times 10^7$-fold smaller than those of ozone, chlorine dioxide and free chlorine, respectively. This result reflects the overall proportional relationship between the inactivating ability and the oxidation potential of the tested disinfectants (Table 3). *Bacillus subtilis* spores and *E. coli* exhibited a similar tendency (Table 3). These remarkably small OH radical CT values confirm the potential of the OH radical as a significantly powerful agent for microbial inactivation.

On the other hand, the OH radical CT values did not differ markedly between *C. parvum* and *E. coli*, when compared with the wide variation in the CT values of ozone, chlorine dioxide and free chlorine. The OH radical CT value required for 2 log inactivation of *C. parvum* obtained from the photo/ferrioxalate system was only 6·2 fold more resistant to that of *E. coli*. While, in the case of free chlorine, the resistance for *C. parvum* inactivation was $3{\cdot}2 \times 10^4$-fold greater than that for *E. coli* (Table 3). This observation clearly reflects the extremely nonselective and oxidative properties of the OH radical in its reaction with organic compounds.

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References

Ali, S. H. (2004) A socio-ecological autopsy of the *E. coli* O157:H7 outbreak in Walkerton, Ontario, Canada. *Soc Sci Med* **58**, 2601–2612.

Cho, M., Chung, H. and Yoon, J. (2002) Effect of pH and importance of ozone initiated radical reactions in inactivating *Bacillus subtilis* spore. *Ozone Sci Eng* **24**, 145–150.

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

Cho, M., Chung, H., Choi, W. and Yoon, J. (2004a) Linear correlation between inactivation of *E. coli* and OH radical concentration in TiO_2 photocatalytic disinfection. *Wat Res* **38**, 1069–1077.

Cho, M., Chung, H. and Yoon, J. (2004b) Inactivation of *Escherichia coli* by photochemical reaction of ferrioxalate at slightly acidic and near neutral pH. *Appl Environ Microbiol* **70**, 1129–1134.

Cho, M., Chung, H., Choi, W. and Yoon, J. (2005) Different inactivation behaviors of MS-2 phage and *Escherichia coli* in TiO_2 photocatalytic disinfection. *Appl Environ Microbiol* **71**, 270–275.

Cho, M., Kim, J. and Yoon, J. (2006) Investigating synergism during sequential inactivation of *Bacillus subtilis* spores with several disinfectants. *Wat Res* **40**, 2911–2920.

Corona-Vasquez, B., Rennecker, J.L., Driedger, A.M. and Mariñas, B.J. (2002) Sequential inactivation of *Cryptosporidium parvum* oocysts with chlorine dioxide followed by free chlorine or monochloramine. *Wat Res* **36**, 178–188.

Craunm, G.F., Hubbs, S.A., Frost, F., Calderon, R. and Via, S. (1998) Waterborne outbreaks of cryptosporidiosis. *J Am Wat Works Assoc* **90**, 81–91.

Curtis, T.P., Walker, G., Dowling, B. M. and Christensen, P.A. (2002) Fate of *Cryptosporidium* oocysts in an immobilized titanium dioxide reactor with electric field enhancement. *Wat Res* **36**, 2410–2413.

Driedger, A.M., Rennecker, J.L. and Mariñas, B.J. (2000) Sequential inactivation of *Cryptosporidium parvum* oocysts with ozone and free chlorine. *Wat Res* **34**, 3591–3597.

Elovitz, M.S., von Gunten, U. and Kaiser, H.P. (2000) Hydroxyl radical/ozone ratios during ozonation processes. II. The effect of temperature, pH, alkalinity, and DOM properties. *Ozone Sci Eng* **22**, 123–150.

EPA Guidance Manual. (1999) *Alternative Disinfectants and Oxidants Guidance Manual*. US EPA 815-R-99-014, Washington, DC: Environmental Protection Agency.

Facile, N., Barbeau, B., Prevost, M. and Koudjonou, B. (2000) Evaluating bacterial aerobic spores as a surrogate for *Giardia* and *Cryptosporidium* inactivation by ozone. *Wat Res* **34**, 3238–3246.

Finch, G.R., Black, E.K., Gyurek, L. and Belosevic, M. (1993) Ozone inactivation of *Cryptosporidium parvum* in demand-free phosphate buffer determined by In Vitro Excystation and Animal Infectivity. *Appl Environ Microbiol* **59**, 4203–4210.

Guittonneau, S., Thibaudeau, D. and Meallier, P. (1996) Free radicals formation induced by the ozonation of humic substances in aqueous medium. *Catalysis today* **29**, 323–327.

Haas, C.N., Joffe, J., Anmangandla, U., Hornberger, J.C., Heath, M.S., Jacangelo, J. and Glicker, J. (1995) *Development and Validation of Rational Design Methods of Disinfection*. Denver, CO: American Water Works Association (AWWA) Research Foundation.

Hatchard, C. G. and Parker, C. A. (1956) A new sensitive chemical actinometer. II. Potassium ferrioxalate as a standard chemical actinometer. *Proc R Soc Lond A* **235**, 518–536.

Hislop, K.A. and Bolton, J.R. (1999) The photochemical generation of hydroxyl radicals in the UV-vis /Ferrioxalate.H_2O_2 system. *Environ Sci Technol* **33**, 3119–3126.

Ireland, J.C., Klostermann, P., Rice, E.W. and Clark, R.M. (1993) Inactivation of *Escherichia coli* by titanium dioxide photocatalytic oxidation. *Appl Environ Microbiol* **59**, 1668–1670.

Jenkins, M.B., Bowman, D.D. and Ghiorse, W.C. (1998) Inactivation of *Cryptosporidium parvum* oocsyts by ammonia. *Appl Environ Microbiol* **64**, 784–788.

Kikuchi, Y., Sunada, K., Iyada, T., Hashimoto, K. and Fujishima, A. (1997) Photocatalytic bactericidal effect of TiO_2 thin films: dynamic view of the active oxygen species responsible for the effect. *J Photochem Photobiol A Chem* **60**, 51–56.

Kim, S. and Choi, W. (2002) Kinetics and Mechanisms of Photocatalytic degradation of $(CH_3)_nNH_{4-n}^+$ $(0 \le n \le 4)$ in TiO_2 suspension: the role of OH radicals. *Environ Sci Technol*. **36**, 2019–2025.

Korich, D.G., Mead, J.R., Madore, M.S., Sinclair, N.A. and Sterling, C.R. (1990) Effect of ozone, chlorine dioxide, chlorine, and monochloramine on *Cryptosporidium parvum* oocyst viability. *Appl Environ Microbiol* **56**, 1423–1428.

Lee, Y.H., Jeong, J.S., Lee, C.H. and Yoon, J. (2003) Influence of various reaction parameters on 2,4-D removal in photo/ferrioxalate/H_2O_2 process. *Chemosphere* **51**, 901–912.

Nakayama, A., Yano, Y., Kobayashi, S., Ishikawa, M. and Sakai, K. (1996) Comparison of pressure resistances of spores of six *Bacillus* strains with their heat resistances. *Appl Environ Microbiol* **62**, 3897–3900.

Parshionikar, S. U., Willian-True, S., Fout, G. S., Robbins, D. E., Seys, S. A., Cassady, J. D. and Harris, R. (2003) Waterborne outbreak of gastroenteritis associated with a norovirus. *Appl Environ Microbiol* **69**, 5263–5268.

Radziminski, C., Ballantyne, L., Hodson, J., Creason, R., Andrews, R.C. and Chauret, C. (2002) Disinfection of *Bacillus subtilis* spores with chlorine dioxide: a bench-scale and pilot-scale study. *Wat Res* **36**, 1629–1639.

Roach, P. D., Olson, M. E., Witley, G. and Wallis, P. M. (1993) Waterborne giardia cysts and *Cryptosporidium* oocysts in the Yukon, Canada. *Appl Environ Microbiol* **59**, 67–73.

Schoenen, D. (2002) Role of disinfection in suppressing the spread of pathogens with drinking water: possibilities and limitations. *Wat Res* **36**, 3874–3888.

Shin, G., Linden, K.G., Arrowood, M.J. and Sobsey, M.D. (2001) Low-pressure UV inactivation and DNA repair potential of *Cryptosporidium parvum* oocysts. *Appl Environ Microbiol* **67**, 3029–3032.

Sjogren, J.C. and Sierka, R.A. (1994) Inactivation of phage MS2 by iron-aided titanium dioxide photocatalysis. *Appl Environ Microbiol* **60**, 344–347.

Watts, R.J., Kong, S., Orr, M.P., Miller, G.C. and Henry, B.E. (1995) Photocatalytic inactivation of coliform bacteria and viruses in secondary wastewater effluent. *Wat Res* **29**, 95–100.

Wei, C., Lin, W., Zainal, Z., Zhu, N.E., Kruzic, K., Smith, R.L. and Rajeshwar, K. (1994) Bactericidal activity of TiO_2 photocatalyst in aqueous media: toward a solar-assisted water disinfection system. *Environ Sci Technol* **28**, 934–938.

Wolfe, R.L., Stewart, M.H., Scott, K.N. and McGuire, M.J. (1989) Inactivation of *Giardia muris* and indicator organisms seeded in surface water supplies by peroxone and ozone. *Environ Sci Technol* **23**, 744–745.

Young, S.B. and Setlow, P. (2003) Mechanisms of killing of *Bacillus subtilis* spores by hypochlorite and chlorine dioxide. *J Appl Microbiol* **95**, 54–67.