Inactivation of *Nitrosomonas europaea* and pathogenic *Escherichia coli* by chlorine and monochloramine

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ABSTRACT

The purpose of this study was to measure the chlorine and monochloramine inactivation kinetics of *Nitrosomonas europaea* at 21°C in the presence and absence of particles. The inactivation kinetics rates were compared with those obtained with *Escherichia coli* O157:H7. The results show that, in pure water, the use of free chlorine produced 4 $\log_{10}$ of *N. europaea* inactivation at a *CT* value of 0.8 mg.min l^{-1}, whereas monochloramine yielded 4 $\log_{10}$ of inactivation at *CT* values of approximately 9.9–16.4 mg.min l^{-1}. With *E. coli*, chlorine produced approximately 4 $\log_{10}$ of inactivation at a *CT* of 0.13 mg.min l^{-1}, whereas monochloramine resulted in 4 $\log_{10}$ of inactivation at a *CT* of approximately 9.2 mg.min l^{-1}. These results suggest that *N. europaea* is more resistant to monochloramine and chlorine than *E. coli*. Corrosion debris, soil material and wastewater had no statistically significant ($p < 0.05$) impact on the inactivation of *N. europaea* by either chlorine or monochloramine. It seems likely that the *CT* values present in distribution systems would be sufficient to control suspended cells of these two organisms, especially under conditions of breakpoint chlorination, which could be used to control nitrification. Adequate disinfection should prevent the growth of these organisms in a distribution system.

Key words | ammonia oxidizing bacteria, chlorine, drinking water, monochloramine

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INTRODUCTION

Treating drinking water to meet regulatory requirements at the treatment plant effluent does not necessarily ensure high quality water at the consumer's tap. During distribution, water quality degrades, which may lead to increased bacterial counts with possible occurrences of pathogens and pathogen indicators and depletion of disinfectant residual. The maintenance of a disinfectant residual in distribution systems has traditionally been used to protect microbial water quality. It is necessary to understand the reasons for the presence and survival of microorganisms in distribution systems despite the continuing presence of disinfectant residuals.

The use of monochloramine as a water disinfectant has led to some concerns regarding the growth of ammonia oxidizing bacteria (AOB) in distribution systems (Cunliffe 1991). For example, nitrifying bacteria were detected in 64% of samples from five chloraminated water supplies in Australia (Cunliffe 1991). In drinking water, AOB grow using free ammonia, which is associated with chloramination, as an electron donor and produce nitrite by oxidation. The presence of AOB in drinking water is a potential health risk mostly because nitrification degrades chloramines and leaves distributed water with a low disinfectant residual. This can lead to increased heterotrophic plate counts, coliform regrowth and water quality deterioration (Regan *et al.* 2002; Pintar & Slawson 2003). Accordingly, nitrification must be controlled in distribution systems or it may potentially lead to violations of various US regulations such as the Surface Water Treatment Rule and the Total Coliform Rule (Oldenburg *et al.* 2002).

Nitrification is carried out by two different types of bacteria. The first ones are the AOB, which oxidize ammonia to nitrite ($NH_4^+ + 1\frac{1}{2}\ O_2 \rightarrow NO_2^- + 2H^+ + H_2O$).

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Nitrite can then be converted to nitrate ($NO_2^- + \frac{1}{2}O_2 \rightarrow NO_3^-$) by nitrite-oxidizing bacteria (NOB). AOB are aerobic chemotrophic Gram-negative bacteria. Most of them are rod-shaped and found as single cells or in small clusters. Examples of AOB include members of the genera *Nitrosomonas* and *Nitrosospira*. In drinking water distribution systems, *Nitrosomonas oligotropha* appears to be the dominant AOB, as reported by Regan *et al.* (2002).

A few inactivation studies on AOB have been published. For example, Wolfe *et al.* (1990) reported that 2 log of AOB was inactivated by a monochloramine dose of 1.0 mg l^{-1} (pH 8.2, 23°C) in 33 minutes. The same study reported 3.9 log of AOB inactivation by a free chlorine dose of 1.0 mg l^{-1} (pH 9.2, 23°C) in 5 minutes. Cunliffe (1991) reported that 2 log of AOB inactivation was achieved by a monochloramine dose of 1.0 mg l^{-1} (pH 8.0, 30°C) in 760 minutes. Oldenburg *et al.* (2002), using two different 'viability' assays, reported that AOB inactivation followed Chick-Watson kinetics and that widely different results were obtained with the different assays.

Another bacterium of concern in drinking water is *Escherichia coli* O157:H7, which is an enterohaemorrhagic strain of *E. coli*. It produces a shiga-like toxin and causes diarrhoea characterized by bloody stools, especially in children and the elderly. *E. coli* O157:H7 is ubiquitous in cattle farms, where it can persist for years (Hancock *et al.* 2001). The infection is often spread through contaminated foods, but waterborne transmission of this agent has also been documented, often in cases in which cow manure contamination of water took place (Swerdlow *et al.* 1992; Anonymous 2000). *E. coli* O157:H7 inactivation kinetics were studied by Rice *et al.* (2001) who found that the average chlorine inactivation rates at 5°C (pH 7.0) for various *E. coli* O157:H7 isolates were very rapid (approximately 3.00 $\log_{10}$ min^{-1}).

An important issue in distribution systems is the potential shielding effect associated with particles and turbidity. Particles that can affect water quality and disinfection efficacy can be in the form of iron pipe corrosion debris, soil contamination (i.e. from a pipe break), and municipal wastewater solids (i.e. from cross-contamination and pipe leaks). The impact of particles on inactivation efficiency in drinking water is poorly understood. In addition, only a few studies have addressed the inactivation kinetics of both AOB and *E. coli* O157:H7 in drinking water. Based on this information, one of the goals of this study was to measure the inactivation kinetics of *Nitrosomonas europaea* (an AOB) in bench-scale experiments in the presence of chlorine or monochloramine and to generate additional chloramine *CT* values for this organism as well as *CT* values for free chlorine. Another objective was to explore the disinfection kinetics of this AOB in the presence of corrosion debris, soil material and wastewater, and compare those results to the kinetics obtained in particle-free water. Finally, the inactivation kinetics of *Nitrosomonas europaea* were compared with those of a strain of *Escherichia coli* O157:H7 to compare the sensitivity of these two different bacteria to chlorine and chloramines.

MATERIALS AND METHODS

Cultivation of *Nitrosomonas europaea* and enumeration by the MPN cultivation technique

Nitrosomonas europaea ATCC 19718 was grown in 500 ml of ATCC Medium 2265 (a liquid mineral medium for *Nitrosomonas europaea*) at 26°C with shaking for 20 to 30 days. Following incubation, cultures were harvested by filtration (25 mm polycarbonate membranes, 0.2 μm pore size, Whatman, Florham Park, New Jersey). Each membrane was suspended in 5 ml of sterile, deionized, distilled water adjusted to pH 7.0 and gently vortexed for approximately one minute to detach the cells. Cell suspensions were immediately used in inactivation experiments.

N. europaea cells in inactivation experiments were enumerated using a most-probable-number (MPN) method as previously described (Donaldson & Henderson 1989). The growth medium was the ATCC 2265 *Nitrosomonas europaea* medium. Aliquots (0.1 ml) of diluted water samples (at least 3 or 4 consecutive dilutions, typically 10^{-1} to 10^{-4}) were incubated in five replicate wells using 24-well plates (Becton Dickinson, Franklin Lakes, New Jersey). Each well contained 1.5 ml of medium and the plates were incubated at 26°C for 25 to 30 days (Aakra *et al.* 1999). Positive controls consist of inoculating a series of wells with a pure culture of *Nitrosomonas europea* ATCC

19718 not exposed to any disinfectant. Negative controls (at least five wells without bacteria) were included in each experiment. After incubation, 3 drops of each nitrite reagent (sulfanilic acid reagent and dimethyl-α-naphthylamine reagent) were added to each well and the colour development was read within one minute. Pink to red wells were scored positive. The EPA MPN calculator version 1.0 (www.epa.gov/nerlcwww/other.htm) was used to calculate the MPN values (MPN ml^{-1}) with the Salama correction (Garthright 1993). Upper and lower confidence levels were also recorded.

Cultivation and enumeration of a pathogenic strain of *Escherichia coli*

Escherichia coli O157:H7 ATCC 35150 was used in this study. The bacterial cells were stored in 10% glycerol at −50°C and on nutrient agar slants kept at 4°C. For inactivation experiments, a loopful from a slant culture was inoculated into 10 ml of tryptic soy broth (TSB) (BD) and incubated at 37°C for 18 hours. Another 10 ml of TSB was inoculated with 1 ml from this culture. After incubation at 37°C for 18 hours, this culture was washed three times by centrifugation (10,000 x g, 10 minutes, 4°C) and resuspended in 5 ml of sterile, deionized, distilled water adjusted to pH 7.0. This washed cell suspension was immediately used in inactivation experiments. In inactivation experiments, *E. coli* samples were appropriately diluted and enumerated by duplicate plate counting on nutrient agar (BD) plates with incubation at 37°C for 24 hours.

Experimental protocol for inactivation experiments

Bench-scale microbial inactivation experiments were conducted in sterile 250–ml chlorine demand-free glass bottles, containing sterile, buffered, deionized, distilled water. Chlorine experiments were conducted at pH 7.0 in demand-free water, whereas monochloramine experiments were conducted at pH 8.0 in demand-free water. Approximately 10^7 to 10^8 bacterial cells (from a washed cell suspension) were added to each bottle. Free chlorine or monochloramine was then added to all vials (except to control vials) at a desired disinfectant dose (0.4–0.5 mg l^{-1} Cl_2 for free chlorine, and 1.25–1.50 mg l^{-1} Cl_2 for monochloramine). The samples were mixed on a magnetic stir plate. Microbial samples (1 ml) were collected at selected time intervals (every 15–30 seconds for chlorine, and every 1–5 minutes for monochloramine, respectively) and placed in sterile microtubes containing 10 μl of 5% (w/v) sterile sodium thiosulfate ($Na_2S_2O_3$) to quench any residual disinfectant. Samples (2 ml) for chlorine or monochloramine residual measurements were collected at each sampling time and immediately analysed. All experiments were conducted in duplicate (duplicate samples of duplicate experiments) at 21 ± 1°C.

Isolation of particles

A soil sample was collected near an excavated pipe in Toronto, Canada. The soil sample was dried at 150°C for moisture removal and sterilization and stored in a sterile bottle until use in experiments. The corrosion debris were artificially produced using a method originally developed for performing bench-scale corrosion rate testing (Method G31-72, ASTM 1996). A metal coupon was suspended in a flask filled with tap water and corrosion material allowed to accumulate for a week. The water was then filtered through a 0.45 μm membrane filter to isolate debris. This material was dried, sterilized, and stored in the same way as the soil samples. The corrosion debris were sterilized by autoclaving prior to each experiment. Raw wastewater was collected from the North Toronto (Canada) wastewater treatment plant and sterilized by autoclaving.

Inactivation experiments with particles

The basic protocol for the inactivation experiments with the particles was the same as described above with the following modifications. All disinfection experiments were conducted in chlorine demand-free glass bottles containing 250 ml of sterile, pH adjusted, deionized, distilled water and a sterile stirrer bar. Particulate matter and microbial culture were added to the reactor and mixed thoroughly for at least one hour. Three different particulate matters were tested. This included the addition of corrosion debris at 50 mg l^{-1} to the reactors; this concentration was selected because the water was noticeably 'orange' with this concentration of corrosion debris in it, which arguably represents

a worst-case scenario. Second, a sufficient amount of wastewater was added to the reactors to reach a turbidity level of 2.0 NTU, which also represents a worst-case scenario. The third treatment consisted of adding 50 mg l^{-1} of soil. This concentration of soil was selected to be consistent with the corrosion debris concentration.

Chemical disinfectants

The disinfectants used were free chlorine and monochloramine. Chlorine stock solution (about 120 mg l^{-1} free chlorine) was prepared by adding 1.0 ml of sodium hypochlorite (6% solution, conforms to APHA and ASTM requirements) to 500 ml with distilled, deionized water. The stock solution was then used in inactivation experiments to reach the target concentration of free chlorine (approximately 0.4–0.5 mg l^{-1}) in buffered, distilled, deionized water (pH 7.0). Solutions were freshly prepared for each experiment. During inactivation experiments, chlorine concentrations were measured spectrophotometrically by using the DPD ferrous titrimetric method (*Standard Methods* 1998).

For monochloramine, a pH 9.4 buffer was prepared by adding 1.95 g of Na_2CO_3 and 6.86 g of $Na_2(HCO_3)_2$ to 1 l of deionized, distilled water. An ammonium chloride stock solution was prepared by adding 200 mg of NH_4Cl to 500 ml of the pH 9.4 buffer. The chlorine stock solution was prepared by adding 0.43 ml of 6% sodium hypochlorite to 75 ml of the buffer. Monochloramine solution was produced by mixing equal volumes of the ammonium chloride stock solution and the chlorine stock solution (chlorine-to-ammonia-nitrogen ratio of approximately 4:1). This stock solution was then used in inactivation experiments to reach the target concentration of monochloramine (approximately 1.25–1.50 mg l^{-1}) in buffered, distilled, deionized water (pH 7.0). Solutions were freshly prepared for each experiment. On average, this method produces about 5.5% of chlorine as Cl_2, 84% as monochloramine and 10.5% as dichloramine, as measured by amperometric titration (*Standard Methods* 1998). During inactivation experiments, monochloramine concentrations were measured spectrophotometrically by using the DPD ferrous titrimetric method (*Standard Methods* 1998).

Statistical analyses

For each microbial data point, two replicate measurements per sampling time were performed. The experiments were independently repeated at least twice on separate days. Comparisons were made between the slopes of two linear regressions of inactivation data to see if they belong to the same population with a 95% confidence level (t-test) using the following equation:

$$t = \frac{b_1 - b_2}{\mathrm{SE}_{b1\,-b}} \tag{1}$$

where b_1 and b_2 are the slopes of the two regressions and,

$$\mathrm{SE}_{b1-b2} = \sqrt{\frac{\mathrm{MS}_{\text{pooled error}}}{\Sigma(x-\bar{x})_1^2} + \frac{\mathrm{MS}_{\text{pooled error}}}{\Sigma(x-\bar{x})_2^2}} \tag{2}$$

and,

$$MS_{\text{pooled error}} = \frac{SS_{\text{error1}} + SS_{\text{error2}}}{\mathrm{DF}_1 + \mathrm{DF}_2} \tag{3}$$

and,

$$SS_{error} = \sum (y - \bar{y})^2 \tag{4}$$

and,

DF = degrees of freedom (n-2 for each data set).

CT and inactivation calculations

Microbial inactivation was measured as the $\log_{10}$ (N_t/N_0), where N_t is the number of viable bacterial cells (in MPN ml^{-1} or CFU ml^{-1}) at time t and N_0 is the number of viable bacterial cells (MPN ml^{-1} or CFU ml^{-1}) at the beginning of the experiment. *CT* values were calculated by integration of the disinfectant residual concentration (C) up to the given sampling time (T). For each time point, the *CT* value was calculated by multiplying the measured chlorine or monochloramine concentration by the time period since the previous measurement. This value was then added to the *CT* value calculated at the previous time point to give the overall *CT* value for a desired sampling time. Therefore, for a sample n and a sampling

time T_n with a disinfectant concentration C_n, the $(CT)_n$ is calculated as follows:

$$(CT)_n = C_n^*(T_n - T_{n-1}) + (CT)_{n-1} \quad (5)$$

RESULTS

Figures 1–3 show the inactivation by monochloramine (pH 8.0, 21°C) of *N. europaea* in particle-free pure water reactors or in the presence of various particulates. Corrosion (50 mg l^{-1}), soil (50 mg l^{-1}) and wastewater (at 2.0 NTU) debris had no statistically significant ($p < 0.05$) impact on *N. europaea* inactivation by monochloramine (Figures 1–3). Monochloramine yielded 4 $\log_{10}$ of inactivation at *CT* values of approximately 9.9–16.4 mg.min l^{-1}. With monochloramine, wastewater and corrosion debris exerted a demand (measured over two minutes) of approximately 0.32–0.38 mg l^{-1} and 0.21–0.30 mg l^{-1}, respectively, whereas there was no disinfectant demand with the soil treatment and in particle-free reactors (data not shown). The same treatments were evaluated with free chlorine (pH 7.0, 21°C) as a disinfectant. Figure 4 represents the inactivation of *N. europaea* by chlorine in control reactors or in the presence of 50 mg l^{-1} of corrosion debris. The results show that the use of free chlorine produced approximately 4 log of *N. europaea* inactivation at a *CT* value of 0.8 mg.min l^{-1}. Similar results were observed with soil and wastewater

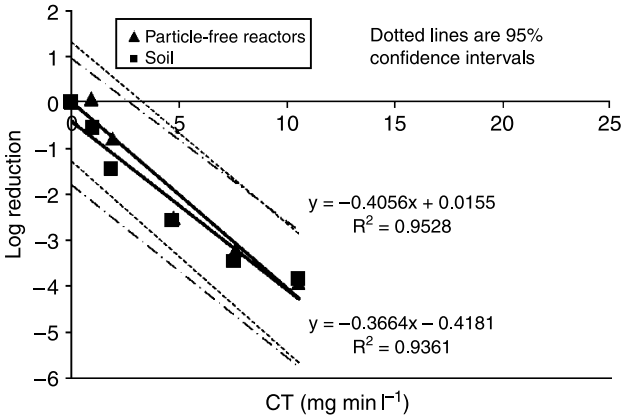

Figure 2 | Inactivation of *Nitrosomonas europaea* ATCC 19718 by monochloramine (pH 8.0, 21°C) in particle-free reactors or in the presence of 50 mg l^{-1} of soil. The slopes of the inactivation kinetics of the two conditions were statistically the same within a 95% confidence level (*t*-test).

particulates (data not shown). As with monochloramine, none of the various particulate suspensions had a statistically significant ($p < 0.05$) impact on *N. europaea* inactivation by free chlorine. The chlorine demand (measured over 30 seconds) exerted by the various particles ranged from 0.09 to 0.22 mg l^{-1}, whereas the demand in particle-free reactors was less than 0.05 mg l^{-1} (data not shown).

The inactivation of *E. coli* O157:H7 ATCC 35150 by free chlorine and monochloramine was also tested in bench-scale experiments (Figures 5 and 6). With monochloramine, 4 $\log_{10}$ of *E. coli* O157:H7 inactivation was observed at an approximate *CT* value of 9.2 mg.min l^{-1} (Figure 5). A comparison of the monochloramine

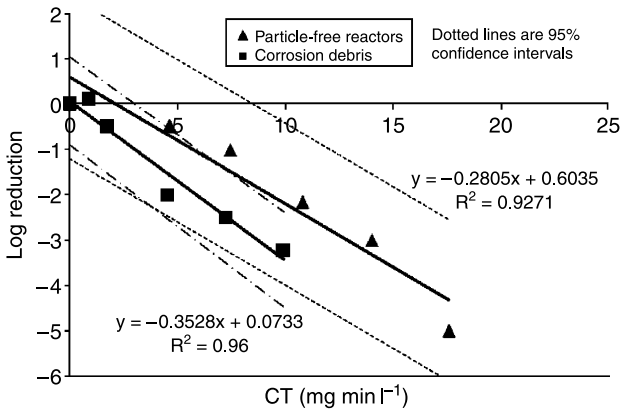

Figure 1 | Inactivation of *Nitrosomonas europaea* ATCC 19718 by monochloramine (pH 8.0, 21°C) in particle-free reactors or in the presence of 50 mg l^{-1} of corrosion debris. The slopes of the inactivation kinetics of the two conditions were statistically the same within a 95% confidence level (*t*-test).

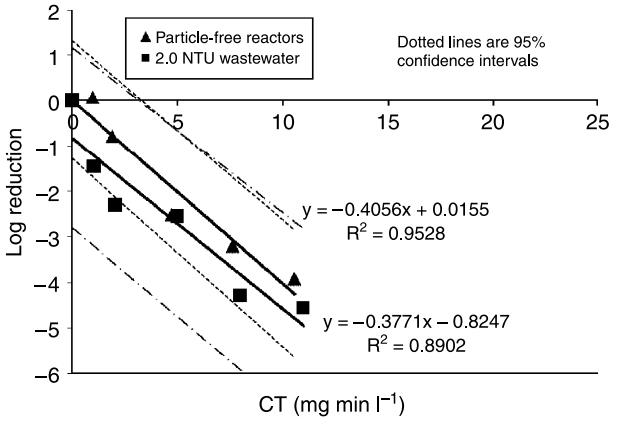

Figure 3 | Inactivation of *Nitrosomonas europaea* ATCC 19718 by monochloramine (pH 8.0, 21°C) in particle-free reactors or in the presence of 2.0 NTU wastewater. The slopes of the inactivation kinetics of the two conditions were statistically the same within a 95% confidence level (*t*-test).

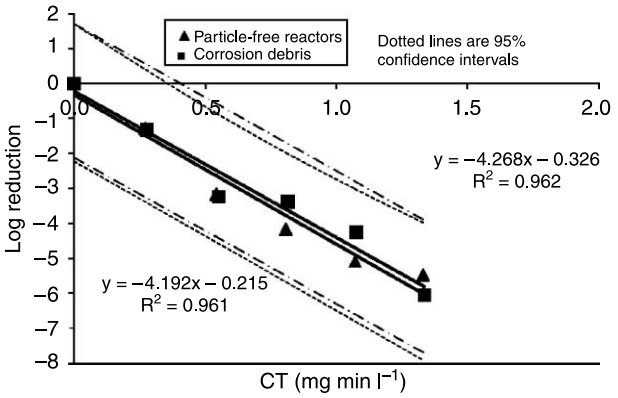

Figure 4 | Inactivation of *Nitrosomonas europaea* ATCC 19718 by chlorine (pH 7.0, 21°C) in particle-free reactors or in the presence of 50 mg l^{-1} of corrosion debris. The slopes of the inactivation kinetics of the two conditions were statistically the same within a 95% confidence level (*t*-test).

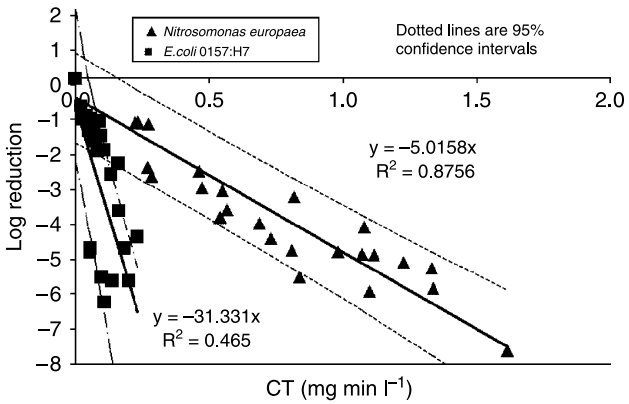

Figure 6 | Chlorine inactivation of *Nitrosomonas europaea* ATCC 19718 and *Escherichia coli* O157:H7 in particle-free reactors (pH 7.0, 21°C).The slopes of the inactivation kinetics of the two conditions were statistically different within a 95% confidence level (*t*-test).

inactivation of *E. coli* O157:H7 to that of *N. europaea* shows that *E. coli* was more sensitive than *N. europaea*, and that this difference was statistically significant ($p < 0.05$). *E. coli* O157:H7 was very sensitive to free chlorine; *CT* values less than 0.13 mg.min l^{-1} were sufficient to inactivate approximately 4 $\log_{10}$ of this organism (Figure 6). Therefore, chlorination appears to adequately control this pathogen, at least in buffered, deionized, distilled water. A comparison of the chlorine inactivation of *E. coli* O157:H7 with that of *N. europaea* shows that *E. coli* was much more sensitive than *N. europaea*, and that this difference was statistically significant with $p < 0.05$ (Figure 6).

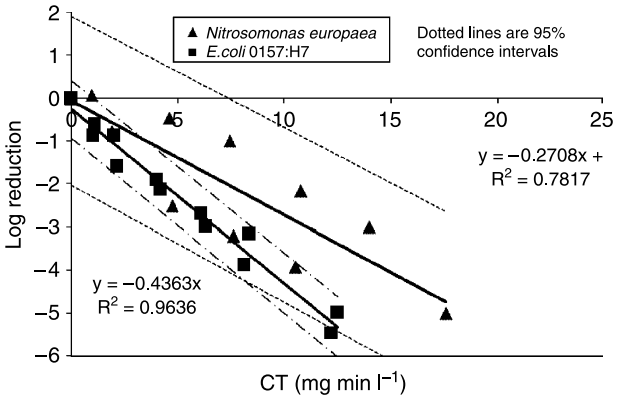

Figure 5 | Monochloramine inactivation of *Nitrosomonas europaea* ATCC 19718 and *Escherichia coli* O157:H7 in particle-free reactors (pH 8.0, 21°C). The slopes of the inactivation kinetics of the two conditions were statistically different within a 95% confidence level (*t*-test).

DISCUSSION

This study evaluated the bench-scale inactivation kinetics of both the ammonia-oxidizing bacterium *Nitrosomonas europaea* and *Escherichia coli* O157:H7 in the presence of either chlorine or monochloramine. For *N. europaea*, the viability assay used in this study was the MPN-cultivation assay whereas *E. coli* viability was assessed by a plate-count method. *E. coli* O157:H7 was shown to be very sensitive to free chlorine since *CT* values of less than 0.13 mg· or ·min l^{-1} were sufficient to inactivate at least 4 $\log_{10}$ of this bacterium. These data are similar to those obtained by Rice *et al.* (2001)who studied the chlorine inactivation kinetics of several strains of *E. coli* O157:H7. On the other hand, *N. europaea* was significantly more resistant to chlorine and monochloramine than *E. coli* O157:H7. *N. europaea* produces extensive layers of stacked intracytoplasmic membranes (Wolfe *et al.* 1990). It is possible that these layers are responsible for the greater resistance to oxidants observed with *N. europaea* in comparison with *E. coli* O157:H7. In other studies, intracytoplasmic membranes have been associated with bacterial resistance to antimicrobial peptides (Brodsky *et al.* 2005), toxic heavy metals (Itoh *et al.* 1998), and to the toxic oxidant oxyanion tellurite (Borsetti *et al.* 2003). The tellurite resistance in one bacterial species was characterized by the presence of a modified plasma-membrane-associated electron transport system (Borsetti *et al.* 2003). It is also known that AOB, under certain conditions,

can form cell aggregates referred to as zoogloea or cysts (Holt 2000). Zoogloea are composed of loosely associated cells embedded in and surrounded by a soft slime layer. In zoogloea, the cells are not compressed or distorted. On the other hand, AOB cysts are made up of densely packed clusters of cells embedded in and surrounded by a firm slime layer, which can distort or compress the cells (Watson *et al.* 1989; Holt 2000). In AOB cysts, these slime layers are known to protect the bacterial cells against disinfectants (Stewart & Lieu 1997). It is possible that the culture conditions in the present study could have favoured the formation of aggregates with a slime layer, which could also explain the greater resistance of *N. europaea* to disinfectant than was observed with *E. coli* O157:H7.

The most comprehensive study so far on AOB inactivation kinetics was performed to generate *CT* values for chloramines and *Nitrosomonas europaea* in a phosphate-buffered (PBS) water (Oldenburg *et al.* 2002). The present study complements the study of Oldenburg *et al.* by providing additional data on chloramines as well as new data on chlorine inactivation. In addition, data were generated in bench-scale reactors that contained different types of particle. When using the MPN assay, Oldenburg *et al.* (2002) obtained *CT* values for chloramines that were similar to those observed in the present study. For example, Oldenburg *et al.* (2002) reported a *CT* value of 14 mg.min l^{-1} for 2 $\log_{10}$ of inactivation at a temperature of 20°C and a pH of 8.6, whereas the present study measured approximately 4 $\log_{10}$ of inactivation at *CT* values of 9.9–16.4 mg.min l^{-1} under similar conditions (MPN assay, pH of 8.0, temperature of 21°C). It is possible that the cell harvesting procedure used by Oldenburg *et al.* (centrifugation followed by a 24 hour recovery period in PBS) resulted in the formation of large cell clusters and, accordingly, more slime layers. This would explain why these researchers observed slightly greater *Nitrosomonas* resistance to chloramines than was seen in the present study. In the present study, *N. europaea* cells were harvested by filtration (as in Wolfe *et al.* 1990) in preparation for inactivation experiments and not by centrifugation. This is more likely to produce fewer large clusters of cells than a centrifugation procedure would because of sedimentation.

CT values for free chlorine were generated in this study. Although more resistant than *E. coli* O157:H7, *N. europaea* inactivation with free chlorine was nevertheless fairly rapid (e.g. a *CT* of 0.8 mg.min l^{-1} yielded approximately 4 $\log_{10}$ of inactivation at 21°C). This information should be useful to water utilities with nitrification problems and considering and/or already practising periodic breakpoint chlorination (superchlorination) as a means of controlling AOB in distribution systems (Odell *et al.* 1996).

Particles were also used to evaluate whether or not they had an impact on disinfection kinetics. The types of particle used in the experiments included iron pipe corrosion debris (at 50 mg l^{-1}), soil contamination to simulate a pipe break (at 50 mg l^{-1}), and municipal wastewater solids to simulate cross-contamination (added to reach a water turbidity of 2.0 NTU). In experiments with either chlorine or monochloramine, there was no significant difference between inactivation in control water versus inactivation in water containing debris, suggesting that the particles tested (at the tested concentrations) did not have an impact on disinfection, regardless of which disinfectant was tested. Particulate matter obviously exerted a demand on the disinfectant residuals; however, when the results were normalized using *CT* values, the inactivation kinetics of the various treatments were identical.

Finally, considering these various results, it is interesting that, even though the *CT* values for *N. europaea* and AOB in general are relatively low, these bacteria are still present in drinking water distribution systems, even at high concentrations at times. Future research should address the issues of aggregation and biofilm formation and how they might influence disinfection resistance in these bacteria in drinking water distribution systems.

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