STRATEGIES FOR INTEGRATED CONTROL OF BIOLOGICAL AGENTS BY SMALL UTILITIES

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CHAPTER 1: INTRODUCTION

Small surface water utilities in the United States are faced with a challenge to meet the demands of the forthcoming Long Term 2 Enhanced Surface Water Treatment Rule (LT2 ESWTR) and to protect against the threat of terrorism via water-borne biological agents. The combination of these two problems has resulted in the need for an efficient disinfection upgrade for all surface water utilities that can inactivate even the most resilient microorganisms. For small utilities in particular, upgrade options are significantly constrained by economic factors.

The LT2 ESWTR is being established to require all surface water utilities to treat adequately for *Cryptosporidium parvum* (*C. parvum*) oocysts and enteric viruses while maintaining Disinfection By-Product (DBP) compliance (US EPA, 2003). Before the LT2 ESWTR, utilities serving populations of less than 10,000 were not required to monitor or treat for *C. parvum* oocysts. Small utilities may soon be required to monitor for *C. parvum* in their source waters if fecal contamination is detected, and depending on the results, may be required to inactivate up to 2.5 logs of *C. parvum* oocysts. Meeting the LT2 ESWTR will be a problem for the majority of small surface water utilities because they rely on free chlorine for primary disinfection, which has been proven highly ineffective against *C. parvum* oocysts (Rennecker *et al*., 2000). Inactivation of *C. parvum* oocysts with free chlorine would result in an impractical contact time due to associated higher cost and DBP formation.

UV disinfection systems have recently been proven highly effective against *C. parvum* oocysts (Kashinkunti *et al*., 2002, Clancy *et al*., 2004), making them a viable upgrade for both small and large utilities (Cornwell *et al*., 2003, Kashinkunti *et al*., 2002, US EPA, 1996). UV systems also have the additional benefits of reducing DBP formation and requiring a relatively small footprint. The major limitation of UV systems is their relative inefficacy against certain pathogenic viruses (Shin *et al*., 2001). However, pathogenic viruses are susceptible to free chlorine disinfection (Engelbrecht *et al*., 1978). Thus, the UV/free chlorine sequential disinfection process is a viable upgrade that will allow small utilities to adequately inactivate both (oo)cysts and viruses.

Small utilities must also protect consumers from acts of bioterrorism that may occur via the drinking water supply. Due to the large dilution factors associated with drinking water terrorism, biological agents are thought to be the greatest threat for utilities because a low concentration of a biological agent can be lethal (Luthy, 2001). This threat is augmented for small utilities treating surface waters due to physical vulnerability of the source water and the inherently smaller dilution factors.

The spore-forming *Bacillus anthracis* (a.k.a. anthrax) would be the most probable biological weapon used by a terrorist due to its natural resistance to a range of disinfection methods and its ability to be reproduced efficiently in high quantities (Burrows, 1999, Kortepeter, 1999). Most treatment plants are designed with common, naturally occurring pathogens in mind, and *Bacillus anthracis* spores are neither regulated or being considered for regulation. However, as small utilities consider

upgrading to the UV/free chlorine sequential disinfection process, it will be important to know how the upgrade will perform with respect to bioterrorist agents.

There are few published studies regarding the inactivation of *B. anthracis* spores in drinking water. In 1958, Brazis *et al.* found that a free chlorine *CT* (product of disinfectant concentration and contact time) of 204 mg*min/L at pH 6.2 and 20^{o}C was required for 4 logs of inactivation of *B. anthracis* spores. The *CT* for the same reduction at pH 8.6 and 20^{o}C was 912 mg*min/L. In a different study on UV disinfection, a UV dose, or *IT* (product of average irradiance and exposure time), of 60 mJ/cm^2 was required for the same reduction of *B. anthracis* spores (Nicholson and Galeano, 2003).

Bacillus subtilis spores are a commonly used surrogate for *B. anthracis* spores. Previous studies have shown that 4 logs of inactivation of *B. subtilis* spores can be achieved with a free chlorine *CT* of 145 mg*min/L at pH 6.5 and 20^{o}C (Mysore et al., 2003) and 170 mg*min/L at pH 5.6 and 20^{o}C (Cho *et al*., 2003). When assessing UV disinfection, Chang *et al.* (1985) determined that a UV dose of 80 mJ/cm^2 was required for 4 logs of *B. subtilis* spore inactivation. Nicholson and Galeano (2003) found that the same inactivation could be achieved at a UV dose of 60 mJ/cm^2

The objectives of this study were to characterize the inactivation of *B. subtilis* spores by the UV/free chlorine sequential disinfection process. Experiments were conducted to assess the influence of water quality parameters and UV pre-treatment levels on the inactivation kinetics. An additional goal was to develop a model that could help small

utilities predict and optimize the inactivation of *B. subtilis* spores at any pH, temperature, and ionic strength conditions encountered in surface water treatment.

CHAPTER 2: MATERIALS AND METHODS

2.1 Spore preparation and viability assessment

B. subtilis spores strain ATCC 6051 were obtained from the American Type Culture Company (Manassas, Virginia) in freeze-dried pellet form. Upon arrival, the pellet was re-hydrated in autoclaved, pH 7 0.01 M phosphate buffer solution (PBS). Droplets of this suspension were incubated on Difco Nutrient Agar plates for 15 h at 37.5^{o}C, resulting in vegetative cells of *B. subtilis*. Replication and sporulation were induced by scraping the vegetative cells from the plates, suspending them in pH 7 PBS, seeding R2A agar flasks with the solution, and then incubating the flasks for ten days at 37.5^{o}C. During this time, the cells experienced an exponential growth phase followed by starvation and spore formation. The newly formed spores were suspended in pH 7 PBS and cleaned repeatedly via centrifugation. Any remaining vegetative cells were removed by heating the suspension in a water bath at 80^{o}C for 10 minutes. The resulting stock solution of spores contained 10^{8} colony forming units/ml (cfu/ml) and was stored at 4^{o}C.

B. subtilis spore viability was determined using ten-fold serial dilutions (10ml:90ml) in pH 7 PBS followed by vacuum filtration of 10 ml samples through a .45 micron membrane filter. pH 7 PBS solution was poured over the filter to distribute the spores evenly on the membrane filter. The captured spores were then incubated on Bacto Nutrient Agar at 37.5^{o}C for 12 to 15 h. For statistical consistency, only plates with a number of colonies between 10 and 200 were included in the data presented in this study.

2.2 Experimental matrix

Inactivation of *B. subtilis* spores with free chlorine was conducted at 20^{o}C in synthetic buffer solutions at pH 6, 7.5, 8, and 9, and in natural water at pH 8.2. Temperature dependence was elucidated at 4, 10, 20, and 30^{o}C in 0.01 M PBS at pH 6. UV inactivation was investigated in 0.01M PBS at pH 6 and 8 and in natural water at pH 8.2. The UV/free chlorine disinfection inactivation was characterized in .01 M PBS at pH 6 and 8 and in natural water at pH 8.2 and 7.73. All of these experiments were performed using the same lot of spores, which was cultured in May of 2004. Additional experiments, designed to assess the variability in spore resistance to free chlorine and UV light, were conducted using two different lots grown under the same conditions from strain ATCC 6051. A summary of all experimentation is presented in Table 1.

2.3 Test Waters

The majority of the experiments were conducted in 0.01 M buffered solutions, prepared from 1 M stock solutions diluted in distilled de-ionized (DDI) water. Phosphate buffer solution (PBS) was used for synthetic water experiments in the pH range of 6-8. Borate buffer solution (BBS) was used for synthetic water experiments with pH greater than 8. The ionic strength of the PBS was 0.012 at pH 6, 0.025 at pH 7.5, and 0.029 at pH 8. The ionic strength of the BBS was 0.003 at pH 9.

The natural water used in this study was obtained from a small surface water utility in east-central Illinois. The water was taken directly from the influent of the plant during July of 2003 and stored at 4^{o}C. The water was equilibrated with the atmosphere prior to

all experiments to avoid carbonate-associated pH changes during the experiments. General water quality parameters for the natural water are shown in Table 2. The alkalinity was estimated based on the ions present, and the ionic strength was calculated based on the ions present and the alkalinity. The ionic strength of the natural water was 0.007.

2.4 Primary disinfection with free chlorine

All free chlorine disinfection experiments were conducted in an amber glass, continuously-stirred, batch reactor. Temperature was controlled using a recirculating water bath (Model 2002, CH/P Temperature Control System, Forma Scientific, Inc). For synthetic water experiments, pH was controlled using 0.01 M phosphate (pH 6-8) or borate (pH 9-10) buffer solutions. The reactor was filled with 400 ml of test water and submerged in the water bath at least one hour prior to experimentation until the desired temperature in the reactor was achieved. After temperature stabilization, a small volume of the spore stock solution was added to the reactor, and a 5-ml viability control sample was taken prior to free chlorine addition. At time zero, a predetermined volume of sodium hypochlorite solution (5%, Fisher Scientific, Itasca, IL) was added to the reactor. 5-ml viability samples were taken periodically and quenched with 5-ml of 1% sodium thiosulfate solution with vigorous shaking. The reactor was only opened to the atmosphere during sampling times in order to minimize carbonate-induced pH changes during the experiment. The pH in the reactor was measured before and after each experiment.

Free chlorine concentration was measured at the beginning and end of the experiment using the DPD-FAS Titrimetric Method as described by Standard Method 4500-Cl F (APHA et al., 1992). For experiments with a free chlorine concentration of more than 5 mg/L as Cl_2, solutions were diluted by a factor of two in DDI water prior to free chlorine measurement.

2.5 Primary disinfection with UV light

UV disinfection experiments were conducted using a 1-kW collimated beam reactor (Model PS1-1-120, Calgon Carbon Corp., Pittsburg, Pennsylvania). Only low-pressure mercury lamps were used in this study. For each data point, a 15-ml sample in a 6-cm Petri dish was continuously-stirred using a magnetic stirrer and was exposed to a predetermined UV dose. Each Petri dish was seeded with the same volume of spore stock solution and stirred for one minute prior to UV exposure. The spore stock solution was mixed using a vortex for one minute prior to spore addition to ensure a consistent initial spore concentration in each dish. The control was obtained by seeding a dish and stirring it for one minute. All UV experiments were performed at room temperature with the exception of UV/FC5, in which the UV portion of the experiment was conducted at an average temperature of 8.5^{o}C. The effect of temperature was not investigated in this study because it was found to be negligible in previous studies (Severin *et al.*, 1983).

Prior to each experiment, the average irradiance in the sample was measured using a radiometer (Model IL1400A, International Light Co., Newburyport, Massachusetts) that measured irradiance at a wavelength of 254 nm and methods developed by Bolton and

Linden (2003). Calculating the average irradiance in the Petri dish involved measuring the irradiance at 16 points 0.5-cm apart along the x and y-axes of the plane perpendicular to the incident beam, the path length from the lamp to the sample, the water depth, and the absorbance of the water at a wavelength of 254 nm. These parameters were used to calculate the average irradiance in the sample and the exposure time required for a given UV dose. This radiometric method was initially cross-checked with the actinometry method developed by Rahn *et al*. (1997) and found to be accurate.

2.6 Sequential disinfection with UV light followed by free chlorine

UV/free chlorine sequential disinfection experiments were conducted by pretreating the spores in the collimated beam UV apparatus and then seeding a batch reactor with the UV-attenuated spores. Viability was measured before and after UV pretreatment, and the subsequent free chlorine secondary disinfection experiment was conducted following the procedures described in Section 2.4. A control experiment using only single-step free chlorine disinfection was simultaneously conducted under the same conditions for direct comparison of primary and secondary free chlorine disinfection.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Effect of temperature on primary disinfection with free chlorine

Experimental results from the inactivation of *B. subtilis* spores with free chlorine in PBS at pH 6 and 4-30^{o}C are shown in Figure 1. Each curve was characterized by a lag phase in which no inactivation occurred followed by a pseudo-first order inactivation phase. As depicted in the figure, the lag phase and pseudo-first order rates of inactivation were found to decrease and increase, respectively, with increasing temperature. Each curve was fitted using the delayed Chick-Watson model used by Rennecker et al. (1999) to characterize the inactivation of *C. parvum* oocysts with ozone:

$$\frac{N}{N_0} = \begin{cases} 1 & \text{if } CT \le CT_{\text{lag}} = \dfrac{1}{k}\ln\left(\dfrac{N_1}{N_0}\right) \\ \dfrac{N_1}{N_0}\exp(-kCT) & \text{if } CT > CT_{\text{lag}} \end{cases} \qquad (1)$$

where C is the free chlorine concentration (mg/L as Cl_2), T is the contact time (min), CT_{lag} is the lag phase CT (mg*min/L), and k is the post-lag phase inactivation rate constant ($mg^{-1}min^{-1}L$). N_1/N_0 is the projected intercept of the pseudo-first order inactivation curve with the ordinate axis (i.e., at CT=0). An initial investigation in which each curve was fitted independently revealed that N_1/N_0 was approximately the same for all four curves. Under this assumption, the four data sets were fitted simultaneously with a least-square regression analysis using Equation 1 in conjuction with the Arrhenius expression:

$$k = A\exp\left(-\frac{E_a}{R\mathrm{T}}\right) \quad (2)$$

where A is the frequency factor ($mg^{-1}min^{-1}L$), E_a is the activation energy (J/mol), R is the ideal gas constant (J/mol*K) and T is the absolute temperature (K). The resulting fitting parameters were N_1/N_0 = 3,060, A = 4.42 x 10^6 L/(mg*min), and E_a = 45,300 J/mol. As depicted in Figure 1, excellent agreement was found between the data and the fitting curves.

3.2 Effect of pH on primary disinfection with free chlorine

The effect of pH on the inactivation kinetics of *B. subtilis* spores with free chlorine was investigated by performing experiments at pH 6-9 and 20°C. Experimental results, shown in Figure 2, revealed that pH had a strong effect consistent with hypochlorous acid being a much stronger disinfectant than the hypochlorite ion. A preliminary analysis of the results revealed that hypochlorous acid was the only free chlorine species responsible for spore inactivation. The *CT* required to achieve an inactivation efficiency of three logs, or 99.9 percent, was 400 mg*min/L at pH 6 and 14,400 mg*min/L at pH 9. The corresponding *CT* ratio 14,400/410 = 35.1, was almost exactly the inverse of the hypochlorous acid concentration ratio $[HOCl]_{pH\ 6}/[HOCl]_{pH\ 9}$ = .0286. Thus, the hypochlorous acid was assumed to be the only free chlorine species responsible for inactivation in the pH range of 6-9. Accordingly, the frequency factor obtained based on total free chlorine concentration ($A_{\text{Total Free Chlorine}}$) for the pH 6 data sets in Figure 1 was corrected to obtain the frequency factor based on the concentration of inactivating species hypochlorous acid (A_{HOCl}) using the following expression:

$$A_{\mathrm{HOCl}} = \frac{\gamma 10^{-\mathrm{pH}} + K_a}{\gamma 10^{-\mathrm{pH}}} A_{\text{Total Free Chlorine}} \qquad (3)$$

in which K_a is the acid-base equilibrium constant of hypochlorous acid, and is the activity coefficient of OCl^- calculated using the Guntelburg approximation of the DeBye-Huckel equation (Snoeyink and Jenkins, 1980). The resulting value was $A_{\mathrm{HOCl}} = A = 4.59\text{x}10^6$ L/(mg*min).

Inactivation curves at the experimental pH values of 7.5, 8 and 9 were then predicted by assuming that, similar to the case of the temperature dependence curves, the coefficient N_1/N_0 was not dependent on temperature or pH. Because the data is plotted in terms of *CT* values calculated with the total free chlorine concentration, the apparent inactivation rate constant (k_{app}) needed to predict the curves in Figure 2 was obtained with the expression:

$$k_{\mathrm{app}} = \frac{\gamma 10^{-\mathrm{pH}}}{\gamma 10^{-\mathrm{pH}} + K_a} A_{\mathrm{HOCl}} \exp\left(-\frac{E_a}{R\mathrm{T}}\right) \qquad (4)$$

A comparison of the experimental and predicted inactivation curves in Figure 2 supported the assertion that hypochlorous acid was the only free chlorine species inactivating spores at measurable levels within the range of experimental conditions investigated. The relatively small discrepancies could have been due to variability of ± 0.01 units in pH or the estimation of activity effects. A considerable increase in the ionic strength of the PBS and a related decrease in activity of monovalent ions occurred as the pH increased due to

the dominance of the less protonated and thus more negatively-charged phosphate species. This was not the case at pH 9, where BBS was used.

3.3 Effects of chlorine concentration, initial spore concentration, and spore age

Additional experiments were performed to evaluate the validity of the *CT* concept (or the lack of free chlorine concentration effect) and to assess if the effects of initial spore concentration on the inactivation kinetics of *B. subtilis* spores with free-chlorine at pH 6 and 20^{o}C. The results from experiments performed with initial total free chlorine concentrations of 1-10 mg/L as Cl_2 and spore concentrations of 1×10^{4}-2×10^{5} cfu/mL are shown in Figure 3. As depicted in the figure, the *CT* concept was found to be valid and the spore concentration did not affect the inactivation kinetics. The slight variability in the curves did not follow any trend with respect to free chlorine or spore concentration and was probably the result of inaccuracies in the measurement of the free chlorine concentration or spore viability. In addition, spore age differences in excess of three months did not appear to affect their resistance to free chlorine.

3.4 Primary disinfection with UV light

The inactivation kinetics of *B. subtilis* spores with UV light is shown in Figure 4. The inactivation kinetics was first order with respect to the spore concentration. Three-log or 99.9 percent inactivation was consistently achieved at a UV dose of 35 mJ/cm^2 independently of pH and ionic strength. It is important to note that the UV dose calculation did account for absorbance factors, so waters with higher absorbance values

were exposed for longer times to achieve the same UV dose. For the waters used in this study, the relative increase in exposure times due to absorbance factors was less than 3%.

3.5 Sequential disinfection with UV light followed by free chlorine

The inactivation kinetics of *B. subtilis* spores with UV light followed by free chlorine at 20^{o}C in PBS at pH 6 and 8 and in natural water at pH 8.2 with and without UV pretreatment is shown in Figure 5. The level of UV pretreatment applied was 12 mJ/cm^2 and resulted in a viability reduction of 1 log. For effective presentation of the data, the UV/FC disinfection curves in Figure 5 were normalized to the control obtained in the free chlorine disinfection phase of the experiment. Thus, the cumulative inactivation is not presented. The inactivation kinetics after UV pretreatment (UV/FC) correlated well with those found when applying free chlorine alone (FC), implying that the effects of the two processes were just additive with no occurrence of synergy. In addition, the previously generated model for free chlorine primary disinfection was still applicable after UV treatment.

3.6 Effect of UV pretreatment level

An additional investigation was conducted with varying levels of UV pretreatment at 20^{o}C in PBS at pH 6. The results, shown in Figure 6, revealed that the secondary inactivation kinetics of *B. subtilis* spores with free chlorine was not affected by the level of UV pretreatment. The inactivation curves in Figure 6 were normalized to the control obtained in the UV phase of the experiment and thus present the total inactivation achieved by the UV/free chlorine sequential process.

3.7 Model performance

The model of *B. subtilis* spore inactivation with UV light followed by free chlorine was tested at pH 7.73 and 8.5^{o}C in natural water, and the predicted curve versus actual data can be seen in Figure 7. Based on this test, the model generated in this study appears to extrapolate reasonably well when applied at other pH and temperature combinations found in surface water treatment but not specifically investigated in this study.

3.8 Lot Variability

Additional experiments were performed to investigate variability between two lots of *B. subtilis* spores. The two lots (101504MP and 101504OC) were grown from the same parent strain (ATCC 6051) simultaneously under the same conditions. Figure 4 includes the inactivation curves of these two lots with UV light, and the inactivation rates were the same as those found previously in this study with Lot 052104MP. Figure 8 presents the inactivation of the two newly grown lots with free chlorine, and the inactivation kinetics for both lots were very similar. However, the lag phase was shorter than the lag phase found originally in this study, and the inactivation rates were also slightly faster.

3.9 Comparison to previous studies

Figure 9 compares the inactivation kinetics of *B. subtilis* spores with free chlorine found in this study to those found in previous studies. Immediately evident is the higher free chlorine resistance of the spores used in this study. This discrepancy may be attributed to the use of a different *B. subtilis* strain in this study (ATCC 6051). The strain investigated

in previous free-chlorine disinfection studies was ATCC 6633 (Mysore *et al.*, 2003 and Cho *et al*., 2003).

To the contrary, the UV disinfection kinetics characterized in this study was faster than those found in previous studies (Figure 10). The inactivation curves from this study lacked a lag phase, which was a common characteristic of the inactivation kinetics of *B. subtilis* spores with UV light in previous studies. However, after the lag phase, it appears that the rates of inactivation were quite similar to the rate found in this study. The strains investigated in previous UV disinfection studies were ATCC 6633 (Nicholson and Galeano, 2003 and Chang et al., 1985), and WN 624 (Nicholson and Galeano, 2003).

CHAPTER 4: DESIGN RECOMMENDATIONS

Table 3(a) presents the inactivation of *B. subtilis* spores that would be achieved when applying the UV dose required for 3 logs of *C. parvum* inactivation (US EPA, 2003) coupled with the free chlorine *CT* required for 3 logs of *Giardia* inactivation (US EPA, 1989). Since most small surface water utilities were designed and are likely operated to meet *Giardia* inactivation requirements, it was assumed that these *CT*'s were the standard operating and design conditions for the treatment plant. Clearly, at these *CT* values, the resulting inactivation of spores would not be sufficient. In fact, only the UV system would contribute to the overall spore reduction because the long lag phase associated with free chlorine disinfection, and only one log of total spore inactivation would be achieved. The doses in Table 3(a) are suggested for a small utility where bioterrorism is not an issue. Though *Giardia* inactivation may occur in the UV disinfection step, it was decided that the *CT* values for *Giardia* inactivation should still be applied to ensure virus inactivation. However, these *CT* values should be further optimized by future research on virus inactivation with free chlorine.

Table 3(b) presents the optimal UV/free chlorine operation to achieve 3 logs of *B. subtilis* spore inactivation when there is a low threat of bioterrorism. Based on the relative total costs of each treatment technology for small communities and the data from this study, it was decided that UV disinfection should be used to provide the spore inactivation while free chlorine should still be applied at previous standard levels to ensure virus inactivation. An EPA study in 1996 indicated that the total cost of UV disinfection when

applying a UV dose of 40 mJ/cm^2 was about half the total cost of free chlorine disinfection (5 mg/L) for communities with populations ranging from 3301-10000. UV disinfection became relatively more cost-effective as population decreased, with the total cost ratio of 1:4 for populations of 1001-3300 and 1:5 for populations of 501-1000. Since this study was based on free chlorine contact times required for *Giardia* treatment only and did not consider the high *CT*'s required for spore inactivation, these total cost ratios are likely very conservative for this discussion.

Table 3(c) presents the suggested operation of the UV/free chlorine sequential process for a small utility that is faced with a high terrorism threat level. The goal in this scenario would be to have multi-barrier protection in order to maximize bioterrorism deterrence. However, the financial impracticality of the *CT*'s needed at high pH values and low temperatures would likely limit a small utility's ability to provide multi-barrier protection against spores in these water conditions. Most small utilities would have to increase the size of free chlorine contact basins and/or control pH in order to provide multi-barrier disinfection. For instance, a small utility treating a highly alkaline water could consider increasing free chlorine contact times (by as much as 68 times for the extreme case of pH 9 and 0.5^{o}C) or lowering the pH in order to optimize disinfection with free chlorine. Increasing chlorine contact time would likely result in more DBP formation. Lowering pH could create corrosion problems in the distribution system if pH is not raised again before distribution, but lowering pH would also have the benefit of enhancing the conventional filtration process through advanced coagulation. Increasing contact times and/or controlling pH to optimize disinfection would be costly, but providing optimal

protection against bioterrorism may be worth these costs for a small community that anticipates a terrorist attack via the drinking water supply.

It is important to note that the inactivation presented in Table 3 is based only on data collected in this study for ATCC 6051 *B. subtilis* spores and does not reflect safety factors. However, the data collected in this study elucidates important trends that will enable small utilities to design and operate UV/free chlorine systems optimally with respect to bioterrorism deterrence.

CHAPTER 5: CONCLUSIONS

The primary inactivation of *B. subtilis* spores by free chlorine was characterized by a lag phase in which no significant inactivation occurs followed by a pseudo first-order inactivation phase. Both of these phases were highly dependent on pH and temperature. Free chlorine disinfection efficacy improved as temperature increased and as pH decreased. The primary inactivation kinetics of *B. subtilis* spores by UV light was first-order and was independent of pH and ionic strength. In addition, the application of UV light prior to free chlorine did not change the free chlorine disinfection kinetics, indicating that the model developed for primary disinfection with free chlorine could still provide accurate predictions after UV pretreatment. The cumulative inactivation achieved by the UV/free chlorine sequential disinfection process revealed that multi-barrier protection against spores can be achieved with high enough *CT* values in the lower pH and higher temperature ranges.

Small utilities concerned about bioterrorism issues would benefit considerably by upgrading to a UV/free chlorine sequential disinfection system. Applying a UV dose of 40 mJ/cm^2 upstream of free chlorine addition would provide significant protection against spores that would not be achieved by primary disinfection with free chlorine under traditional operating conditions. For utilities seeking multi-barrier protection against spores, the options are to increase free chlorine contact time significantly and/or to control pH such that free chlorine disinfection is optimized. Multi-barrier protection becomes increasingly more costly as pH increases.

In addition, the strain of *B. subtilis* used in this study (ATCC 6051) was significantly more resistant to free chlorine disinfection than the strain used by previous researchers (ATCC 6633). It was also less resistant to UV disinfection. Since different *B. anthracis* strains may exhibit similar variability, different *B. subtilis* strains should be used in UV and free-chlorine disinfection studies in order to challenge each technology with the most resistant strain. In addition, these observations of variability may have interesting implications for mechanistic studies of the inactivation of *B. subtilis* spores with UV light and free chlorine. Genetic and structural differences between the strains ATCC 6051 and 6633 may be directly associated with both UV and free chlorine resistance. .

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TABLES

Test	Lot	pH	Temp (^oC)	N_o (cfu/ml)	C_o (mg/L)	Water	Date 2004
FC 1	052104MP	6	20	2E+5	10	.01M PBS	6/8
FC 2	052104MP	7.5	20	1E+5	7.3	.01M PBS	7/9
FC 3	052104MP	8	20	1E+5	8.4	.01M PBS	6/10
FC 4	052104MP	9	20	2E+5	9.8	.01M BBS	8/21
FC 5	052104MP	6	4	2E+5	6.7	.01M PBS	6/29
FC 6	052104MP	6	10	2E+5	9.4	.01M PBS	6/23
FC 7	052104MP	6	30	1E+5	9.8	.01M PBS	6/23
FC 8	052104MP	6	20	2E+5	1.0	.01M PBS	6/16
FC 9	052104MP	6	20	2E+5	2.0	.01M PBS	6/16
FC 10	052104MP	6	20	2E+5	4.0	.01M PBS	6/16
FC 11	052104MP	6	20	1E+4	8.3	.01M PBS	7/20
FC 12	052104MP	8.2	20	5E+4	7.6	Natural	10/6
FC 13	101504MP	6	20	2E+5	8.2	.01M PBS	10/21
FC 14	101504OC	6	20	2E+5	8.4	.01M PBS	10/21
UV 1	052104MP	6	21	2E+6	-	.01M PBS	6/4
UV 2	052104MP	8	21	2E+6	-	.01M PBS	6/4
UV 3	052104MP	8.2	23	2E+6	-	Natural	10/6
UV 4	101504MP	6	22	3E+5	-	.01M PBS	10/23
UV 5	101504OC	6	22	1E+5	-	.01M PBS	10/23
UV/FC 1	052104MP	6	20	1e+7/6e+4	8.7	.01M PBS	9/7
UV/FC 2	052104MP	8	20	1e+7/6e+4	8.6	.01M PBS	9/17
UV/FC 3	052104MP	8.2	20	3e+7/2e+4	8.2	Natural	10/6
UV/FC 4*	052104MP	6	20	7e+6/4e+4	8.3	.01M PBS	9/23
UV/FC 5*	052104MP	6	20	7e+6/4e+3	8.7	.01M PBS	9/23
UV/FC 6	052104MP	7.73	8.5	7e+7/4e+5	6.8	Natural	10/29

Table 1. Experimental Matix. Grey Boxes Indicate the Parameter(s) of Interest for that Particular Experiment. * *Parameter of interest was UV Pretreatment Level.*

NATURAL WATER CONSTITUENTS (mg/L)	
Calcium	31
Magnesium	28
Sodium	20
Potassium	2.3
Aluminum	.029
Copper	.008
Barium	.035
Arsenic	.0015
Bromide	< .4
Ammonia	< 1
Chloride	25
Nitrate	21
Sulfate	51
Hardness (as $CaCO_3$)	133*
Ionic Strength	.007*

Table 2. Characteristics of the Natural Surface Water Influent used in this Study.

* *Calculated values based on data above.*

3(a) SUGGESTED UV/FREE CHLORINE OPERATION FOR CASE OF *NO* BIOTERRORISM THREAT LEVEL						
pH	Temp (^oC)	UV Dose (mJ/cm^2)	CT (mg x min/L)	Logs UV Reduction	Logs FC Reduction	Logs Total Reduction
6	.5	12	165	1	0	1
7	.5	12	236	1	0	1
8	.5	12	346	1	0	1
6	10	12	87	1	0	1
7	10	12	124	1	0	1
8	10	12	182	1	0	1
6	20	12	44	1	0	1
7	20	12	62	1	0	1
8	20	12	91	1	0	1

3(b) SUGGESTED UV/FREE CHLORINE OPERATION FOR CASE OF *LOW* BIOTERRORISM THREAT LEVEL						
pH	Temp (^oC)	UV Dose (mJ/cm^2)	CT (mg x min/L)	Logs UV Reduction	Logs FC Reduction	Logs Total Reduction
6	.5	40	165	3.5	0	3.5
7	.5	40	236	3.5	0	3.5
8	.5	40	346	3.5	0	3.5
6	10	40	87	3.5	0	3.5
7	10	40	124	3.5	0	3.5
8	10	40	182	3.5	0	3.5
6	20	40	44	3.5	0	3.5
7	20	40	62	3.5	0	3.5
8	20	40	91	3.5	0	3.5

3(c) SUGGESTED UV/FREE CHLORINE OPERATION FOR CASE OF *HIGH* BIOTERRORISM THREAT LEVEL						
pH	Temp (^oC)	UV Dose (mJ/cm^2)	CT (mg x min/L)	UV Reduction	FC Reduction	Total Reduction
6	.5	40	1520	3.5	3	6.5
7	.5	40	1820	3.5	3	6.5
8	.5	40	4750	3.5	3	6.5
6	10	40	785	3.5	3	6.5
7	10	40	984	3.5	3	6.5
8	10	40	2970	3.5	3	6.5
6	20	40	410	3.5	3	6.5
7	20	40	539	3.5	3	6.5
8	20	40	1830	3.5	3	6.5

Table 3 (**a**,b,c) UV/Free Chlorine Application Scenarios and the Resultant Inactivation of ATCC 6051 *B. subtilis* Spores. Recommended Disinfectant Doses Based on US EPA Regulations (US EPA, 1989 and US EPA 2003) and the Model Generated in This Study. Refer to *Chapter 4: Design Recommendations* in Text for Details.

FIGURES

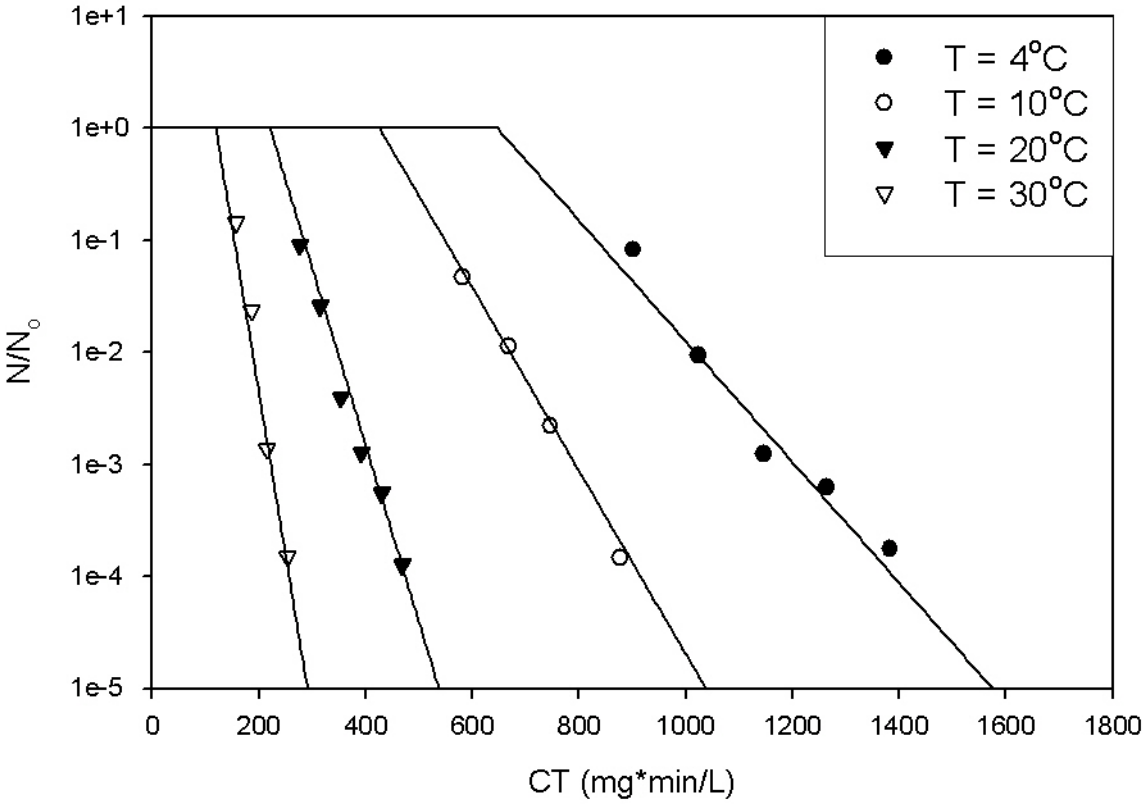

Figure 1. Effect of Temperature on the Primary Inactivation of *B. subtilis* Spores with Free Chlorine at pH 6 in .01 M Phosphate Buffer Solution (PBS).

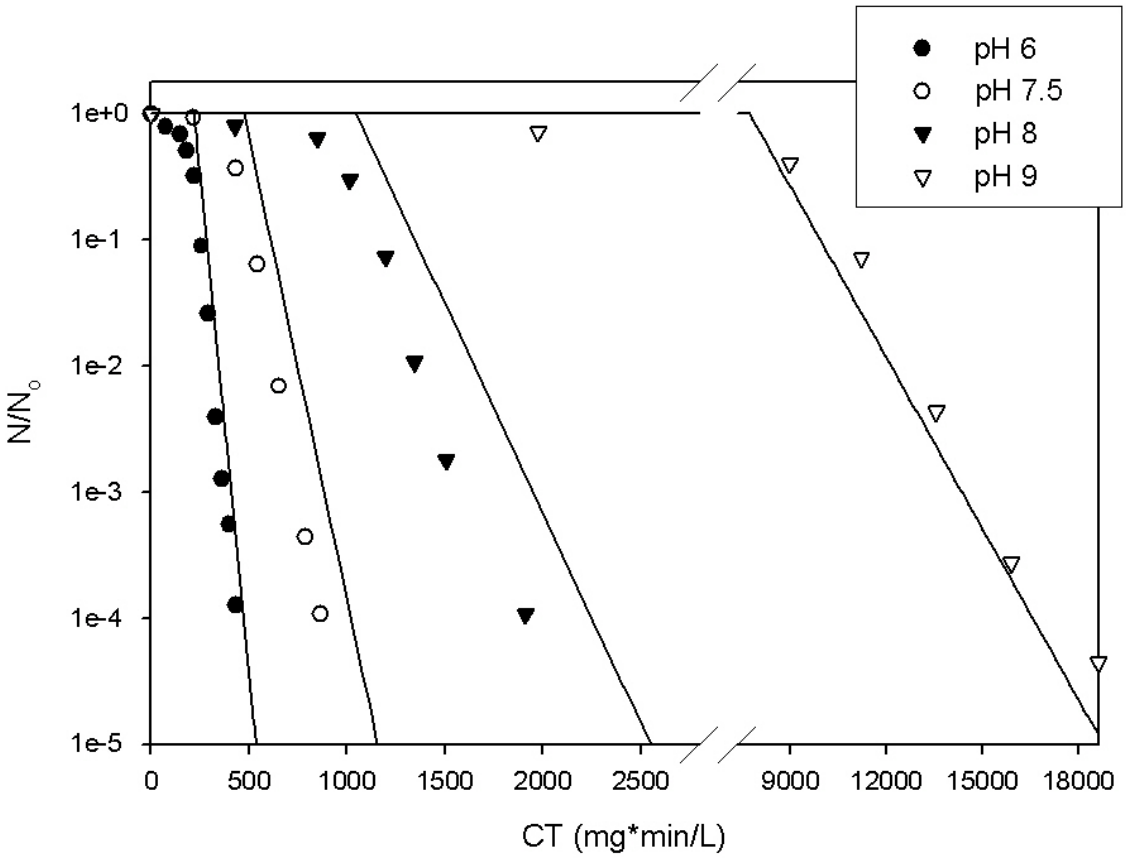

Figure 2. Effect of pH on the Primary Inactivation of *B. subtilis* Spores with Free Chlorine at 20^{o}C in .01 M Phosphate (pH 6, 7.5, 8) and Borate (pH 9) Buffer Solutions. *Note break in x-axis after 2500 mg*min/L.*

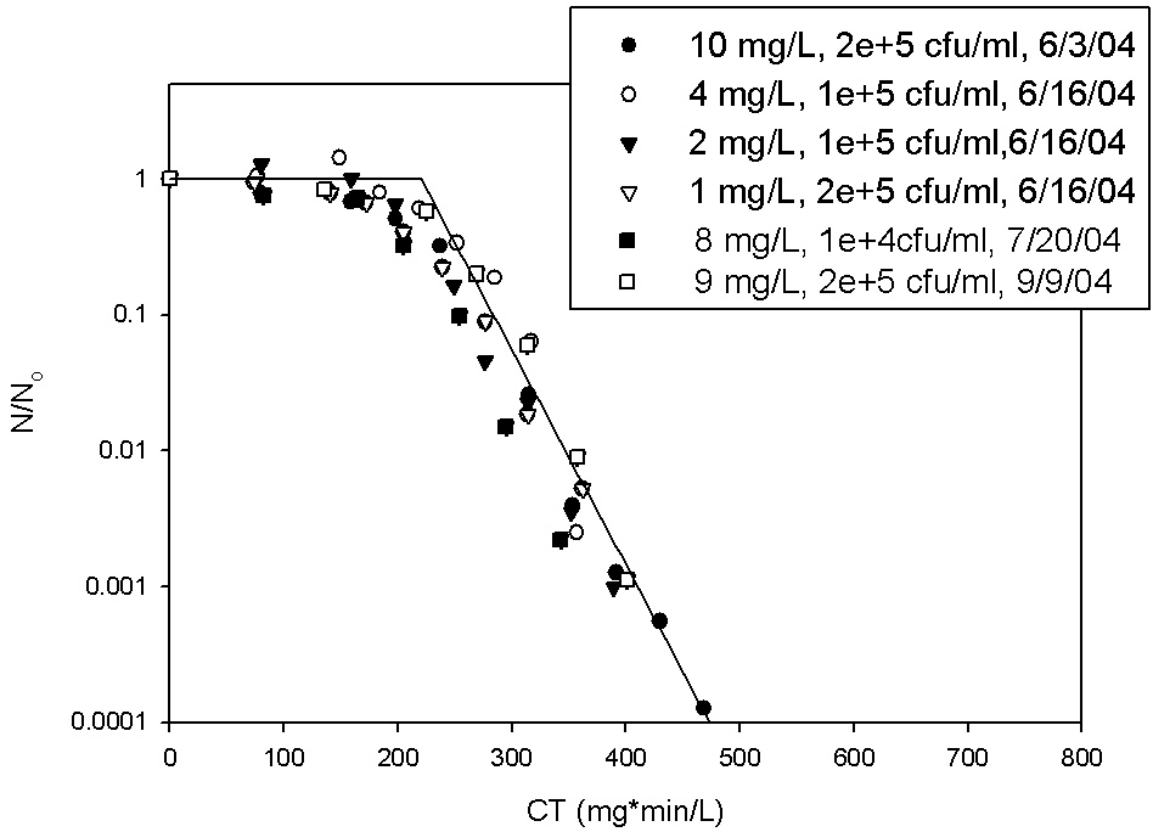

Figure 3. Effect of Disinfectant Concentration, Initial Spore Concentration, and Spore Age on the Inactivation of *B. subtilis* Spores with Free Chlorine in .01M PBS at pH 6 and 20°C.

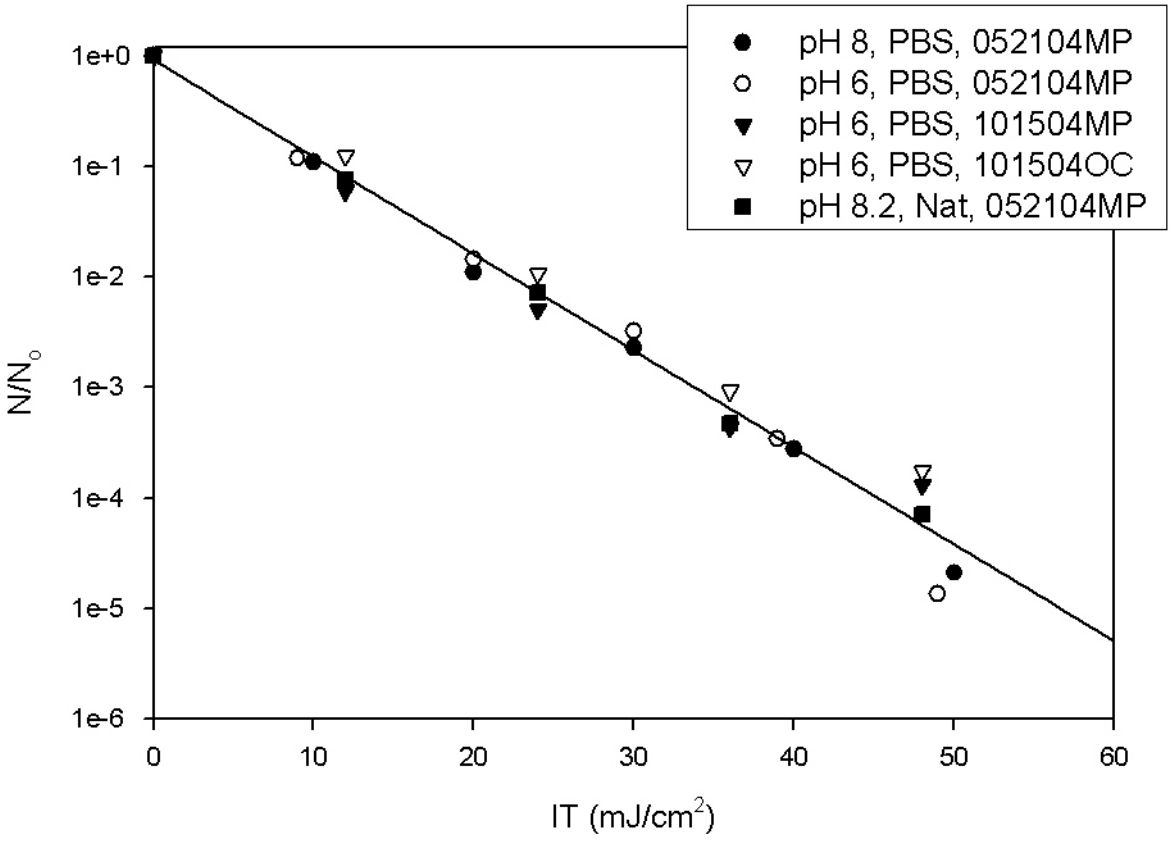

Figure 4. Inactivation of *B. subtilis* Spores with UV light in .01 M PBS (pH 6, 8) and in natural water (pH 8.2). Graph Includes Inactivation of Three Different Lots of ATCC 6051 *B. subtilis* Spores at pH 6.

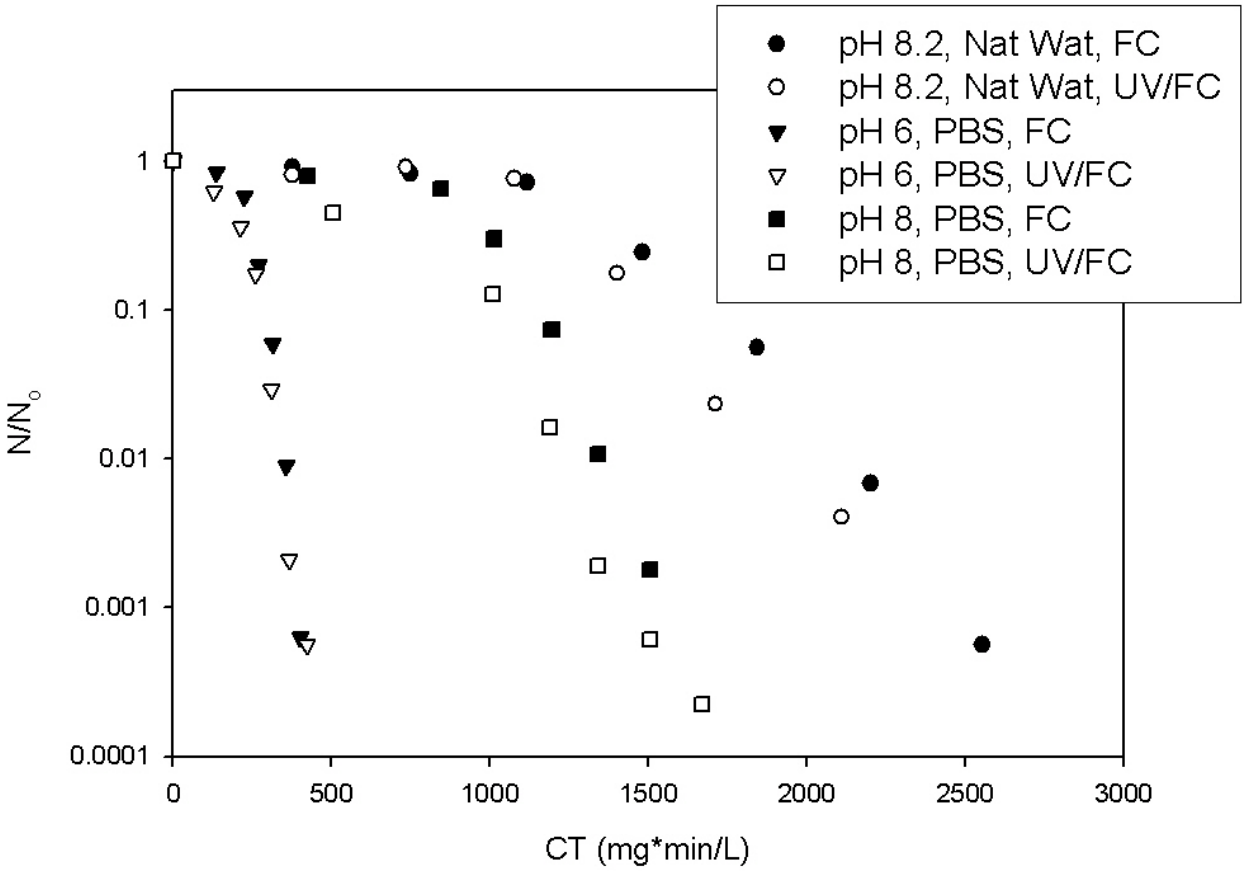

Figure 5. Primary versus Secondary Inactivation of *B. subtilis* Spores with Free Chlorine in .01 M PBS at 20^{o}C. The UV Dose Applied was 12 mJ/cm^2 in UV/FC cases.

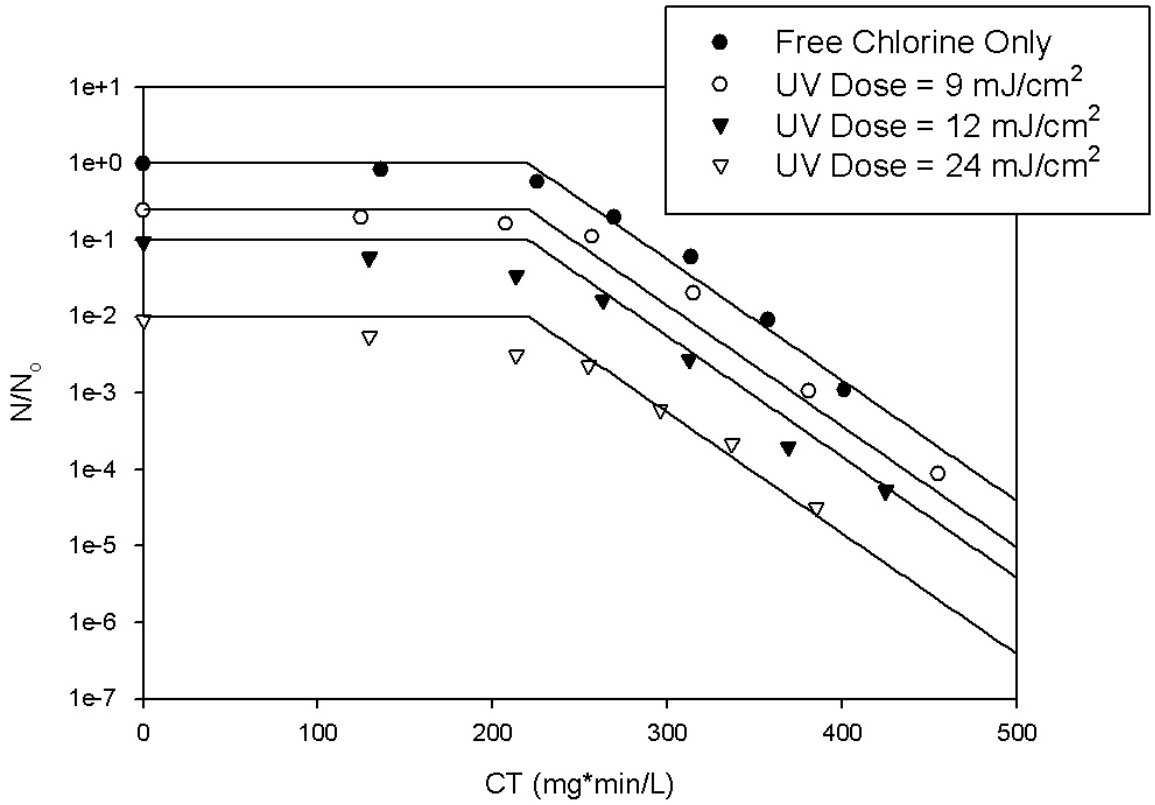

Figure 6. Effect of UV Dose on the Secondary Inactivation of *B. subtilis* Spores with Free Chlorine after UV Pretreatment in PBS at pH 6 and 20°C. The UV Doses Applied were 9, 12 and 24 mJ/cm^2.

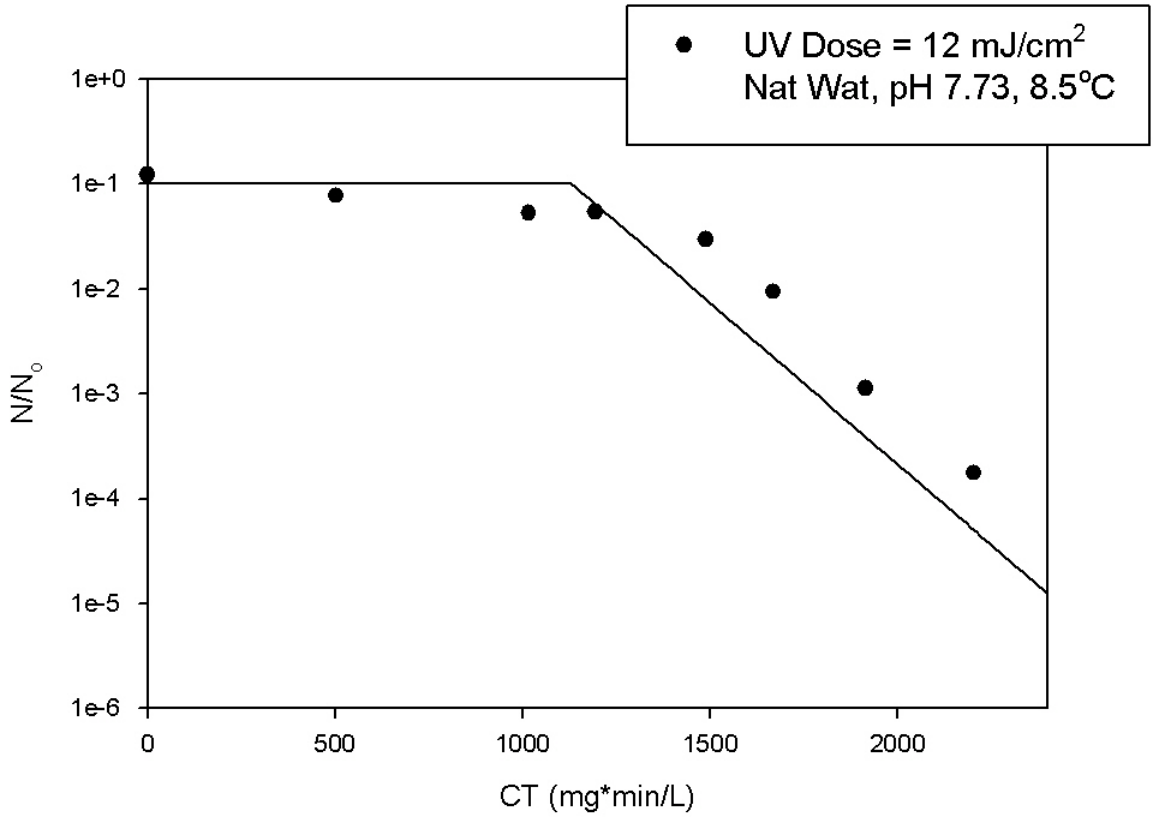

Figure 7. Model Performance: Inactivation of *B. subtilis* Spores with Sequential UV/Free Chlorine in Natural Water at pH 7.73 and 8.5°C. The UV Dose Applied was 12 mJ/cm^2.

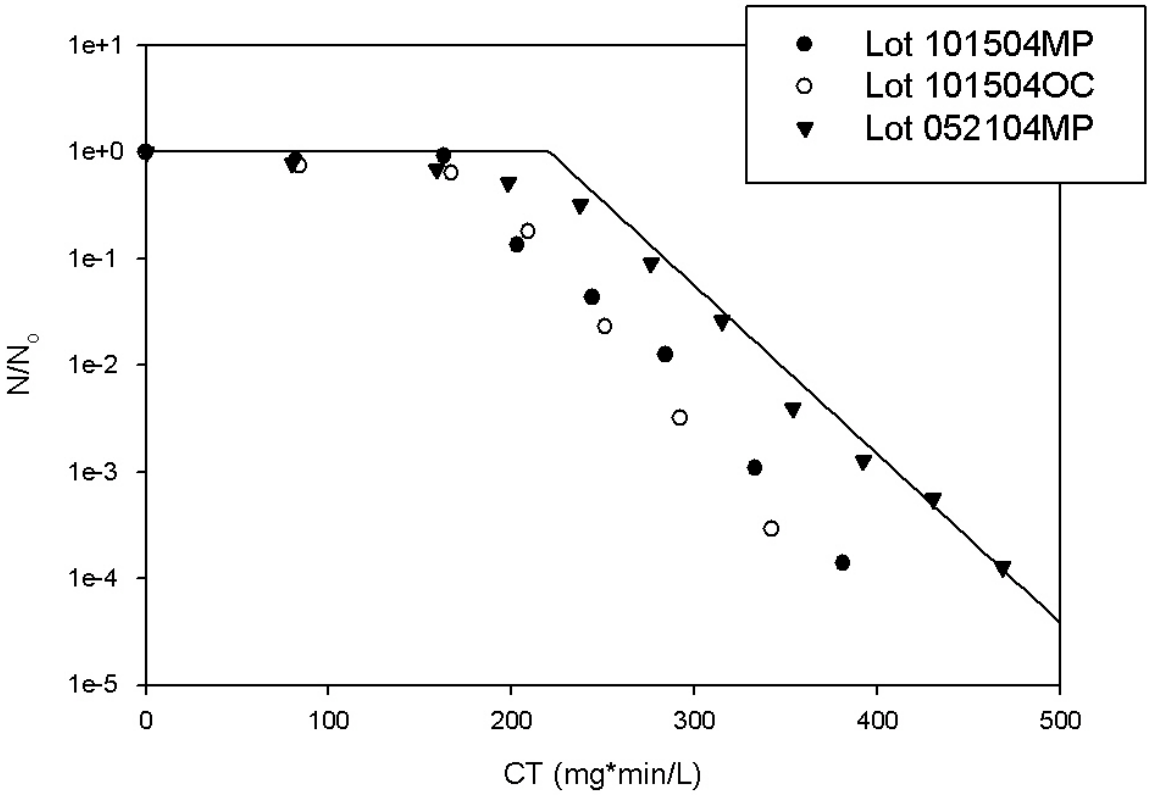

Figure 8. Variability in the Inactivation of Three Different Lots of ATCC 6051 *B. subtilis* Spores with Free Chlorine in PBS at pH 6 and 20°C.

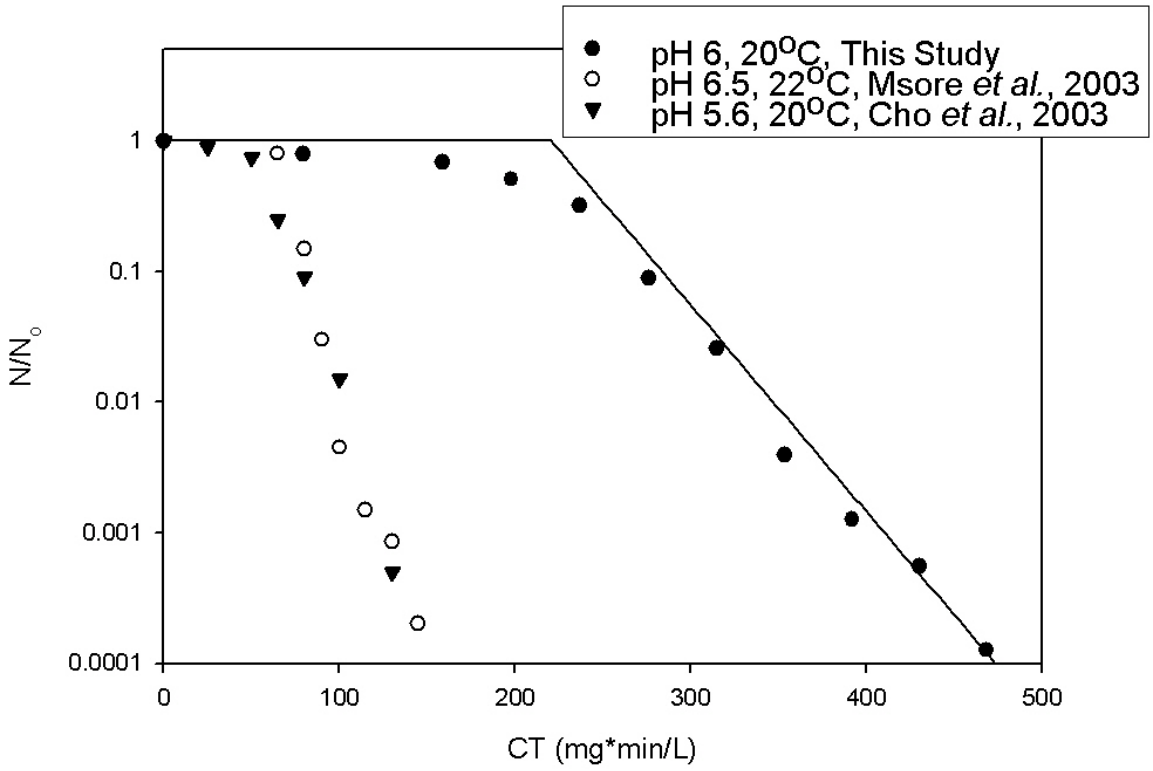

Figure 9. Variability in the Inactivation of Two Different Strains of *B. subtilis* Spores with Free Chlorine. Inactivation Data for Strain ATCC 6633 was Obtained from Mysore *et al.* (2003) and Cho *et al.* (2003).

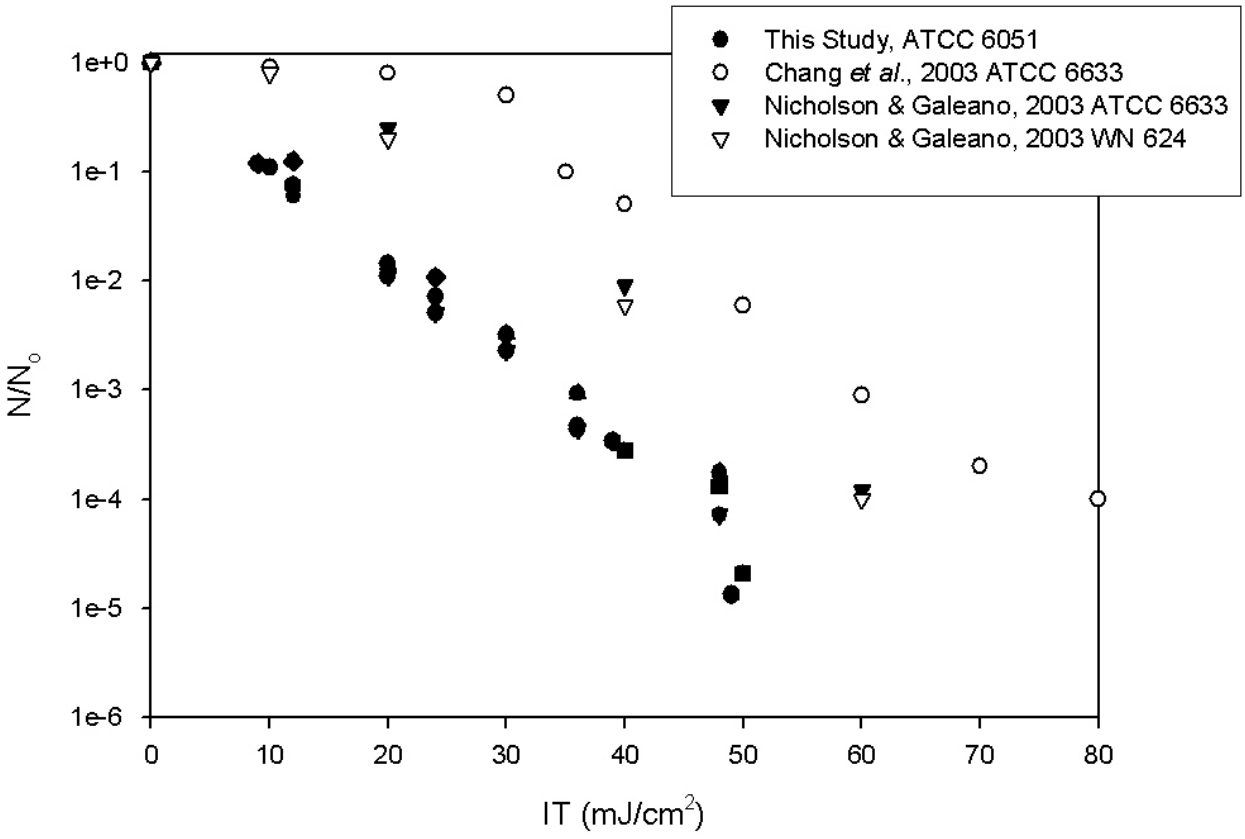

Figure 10. Variability in the Inactivation of Three Different Strains of *B. subtilis* Spores with UV light. Inactivation Data for Strain ATCC 6633 was Obtained from Nicholson and Galeano (2003) and Change *et al*. (1985). Inactivation Data for Strain WN624 was Obtained from Nicholson and Galeano (2003).