Comparative Inactivation of *Bacillus Subtilis* Spores and MS-2 Coliphage in a UV Reactor: Implications for Validation

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Abstract: Biodosimetry is the currently accepted method for validation of fluence delivery in ultraviolet (UV) reactors for water disinfection. This method utilizes the inactivation of a surrogate microorganism to predict the reduction equivalent fluence and subsequent inactivation of a target pathogen. Two common surrogates—*Bacillus subtilis* spores and MS-2 coliphage—were used to examine the relationship between surrogate type and biodosimetry results. A pilot-scale LP UV reactor was investigated at two flow conditions (7.5 and 15 gpm) and four different UV 253.7 nm water transmittance (UVT, 1 cm) values between 82 and 91%. The calculated reduction equivalent fluence differed from a maximum of 30% at 7.5 gal./min and 15% at 15 gal./min between the surrogates tested, depending on the UVT. These differences were attributed to the sensitivity of organisms used, hydraulic inefficiences, and UV fluence distribution in the reactor, thus the choice of validation microbe may impact the determination of reduction equivalent fluence in UV reactors.

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Introduction

Ultraviolet (UV) treatment of water is being increasingly used for disinfection of wastewater and drinking water in North America, Europe, and numerous other countries around the world. Direct measurement of UV fluence (commonly referred to as UV dose) delivered in a UV disinfection reactor is difficult, therefore the performance of a UV reactor is currently validated using a technique called biodosimetry. In biodosimetry, the log inactivation of a pathogen surrogate through a UV reactor is measured and the average delivered UV fluence is back-calculated from a known UV fluence–response curve (Mackey et al. 2002; Qualls and Johnson 1983).

However, biodosimetry has its challenges because natural variations in microbial physiology and chemistry dictate an organism's response to UV irradiation. Therefore, specific knowledge of microbial responses to UV irradiation is critical for predicting UV reactor fluence. The surrogates that commonly serve as a tool for validating UV reactor treatment efficiency are MS-2 coliphage and *Bacillus subtilis* spores. In North America, MS-2 is typically used for validation of UV reactors, whereas in Europe *Bacillus subtilis* spores are more common. MS-2 is more resistant to UV disinfection than most human enteric viruses (Wilson et al. 1992) other than adenovirus and is similar to many viruses in size and shape. Consequently, MS-2 is considered an appropriate surrogate organism for indicating virus inactivation or removal from water (Meng and Gerba 1996; Wok 2001). The fluence-response curve for MS-2 is a log-linear function between the $\log_{10}$ of the inactivation ratio and the UV fluence (Jolis 2002; Sommer et al. 1998). The other commonly used test organism for UV biodosimetry studies is spores of *Bacillus subtilis*, due to their comparatively high degree of UV resistance, reproducibility in inactivation response, and ease of use (Marshall et al. 2003; Nicholson and Galeano 2003). A semilogarithmic plot of spore inactivation versus UV fluence produces a characteristic curve, consisting of a shoulder at low UV fluences, followed by exponential inactivation at higher UV fluences (Nicholson and Galeano 2003), and possibly tailing. Therefore the kinetics of UV inactivation of *Bacillus subtilis* spores is more complex than the simple log-linear inactivation exhibited by MS-2. Additionally, *Bacillus subtilis* spore cultivation methods result in different susceptibilities to UV irradiation depending upon the cultivation approach used (Sommer and Cabaj 1993). *B. subtilis* spores cultivated in liquid broth and used as a biodosimeter for reactor validation were previously investigated by Cabaj et al. (2002). *B. subtilis* spores cultivated on an agar plate surface were found to be more sensitive to UV compared to the liquid cultivated spores (Sommer and Cabaj 1993).

It has been shown that a surrogate with similar sensitivity to the target pathogen will provide more precise fluence determination (Mackey et al. 2002), and reduce the reduction equivalent fluence (REF) bias that results from reactor hydraulic inefficiencies. Ideally, the pathogen and surrogate will have similar UV fluence–inactivation functions. Thus, using both sensitive and re-

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sistant surrogate organisms mixed in the same UV reactor may provide an indication as to the effect of the UV fluence–inactivation function of an organism on the REF determination and cover a validation for pathogens over a range of UV sensitivities. The fluence delivered to microorganisms in a reactor can be described by a fluence distribution, which is currently not measured directly but can be modeled for specific reactors (Ducoste et al. 2005a,b; Lawryshyn and Cairns 2003; Severin et al. 1983). The biodosimetry approach can only measure the average log inactivation of an organism through a reactor. Therefore the resulting REF calculated from the effluent log inactivation has a value between the minimum and the average of the fluence distribution curve (Cabaj et al. 1996; Mackey et al. 2002). Measuring and understanding the impact of microbial UV response on the REF estimation may improve the accuracy in calculating the REF from surrogate organisms. In this study, a dual biodosimetry test was performed using MS-2 phage and the more sensitive *B. subtilis* spores in a pilot LP UV reactor. The aim was to compare the RED measured by these two biodosimetry testing methods to gain insights into the sensitivity of the REF method to the type of organism used for reactor validation. The results were also compared to numerical simulations of the average reactor fluence.

Material and Methods

Experiments were performed using dechlorinated (with sodium bisulfite or thiosulfite) tap water at North Carolina State University, Raleigh, N.C. By quenching the initial tap water using sodium thiosulfite, the water transmittance dropped 4%, while using sodium bisulfite the drop in transmittance was lower (2%). Further, the water transmittance was artificially adjusted with instant coffee. Two runs with spores and MS-2 mixed together were performed and two other runs were performed: one only with more resistant *B. subtilis* spores, the other one only with MS-2. The separate run for MS-2 was chosen as a control run to assure that there was no unpredictable interference in the reactor due to using the mixture of microorganisms. In the case of *B. subtilis* spores, the separate run was done for liquid cultivated spores, which are commonly used for reactor validation in Europe as compared to surface cultivated spores in our study. These runs will be denoted as MS2Bac1 (spores+MS-2 mixture), MS2Bac2 (spores+MS-2 mixture), Bac (spores only), and MS2 (MS-2 only). Mixed microbial suspensions were spiked into a 400 gal. water reservoir containing dechlorinated tap water and mixed for 10 min. The UV lamp was turned on prior to the start of the experiment to allow the lamp to warm up. The flow rate was then adjusted to the desired values prior to UV exposure and sampling. At least three influent and effluent samples were taken for each test condition. In addition to the UV exposure test, a control was performed with the UV lamp off while one influent and three effluent volumes were sampled. A schematic of the pilot UV reactor and experimental settings is displayed in Fig. 1. The experimental test conditions included flow rates of ~7.5 gal./min and ~15 gal./min, and four UV water transmittances (UVT at 253.7 nm, 1 cm) 91.4, 88, 85.5, and 82.5%. Additional water samples were collected to perform the bench scale quasi-collimated beam (CB) tests and for water analyses. These items are described below.

Water Analyses

Analyses of tap water, water spiked with *B. subtilis* spores and MS-2, and water after addition of coffee were performed within 48 h after the experimental run. Absorbance, conductivity, pH, alkalinity, total organic carbon, turbidity, and transmittance at 253.7 nm were measured according to standard methods for examination of water and wastewater (APHA 1998).

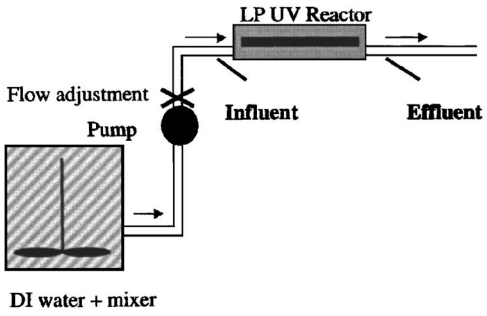

Fig. 1. Schematic figure of pilot test LP UV reactor and experiment settings. Arrows present direction of water flow.

Microbe Propagation and Enumeration

MS-2 Coliphage

The method described in International standard ISO 10705-1 (ISO 1995) was used for the propagation and enumeration of phage. In short, *E. coli* host C3000 (ATCC 15597) was cultivated using the recommended tryptone based broth in incubator at 37°C shaking for 4 h until the concentration was 10^{+08} cells/mL. 1 mL of *E. coli* host and 0.1 mL of concentrated MS-2 (ATCC 15977-B1) were spiked into 4 mL of soft agar tempered to 43–45°C, prior to the solution being immediately poured on the surface of Petri dish with TYGA (tryptone based) agar. After 24 h of incubation, the soft agar was scraped off the surface of the agar plates and centrifuged with phosphate buffer saline (PBS) at 1,000 rpm for 25 min to settle the cellular debris and agar. The supernatant was filtered through 0.20 μm Millipore filter and the filtrate was stored in the refrigerator at 4°C. A similar method was used for MS-2 enumeration with the exception that the *E. coli* host was quickly cooled down on melting ice after cultivation in the incubator. The sample containing MS-2 was diluted as desired and 0.1 mL was added into a test tube with 4 mL of soft agar and 0.5 mL of host. The mixture was poured on TYGA agar plate and allowed to solidify. The mixture was then incubated upside down at 37°C for 18 h, after which the MS-2 plaques were counted. The same MS-2 parent stock was used in all experimental runs. For MS-2 enumeration, the samples were first filtered through a 0.45 μm filter to avoid *B. subtilis* spores contamination on the overlay plate. In control experiments, there was no MS-2 loss due to filtering (data not shown) and no interference from the *Bacillus* spores in the mixture when used in MS-2 enumeration.

Surface and Liquid Cultivated *Bacillus Subtilis* Spores

Sommer and Cabaj (1993) proposed two methods for cultivation of *Bacillus subtilis* spores (ATCC 6633); one involved production of spores in a broth liquid, termed liquid cultivated spores, and the other involved production of spores on a solid medium termed surface cultivated spores. Liquid cultivated spores were kindly provided by Dr. R. Sommer (University of Vienna, Austria), freeze dried, and sent to Duke University laboratories for analysis. These spores were cultivated in liquid enrichment media (Sommer 1991) in a fermentation device with air and constantly

stirred. The cultivation process of surface cultivated spores consisted of streaking spores on Columbia agar plates (Oxoid CM 331) prepared with 1% $CaCl_2$ for 24 h to assure isolated colonies. Pure colonies were then inoculated into liquid tryptic soy broth. After 4–5 h incubation, the bacteria were inoculated on Columbia plates and left to resporulate for 7 days at 37°C. Spores were collected and harvested by washing with sterile, deionized water three times, centrifuged at 5,000 *g* for 15 min. To enumerate spores, it was necessary to eliminate the vegetative bacteria by pasteurization. The suspension was placed in a water bath at 80°C for 10 min to pasteurize and then refrigerated at 4°C. Spore concentration of nonirradiated and UV irradiated samples was determined after serial tenfold dilutions, followed by distributing 1 mL aliquots of the suspension on empty agar plates, pouring the agar medium (plate count agar) at 45°C into the plates (about 15–20 mL) and incubating 48±4 h at 37°C. For each experimental run, fresh surface cultivated spores stocks were prepared. Preliminary experiments verified that there was no interference in enumeration of the spores in the presence of MS-2 coliphage.

UV Test Reactor and Modeling

The test reactor contained one low pressure (LP) UV lamp oriented parallel to the flow, with output principally at 254 nm. The lamp sleeves were chemically cleaned regularly to reduce unnecessary reversible fouling. In this study, a finite volume based commercial computational fluid dynamics (CFD) code PHOENICS (CHAM, U.K.) was used for modeling. The transport of the microorganisms through the UV reactors was simulated using a Lagrangian particle tracking approach (Ducoste et al. 2005a,b). The Lagrangian particle tracking approach requires the solution of the mass, momentum, and turbulence equations. In this study, the renormalized group (RNG) two-equation k-ε model was used to characterize the turbulence in the UV reactor (Wilcox 1998). A spatial homogeneous concentration of 2,500 particles was released at the UV system influent. Each particle was tracked until it exited the reactor. The fluence, as seen by each particle, was calculated by integrating the fluence rate (commonly referred to as irradiance) values over the particle track time history using the following equation:

$$H'(P) = \int_0^T E'(t)dt \approx \sum E'\Delta t \tag{1}$$

where T=total particle time spent within the reactor; Δt=particle time step along an individual particle track; $H'(P)$ =UV fluence for particle P (J/m^2 or mJ/cm^2); and E'=fluence rate (W/m^2 or mW/cm^2).

Using the Lagrangian particle tracking method, the overall microbial inactivation can then be computed as

$$\left(\frac{N}{N_0}\right)_{\text{effluent}} = \int_0^\infty f(H')E(H')dH' \tag{2}$$

where $f(H')$ represents bench-scale microbial inactivation function determined for a specific organism; $(N/N_0)_{\text{effluent}}$ represents effluent normalized concentration of surviving organisms; and $E(H')$ represents particle number fraction. The bench scale microbial inactivation kinetics equation will be described in a later section.

The local fluence rates in the UV reactor were computed using the RADial line source integration (RAD-LSI) model (Liu et al. 2004). The RAD-LSI model accounts for the reduction in transmittance due to reflection, refraction (bending effect only), and absorption (both in the fluid and through the quartz sleeve) and UV-C lamp efficiency. In addition, the RAD-LSI includes terms to help reduce the high fluence rate values that the LSI has been shown to predict at distances close to the lamp surface (Liu et al. 2004).

Convergence of the numerical solution was based on: (1) the sum of the absolute residual sources over the whole solution domain must be less than 0.1% of the total inflow quantity; and (2) the values of the monitored dependent variables at several locations must not change by more than 0.1% between successive iterations. The grid size was determined through successive refinement and evaluating its impact on the concentration, turbulence, and mean velocity profiles at selected points in the UV reactor. The final grid size was determined once these profiles were insensitive to further grid refinements. UV simulations were done with a structured grid. Irregular boundaries were handled using a cut-in cell method (Yang et al. 1997).

Quasi-Collimated Beam Settings

UV exposures for producing standard UV fluence-response curves were performed in a bench scale "collimated beam" LP UV system (monochromatic output, 253.7 nm). Experiments were performed in completely mixed Petri dishes with 10 mL sample at UV fluences of 5, 10, 20, 30, 40, and 50 mJ/cm^2. UV absorbance (200–400 nm) of the samples spiked with microbes was measured in a spectrophotometer (Cary 100-bio, Varian corp., Houston). UV fluence (mJ/cm^2) was calculated as the average irradiance multiplied by the exposure time. UV incident irradiance (mW/cm^2) was measured at the surface of the liquid suspension using a radiometer (International Light IL1700 SED240/W) calibrated at 254 nm using methods traceable to the National Institute of Standards and Technology. The average UV irradiance in the mixed sample was determined using the protocol described by Bolton and Linden (2003).

Kinetic Analyses for Bacillus Subtilis Spores

Shoulder and tailing in a fluence-inactivation curve are descriptions of nonlog-linear responses. A shoulder refers to the section of the curve in which survival is higher than the expected linear response at low UV fluences, while tailing refers to extended survival at high UV fluences. The survival function of microorganisms with shoulder and tailing can be modeled using an equation with four parameters (k_1, k_2, d, a) developed by Cabaj and Sommer (2000) and experimentally validated by Mamane-Gravetz and Linden (2005). Spores can exhibit two different fluence-based kinetic zones where the first-order inactivation zone corresponds to the sensitive portion of the spore population (k_1) and the tailing zone corresponds to the less sensitive portion of the population (k_2)

$$\frac{N}{N_0} = \frac{1-(1-10^{-k_1\cdot H'_0})^{10^d} + a\cdot 10^{-k_2\cdot H'_0}}{1+a} \tag{3}$$

$$a = \frac{N_{0,2}}{N_{0,1}} \tag{4}$$

where N/N_0=survival rate; d=y axis intercept of the first order linear zone in the fluence–response curve; N_0=initial concentration of sensitive ($N_{0,1}$) and less sensitive ($N_{0,2}$) microorganism; a=y axis intercept in logarithmic scale of the fluence–response

Table 1. Analyses of Water Spiked with Microorganisms and with Addition of Coffee Used for Experiments Performed in February (MS2Bac1) and March (MS2Bac2) 2004

Water characteristic	MS2Bac1	MS2Bac2
Transmittance (%)	82.5	85.5
Absorbance (254 nm)	0.0830	0.0805
Turbidity (NTU)	0.49	0.62
pH	7.7	7.0
Alkalinity (mg/L $CaCO_3$)	29	23
Conductivity (μS)	191	174
TOC (mg/L)	10.3	9.9

curve in the tailing zone (where $N_{0,2} \ll N_{0,1}$); and H_0'=fluence at 253.7 nm (mJ/cm^2).

The survival function of microorganisms with first-order inactivation such as MS-2 without shoulder and tailing (the parameters k_2, d, and a are assumed equal to zero), can be modeled as follows:

$$\frac{N}{N_0} = 10^{-k_1 \cdot H_0'} \tag{5}$$

Data Analysis

The data are presented as function of UV fluence and fluence rate, terms commonly referred in engineering literature as "UV dose" and "irradiance," respectively. Analysis of covariance (ANCOVA) was used for data comparison and linear and nonlinear regressions for analysis of fluence–response behavior and calculation of regression equation for REF (software: JMP 3.1.2, SAS institute Inc., Cary, N.C., 1995).

Results and Discussion

Effect of Water Quality

Results of the analyses of the tap water after spiking the microorganisms and after addition of coffee for each run are presented in Table 1. Addition of microorganisms reduced the UV 253.7 nm transmittance by about 2% while coffee was added to reduce the UV transmittance further by 6%. The water absorbance, turbidity, and total organic carbon (TOC) increased with added spores and MS-2, while other measured water characteristics (pH, alkalinity, and conductivity) were not affected. The addition of coffee did not affect any of the measured characteristics of the water except transmittance and TOC. The turbidity increased from 0.29 to 0.49 after spiking microorganisms in the MS2Bac1 run while in the MS2Bac2 run the turbidity went from 0.15 to 0.62 NTU. These differences between runs can be explained by the fact that in run MS2Bac2, higher initial concentration of *B. subtilis* spores and MS-2 were spiked to the water. In MS2Bac1 experiment, surface cultivated *B. subtilis* spores and MS-2 phage were spiked in concentrations of $4.8 \times 10^{+04}$ and $2.4 \times 10^{+04}$ cfu/mL, respectively. In MS2Bac2 experiment, the initial concentration of *B. subtilis* spores and MS-2 were $6.5 \times 10^{+04}$ and $2.7 \times 10^{+05}$ cfu/mL.

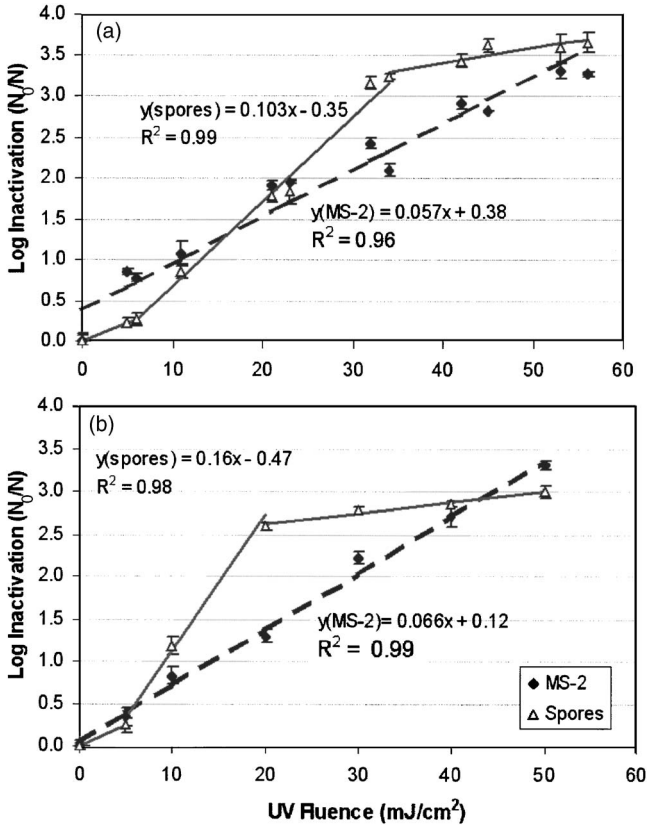

Fig. 2. Bench scale LP UV fluence–response curves for MS-2 and *B. subtilis* spores: (a) in MS2Bac1 experiment run; (b) MS2Bac2 experiment run. Regression equations on graph are presented only for linear fits of regression. Points represent averages of four repeated UV exposures (two repetitions for each influent) and plating of each exposure in triplicate; error bars represent standard deviations.

Log Inactivation of MS-2 Compared to Surface and Liquid Cultivated Spores

The collimated beam experiments were performed using samples from the influent to the LP UV reactor containing mixed *B. subtilis* spores and MS-2. Fig. 2 illustrates the log–inactivation curves for spores and MS-2 as a function of UV fluence. The two experiments are presented separately due to the different batches of surface spores that were cultivated for each experimental run and they showed different fluence–response relationships. In the UV fluence–response curve for surface spores, there is presence of a shoulder at UV fluences of 0–5 mJ/cm^2 for both MS2Bac1 and MS2Bac2 runs and tailing was observed beyond fluence of 32 mJ/cm^2 for the MS2Bac1 run and 20 mJ/cm^2 for the MS2Bac2 run. The reasons for the tailing of these spores are not clear as the counts were around or above detection limit for most tested UV fluences. Similar nonlinear UV fluence–response relationships for *B. subtilis* spores was described in previous studies (Nicholson and Galeano 2003; Harm 1980; Severin et al. 1983). The fluence–response data of the log–linear phase were fitted using a linear regression approach and the difference in inactivation between spore or MS-2 samples was determined by comparing the rate coefficients.

The inactivation rates of the log-linear phase in the fluence–response curve for the different batches of spores were significantly different ($p < 0.05$). In the MS2Bac1 run, a linear relationship was observed between fluence of 5 and 32 mJ/cm^2 with

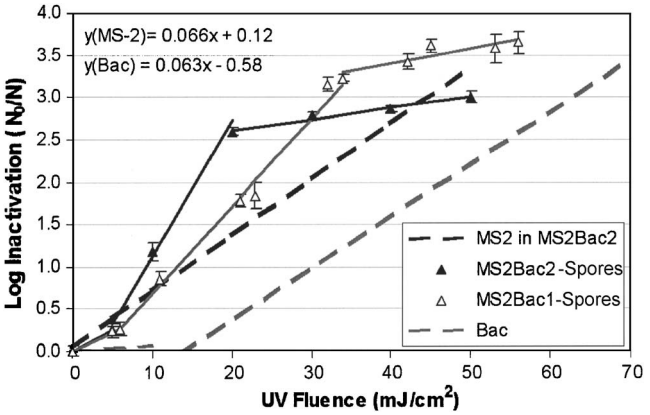

Fig. 3. LP UV fluence–response curves for *B. subtilis* spores liquid cultivated (separate run Bac) and surface cultivated (Runs MS2Bac1 and MS2Bac2). Regression equations on graph are presented only for linear fits of regression function. Points represent averages of four repeated UV exposure (two repetitions for each influent) and plating of each exposures in triplicate; error bars represent standard deviations.

inactivation rate constant of 0.106 cm^2/mJ. The spores MS2Bac2 run had inactivation rate constant 0.16 cm^2/mJ in the area of linear relationship between fluences 5 and 20 mJ/cm^2. Sommer et al. (1995) found that a fluence of 16.9 mJ/cm^2 was needed for 2-log inactivation of *B. subtilis* spores by LP while Nicholson and Galeano (2003) observed that a LP UV fluence of 24.5 mJ/cm^2 was needed for 2-log reduction of these spores. In our experiment, spores in the MS2Bac1 and MS2Bac2 runs showed 2-log inactivation after fluences 22.8 and 15.4 mJ/cm^2, respectively. Differences within the same species of bacterial spores are known to occur due to cultivating methods (Nicholson and Galeano 2003; Sommer and Cabaj 1993). The difference between inactivation of surface cultivated spores with and without coffee addition in the collimated beam experiment was not statistically significant ($p=0.85$, 0.98 in MS2Bac1 and MS2Bac2 run, respectively).

The MS-2 fluence-response relationship followed first-order kinetics with inactivation rate constants of 0.066 and 0.054 cm^2/mJ for the MS2Bac1 and MS2Bac2 runs, respectively. The inactivation rate constant for MS-2 described previously were 0.04 (Mackey et al. 2002) and 0.058 (Meng and Gerba 1996). The measured inactivation of MS-2 due to LP UV radiation was within the 90% prediction interval for MS-2 fluence–response published in the EPA UV *Disinfection Guidance Manual* (UVDGM) draft (USEPA 2003). No significant difference was observed between MS-2 inactivation in MS2Bac1 and MS2Bac2 experimental runs ($p=0.67$) and in inactivation of MS-2 between samples with and without coffee ($p=0.37$, 0.65 for MS2Bac1 and MS2Bac2, respectively).

In both bench scale experiments, there was a difference in LP UV inactivation between spores and MS-2. Due to the shoulder, spores showed slower inactivation rate up to the fluences 5.7 and 15.9 mJ/cm^2 in the MS2Bac1 and MS2Bac2 runs, respectively. At higher fluences spores were inactivated faster than MS-2. Overall, in the fluence interval from 0 to 60 mJ/cm^2, MS-2 displayed a higher resistance to LP UV irradiance than surface cultivated *B. subtilis* spores.

Log survival of surface cultivated spores, liquid cultivated spores, and MS-2 as a function of UV fluence is presented in Fig. 3. For comparison, spores were also cultivated by the liquid technique and used in the reactor tests (Bac run) as presented in Fig. 2. For liquid cultivated *B. subtilis* spores, the inactivation rate constant in the collimated beam experiment was 0.063 cm^2/mJ and shoulder up to 10 mJ/cm^2. The fluence–response curve of the liquid cultivated spores was within the 90% prediction interval for *B. subtilis* spores published in the EPA UVDGM draft (USEPA 2003). Compared to the other results, liquid cultivated spores were more resistant to UV irradiation than surface cultivated spores and MS-2. This is consistent with findings of Sommer et al. (1998) that described liquid cultivated *B. subtilis* spores as more resistant to LP UV than MS-2. Although the MS-2 inactivation rate constant is only slightly higher (0.066 cm^2/mJ) than that of liquid cultivated spores (0.063 cm^2/mJ) in the log-linear phase of the inactivation curve, the liquid cultivated spores were overall more resistant to UV due to the shoulder observed at low UV fluence. For instance, the liquid cultivated spores are inactivated less than 2 log after irradiation by UV fluence of 40 mJ/cm^2, while the MS-2 is inactivated almost 3 logs at this fluence. The results of the present study show that a larger inactivation rate constant value does not necessarily lead to a lower UV resistance because the shape of UV response curve can alter the result.

Table 2. Average Log Inactivation (± Standard Deviation in Parentheses) of MS-2 and *Bacillus Subtilis* Spores in Reactor for Each Experimental Setting. Modeled Log Inactivation Is Also Shown for Comparison.

Flow (gal./min)	Water transmittance (%) (1 cm)	Log reduction (N_0/N_d)		Modeled log reduction (N_0/N_d)	
		MS-2	Spores	MS-2	Spores
7.5	91.4	3.04(0.05)	3.09(0.05)	2.86	2.99
	88.1	2.93(0.03)	3.67(0.09)	2.77	3.21
	85.5	2.80(0.04)	2.92(0.03)	2.40	2.93
	82.5	2.79(0.06)	2.72(0.03)	2.17	2.66
15	91.4	2.11(0.05)	2.81(0.04)	1.65	2.62
	88.1	2.07(0.03)	2.11(0.12)	1.71	1.71
	85.5	1.99(0.02)	2.72(0.04)	1.40	2.32
	82.5	1.90(0.02)	1.59(0.06)	1.35	1.34

Implications for Reactor Validation

The experimental design consisted of a mix of *B. subtilis* spores and MS-2 flowing through the LP UV reactor. The average log reduction of MS-2 and surface cultivated spores in the LP UV pilot reactor for all the experimental settings are presented in Table 2. Table 2 also displays the numerical model predictions for the microbial log inactivation. The modeled fluence distributions associated with each operating condition are displayed in Fig. 4. Both the numerical and experimental inactivation data displayed a decrease in inactivation with increasing flow as seen in previous experiments (Mackey et al. 2002).

When the flow through the reactor was 7.5 gal./min, the *B. subtilis* spores were always inactivated at a higher rate compared to MS-2 ($p<0.05$), which indicates higher sensitivity of the surface cultivated spores to LP UV. This higher sensitivity of surface spores was confirmed by the collimated beam experiment (Fig. 2). According to the CFD modeled fluence distribution (Fig. 4), the lowest fluence in the LP UV reactor with flow of 7.5 gal./min was 25, 23, 19, and 17 mJ/cm^2 for water transmittances 91, 88, 86, and 83%, respectively. These fluences are high enough such

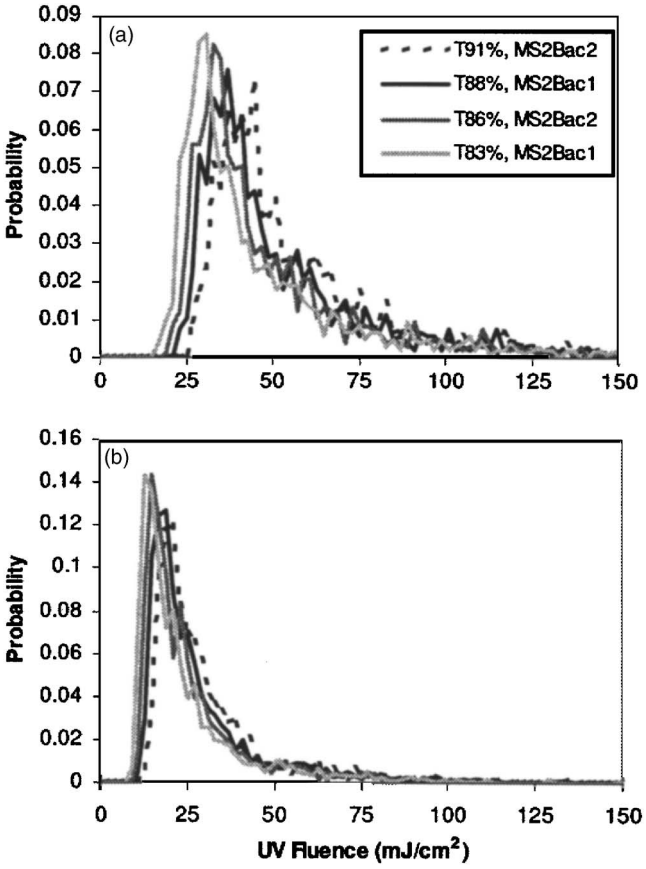

Fig. 4. Modeled fluence distribution in LP UV reactor used for experimental runs: (a) flow ~7.5 g; (b) flow ~15 gal./min. Four different water transmittances (UVT) 91, 88, 86, and 83% were examined.

that the result of the inactivation of spores is not affected by the shoulder of the fluence–response curve.

The log inactivation of *B. subtilis* spores at the flow of 15 gal./min in the LP UV reactor was significantly higher ($p < 0.05$) compared to MS-2, with the exception of the run with lowest water transmittance (82.5%). In the experimental run MS2Bac1, where more UV sensitive surface spores were used, and at the low water transmittance, the spores were inactivated slower than MS-2. The lowest modeled fluence in the reactor at these conditions (flow 15 gal./min and water transmittance 83%) was 9 mJ/cm^2. This contradictory result to previous studies maybe due to the shoulder in the UV fluence–response of *B. subtilis* spores, which is apparently making the spores more resistant to UV than MS-2 in low UV fluence range.

Overall, the modeling results displayed reasonable agreement with the experimental log inactivation. As can be seen in Table 2, the model results were generally within 20% of the experimental results with the exception of the MS-2 high flow (15 gal./min), low UV water transmittance (UVT) (i.e., 82.5 and 85.5%). These two cases deviated from the experimental results by ~30%. One explanation is that the higher flow rate cases may have experienced additional mixing due to transient turbulent structures that brought a higher fraction of the microorganism tracks closer to the lamp surface. This additional transient mixing would be more critical under low UVT conditions due to significant variation in the fluence rate field. These transient turbulent structures are not described with the steady-state turbulent fluid flow model used in this study.

Table 3. REF Measured in Reactor Using MS-2 and *Bacillus Subtilis* Spores and Calculated REF for Target Pathogen *Cryptosporidium*

Flow gal./min	Water transmittance (%)	REF (REF bias) MS-2	Spores	Cryptosporidium calculated
7.5	91.4	44(1.07)	~50(~1.22)	41
	88.1	40(1.08)	~53(~1.43)	37
	85.5	41(1.21)	43(1.27)	34
	82.5	43(1.43)	30(1.00)	30
15	91.4	30(1.43)	33(1.57)	21
	88.1	27(1.42)	23(1.22)	19
	85.5	28(1.65)	27(1.59)	17
	82.5	28(1.75)	19(1.19)	16

Note: REF bias is included in parentheses.

Reduction Equivalent Fluence

Table 3 displays the REF for MS-2 and *B. subtilis* spores for the different test conditions. In addition, Table 3 also displays the REF for *Cryptosporidium* using the UV response kinetics from the EPA UVDGM draft (USEPA 2003) and the computed REF bias based on the fluence distribution illustrated in Fig. 4. In Table 3, the MS-2 REF was 40–44 mJ/cm^2 at the low flow rate (7.5 gal./min) while 27–30 mJ/cm^2 at the high flow rate (15 gal./min). The *B. subtilis* REF was 30–53 mJ/cm^2 for the low flow rate and 19–33 mJ/cm^2 for the high flow rate, as calculated by the nonlinear equation. On average, the MS-2 REF dropped 33% and the *B. subtilis* REF dropped in excess of 42% when the flow rate doubled.

The REF measured with the two biodosimeters differed from 1 to >13 mJ/cm^2. This range is likely due to the wide range of the fluence distribution in the reactor (Cabaj et al. 1996; Ducoste et al. 2005a,b). The flow pattern in the reactor is therefore very important, because the broad fluence distributions give lower biodosimetry results (Cabaj et al. 1996). The fluence distribution in the reactor was modeled (Fig. 4) and a wider distribution was found in the system when the flow was 7.5 gal./min compared to the distribution with flow of 15 gal./min. This wider distribution at the lower flow rate was also experimentally indicated by Bohrerova et al. (2005). Therefore the differences in REF value calculated using either MS-2 or *B. subtilis* spores were lower in the 15 gal./min flow experiments (average difference was 4.3 mJ/cm^2) as compared to the 7.5 gal./min flow experiments (average difference in REF values was 8.5 mJ/cm^2).

Although a biodosimeter with a shouldered curve would result in higher REF values compared to nonshouldered curves under similar sensitivity (Cabaj and Sommer 2000), the *B. subtilis* spores used in this study were more sensitive to UV compared with MS-2. With the exception of the two highest UVTs at the low flow condition where the tailing in the spore UV response curve affected the results, the *B. subtilis* biodosimeter displayed a lower or similar REF compared to MS-2. The lower REF for spores was pronounced especially in the MS2Bac1 experiment (average difference in REF 9 mJ/cm^2), where work was performed with a more UV sensitive spore batch than in the MS2Bac2 run (average difference in REF 3 mJ/cm^2). The higher REF measured with more resistant organisms compared to more

Table 4. Comparison of REF for *B. Subtilis* Spores Using Nonlinear Fluence–Response Curve for Calculation, or Linear Fluence–Response Curve. REF Values Are Added Also for Liquid Cultivated Spores (Run Bac).

Flow (gal./min)	Water transmittance (%)	REF	
		Nonlinear	Linear
7.5	91.4	>50	23
	88.1	>53	31
	85.5	43	22
	82.5	30	24
	Bac N/A	56	55
	Bac N/A	37	41
15	91.4	33	21
	88.1	23	17
	85.5	27	21
	82.5	19	13
	Bac N/A	30	29
	Bac N/A	23	25

Note: Bac N/A=water transmittance is not available.

sensitive ones was also described previously (Cabaj et al. 1996).

Two of the experimental REFs for the spores at the low flow (7.5 gal./min) and high UVT conditions (i.e., 88.1 and 91.4%) were found to exceed the maximum measured fluence in the *Bacillus subtilis* UV response kinetics curve. In this case, the UV fluence in the reactor was in the tailing region of the spores' fluence-response curve and the REF was higher for *B. subtilis* spores than MS-2. The calculated REF in this region was found to be very inaccurate, even when the nonlinear equation was used. An error analysis was performed that involved the calculation of the REF using averages of log inactivation ± standard deviations in the tailing region of the fluence-response curve for the spores and the resulted REF was found to vary by ±4 mJ/cm^2. Consequently, an approximation sign (~) was placed in front of this value in Table 3 due to the uncertainty in the UV fluence measured. Moreover, the impact of ignoring spore tailing on REF was determined by assuming the parameters k_2 and a are equal to zero [Eq. (3)], resulting in a linear fluence–response curve including the shoulder. The comparison between a nonlinear fluence–response curve to this linear fluence–response curve is presented in Table 4. This comparison confirms that ignoring tailing of microorganisms that have a nonlinear fluence–response curve results in erroneous REF values. Thus, the tailing and the shoulder complicate the physical explanation of the REF phenomenon.

Table 3 also displays the computation of the REF bias for the two biodosimeters. The REF bias was proposed by the EPA UVDGM draft (USEPA 2003) to account for the differences in computed REF using a challenge microorganism that is significantly different in UV response than the target microorganism, such as *Cryptosporidium* is to MS-2 coliphage. As can be seen in Table 3, the *Cryptosporidium* REF values are generally smaller than both the MS-2 and *B. subtilis* REF values, resulting in an REF bias that is greater than 1. The MS-2 REF bias was found to decrease with increasing UVT and with decreasing flow rate. However, no clear trend was found with *B. subtilis* spores REF bias, although the average bias did decrease with decreasing flow rate. One possible explanation for the unclear trend with the *B. subtilis* spores REF bias is due to the complex nonlinear shape of the UV fluence–response kinetics. In Table 3, the results show that the REF bias was slightly larger using the *B. subtilis* spores when the UVT was 85.5, 88.1, and 91.4% at the low flow rate and 91.4% at the high flow rate compared to the REF bias using MS-2. The MS-2 REF bias was larger than the *B. subtilis* REF bias when the UVT was 82.5, 85.5, and 88.1 at the high flow rate and 82.5% at the low flow rate. In particular, the MS-2 REF bias was significantly larger (i.e., greater than 40%) when the UVT was 82.5% regardless of flow rate. Again, the differences between the two dosimeter REF bias values is due to the *B. subtilis* UV response kinetics, suggesting that it may be difficult to determine whether a more sensitive challenge microorganism will significantly reduce the REF bias if the dose–response is nonlinear. Given the clear trend with the MS-2 REF bias, a reduction in the REF bias may be achieved with a challenge microorganism that has a linear UV response with a similar inactivation rate constant or similar sensitivity to UV light as the target microorganism.

Conclusions

Biodosimetry is a widely accepted method used for validation of UV reactors, although the dependence of this method on the linearity and/or nonlinearity of the UV fluence–response curve for test organisms have scarcely been investigated. A pilot scale LP UV reactor was tested using a mixture of two common biological surrogates MS-2 and *B. subtilis* spores. *B. subtilis* spores were more sensitive to LP UV than MS-2 and the fluence–response curve for spores showed nonlinear kinetics in contrast to the linear fluence–response curve for MS-2. Although the spores in this study were generally more sensitive to UV than MS-2 and exhibited higher log inactivation in the reactor, the results confirmed that due to the shoulder in the spores UV fluence–response curve, this trend was reversed at low UV fluences. The REF differed between 1 and 13 mJ/cm^2 when using MS-2 or *B. subtilis* spores for calculation (depending on flow and transmittance) and was higher when calculated using MS-2 measured results. When the UV fluence in the reactor was high, the calculated REF using *B. subtilis* spores was inaccurate since the inactivation readings were in the tailing region of the spore UV fluence–response curve. REF bias relative to *Cryptosporidium* calculated from *B. subtilis* data was not consistently lower than the bias calculated from MS-2 data, despite that MS-2 was more resistant to LP UV. The mathematical model showed reasonable agreement with all measured data.

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