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UV disinfection of indigenous aerobic spores: implications for UV reactor validation in unfiltered waters

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Abstract

Conventional validation testing of UV reactors use cultured microorganisms spiked into test water flowing through a reactor. These tests are limited by the microbe titer it is possible to grow, thus limiting the size of the reactor it is possible to validate. The goal of this study was to examine the UV inactivation of indigenous aerobic spores naturally occurring in raw/unfiltered water supplies and to assess their use as an alternative indicator for validation testing of UV reactor performance, specifically for unfiltered water supplies planning large UV reactors. These spores were found in all raw waters tested in concentrations ranging between 20 and 12,000 CFU/100 mL and were very resistant to UV irradiation compared to a range of different microbes in the literature (i.e. adenovirus, MS-2 coliphage, and *Cryptosporidium parvum*). The inactivation of indigenous natural aerobic spores followed first-order kinetics with an inactivation coefficient ranging between 0.013 and 0.022 cm^2/mJ with a high correlation coefficient. It was determined that naturally occurring aerobic spores, well characterized with respect to UV 253.7 nm inactivation, can be a useful tool when validating plant performance, and might also be used as a regular monitor of UV fluence and performance in a water treatment plant.
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1. Introduction

Ultraviolet (UV) disinfection is now considered an acceptable process for inactivation of pathogens in drinking water. Because it is difficult to directly measure the UV fluence delivered in an UV disinfection reactor, the current state-of-the-art to validate an UV reactor is to measure the inactivation of a pathogen surrogate through an UV reactor and back-calculate the delivered UV fluence using the biodosimetry method [1,2,15]. All validation tests utilize spiked "challenge" microorganisms into a flowing test reactor. The biodosimetry approach has been effectively utilized for reactors rated at flows less than 20 MGD, but has not been widely evaluated for testing reactors at flows much greater than this. Although possible, it may not be always practical to grow large quantities of laboratory microbe titer for validation of large reactors over extended periods. This has implications for the concentration of microorganisms achieved in the reactor flow and the range of UV fluence it is possible to measure in the reactor. Furthermore, culture conditions of many organisms can affect their sensitivity to UV [3,4] or chlorine dioxide disinfection [5]. For instance, the sporulation method of bacterial spores plays a role in determining the resistance of purified spores to UV disinfection in soil suspensions [4] and in drinking water [6]. Therefore use of monodispersed laboratory strains can also be criticized due to

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the potential discrepancies within the same strain. Pure spore strains are used in biodosimetry to measure the fluence in a reactor during reactor type testing, however there is a need for a microbial indicator which can be used for monitoring the efficiency of UV disinfection during practical operation. Therefore, use of an indigenous microbe for reactor validation may offer a realistic approximation of the efficiency of UV disinfection in practice.

Indigenous and cultured aerobic spores have been evaluated as surrogates for pathogen removal for different disinfection strategies. Isolated natural aerobic spores were evaluated as a surrogate for *Cryptosporidium parvum* inactivation with ozone disinfection, and did not prove to be a sufficient indicator [7]. With chlorine dioxide disinfection, it was also found that *Bacillus subtilis* spores were insufficient indicators of *C. parvum* [5]. Although there was no direct linear correlation between removal of *C. parvum* and indigenous aerobic spores studied in conventional water treatment plant processes, removal of 3–4 log of aerobic spores in addition to other surrogates did reliably indicate removal of pathogens such as *C. parvum* [8–10]. Because UV disinfection is very effective for inactivating *C. parvum* [11,12], researchers have focused on finding a sensitive surrogate for protozoan cysts in UV treatment. A UV-sensitive *B. subtilis* strain was suggested as a biodosimetry surrogate for *C. parvum* [13], but this strain was constructed with antibiotic resistance markers and should not be used in studies where it could enter the natural environment.

Several researchers have proposed indigenous aerobic spores naturally occurring in raw water as an indicator for evaluating water treatment plant performance. Aerobic spores occur in sufficient concentration in raw water to provide measurable removal through treatment processes, they are nonpathogenic, they do not propagate under ambient conditions, they are evenly distributed in water, the analysis is simple and inexpensive, and consequently they can be a useful measure of plant performance [8,9]. Indigenous aerobic spores in water correlated to the removal of particles through coagulation, sedimentation and filtration treatments, however they were very resistant to chlorine disinfection [10]. Further research was suggested to test whether the heterogeneous population of indigenous spores could be a general indicator or a local indicator of chlorine disinfection in raw water [14].

Studies investigating the UV inactivation of indigenous aerobic spores in unfiltered water systems have not been previously reported. Microbial surrogate indicators currently used to validate UV reactors are typically laboratory cultivated strains of *B. subtilis* spores [15] and MS2 coliphage [2]. The use of a source water indigenous microbe to validate UV reactors may offer advantages for validation of large UV reactors such as those under consideration by some unfiltered water utilities. Unfiltered water supplies are particularly amenable to using indigenous biodosimeters because these microbes would not be filtered out during the water treatment process upstream of the disinfection reactors.

The goal of this study was to examine the UV inactivation of indigenous aerobic spores in unfiltered water supplies and assess their use as an alternative indicator for validation testing of UV reactor performance. The specific objectives were to (1) develop a standard method for testing the UV inactivation of indigenous aerobic spores in unfiltered drinking water sources, (2) compare the inactivation of laboratory strain *B. subtilis* spores and indigenous aerobic spores, and (3) assess the usefulness of these spores for validation testing of UV reactors.

2. Materials and methods

Experiments were performed both on raw waters serving conventional filtration treatment plants and on intake waters for unfiltered water treatment plants. Inactivation of indigenous spores was conducted using a bench scale low-pressure Hg lamp apparatus emitting monochromatic (254 nm) UV light.

2.1. Water sources

Six water sources were evaluated. Raw water from Lake Michie, NC, a source for the Brown Water treatment Plant in Durham NC, was collected prior to any water treatment, on three occasions in March and April of 2002. Raw water from Little River Lake, NC (also a source for Brown Water Treatment Plant) was collected in June, 2002. Intake waters for unfiltered treatment plants from the Kensico Reservoir, and Croton Reservoir both serving New York City, NY were collected in May 2002 and August 2002, respectively, and shipped overnight on ice to the Duke University UV Research laboratory for analysis. Intake waters for unfiltered treatment plants from the Seattle Cedar Supply and Tolt Supply near Seattle Washington were collected in June 2002 and July 2002 and shipped overnight to the Duke University laboratory. All samples were kept at 4°C and analyzed within 2–24 h from collection time.

2.2. Indigenous aerobic spore enumeration

To enumerate spores, it is necessary to eliminate the vegetative bacteria by pasteurization. Three methods were evaluated:

UV-count (method A): UV irradiation of water samples at different fluence rates and then enumeration

of the colonies. This method does not include pasteurization of the water sample.

Pas-UV-count (method B): Pasteurization of the bulk water sample, UV irradiation and then enumeration of the colonies.

UV-pas-count (method C): Irradiation of water sample, pasteurization of each sample and then enumeration of the colonies.

Isolating spores followed one of two methods from the literature termed here direct pasteurization or indirect pasteurization. Direct pasteurization consisted of pasteurizing samples for 15 min at 75°C [16], whereas indirect pasteurization consisted of initial incubation at 35–37°C for 30 min, followed by pasteurization for 15 min at 65°C with a shaking water bath, and then placing the flasks in ice water [9]. With method B samples, the bulk water was directly pasteurized (75°C, 15 min) in 1 L bottles, and then distributed to 100 mL vials for UV exposure experiments. However, method C samples were initially distributed to 100 mL vessels, UV irradiated and then indirectly pasteurized in a shaking water bath in 100 mL flask. For enumeration, samples were membrane filtered (0.45 μm, 47 mm, Pall Corporation, Ann Arbor, MI, USA) and placed on a pad to which 1.45 mL tryptic soy broth (TSB) had been added [16]. Colony counts were determined within 22–24 h of incubation at 35°C and all the various colonies that grew on the plate were counted as indigenous spores. Total organic carbon (TOC) and dissolved organic carbon (DOC) of the waters were measured by a TOC analyzer (Tekmar, Dohrmann, Apollo 9000, OH, USA) Turbidity was measured by turbidimeter (Hach, model 2100N, Loveland, CO, USA). Standard Methods [17] were used for conductivity [Method 2510A] and pH [Method 4500-H] measured via probes, and alkalinity was measured by titration [Method 2320B].

2.3. B. Subtilis ATCC 6633 spore preparation and enumeration

B. subtilis spores (ATCC 6633) used as a reference strain were produced by fermentation technique and obtained freeze-dried. Working solutions were produced by resuspending the spores in water to a concentration of 10^6 CFU/mL. Ten-fold serial dilutions of all the spore samples were determined 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 (*B. subtilis* spores and working methods were provided by Sommer [18]).

2.4. Low-pressure UV irradiation system and radiometry

A quasi-parallel beam bench scale UV apparatus consisted of four 15-W low-pressure mercury vapor germicidal lamps emitting monochromatic UV radiation at 253.7 nm that was directed through a circular opening to provide incident radiation normal to the surface of the test suspension. UV irradiance (mW/cm^2) was measured with a radiometer and a UV detector (International Light IL1400, Newburyport, MA, SEL 240 detector) that had been factory-calibrated, traceable to National Institute of Standards and Technology standards with accuracy of $\pm 7\%$.

2.5. UV fluence determination and exposures

The measured incident irradiance at the surface of the test liquid was corrected for non-homogeneity of irradiation across the surface area of the petri dish to provide the average incident irradiance. The average irradiance in the mixed suspension was determined mathematically from an integration of the Beer–Lambert Law over the sample depth, accounting for UV absorbance of the test suspension and incident average irradiance [19]. UV absorbance was measured by UV–VIS spectrophotometer (Varian, Model Cary 100BIO, Victoria, Australia). Exposure times were calculated by dividing the desired UV fluence by the average UV irradiance. 100-mL volumes of sample placed in 66 mm diameter irradiation vessels resulting in a sample depth of 32 mm were irradiated using the bench scale UV system. Each exposure was performed in duplicate, at UV fluence ranging from 0 to 110 mJ/cm^2.

2.6. Data presentation

Concentration (colony forming units—CFU/100 mL) of indigenous spores in the control sample (without UV) was taken as the initial concentration, N_o. The control sample was the raw water enumerated for indigenous spores without exposure to UV light according to each pasteurization method. For each UV fluence the arithmetic mean of three replicates of aerobic spore concentration was calculated, and was taken as N_d. The $\log_{10}$ transformation for N_o/N_d was plotted as a function of the UV fluence (H). A linear regression was used to fit the linear portion of the log-inactivation curve. The linear curve was described by the following equation:

$$\log_{10}(N_o/N_d) = k*H. \quad (1)$$

The fluence-based inactivation rate coefficient (k) was determined for each experimental run based on a linear regression on the data.

3. Results and discussion

3.1. Concentration and morphology of indigenous spores from different water sources

The concentration of indigenous spores examined in unfiltered waters varied from 10^2–10^4 spores/100 mL (Table 1). The population of indigenous aerobic spores present in source water is heterogeneous in species, as observed by the different colony types growing on the membrane filter after incubation. For example, colonies of indigenous spores were observed with various morphologies as: white 2.5 mm with fuzzy margins; brown 1–2 mm colonies; white, flat, wrinkled big colonies and yellow small 1 mm colonies. Interestingly, at the higher UV fluences (90–110 mJ/cm^2) the variety of different colony morphology decreased indicating that different indigenous spore strains may have different resistance to UV inactivation. With chlorine disinfection, researchers obtained a five-fold difference in CT values necessary for 2-log inactivation of isolated aerobic spores from water sources [14]. The relative UV susceptibility of different isolated indigenous spores is currently being investigated.

3.2. Comparison of different pasteurizing methods

Pasteurization is used to destroy vegetative bacteria and thus more clearly enumerate the indigenous aerobic spores in water. Different pasteurization methods were evaluated to examine if changing the sequence of pasteurization and disinfection affected the inactivation of spores. Three options were identified for testing the indigenous spores within the bench scale apparatus: UV irradiation of water followed by enumeration of spores without pasteurization (method A), pasteurization of the water sample, UV irradiation, and then enumeration (method B), and UV irradiation, pasteurization of each sample, and then enumeration (method C). These three methods were assessed by comparing the UV inactivation for each method using a single water source. Because method A does not include pasteurization, it is expected to enumerate all the aerobic bacteria present in the sample.

The inactivation of indigenous spores as a function of UV fluence and pasteurization methods for Lake Michie water is plotted in Fig. 1. The slopes of the regression lines for the three methods are not statistically different and each fluence level applied resulted in less then 0.3 log difference in inactivation between the different methods. Since the media used for counting indigenous spores is non selective, it is advisable to pasteurize the water samples as a general rule to avoid potential interference from surviving vegetative bacteria. Therefore, the highest initial counts obtained by method A are due to enumeration of vegetative bacteria in addition to the indigenous spores and used as a base-line comparison to emphasize the significance of pasteurization applied with methods B and C. The higher initial spore count obtained by method B vs. C may be due to differences in the pasteurization protocol used. In method B, the bulk liquid was pasteurized in 1 L bottles, and then distributed to 100 mL vials for irradiation, whereas for method C, samples were distributed to 100 mL vessels,

Table 1
Initial indigenous aerobic spore count and UV fluence-based inactivation rate coefficient for each water tested

Water source	Enumeration method	Initial spore count (CFU/100 mL)	Fluence-based inactivation rate coefficient (cm^2/mJ)	R^2
Lake Michie, NC[a]	B	12,000 ± 530	0.021	0.98
Lake Michie, NC[b]	A	12,500 ± 1400	0.019	0.96
	B	8600 ± 1100	0.019	0.99
	C	5100 ± 300	0.017	0.93
Lake Michie, NC[c]	A	5600 ± 720	0.019	0.93
	B	4500 ± 100	0.018	0.97
	C	3800 ± 530	0.019	0.92
Kensico, NY	A	1300 ± 450	0.022	0.85
	C	990 ± 80	0.022	0.90
Tolt, Seattle	C	19 ± 5	—	—
Cedar, Seattle	C	160 ± 40	—	—
Little River Lake, NC	C	2700 ± 40	0.014	0.85
Croton, NY	C	590 ± 110	0.013	0.92

± Represents the standard deviation of the mean.
Significant at $p < 0.001$ level for all rate coefficients.
[a] Experiment performed on 17 March.
[b] Experiment performed on 5 April.
[c] Experiment performed on 19 April.

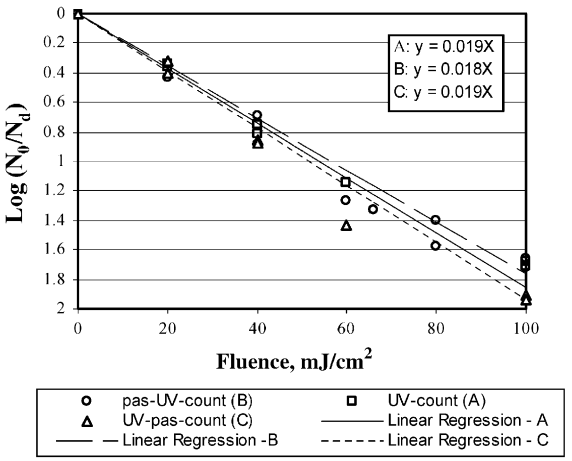

Fig. 1. Log inactivation of indigenous aerobic spores as a function of UV fluence utilizing enumeration treatments A, B, and C, for Lake Michie water (sampled April 19).

irradiated and then pasteurized in a shaking water bath in the 100 mL flasks. These differences in preparation methods may have led to the differences in the initial spore counts (initial counts by methods A–C are presented in Table 1). For instance, the larger pasteurization sample size for method B could have resulted in insufficient temperature levels at the core of the 1 L vessel, as there was no active mixing during pasteurization. Lower temperature levels at the core of the vessel could result in subsequent survival of some vegetative bacteria during pasteurization in method B, thus would lead to falsely higher plate counts. Previous research has shown that laboratory cultured spores were sensitive to high pasteurization temperatures [9] and because the average temperature of method C (30 min at 35–37°C then 15 min at 65°C, 100 mL flask) was likely different than in method B (15 min at 75°C, 1 L bottles), it could have affected the spore concentration. However, the differences between methods B and C probably occurred due to the volume of sample pasteurized and therefore it is recommended to pasteurize in small volumes (100 mL or less). For practical purposes of reactor validation, it is more suitable to pasteurize the UV exposed sample collected from the reactor outlet and enumerate the spores (method C). This method is more reliable because of the small volumes (100 mL) used for pasteurization. Furthermore, in a water treatment plant, it is not practical to pasteurize all the raw water (million of gallons per day) and then irradiate and count (as in method B).

The effect of pasteurization on water quality was examined before and after pasteurization of the Lake Michie water, as presented in Table 2. There was no appreciable difference in water quality due to pasteurization protocol.

Table 2
Water quality before and after pasteurization, Lake Michie (March 17)

Parameter	Raw water	After pasteurization
Conductivity[a] (μs/cm)	77±1	78±1
pH[a]	6.51±0.02	6.48±0.02
Turbidity[a] (NTU)	10.0±0.05	9.80±0.05
Alkalinity[b] (mg /L)	22.5	20
TOC (mg C/L)	18.5±0.4	19.6±0.3
DOC (mg C/L)	19.7±0.1	19.3±0.3

[a] Instrument accuracy
[b] Only one sample analyzed

3.3. UV fluence–response curves of indigenous aerobic spores

Fig. 2 illustrates the log inactivation of indigenous aerobic spores as a function of UV fluence for all raw waters sampled. The reduction of 1-log inactivation of indigenous spores as measured by method C for all water samples was within a span of 47–77 mJ/cm^2. However, within the same water source (Lake Michie), the span of UV fluence for 1-log inactivation was between 50 and 58 mJ/cm^2. Based on the microbial assay sensitivity, it was possible to follow approximately 3 logs of inactivation for this water sample. However, at the UV fluence tested, up to 110 mJ/cm^2, only 2–2.5 logs of inactivation were achievable with any water. Indigenous spores measured in the Seattle WTP showed very low counts and thus this water was unsatisfactory for determining log-removal based on 100 mL samples used in the lab bench scale apparatus. Larger volumes could be filtered (e.g. 1 L) when the concentration of spores in 100 mL samples is not sufficient [9] for evaluation of treatment process performance, however, for practical purposes, use of 1 L of sample was not possible in the bench scale UV apparatus used.

3.4. Fluence-based inactivation kinetics

The fluence–response data were fitted using a linear regression approach (Fig. 1 and 2) and the difference in inactivation between different water samples was determined by comparing the fluence-based inactivation rate coefficient as presented in Table 1. The linear correlation coefficient (R^2) ranged between 0.85 and 0.98, indicating a strong linear relationship between the log inactivation of the indigenous spores and the UV fluence. The inactivation rate coefficient followed first-order kinetics with values between 0.013 and 0.022 cm^2/mJ.

The water quality parameters measured on the waters collected are presented in Table 3. The quality of the

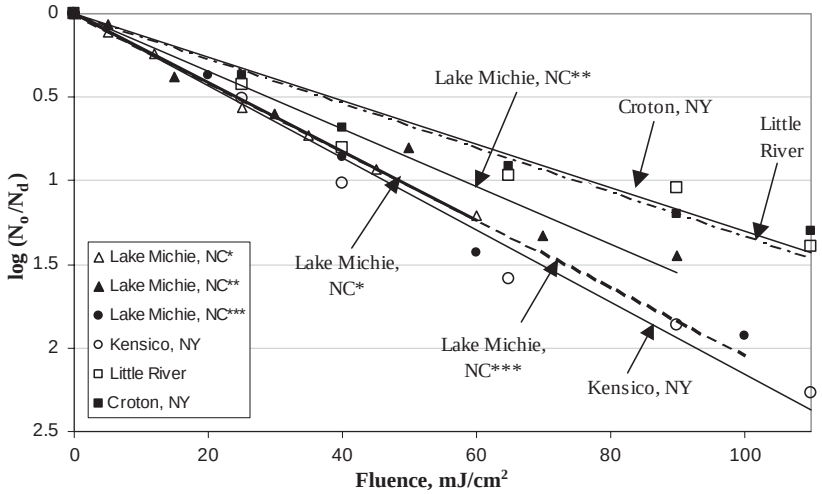

Fig. 2. Fluence-response curves for unfiltered surface water samples utilizing enumeration method C. * 17 March; ** 5 April; *** 19 April, (Note: 17 March sample utilized enumeration method B).

Table 3
Summary of water quality for all waters tested

	Lake Michie (19 April)	Little-River (12 June)	Kensico, NY (15 May)	Cedar, Seattle (5 June)	Croton, NY (2 August)
TOC (mg C/L)	6.31	5.40	1.65	0.84	N/A
Turbidity (NTU)	4.6	3.19	0.89	0.66	1.84
Conductivity (μs)	86	92	73	37	340
254 nm Transmittance (%)	61.32	69.45	91.83	96.80	80.04
pH	6.87	6.75	6.65	6.98	6.56
Alkalinity (mg/L as $CaCO_3$)	25	26	15	18	67

waters tested varies widely. For instance the TOC ranges from 6.3 to 0.8 mg/L and turbidity ranges from 4.6 to 0.66 NTU. The highest number of indigenous spores present in the waters tested was found in the waters with high levels of turbidity and TOC. Thus, the better the water quality, the lower the numbers of aerobic spores. This finding is similar to that reported by Nieminski and Bellamy [9] who found that increased levels of aerobic spores were directly related to increases in turbidity and particles.

3.5. Comparison of laboratory and indigenous spore inactivation

B. subtilis ATCC 6633 spores were spiked to filtered raw water, such that the mixed suspension contained viable spores at a concentration of 10^6 CFU/mL. Inactivation of the ATCC spore strain compared to the indigenous spore at identical delivered UV fluence is presented in Fig 3. The inactivation of spiked spores was much more rapid than the indigenous spores, reaching 3.5-$\log_{10}$ inactivation at a UV fluence of 60 mJ/cm^2

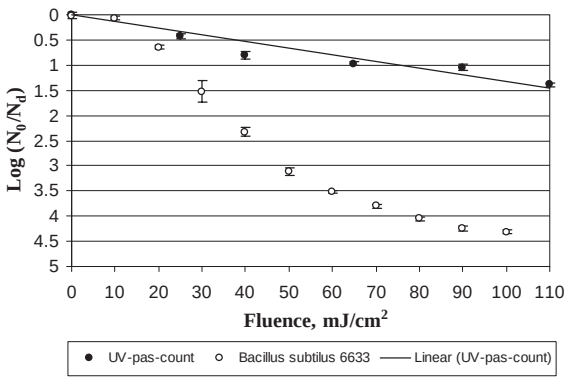

Fig. 3. Log inactivation of indigenous aerobic spores and spiked *B. subtilis* spores (ATCC 6633) as a function of UV fluence, for Little River raw water.

compared to less than 1-log inactivation for the indigenous spores at this UV fluence. As stated previously, spiked spores inactivation depends on the sporulation method [4, 6] compared to the indigenous

spores that naturally occur as spores in the unfiltered water samples.

At UV fluence greater than 60 mJ/cm^2 the inactivation response exhibited tailing for the ATCC strain. Tailing could occur due to microbe enumeration sensitivity at higher fluence since few viable microbes that remain in solution can make the enumeration inaccurate. Otherwise, tailing could occur due to residual microbes that are clumped with each other and protected from UV light. The exact reason for the tailing is unknown. Typically a shoulder or lag in inactivation of *B. subtilis* spores occurs at a fluence up to about 10 mJ/cm^2 [6] which was not apparent in the indigenous spores of lake Michie samples and therefore the fluence based kinetics was assumed to be linear throughout all the UV fluence tested.

UV inactivation of other microbes reported in the literature place the *B. subtillis* data presented here into perspective. A fluence of 20 mJ/cm^2 resulted in 1-log$_{10}$ inactivation of MS-2 [2] while a UV fluence of 3 mJ/cm^2 resulted in more then 2.6-log$_{10}$ inactivation for *C. parvum* [12]. Adenovirus 40 was capable of 1-log$_{10}$ inactivation at a fluence of 30 mJ/cm^2 and 4-log$_{10}$ inactivation at a fluence of 124 mJ/cm^2 [20]. Compared with these microbes, the indigenous spores are much more resistant to UV radiation. With chlorine disinfection, a similar phenomenon of decreased resistance of pure *B. subtilis* spores relative to indigenous spores was attributed to the consistent nature of the purified spores preparation in contrast to the different stages of dormancy and metabolism that are characteristic of the indigenous spores in their natural state [10]. Another explanation for the increased resistance of the indigenous spores may be attributed to association with particles in the water. All of the waters tested were from raw water sources, some typical for what would be used by unfiltered treatment facilities. Particles, such as those in wastewater, are known to protect microbes from UV inactivation [21, 22], however the impact of particles on disinfection of unfiltered waters has not been sufficiently investigated. To achieve the log-linear inactivation exhibited in this study, all the indigenous spores would have to have been particle associated, which may not be a practical assumption.

3.6. Indigenous aerobic spores as a UV performance indicator

In order to test the applicability of indigenous spores to validate UV fluence in UV disinfection reactors, it is helpful to evaluate the fluence required to reduce 1-log$_{10}$ of spores, given the differences in initial spore concentrations as presented in Table 4. While the initial concentration of indigenous spores varies significantly between Kensico sample and Lake Michie sampled on March 17, the fluence required to attain 1-log$_{10}$ inactivation varies in between 47 and 49 mJ/cm^2. The Kensico indigenous spore concentration did not vary significantly from the Croton spore concentration, however, the fluence required to attain 1-log$_{10}$ inactivation is between 47 and 77 mJ/cm^2. Therefore initial count by itself is not an indicator of inactivation. Changes that might occur spatially and temporally in initial spore counts, in the diversity of spore species, and in maturity of the spores may be reflected in the fluence-based kinetic rate coefficients. Given the difference in inactivation rate coefficients between different sites, it is clear that the response of spores to UV radiation can differ from site to site.

Table 4
UV fluence to achieve 1-log inactivation for different water sources with different initial aerobic spores concentration

	Initial concentration (CFU/mL)	Fluence of 1-log inactivation[d] (mJ/cm^2)
Lake Michie[a]	12 000	49
Lake Michie[b]	5100	58
Lake Michie[c]	3800	50
Kensico, NY	990	47
Croton, NY	590	77
Little River, NC	2700	65

[a] Experiment performed on 17 March.
[b] Experiment performed on performed 5 April.
[c] Experiment performed on 19 April.
[d] Fluence as measured by using method C, except for Lake Michie (experiment performed on 17 March) sample using method B.

3.7. Confidence and prediction intervals

To estimate the 95% confidence interval and prediction interval for the inactivation of spores in a given water sample, a fluence–response curve was performed with five replicate UV exposures each at fluence of 25, 65, 90 and 110 mJ/cm^2 (Fig 4). The 95% confidence intervals indicate a 0.95 probability of containing the mean of a log inactivation for a set of data at a specified fluence. This graph shows the variability in the bench testing that can be expected within one sample. For example, at a UV fluence of 65 mJ/cm^2 delivered to a reactor there is 95% probability that the mean log inactivation will be between 0.86 and 0.96. The confidence level for the water sample was narrow indicating a high accuracy of the mean UV response to the analytical methods. The 95% prediction interval which indicates the 95% probability range of containing all log inactivation data points resulting from exposure to a specific fluence, is shown for comparison.

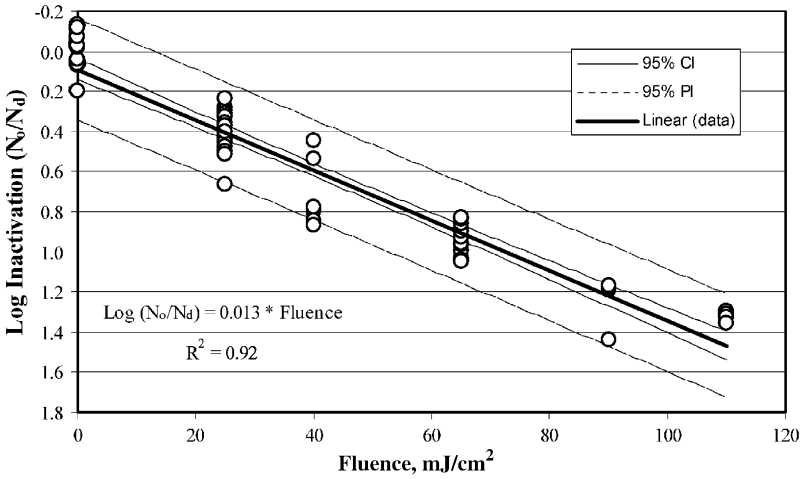

Fig. 4. Log inactivation of indigenous aerobic spores as a function of UV fluence, for Croton NY raw water. Upper and lower limits for the 95% confidence (CI) and prediction intervals (PI) are shown.

3.8. Practical application of indigenous spores

Validation of UV reactors on-site with indigenous spores could be a convenient means for water utilities. For such validation testing, a side stream of the raw or minimally treated water can be introduced into a UV reactor and samples collected at the inlet and outlet of the reactor. The reactor could be tested over extended periods or at regular intervals during the lifetime of the UV reactor for periodic validation testing. Reactor testing costs would be reduced because the testing would not require use of spiking pumps, mixers, and the disposal of microbe spiked water that may otherwise pollute the environment. The conventional alternative of spiking microorganisms into the flow to evaluate reactor performance comes with drawbacks, such as unbalanced plant operation, regulatory considerations, excess costs, and public sensitivity [9].

4. Conclusions

A number of raw and unfiltered drinking water sources were evaluated for the presence of indigenous aerobic spores and for use in validation studies for UV disinfection reactors. Indigenous aerobic spores were found in all raw/unfiltered waters tested. The initial concentration of spores varied among the waters, and all spores evaluated were very resistant to UV disinfection compared to ATCC strain spores and other microorganisms.

The fluence-based inactivation rate coefficient for the indigenous spores varied with the different water sources, but was relatively consistent within the same water source (e.g. Lake Michie) over time. The inactivation rate was not dependent on the initial spore starting concentration, nor did it vary consistently with water quality.

Of the three methods of spore enumeration tested, method C—sampling the water after UV exposure, pasteurizing and then enumerating—is suggested when using indigenous spores for reactor validation testing. For accurate reactor validation testing, it is essential that the water sample used to develop the bench scale fluence-inactivation relationship be collected at the same time as the reactor testing occurs. The unknown diversity of the indigenous spore population makes the evaluation of a UV disinfection system unique from site to site and over seasons. Thus, the consistency of the bench scale data should be evaluated using sufficient replicates to gain confidence in the relationship between log inactivation and UV fluence.

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References

[1] Qualls RG, Johnson JD. Bioassay and dose measurement in UV disinfection. Appl Environ Microbiol 1983;45(3): 872–7.

[2] Mackey ED, Hargy TM, Wright HB, Malley Jr. JP, Cushing RS. Comparing *Cryptosporidium* and MS-2 bioassays—implications for UV reactor validation. J Am Water Works Assoc 2002;94(2):62–9.

[3] Severin BF, Suidan MT, Engelbrecht RS. Kinetic modeling of UV disinfection of water. Water Res 1983;17(11): 1669–78.
[4] Nicholson WL, Law JF. Method for purification of bacterial endospores from soils: UV resistance of natural Sonoran desert soil population of *Bacillus* spp. with reference to *B. subtilis* strain 168. J Microbiol Methods 1999;35:13–21.
[5] Chauret CP, Radziminski CZ, Lepuil M, Creason R, Andrews RC. Chlorine dioxide inactivation of *Cryptosporidium parvum* oocysts and bacterial spore indicators. Appl Environ Microbiol 2001;67(7):2993–3001.
[6] Sommer R, Cabaj A. Evaluation of the efficiency of a UV plant for drinking-water disinfection. Water Sci Technol 1993;27:357–62.
[7] Facile N, Barbeau B, Prevost M, Koudjonou B. Evaluating bacterial aerobic spores as a surrogate for *Giardia* and *Cryptosporidium* inactivation by ozone. Water Res 2000; 34(12):3238–46.
[8] Nieminski EC, Bellamy WD, Moss LR. Using surrogates to improve treatment plant performance. J Am Water Works Assoc 2000;92(3):67–78.
[9] Nieminski EC, Bellamy WD. Application of surrogate measures to improve treatment plant performance, Project no. 363. Denver, CO: AWWA Research Foundation; 2000.
[10] Rice EW, Fox KR, Miltner RJ, Lytle DA, Johnson CH. Evaluating plant performance with endospores. J Am Water Works Assoc 1996;88(9):122–30.
[11] Clancy JL, Bukhari Z, Hargy TM, Bolton JR, Dussert BW, Marshall MM. Using UV to inactivate *Cryptosporidium*. J Am Water Works Assoc 2000;92(9):97–104.
[12] Shin GA, Linden KG, Arrowood MJ, Sobsey MD. Low-pressure UV inactivation and DNA repair potential of *Cryptosporidium parvum* oocysts. Appl Environ Microbiol 2001;67(7):3029–32.
[13] Nicholson WL, Hargy TM, Marshall MM, Durda JP. UV sensitive spores of *Bacillus subtilis* strain provide a biodosimetry surrogate for *Cryptosporidium*, 2001, Proceedings of the First International Congress on UV Technologies, International Ultraviolet Association, Washington DC, June 14-16, 2001.
[14] Barbeau B, Boulos L, Desjardins R, Coallier J, Prevost M. Examining the use of aerobic spore-forming bacteria to assess the efficiency of chlorination. Water Res 1999; 33(13):2941–8.
[15] Anonymous. Austrian National Standard ONORM M 5873-1. Plants for the Disinfection of Water Using Ultraviolet Radiation, Requirements and Testing. Part 1: Low Pressure Mercury Lamps, 2001.
[16] Barbeau B, Boulos L, Desjardins R, Coallier J, Prevost M, Duchesne D. A modified method for the enumeration of aerobic spore-forming bacteria. Can J Microbiol 1997;43: 976–80.
[17] APHA. Standard methods for the examination of water and wastewater, 19th ed. Washington, DC: American Public Health Association; 1995.
[18] Sommer R. Clinical Institute of Hygiene, Medical Microbiology University, Vienna, Austria, 2002, personal communication.
[19] Morowitz HJ. Absorption effects in volume irradiation of microorganisms. Science 1950;111:229–30.
[20] Meng QS, Gerba CP. Comparative inactivation of the enteric adenoviruses, poliovirus and coliphages by ultraviolet irradiation. Water Res 1996;30(11):2665–8.
[21] Emerick RW, Loge FJ, Ginn T, Darby JL. Modeling the inactivation of particle-associated coliform bacteria. Water Environ Res 2000;72(4):432–8.
[22] Jolis D, Lam C, Pitt P. Particle effects on ultraviolet disinfection of coliform bacteria in recycled water. Water Environ Res 2001;73(2):233–6.