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Spectral Sensitivity of *Bacillus subtilis* Spores and *MS2 Coliphage* for Validation Testing of Ultraviolet Reactors for Water Disinfection

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The microbicidal UV fluence under polychromatic radiation from UV lamps is typically measured using the DNA absorbance spectrum as a weighting factor for the relative wavelength effectiveness. However, this DNA-based weighting does not necessarily match the spectral sensitivity of the microorganism being tested. *Bacillus subtilis* spores are often used for UV reactor validation in Europe, while *MS2 coliphage* is typically used for validation testing in the United States. These organisms were exposed to quasi-monochromatic UV irradiation across the microbicidal spectrum at wavelengths of 214, 230, 240, 254, 265, 280, and 293 nm. MS2 was three times more sensitive to wavelengths near 214 nm compared to the 254 nm output of low-pressure lamps, while *B. subtilis* spores were most sensitive to wavelengths around 265 nm. Use of these action spectra, compared to the DNA-based weighting, resulted in differences in the calculated polychromatic UV fluence. Consequently, the action spectrum, which is specific for each microorganism, has implications on the uncertainty of UV fluence determination during validation of reactors with polychromatic UV lamps.

Introduction

Ultraviolet (UV) disinfection has grown in acceptance as a primary disinfection process for water since its efficacy for inactivating *Cryptosporidiu*m was revealed (*1*). There is an increased need to develop standardized procedures for evaluating the UV fluence (also commonly called UV dose) in a UV reactor. Because direct determination of the UV fluence delivered in a reactor is not possible, a currently acceptable method is to experimentally measure the average delivered UV fluence in a UV disinfection system using biodosimetry. In this approach, the inactivation of a pathogen surrogate caused by exposure to UV irradiation in a UV reactor is used to back calculate the delivered fluence (*2*) using a laboratory-derived standard UV inactivation curve. The measured reactor fluence is termed the Reduction Equivalent Fluence (REF) (*3*). REF in low-pressure (LP) systems is the average microbicidal fluence at a wavelength of 254 nm (*4*)—the principal wavelength emitted by the LP mercury lamps. Biodosimetric measurements depend on the survival function of the microorganisms and on the shape of the fluence distribution. A broad fluence distribution will result in REF measurements that strongly depend on the microorganism tested (*5*).

The determination of REF in UV disinfection systems with medium pressure (MP) mercury vapor polychromatic UV lamps is even more complex (compared to the LP UV quasi-monochromatic UV systems) because it is necessary to know the spectral sensitivity of the microorganisms toward the various wavelengths emitted by the MP lamp as well as the spectral transmittance of the water. Previous research has indicated different wavelength susceptibilities between single to double stranded DNA and RNA bacteriophages (*6*), as well as for *Cryptosporidium*, MS2, and Herpes simplex virus (*7*). The currently accepted method of determining the UV fluence for polychromatic radiation sources is to use the DNA absorbance spectrum as a microbicidal weighting (*4*).

Because a microorganism action spectrum affects the biodosimetry measured fluence under polychromatic irradiation, it is essential to define the action spectra of the commonly used microorganisms in UV reactor validation. This is especially important because polychromatic MP systems are increasingly being used for UV treatment of water and wastewater as indicated by the development of national standards for the use of MP UV lamps (*7*, *8*). The most commonly used microorganisms for UV disinfection system validation are *Bacillus subtilis* spores and *MS2 coliphage*. *B. subtilis* spore cultivation methods have been shown to result in different susceptibilities to UV LP lamps (*9*). The spectral sensitivity of the liquid-cultivated *B. subtilis* spores regularly used as a biodosimeter for reactor validation in Europe was previously investigated (*10*). In the current research the spectral sensitivity of *B. subtilis* spores grown using an agar plate method, commonly used by other researchers, was investigated. Side by side, the spectral sensitivity for *MS2 coliphage* was examined as this microorganism is also widely used for validation testing of UV reactors in North America (*11*).

Knowledge of the specific spectral sensitivity of important health-related microorganisms can lead to improvements in UV treatment by targeting specific wavelengths for future design. In addition, sensors employed for the surveillance of UV reactors used in practice are not equally sensitive to all wavelengths of light; therefore, the microorganism-specific spectral sensitivity will directly affect the proper use of UV sensors to monitor MP UV systems. The objectives of this study were to determine the spectral sensitivity of *B. subtilis* spores and *MS2 coliphage* for wavelengths between 200 and 300 nm, propose standard procedures for such testing, and demonstrate the importance for validation of polychromatic UV disinfection systems.

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Materials and Methods

Spore Preparation and Enumeration. Spores of *B. subtilis* ATCC 6633 were cultivated on agar plates and termed surface spores (S6633) in this manuscript. The cultivation process consisted of streaking liquid-cultivated *B. subtilis* ATCC 6633 on Columbia agar plates (Oxoid CM 331) prepared with 1% $CaCl_2$ for 24 h to ensure isolated colonies (*9*, *12*). Individual colonies were then inoculated into liquid tryptic soy broth (Oxoid CM 129), and after 4−5 h of incubation, the bacteria were inoculated on Columbia plates and left to re-sporulate for 7 days at 37 °C. Spores were collected and harvested by washing with sterile, deionized water three times, at 5000*g* for 15 min. The suspension was placed in a water bath at 80 °C for 10 min to destroy remaining vegetative cells and was refrigerated at 4 °C. The spore concentrations (of nonirradiated and UV irradiated samples) were determined after serial 10-fold dilutions, using the pour plate technique with PC-Agar (Oxoid CM 325), at 37 °C for 2 d, as colony-forming units (CFU) per mL. The purity of sporulation was between 90 and 95% as tested by phase contrast microscopy.

MS2 Virus Preparation and Enumeration. *MS2 coliphage* (ATCC 15597-B1) was cultivated and enumerated with the double agar layer technique according to the ISO 10705-1 (*13*) using the host strain *Escherichia coli* NCTC 12486. A filtered portion of MS2 stock suspension with a concentration of $\sim 10^{11}$ plaque-forming units (PFU) per milliliter was diluted for UV inactivation experiments. The MS2 concentrations (of nonirradiated and UV irradiated samples) were determined after serial 10-fold dilutions as PFU per milliliter. The enumeration method was modified by adding X-Gal (a chromogenic substrate for the detection of the enzyme beta-galactosidase) to the bottom layer, which improved the counting of the plaques.

Monochromator UV Irradiation System and Radiometry. A 400 W Cermax Xenon lamp together with a single monochromator (Jobin-Yvon HL) and collimating optics provided the monochromatic UV radiation source. The spectral incident irradiance at the surface of the suspension was measured with a calibrated spectroradiometer (Bentham DTM300) equipped with a quartz light guide and a Teflon diffuser as entrance optics. The measurement of the spectral incident irradiance was produced in intervals of 1 nm with an integration band in the wavelength range of 200−400 nm, at the surface of the liquid suspension. For the UV inactivation experiments a suspension of 10^6 CFU/mL of S6633 spores or 10^6 PFU/mL of MS2 was prepared and constantly stirred. The samples were then irradiated for proper times corresponding to the desired delivered UV fluence. Five milliliters of sample was sub-sampled after reaching the respective fluence. For each sample, there were four fluence values tested; therefore, the volume of the sample taken for subsampling changed significantly from the initial volume of 35 mL (termed full Petri dish) to the final 20 mL volume of liquid irradiated (termed final Petri dish). These volume changes resulted in a total sample depth change of 0.94 cm in the 4.5 cm diameter Petri dish (Becton Dickinson Labware, France). After exposure, the spectral incident irradiance was again measured, this time with narrow integration bands closely in the region of the target wavelength at the surface levels of the liquid suspension corresponding to the full and the final Petri dish. The monochromator was set to a specific target wavelength prior to exposures; however, the real wavelength to which the samples were exposed was determined from the measured wavelength corresponding to the peak irradiance and termed central wavelength. The experimental details of irradiation experiments with the monochromator are presented in Table 1.

The total fluence rates for both the full and final Petri dish levels were obtained as a summation of the spectral fluence rate, which was obtained by multiplying the spectral incident irradiance within the integration band by the bandwidth at each wavelength, taking into consideration the spectral transmittance and the water reflection. The spectral absorbance was measured with a spectrophotometer (Hitachi U-3000) in 1 cm cells and converted to spectral transmittance. A linear correlation was obtained to estimate the fluence rate for the intermediate depths of the Petri dish due to subsampling. The total fluence rate for each depth of Petri dish multiplied by the exposure time gave the total fluence. The maximum half-peak bandwidth for the monochromatic system was 10 nm for the wavelengths of 214, 230, 240, 254, 265, 280, and 293 nm.

TABLE 1. Experimental Details of Irradiation Experiment with Monochromator

target WL[a] (nm)	central WL at final[c] (nm)	integration band final	mean fluence rate[d] (W/ m^2)
214	216.6 ± 0.4	200−235	0.038
230	231.3 ± 0.6	210−255	0.198
240	240.8 ± 0.4	200−260	0.370
254	254.2 ± 0.3	235−270	0.610
265	265.5 ± 0.3	240−285	0.886
280	281.0 ± 0.6	260−300	1.353
293	293.5 ± 0.6	270−320	1.644
254 LP[b]			0.827

[a] Wavelength (WL). [b] QPB low pressure (LP). [c] Final (Petri dish). [d] Mean of the full and final fluence rate.

Low-Pressure UV Irradiation System and Radiometry. A quasi-parallel beam (QPB) bench scale UV apparatus was equipped with three low-pressure (LP) mercury vapor lamps (36 W each, ozone-free, EK 36, Katadyn) emitting quasi-monochromatic radiation at 253.7 nm oriented normal to the surface of the test suspension. The UV irradiance (W/m^2) measured with a radiometer and a UV detector (IL 1700 with sensor SED 240, interference filter NS 254, and wide-angle W diffuser (International Light, Newburyport, MA)) was integrated over the irradiation time giving the UV-253.7 nm fluence. Twenty five milliliters of test suspension was placed in a 90 mm diameter Petri dish under constant stirring during UV exposure.

Data Presentation. Concentrations of spores or MS2 in the control sample (without UV) were taken as the initial concentration, N_0. For each UV fluence the mean of three replicates of S6633 or MS2 (N_d) was calculated. The log transformation of N_d/N_0 was plotted as a function of the UV fluence (H_0). A linear regression was used to fit the linear portion of the log inactivation relationship, described by the following equation:

$$\frac{N_d}{N_0} = 10^{-k \cdot H_0} \quad (1)$$

The fluence-based UV sensitivity coefficient (k) was determined for each experimental condition based on a linear regression of the data. For a shouldered survival curve, typical of *B. subtilis* spores (*4*), eq 2, where two parameters (k, d) must be known, was used to calculate the survival function of microorganisms.

$$\frac{N_d}{N_0} = 1 - (1 - 10^{-k \cdot H_0})^{10^d} \quad (2)$$

The shoulder coefficient (d) is the intercept with y-axis (log-(N_d/N_0)) of the linear portion of the fluence response curve.

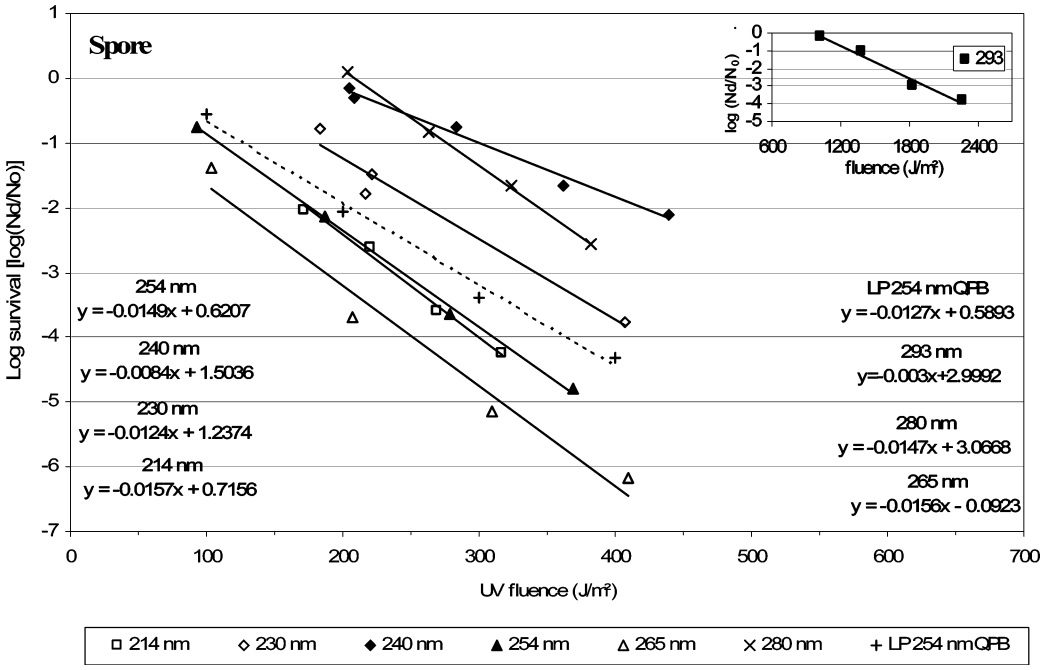

FIGURE 1. Log survival of Surface Spores S6633 as a function of UV fluence, based on a linear fit for all wavelengths.

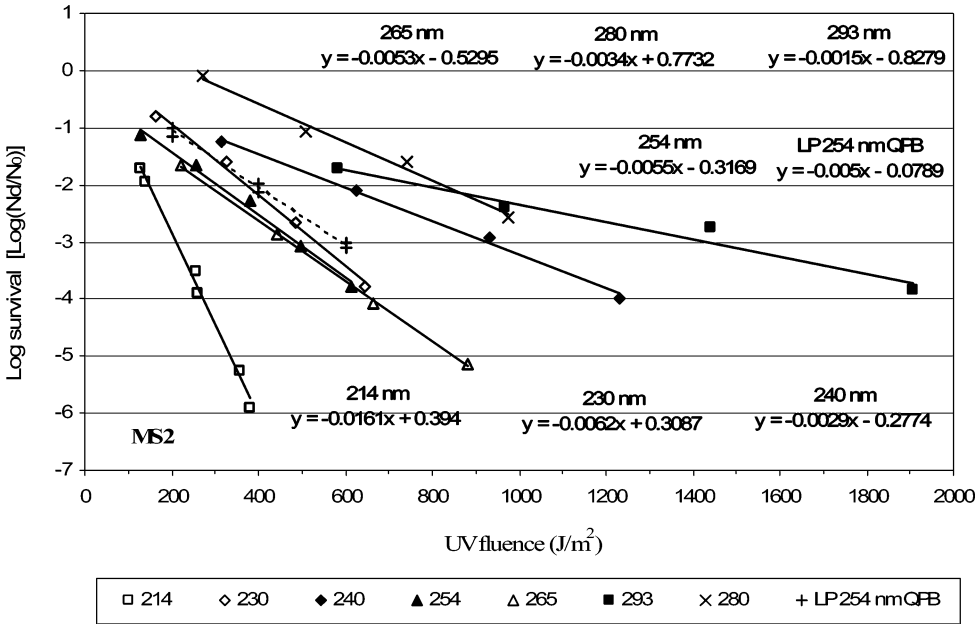

FIGURE 2. Log survival of MS2 as a function of UV fluence, based on a linear fit for all wavelengths.

The parameters k and d were obtained for each wavelength tested, described hereafter as $k(\lambda)$ and $d(\lambda)$.

Simulated Drinking Water. The chemical characteristics of the test water (simulated drinking (SD) water) were modeled after natural surface water. This simulated water was used to provide a consistent water quality over the period of the experiments which could not be assured using a natural water source. Three stock solutions were used to prepare experimental solutions using deionized (DI) water. These were Stock A (4.84 mmol/L $NaNO_3$ and 113 mmol/L NaCl), Stock B (0.25 g/L NOM, Suwannee River from the International Humic Substances Society and 0.53 g/L alginic acid), and Stock C (0.1 mol/L KH_2PO_4). These were diluted with 0.1 mol/L NaOH, augmented with *B. subtilis* spores, and spiked with 1.5 mmol/L $NaHCO_3$ to prepare waters at pH 6.8 (*14*).

Results and Discussion

UV Fluence-Response Curves as a Function of Wavelength. Log survival of *B. subtilis* spores as a function of UV fluence produced by the monochromator at different wavelengths is illustrated in Figure 1. In all cases the presence of a lag phase, or shoulder, was observed at low UV fluences (data not presented), while at higher fluences a first-order relationship was observed. At 293 nm, a shoulder appeared up to a UV fluence of 1000 J/m^2; however, for other wavelengths a shoulder existed up to a fluence of only 200 J/m^2. The presence of a shoulder in *B. subtilis* spore inactivation may be explained by the series-event model (*15*) or by DNA repair (*5*, *16*, *17*) and can be mathematically modeled (*4*).

Log survivals of MS2 as a function of UV fluence for all wavelengths are presented in Figure 2. The fluence-response data in the first-order region were fitted using a linear

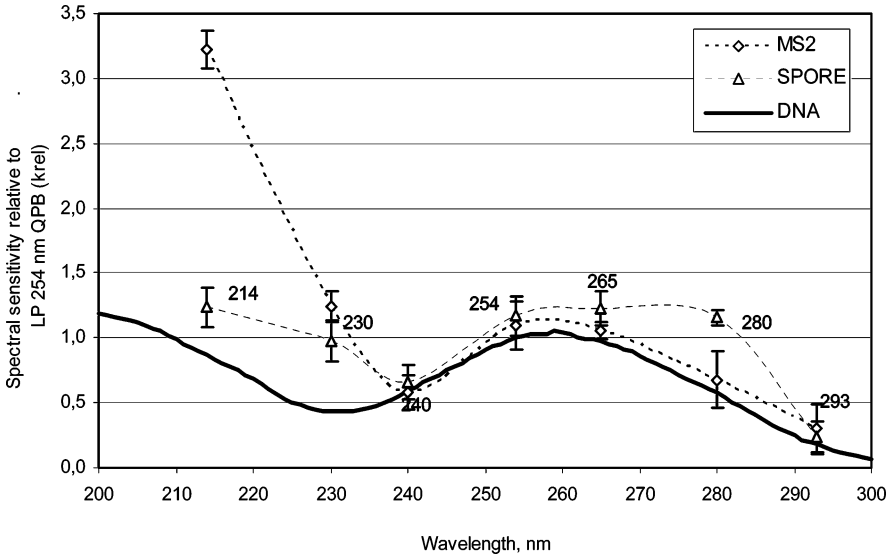

FIGURE 3. Spectral sensitivity of S6633 spores and MS2, as produced using the monochromator, relative to LP 254 nm QPB for spores and MS2, respectively, and DNA relative to 254 nm of DNA. Error bars represent 95% CI for mean sensitivity coefficient.

regression approach resulting in a UV sensitivity coefficient as presented on the graphs. This sensitivity coefficient, at different wavelengths, reflects the difference in inactivation between spore or MS2 samples. With spores, data were not extrapolated through zero point, due to the shoulder in the log-inactivation curve. Nonshouldered curves such as those for MS2 are characterized by first-order inactivation throughout the entire wavelength range, characteristic of a one hit-one target survival curve typical of single-stranded DNA or RNA viruses (*18*).

Linear inactivation kinetics values were calculated using at least four fluences (per wavelength), each averaged from triplicates of microorganism counts. The fluence-based UV sensitivity coefficients, $k(\lambda)$, revealed values from 0.003 to 0.016 m^2/J for spores and values from 0.002 to 0.016 m^2/J for MS2. The coefficient of determination (R^2) for the regression analysis ranged between 0.96 and 0.99 for spores and MS2, indicating a strong first-order relationship between the log survival and the UV fluence. In contrast to spores, repair in viruses depends on the ability of the host cell to repair the virus UV damage, as the virus does not have the repair enzymes by itself. This process was insignificant in RNA viruses (*6*), as indicated by the linear kinetics of MS2. However, recent studies suggested that differences in UV sensitivity of viruses may possibly relate to differences in virus capsid structure, virus transfer of genes to the host that can induce repair, and alteration of host resistance (*19*).

Action Spectra of Spores and MS2. The UV sensitivity coefficient $k(\lambda)$ is a measure of the sensitivity of a microorganism at a particular wavelength, and when plotted as a function of wavelength it represents the action spectrum of a microorganism (*6*, *20*). The action spectrum is typically reported relative to 254 nm, the principal output of the low-pressure Hg vapor lamp. The $k(\lambda)$ from linear regression is transformed to $k_{rel}(\lambda)$, the relative coefficient, by dividing $k(\lambda)$ from a specific band of wavelengths generated from the monochromator by $k(254)$ from exposure to LP 254 nm QPB. A similar transformation is made for $d_{rel}(\lambda)$, the relative shoulder coefficient (*10*). The LP 254 nm factors for spores in this study were $k(254) = 0.0127$ and $d(254) = 0.5893$, while for MS2 they were $k(254) = 0.005$ and $d(254) = 0.0789$.

Figure 3 illustrates the relative spectral UV sensitivity coefficient $k_{rel}(\lambda)$ for surface spores S6633 and MS2, relative to LP 254 nm QPB. In addition, DNA_{rel} is the DNA absorbance relative to DNA absorbance at 254 nm. The actual values determined for $k_{rel}(\lambda)$ can be obtained from data in Figures 1 and 2. The UV action spectra for spores, MS2, and DNA peak at about 260 nm, drop near zero at 293, and feature a minimum around 240 nm for spores and MS2 and nearly 230 nm for DNA. Therefore, both organisms feature a deep minimum at 240 nm that is almost symmetrical toward the lower and higher wavelengths. The spore and MS2 action spectra follow the shape of the DNA spectrum from wavelengths between 240 and 265 nm and at 293 nm. However, at 280 nm the spores are more sensitive than would be expected based on the DNA absorbance. Below 240 nm the spores and MS2 are more sensitive compared to the DNA spectrum. On a relative basis, MS2 is most sensitive to lower wavelengths below 230 nm and *B. subtilis* spores (S6633) are most sensitive to wavelengths around 265 nm. Most notably, the efficiency of MS2 inactivation at 214 nm is 3 times higher compared to that of MS2 LP 254 nm QPB, while the 265 nm sensitivity for spores is slightly more efficient compared to that of spore LP 254 nm QPB. Gates (*21*) discovered that the relative biological effect of *E. coli* toward various UV wavelengths matched the absorbance of the DNA.

Although the same strain of *Bacillus* spores is used for validation in different parts of the world, the sensitivity of these spores to UV irradiation will differ depending upon the cultivation method used; thus, they may differ from site to site. This difference in UV susceptibility may also be related to the individual spectral UV sensitivity of these spores. Sommer and Cabaj (*9*) proposed two methods for cultivation of *B. subtilis* spores, one with sporulation on a solid medium (surface) and the other with the cultivation of spores in a liquid medium. Each cultivation method resulted in different UV-254 nm sensitivities of *B. subtilis* spores. Spores cultivated on the surface were significantly less resistant to UV 254 than those cultivated in liquid. Values for the absolute and relative spectral sensitivity coefficients $k(\lambda)$ and $d(\lambda)$ for the liquid-cultivated spores were published by Cabaj et al. (*4*, *10*) and values for surface-cultivated spores are published in this study. Differences can be quantified using the parameters defined in eq 2. The relative shoulder coefficient $d_{rel}(\lambda)$, which is the intercept of the first-order curve with the y axis, can be calculated from data in Figures 1 and 2. Higher values of $d_{rel}(\lambda)$ obtained for a first-order curve with a similar slope indicate a larger shoulder region. The coefficient d_{rel} is not equal for all microorganisms (*4*) and may differ by an order of 10 in some regions of the UV spectrum (*10*). The overall shape for the action spectra of liquid-cultivated and surface spores was similar, with a maximum effectiveness around

265 nm, rapid decrease toward 293 nm, and a minimum around 240 nm. The *d* values of the liquid-cultivated spores (*10*) increased with increasing wavelengths above 200 nm, whereas for surface spores the *d* values increased up to 240 nm, decreased to 265 nm, and remained constant up to 293 nm. This strongly indicates that for the description of the UV sensitivity of a microorganism with a shouldered UV inactivation curve both parameters, the *k* value as well as the *d* value, have to be known.

The values of the UV-254 nm coefficients *k* and *d* for the *B. subtilis* spores biodosimeter (liquid-cultivated) have been previously reported as $k = 0.0065 \pm 20\%$ m^2/J and $d = 0.7 \pm 30\%$, which helps to ensure consistent and comparable measurements (*22*). Taking into account the *k* values as a measure for the UV sensitivity (*23*), the liquid-cultivated spores are more resistant than the surface spores by a factor of 2 and more resistant than MS2 by a factor of 2.5 at a wavelength of 253.7 nm. Cabaj et al. (*3*) reported that the *k* value at 253.7 nm for spores grown using the surface technique was 0.0134 m^2/J, similar to the results in this research for the surface-grown spores (0.0127 m^2/J).

The specific spectral sensitivity of the microorganism used as a biodosimeter in validation testing has important ramifications. The United States Environmental Protection Agency *UV Disinfection Guidance Manual* (*7*) outlines a number of biases that occur during a UV reactor validation with the biodosimetry test. The REF bias is one bias that accounts for the difference between the fluence delivered to the target pathogen and fluence measured using a challenge microorganism under a given set of reactor conditions. The polychromatic bias is a wavelength-dependent bias that accounts for the action spectra of the challenge microorganism compared to the target pathogen (*7*). Biodosimeters with shouldered inactivation curves may result in higher REF values compared to nonshouldered curves if they have the same *k* value (*24*). Knowledge of the parameters for coefficients *d* and *k* are important when using biodosimeters for the validation of flow-through systems with typical nonideal mixing hydraulics (*24*).

Factors Considered in Fluence Determination. The average microbicidal fluence rate is a function of several factors including the incident fluence rate (W/m^2), the spectral UV absorbance, path length of light traveling through the water, reflection factor, Petri factor, and divergence factor (*25*). The UV fluence (J/m^2) is obtained by multiplication of the average microbicidal irradiance by exposure time. Additional correction factors should be considered for MP lamps such as the radiometer sensor factor and the microbicidal effectiveness correction for the wavelength sensitivity of the target microorganism (*26*), which is conventionally approximated using the DNA absorbance spectrum as a weighting tool.

With the monochromator the divergence factor was close to unity and thus was not taken into consideration. The water factor, which is an integration of Beer−Lambert law over the sample depth, was considered and the reflection factor, which is determined by the change in refractive index from air to water, was taken as 0.975. Petri factor was also unity because the diameter of the UV detector was identical to the exposure dish diameter. Mean fluence rates for each condition, averaged from the full and final depth of water in the Petri dish, are presented in Table 1. The fluence rate delivered by the monochromator increased with wavelength because the Xenon lamp emits more radiation at higher wavelengths and the efficiency of the monochromator increases with wavelength.

To describe polychromatic UV radiation, the coefficients $k(\lambda)$ and $d(\lambda)$ can be weighted with the normalized spectral fluence of the radiation over the UV range and substituted into eq 2. Cabaj et al. (*4*) concluded that polychromatic survival functions of microorganisms are possible to calculate, once the monochromatic wavelength dependence is known. The spectral sensitivity of a given test microorganism (as in Figure 3) is essential to evaluate the true microbicidal irradiance delivered by MP lamps for a specific microorganism; therefore, this procedure is suggested in validation testing of MP UV reactors.

TABLE 2. Comparison of Microbicidal Average Fluence Rate Using Different Action Spectra and Water Types

	microbicidal average fluence rate, W/m^2			
	deionized water (DI)		simulated drinking water (SD)	
microbicidal weighting	spiked with spores	spiked with MS2	spiked with spores	spiked with MS2
DNA	3.7	6.1	5.8	5.9
spore	4.5		7.1	
MS2		7.7		7.4
Abs 254 nm	0.023	0.001	0.066	0.049
Abs 220 nm	0.031	0.004	0.267	0.246

UV fluence determination using the conventional DNA absorbance spectrum was compared to that using the action spectra of MS2 or spores (S6633). The fluence rates are presented in Table 2. A simulation was performed for calculating the fluence by substituting the MS2 or spores (S6633) action spectrum obtained in this study for the DNA absorbance spectrum typically used. For wavelengths below 214 nm the action spectrum was assumed to be similar to the value at 214 nm. The polychromatic microbicidal average irradiance was recalculated for 10^6 CFU/mL of spores or 10^6 PFU/mL of MS2 suspended in DI water or in SD water. The simulation used a MP lamp with polychromatic emission spectrum and apparatus described elsewhere (*27*), incident fluence rate of 7.5 W/m^2, and 10 mL sample volume resulting in a water depth in the Petri dish of 0.453 cm. The SD water highly absorbed at wavelengths below 220 nm compared to the DI water, while the absorbance of water spiked with spores was higher compared to that of water spiked with MS2. Because addition of surface-cultivated spores resulted in increased absorbance in both waters whereas with MS2 it did not, the differences in microbicidal fluence rate of spores was large compared to MS2 (comparison between SD column to DI column of a certain microorganism at any weighting). When using different weighting factors (DNA vs microorganism action spectra) for the calculation of the fluence rate in any water, the differences resulted more from the organism and its spectral sensitivity than from the water type used.

Batch et al. (*28*) evaluated the effect of changing the microbicidal action spectrum used to calculate the polychromatic MP UV fluence in the inactivation of MS2 from a DNA-based weighting to an MS2 action spectrum weighting. Modifying the action spectrum did not result in a statistical difference in the UV inactivation of MS2 in their test system. Based on the results presented in Table 2 for *B. subtilis* spores, for an irradiance time of, e.g., 88 s, a MP fluence of 400 J/m^2 weighted by using the surface spore action spectrum is equivalent to a fluence of 330 J/m^2 if the DNA action spectrum was used. Based on previous investigations of spores with a MP lamp (unpublished data from Duke University), a fluence of 400 and 330 J/m^2 will result in a 3.2 and 2.6 log inactivation respectively for liquid-cultured spores and 4.5 and 3.7 log inactivation respectively for surface spores. This clearly shows the importance of the knowledge of the biodosimeter (spores or MS2) to guarantee reliable measurements of the REF delivered in MP reactors.

Effectiveness Relative to Fluence in Water Treatment Plant. To practically assess the impact of the relative UV wavelength effectiveness for spores and MS2, a fluence of 400 J/m^2 can be evaluated because it is typically the minimum

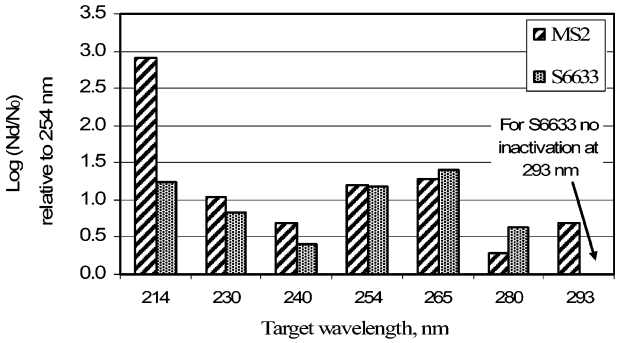

FIGURE 4. Log inactivation of S6633 spores and MS2 at a UV fluence of 400 J/m^2 for different wavelengths, relative to LP 254 nm QPB apparatus.

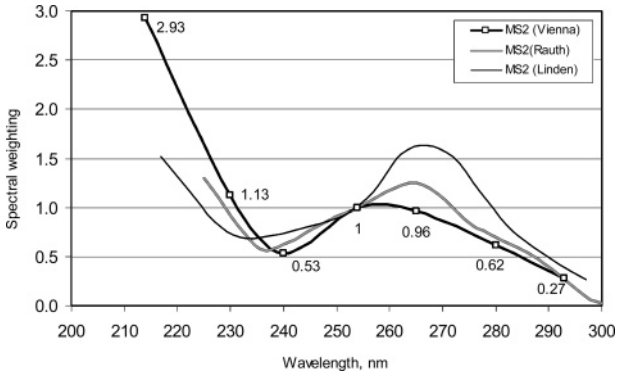

FIGURE 5. Comparative spectral sensitivity of MS2 as measured by different researchers, relative to the data obtained at 254 nm. MS2 (Rauth) from Rauth (*6*). MS2 (Linden) from Linden et al. (*29*). MS2 (Vienna) from current research study.

required averaged fluence delivered by UV disinfection systems (*22*). Figure 4 illustrates the survival of S6633 spores and MS2 at a UV fluence of 400 J/m^2 for different wavelengths relative to 254 nm QPB. With insertion of $k(\lambda)$ and $d(\lambda)$ into eq 2, inactivation (N_d/N_0) at a specific wavelength at a fluence of 400 J/m^2 was obtained. For a UV fluence of 400 J/m^2 the most effective wavelength for inactivation of spores was 265 nm and for MS2 it was 214 nm. The lowest inactivation for spores at a fluence of 400 J/m^2 occurred at wavelengths of 293 followed by 240 and 280 nm. For MS2 the lowest inactivation occurred at wavelengths of 280 nm followed by 240 and 293 nm. Usually the highest sensitivity for microorganisms is between 200 and 290 nm, with a strong decrease from 300 to 400 nm (*10*); however, wavelengths above 300 nm were not evaluated in this study.

Exposure Procedures Influencing MS2 Inactivation. Variability in the existing spectral sensitivity data in the literature for MS2 virus is illustrated in Figure 5. This variability may be attributed to the different procedures used to generate wavelengths for action spectra determination. The spectrum MS2 (Rauth) (*6*) is mostly similar to spectrum MS2 (Vienna) obtained in this study except at 265 nm. The MS2 (Rauth) spectrum was not published for wavelengths less than 225 nm. At 265 nm and at lower wavelengths (below 240 nm) the MS2 (Linden) (*29*) spectrum is different from the MS2 (Vienna) spectrum, perhaps due to the use of band-pass filters for generating wavelength bands in their study. Band-pass filters can be problematic for investigating the wavelength effectiveness because the fluence rate is reduced by the filter and the wavelengths passed are often greater than 10 nm in half-peak bandwidth. Therefore, they require longer exposure times and are broader in bandwidth compared to that in a monochromator system. These differences highlight the need for a standard exposure procedure using a monochromator for the investigation of the spectral sensitivities of test microorganisms in UV systems. In addition, due to differences in exposure time, it is important to consider the time-fluence reciprocity, which could affect the comparison between spectral sensitivities obtained under different experimental designs. Sommer et al. (*30*) investigated the time-fluence reciprocity of MS2 virus compared to *B. subtilis* spores and found that both followed the time-fluence reciprocity law.

Unexpectedly, the coefficients k and d (254 nm) for the inactivation of spores and MS2 resulting from the irradiation in the low-pressure bench system on one hand and the monochromator Xenon lamp at 254 nm on the other hand did not match very well, although both experiments were corrected for all the proper fluence measurement factors. Because the reciprocity law is obeyed with these microorganisms, the differences are likely due to other factors. The monochromator has a half-peak bandwidth of about 10 nm with central wavelength at 254.2 $\pm$ 0.3 (Table 1) but does not produce a narrow emission line as the low-pressure lamp does at 253.7 nm. Changes in the sensitivity curve over a 10 nm region, especially at lower wavelengths, could result in the differences in inactivation between bench scale LP 254 nm QPB and the 254 $\pm$ 5 nm wavelength range of the monochromator.

Biological Factors Influencing Difference in Action Spectra of Surface Spores and MS2. *MS2 coliphage* is a single-stranded RNA virus, with a polyhedral shape at the size of 0.026 μm (*31*), whereas *B. subtilis* spores possess a double-stranded DNA, elliptical in shape with a length of about 1.3 μm and a width of about 0.7 μm (*19*). The nucleotide bases of the DNA are adenine, guanine, thymine, and cytosine, whereas RNA contains uracil instead of thymine (*18*). Photochemical reactions for inactivation are mostly efficient with wavelengths close to the maximum absorbance of pyrimidine (thymine, cytosine, and uracil) and purine (adenine and guanine) nucleobases (*32*). DNA and RNA have similar absorbance spectra while uracil absorbance is slightly shifted to the lower wavelengths compared to RNA, DNA, and thymine, indicating that uracil absorbs less at the lower wavelengths (*31*, *33*). This difference in absorbance is contradictory to the higher UV sensitivity of MS2 at those wavelengths compared to that of spores, indicating that factors other than uracil or thymine absorbance are responsible for the difference in UV inactivation. Furthermore, the difference in uracil and thymine absorbance did not appear to affect the overall absorbance spectrum of DNA or RNA.

B. subtilis spores and MS2 virus exposed to UV radiation above 290 nm exhibit different DNA photochemistry and DNA repair responses compared to 254 nm UVC which may relate to the demonstrated action spectra of these microorganisms. At 254 nm, spores accumulate spore photoproduct (SP) as the major DNA damage (*20*). SP can be repaired during spore germination by two major repair pathways: SP lyase enzyme and to a lesser extent excision repair (NER) (*34*). SP is also produced at longer UV wavelengths (UVB, UVA), however, with a lower quantum yield, resulting in the lower sensitivity of the spores at wavelengths above 270 nm. It is suggested that at the longer wavelengths nonspore photoproducts (as dimers) are induced with distribution of the dimers depending on wavelength, and a relative shift in repair toward NER (*34*, *35*). Spore coat layers are thought to contribute to spore resistance during UVA and UVB (in sunlight) radiation but not to 254 nm UVC radiation (*36*). Therefore, spores exposed to 293 nm irradiation will likely be very UV resistant. Munakata (*20*) suggested that low sensitivity of spores (e.g. at 240 nm in this study) results from lethal damage that can be repaired or from less damage, while high sensitivity (e.g., at 214, 254, and 265 nm in this study) results from damage that cannot be repaired, increased

DNA damage, or possibly different spore photoproducts than those stated above.

Differences in the nucleic acid of viruses as single or double stranded, RNA or DNA, and the UV wavelength these viruses are exposed to might affect their UV spectral sensitivity. Inactivation of viruses at 254 nm is mainly due to damage of nucleic acids as DNA and RNA have the highest absorption coefficient between 200 and 300 nm compared to other viral components at 254 nm. However, this presumption may not be valid if the nucleic acid is a small percent of the total viral material and the UV exposure is mainly at low wavelengths where protein absorbance is not negligible (*6*). Therefore, MS2 virus might be more sensitive at 214 nm due to the relatively high protein absorbance. However, it is important to consider that both the quantum yield for dimer (as with MS2) or SP (as with *Bacillus* spore) formation and quantum yield for dimer or SP repair (*36*) are opposing factors in the overall sensitivity of MS2 and spores at different wavelengths.

Because each organism is unique in the response to UV irradiation at different wavelengths, standardizing the use of DNA absorbance to obtain the average microbicidal fluence for polychromatic lamps can be inaccurate. Consequently, using the action spectrum of the challenge organism will improve the accuracy in the determination of the REF for polychromatic UV sources and thus the validation of those reactors. Knowledge of the spectral sensitivity of the biodosimetry test microorganisms will assist design and optimization of efficient UV disinfection processes as well as provide insights into differences of UV resistance for challenge microorganisms. Determination of the spectral sensitivity via a standardized protocol, such as that suggested in this study, will allow for proper data comparisons between different researchers. Accepted protocols for wavelength sensitivity analysis should be standardized in UV disinfection guidance manuals and considered by regulators when evaluating validation data generated for UV disinfection systems with polychromatic MP lamps.

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