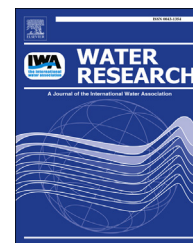


Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

Action spectra for validation of pathogen disinfection in medium-pressure ultraviolet (UV) systems

Sara E. Beck^a, Harold B. Wright^b, Thomas M. Hargy^c,
Thomas C. Larason^d, Karl G. Linden^{a,*}

^a Department of Civil, Environmental, and Architectural Engineering, University of Colorado Boulder, UCB 428, Boulder, CO 80309, USA

^b Carollo Engineers, 12592 West Explorer Drive, Suite 200, Boise, Idaho 83713, USA

^c Corona Environmental Consulting, 318 Overlake Dr, Fairfax, VT 05454, USA

^d National Institute of Standards and Technology, 100 Bureau Dr., Gaithersburg, MD 20899, USA

ARTICLE INFO

Article history:

Received 20 August 2014

Received in revised form

15 November 2014

Accepted 17 November 2014

Available online 28 November 2014

Keywords:

Inactivation

Phage

Surrogate

Water treatment

Polychromatic

ABSTRACT

Ultraviolet (UV) reactors used for disinfecting water and wastewater must be validated and monitored over time. The validation process requires understanding the photochemical properties of the pathogens of concern and the challenge microorganisms used to represent them. Specifically for polychromatic UV systems, the organisms' dose responses to UV light and their sensitivity across the UV spectrum must be known. This research measured the UV spectral sensitivity, called action spectra, of *Cryptosporidium parvum*, and MS2, T1UV, Q Beta, T7, and T7m Coliphages, as well as *Bacillus pumilus* spores. A tunable laser from the National Institute of Standards and Technology was used to isolate single UV wavelengths at 10 nm intervals between 210 and 290 nm. Above 240 nm, all bacteria and viruses tested exhibited a relative peak sensitivity between 260 and 270 nm. Of the coliphage, MS2 exhibited the highest relative sensitivity below 240 nm, relative to its sensitivity at 254 nm, followed by Q Beta, T1UV, T7m and T7 coliphage. *B. pumilus* spores were more sensitive to UV light at 220 nm than any of the coliphage. These spectra are required for calculating action spectra correction factors for medium pressure UV system validation, for matching appropriate challenge microorganisms to pathogens, and for improving UV dose monitoring. Additionally, understanding the dose response of these organisms at multiple wavelengths can improve polychromatic UV dose calculations and enable prediction of pathogen inactivation from wavelength-specific disinfection technologies such as UV light emitting diodes (LEDs).

© 2014 Elsevier Ltd. All rights reserved.

* Corresponding author. Tel.: +1 303 492 4798; fax: +1 303 492 7317.

E-mail address: karl.linden@colorado.edu (K.G. Linden).

<http://dx.doi.org/10.1016/j.watres.2014.11.028>

0043-1354/© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Ultraviolet (UV) disinfection is common for pathogen inactivation in drinking water and wastewater sources. UV systems generally use either low-pressure (LP) or medium-pressure (MP) mercury vapor lamps. In the germicidal spectrum, between 200 and 300 nm, LP lamps emit monochromatic light at 253.7 nm. Photons at this wavelength primarily form lesions in nucleic acids (DNA and RNA), due to their relative peak absorbance at 260 nm. MP lamps emit polychromatic light across the electromagnetic spectrum. The broader wavelength range is responsible not only for damaging nucleic acids, but also for causing photochemical reactions in proteins and enzymes. Most protein damage occurs below 240 nm, where proteins absorb UV light the strongest (Harm, 1980).

Federal guidance provides direction for the validation and dose monitoring of UV systems while regulations define the UV dose requirements for pathogens. The Ultraviolet Disinfection Guidance Manual (USEPA, 2006a) was written to provide guidance to state and federal regulatory agencies and public water systems on the design, operation, maintenance, and validation of UV disinfection systems. The UVDGM provides guidance on reactor validation, dose monitoring and reporting and supplements the UV disinfection requirements listed in the Long Term 2 Enhanced Surface Water Treatment Rule (USEPA, 2006b), which regulates required UV dose. The LT2 also requires that UV reactors use dose monitoring that has been proven through validation testing.

UV disinfection systems in the United States are validated using biosimetry in which the log inactivation of an organism is measured through a UV system and the corresponding applied average UV dose is back-calculated through a dose–response curve. For validation, test microorganisms or surrogates are typically used; ideal surrogates would match the pathogen in terms of dose response and action spectrum. Dose response is a measurement of pathogen inactivation as a function of UV dose. The action spectrum of a microorganism is a measure of its spectral sensitivity or its UV inactivation as a function of wavelength for a given UV dose (USEPA, 2003). The dose responses and action of microorganisms and viruses vary across the UV spectrum; differences in these parameters between the pathogens of concern and the test microorganisms lead to errors in validation. For instance, below 240 nm, MS2 has a greater relative spectral sensitivity than *Cryptosporidium* (Linden et al., 2001; Mamane-Gravetz et al., 2005). Therefore, for MP UV lamp systems that don't absorb light below 240 nm, validation testing using MS2 over-predicts the UV dose delivered to *Cryptosporidium*, raising a potential public health concern. The UVDGM requires validation factors be applied to account for validation uncertainty. Differences between the organisms' dose responses at 254 nm are accounted for with a RED (reduction equivalent dose) bias. Differences in the spectral sensitivity or action spectra of the organisms are accounted for with an action spectra correction factor (ASCF).

Validating MP UV reactors requires calculating ASCFs, which requires accurately knowing the action spectra.

However, existing action spectra data for waterborne pathogens and UV bioassay test organisms typically do not extend below 220 nm and often not below 240 nm. Nevertheless, the wavelengths below 240 nm are considered responsible for the enhanced efficacy of MP UV over LP UV for disinfecting certain pathogens (Malley et al., 2004; Linden et al., 2007; Eischeid et al., 2009; Beck et al., 2014).

Additionally, many of the existing published action spectra were measured using bandpass optical filters, which can introduce an error that impacts the interpretation of the data. Bandpass filters restrict the UV light from an MP lamp to a given range of wavelengths; however, the bandpass filters have a bandwidth on the order of 10 nm at half of the maximum peak transmittance and a bandpass of 20 nm at 10 percent of the peak. This breadth essentially allows polychromatic light from the MP source through the filter and decreases the precise delivery of specific wavelengths during action spectra measurements. Use of bandpass filters can cause errors during the action spectra measurement because the effective wavelength of the bandpass filtered light differs from the nominal wavelength of the bandpass filter. Statistically significant error has been shown when the UV dose response of microbes was measured using monochromatic 254 nm light from an LP lamp compared with 254 nm bandpass filtered light from an MP lamp. The doses required for 3-log inactivation of T7 and Q Beta coliphage were 20–34% less when irradiated with the bandpass-filtered MP UV lamp than with the LP UV lamp emitting at the same wavelength (Wright et al., 2007). Mamane-Gravetz et al. (2005) also noted differences (approximately 9–15%) between the inactivation coefficients for MS2 coliphage and *Bacillus subtilis* spores irradiated with an LP UV lamp compared to irradiation from a xenon lamp monochromator centered at 254 nm with an FWHM of 10 nm.

To address these errors, this research measured the action spectra of *Cryptosporidium parvum* as well as MS2, T1UV, Q Beta, T7, and T7m coliphages from 210 nm to 290 nm using a tunable laser that produced precise monochromatic light (bandwidth $<< 1$ nm). To add to the body of knowledge, the action spectrum of *Bacillus pumilus* spores was also determined; however, an MP UV source with bandpass filters was used due to resource limitations. This work was required for calculating accurate action spectra correction factors for MP UV system validation, for matching appropriate test microorganisms to pathogens, and for defining what wavelengths are important for UV dose monitoring.

Action spectra can be used to calculate germicidal UV dose when irradiating with polychromatic UV sources. Past research has suggested that weighting the MP UV dose calculation by action spectra is more appropriate than the standard practice of weighting by DNA absorption (Malley et al., 2004; Mamane-Gravetz et al., 2005; Hijnen and Medema, 2010; Beck et al., 2014). With a correct MP UV dose calculation, the dose response of the organism to MP UV light would match its response to LP UV light (Bohrerova and Linden, 2006; Guo et al., 2009). In addition to development of the action spectra for numerous microorganisms, in this work the action spectrum for MS2 coliphage was used to weight the average irradiance in an MP UV dose calculation and the results were compared to the LP UV dose response.

1.1. Pathogen tested

1.1.1. *Cryptosporidium parvum*

C. parvum is a protozoan parasite that infects the cell lining of the digestive tract, causing the diarrheal disease, cryptosporidiosis. The pathogen's resistant oocysts, 4 μm –6 μm in size, can infect humans through multiple transmission pathways and have been associated with numerous waterborne disease outbreaks (Fayer et al., 2000), including one that affected the lives of 400,000 people in Milwaukee in 1993 (Mackenzie et al., 1994). *Cryptosporidium* is important to the water treatment industry due to its resistance to chemical disinfection with chlorine.

1.2. Surrogates tested

1.2.1. MS2 coliphage

MS2 coliphage, or male specific 2, is a single-stranded RNA virus that infects the F pilus of male-specific strains of *Escherichia coli*. Frequently used for reactor validation in North America, MS2 is similar in size, shape, and genome length to enteroviruses including poliovirus, echovirus, and coxsackievirus, but it is more UV-resistant than these viruses (Hijnen et al., 2006; Park et al., 2011). MS2 has been used as a reactor validation surrogate for *Cryptosporidium* and adenovirus although it has a different dose response and action spectrum than these pathogens.

1.2.2. T1UV coliphage

T1UV coliphage, an organism with a genome similar to coliphage T1, was isolated from swine manure by GAP Environmental Services (London, Ontario Canada). T1UV is a double-stranded DNA virus with a non-contractile tail (Stefan et al., 2007). T1UV has similar sensitivity as *Cryptosporidium* and *Giardia* to low-pressure mercury vapor lamps (253.7 nm) and is often used as a surrogate microorganism for reactor validation.

1.2.3. Q beta coliphage

Q Beta coliphage is a single-stranded RNA virus similar to MS2 that infects *E. coli* through the F pilus of male-specific strains. Q beta has been used for UV reactor validation (USEPA, 2006a) though not frequently. Q Beta is more sensitive to 254 nm UV light than MS2.

1.2.4. T7 coliphage

T7 coliphage is a double-stranded DNA virus that infects *E. coli*. Like T1UV, T7 exhibits inactivation kinetics similar to *Cryptosporidium* and *Giardia* and is often used for reactor validation. **T7m coliphage**, a strain of T7 with a sequence similar to T3 (Lin et al., 2012), is not currently used for validation, but was used in the past.

1.2.5. *Bacillus pumilus*

B. pumilus is a non-pathogenic spore-forming bacterium, highly resistant to monochromatic UV light. Given its UV resistance, *B. pumilus*, in its spore form, is being considered as a surrogate microorganism for adenovirus (Rochelle et al., 2010). The UV dose–response of spores varies by strain and cultivation method.

2. Materials and methods

2.1. UV irradiations

UV irradiations were conducted using one of three UV sources: a tunable laser, an LP UV lamp, or an MP UV lamp. *Cryptosporidium* and the five coliphage were irradiated with an NT242 series Ekspla tunable laser from the National Institute of Standards and Technology (NIST), as described previously (Beck et al., 2014) at wavelengths of 210 nm, 220 nm, 230 nm, 240 nm, 253.7 nm, 260 nm, 270 nm, 280 nm, and 290 nm. The 2.5 mm diameter laser beam first passed through a computer-controlled shutter, before being reflected off two mirrors. Visible light was filtered out by either mirrors with dielectric coatings for wavelengths from 240 nm to 290 nm or a fused silica prism and slit for the 210 nm, 220 nm, and 230 nm wavelengths. The beam traversed next through a beam splitter and an etched, fused silica diffuser (RPC Photonics, Rochester, NY), specifically engineered to modify the laser beam from a collimated oval shape to a uniform diverging beam (10° half-angle) for irradiating the water samples. A more detailed description of the tunable laser is included in the [Supplementary Info](#).

For comparison, samples were also exposed to UV light from an LP mercury vapor lamp (Atlantic Ultraviolet G12T6L, Hauppauge, NY), emitting at 253.7 nm, with a full width at half maximum (FWHM) of 2 nm. For *Cryptosporidium*, UV light from the tunable laser was attenuated using a neutral density filter to increase the exposure times to at least 30 s to improve the accuracy of the measured UV dose.

UV irradiations of *B. pumilus* spores were conducted using the LP lamp noted above and with a 1-kW Rayox medium-pressure mercury vapor lamp (Calgon Carbon, Pittsburgh, PA) with bandpass filters (Andover Corporation, Salem, NH), which had an FWHM of 10–12 nm. The emission spectra, shown in [Figure S1](#), were measured with an Ocean Optics Maya 2000 Pro spectrometer (Dunedin, FL). The weighted average wavelengths used for irradiation of *B. pumilus* spores were 220, 230, 244, 258, 264, 270, 281, and 292 nm. For each polychromatic irradiation, UV dose was calculated by summing the average UV irradiance over the wavelength range, accounting for absorbance and sample depth, following the Beer–Lambert Law as described previously (Bolton and Linden, 2003; Linden and Darby, 1997). Although the action spectra for *B. pumilus* spores would not be as precise as if it were determined with the NIST tunable laser, the authors chose to include this spectrum with the others because a *B. pumilus* action spectrum has not previously been published in the peer-reviewed literature.

For all test microorganisms, four collimated beam exposures were conducted at each wavelength to generate a dose response curve up to at least 3-log inactivation of each microorganism. Microbial suspensions of 5 mL in a continuously stirred dish (0.6 cm sample depth) were irradiated by one of the three lamp sources. UV irradiance from the NIST laser was measured by a photodiode detector (IRD SXUV 100, Opto Diode Corporation, Thousand Oaks, CA) comprised of 10 mm \times 10 mm windowless silicon and an 8 mm diameter electroformed precision aperture (SK# 030483-1073, Buckbee

Table 1 – Coliphage propagation host and media specifics.

Coliphage (Source/ID)	Host (Source/ID)	Propagation media	Total time to propagate
MS2 (ATCC/15597-B1)	<i>E. coli</i> HS(pFamp)R (ATCC/700891)	Tryptic Soy Broth supplemented with Ampicillin/Streptomycin	5 h
T1UV (Laval University/HER#468)	<i>E. coli</i> CN13 (ATCC/700609)	Modified TYGB	21–27 h
Qβ (ATCC/23631-B1)	<i>E. coli</i> (ATCC/23631)	Tryptone Yeast Glucose Broth (TYGB)	5 h
T7m (ATCC/11303-B38)	<i>E. coli</i> B (ATCC/11303)	Nutrient Broth supplemented with 0.5% Sodium Chloride	7 h
T7 (ATCC/BAA-1025-B2)	<i>E. coli</i> CN13 (ATCC/700609)	Tryptic Soy Broth	24 h

Mears, Cortland, NY) supplied and calibrated by NIST. At each wavelength, the irradiance uniformity (i.e. Petri factor) of the laser was measured at the water surface plane using a radiometer (model 1400A) and detector (SED 240) and W-diffuser (International Light Technologies, Peabody, MA) with a 1 mm aperture in front of the detector. All Petri factors were within the range of 0.94–1.05. UV irradiance from the LP UV and MP UV lamp sources were measured at the water surface using the same calibrated radiometer (model 1400A), detector (SED 240) and W-diffuser. For LP UV exposures, irradiance readings were confirmed using a second radiometer/detector (Gigahertz/Optik X9-11, Newburyport, MA). UV dose was calculated based on average irradiance multiplied by the exposure time where the irradiance accounted for surface reflection, sample depth, UV absorbance, and uniformity across the surface as described previously (Bolton and Linden, 2003). UV doses ranged from 0.5 mJ/cm² to 160 mJ/cm², depending on the pathogen and the wavelength tested. Immediately after exposure and prior to starting the assays, the irradiated samples were refrigerated at 4 °C in the dark.

2.2. Stock preparation and enumeration

2.2.1. *Cryptosporidium parvum*

C. parvum oocysts (Iowa isolate) were purchased from Waterborne, Inc. (New Orleans, LA). These had been produced by experimentally infected mice. Feces were collected and purified using sucrose and Percoll density gradient centrifugation. Oocysts were <1 month old post shedding and had ≥5% infectivity rate for inactivation trials at time of testing.

Cryptosporidium infectivity was quantified using HCT-8 (human ileocaecal adenocarcinoma) cells (American Type Culture Collection, ATCC # CCL-244). The HCT-8 cell stock solutions were prepared by placing the cells in a volume of maintenance media (RPMI), supplemented with antibiotics, an antifungal agent and fetal bovine serum. The flask was incubated at 35 °C ± 1.0 for 2–5 days under 5% CO₂. When the cells formed a confluent monolayer, they were removed from culture flask by trypsinization, concentrated by slow speed centrifugation at 400 g for 2 min, and re-suspended in maintenance media. A small portion of the re-suspended cells was placed in a sterile flask containing fresh media for continued growth. The *Cryptosporidium* infectivity assay was conducted by placing 10⁵ HCT-8 cells in each chamber of a well slide containing 8 chambers and incubating at 35 °C ± 1.0 °C and 5% CO₂ for 2–3 days. When the monolayers in the well chambers

were 80 %–90 % confluent, they were ready to support *C. parvum* infections. HCT-8 cells were discarded after 24 passages.

C. parvum infectivity was measured within 24 h of UV exposure using a protocol described previously (Johnson et al., 2010). Briefly, oocysts were concentrated using immunomagnetic separation (IMS) as described in US EPA Method 1623 (McCuin and Clancy, 2003) within 2 h of exposure. Each sample was transferred to an individual Leighton tube containing 1 mL each of SL-A and SL-B buffers. Centrifuge tubes were rinsed with approximately 1 mL–2 mL of phosphate buffered saline (PBS) to remove residuals and the rinse was transferred to its respective Leighton tube. One hundred microliters each of *Cryptosporidium* IMS beads were added to each tube and were incubated for 1 h at room temperature under constant rotation. Each Leighton tube was then placed in a magnetic particle concentrator (MPC-1) and gently rocked for 2 min, through a 90° angle. The supernatant in each tube was aspirated. The tube was removed from the MPC-1 and the bead-oocyst complex was gently re-suspended in 1 mL PBS. The tube was replaced in the MPC-1 and the bead isolation procedure was repeated and the supernatant discarded. The beads in each tube were re-suspended in 100 µL Hank's Balanced Salts Solution (HBSS) and refrigerated overnight at 4 °C.

The following morning, samples and controls (method blanks, heat-inactivated oocysts and unexposed oocysts) were incubated at 35 °C for 1 h in acidified HBSS containing a concentration of 1% trypsin to stimulate excystation of the oocysts. Sample aliquots were rinsed to remove the acidified HBSS and known concentrations of *C. parvum* oocysts were applied to confluent monolayers of HCT-8 cells and then incubated for 68–72 h under 5% CO₂. After incubation, inoculated monolayers were washed with phosphate buffered saline and stained with fluorescein isothiocyanate (FITC) conjugated polyclonal antibodies. Detection and enumeration of the intracellular reproductive stages of *C. parvum* oocysts were performed using epifluorescence and differential interference contrast (DIC) microscopy.

Quality assurance/quality control samples were analyzed with each analytical batch. Method blanks, positive controls, a negative control consisting of heat-inactivated oocysts, and inoculation process controls were analyzed. Initial concentrations of oocysts used in each experiment were determined by hemocytometer counts. Initial oocyst infection rates were used to calculate the number of oocysts needed for each exposure to accurately measure the log₁₀ inactivation.

2.2.2. Bacteriophage

Stock solutions of coliphage were prepared by inoculating the propagation media listed in Table 1 with the log-phase host bacteria and an aliquot of the phage stock also listed.

The broth was incubated at $35\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$ with constant shaking for the time specified in Table 1. Bacterial debris was removed by centrifugation at 5000 g for 30 min. The clarified supernatant containing the phage was decanted to sterile containers and stored at $4\text{ }^{\circ}\text{C}$. For some exposures (i.e., MS2, T1UV, and T7), coliphage stocks were received from other laboratories involved in UV reactor validations.

Phage concentrations were determined using the double agar layer method as described previously (Fallon et al., 2007). Briefly, log phase host bacteria were added to “soft agar” with an aliquot of the sample containing an estimated 20–200 plaque forming units (pfu). The inoculated soft agar was poured over an agar plate and allowed to harden. Agar plates were incubated inverted for 16–24 h at $35\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$ and then examined for plaque formation. Viral plaques were counted to determine the original concentration of coliphage in the original broth culture and the concentrations after exposure to UV light. Each sample was analyzed at multiple dilutions with three replicates plated at each dilution. Controls were analyzed each day samples were analyzed. Negative controls were analyzed at the beginning of each analytical batch and periodically during the plating procedure to ensure the sterility of plating area. A negative control was also analyzed at the end of the plating. A positive control sample was plated each day to ensure the assay was working correctly.

2.2.3. *Bacillus pumilus*

The ASFUVR strain of *B. pumilus* was isolated from native aerobic spore forming (ASF) bacteria after exposure to high doses of LP UV light. Spore preparation was conducted as described previously (Rochelle et al., 2010). Briefly, spores were cultured for 6 days by incubating at $37\text{ }^{\circ}\text{C}$ in a modified sporulation medium supplemented with 0.1 mM MnSO_4 . Spores were harvested by centrifuging at 4000 g at $4\text{ }^{\circ}\text{C}$ for 15 min. The resulting cell pellets were washed three times by resuspending in sterile phosphate buffered water (PBW) and centrifuging at 4000 g at $4\text{ }^{\circ}\text{C}$ for 15 min. The final cell pellet was resuspended in 10 mL of PBW, pasteurized at $80\text{ }^{\circ}\text{C}$ for 20 min to kill vegetative cells, and then stored at $4\text{ }^{\circ}\text{C}$ prior to UV exposure.

B. pumilus spore assays were conducted as described previously (Rochelle et al., 2010). The stock suspension was serially diluted and then aliquots were filtered using $0.45\text{ }\mu\text{m}$ pore-sized mixed cellulose esters membranes. Membranes were applied to plates of tryptic soy agar (TSA) and incubated inverted for 22–26 h at $35\text{ }^{\circ}\text{C}$. Colonies were enumerated to determine the initial spore concentrations. For exposed samples, sample volumes or dilutions containing and estimated 20–60 colony forming units (cfu) per membrane were analyzed to determine \log_{10} inactivation. Each volume analyzed was plated in triplicate.

Quality control samples were analyzed with each batch of samples and include a negative control of the rinse water, a negative control of vegetative bacteria, *E. coli* stock (ATCC #25922) after pasteurization, and a positive control of a known concentration of *B. pumilus*.

2.3. Statistical analysis

Sutherland (2002) and Coohill (1991) describe an action spectrum as constants that map the UV dose response of an organism at different monochromatic wavelengths to its UV dose–response at a reference wavelength, such as 253.7 nm. To determine the constant at a given wavelength, the UV dose–response at 253.7 nm was modeled empirically using a quadratic function:

$$\log I = A \times D + B \times D^2$$

The quadratic function was used because it accounted for the statistically significant curvature ($p < 0.10$) observed with a majority of the UV dose–response curves measured in this work. The constants were then determined as the values that mapped the UV dose response at each wavelength tested onto the UV dose response at 253.7 nm, minimizing the sum of the squares of the differences between the measured log inactivation and that predicted using the quadratic function for a given UV dose. The mapping approach was used for determining action spectra because it incorporates all of the dose–response data as opposed to one value at a fixed log inactivation. See Figure S5 for a visual illustration of the mapping approach.

The full action spectra were then generated by fitting the averaged values at the wavelength intervals tested with a cubic spline. The 95% confidence intervals were determined by multiplying the t-statistic (1.96) by the standard deviation and dividing by the square root of the number of samples.

With *B. pumilus*, the UV dose response data obtained using the MP UV lamp with bandpass filters was mapped to the UV dose–response data from an LP UV lamp emitting at 253.7 nm. With all other microbes, the UV dose response data obtained from the tunable laser emitting at various wavelengths described above was mapped to the UV dose response data obtained using the tunable laser emitting at 253.7 nm.

The dose response of MS2 to MP UV light was determined given two different MP UV dose calculations. The doses were calculated as described in Linden and Darby (1997) with the spectral weighting being the standard measure of germicidal effectiveness (USEPA, 2006a), the DNA absorption curve, in one calculation and the MS2 action spectrum from this study in the other. The two dose response curves were compared with the dose response of MS2 to LP UV light using a dummy variable analysis.

3. Results and discussion

3.1. *Cryptosporidium*

Results from the three collimated beam trials with *C. parvum* are shown in Fig. 1. At the tunable laser emission of 253.7 nm, 2-log inactivation was attained at an average UV dose of $1.9\text{ mJ}/\text{cm}^2$. This is consistent with the literature in which 1.7-log inactivation was reported at an LP UV dose of $2\text{ mJ}/\text{cm}^2$ (Shin et al., 2001). The required dose for 2-log inactivation of *Cryptosporidium*, stipulated in the Federal Register is a conservative $5.8\text{ mJ}/\text{cm}^2$ (USEPA, 2006b). *Cryptosporidium* dose

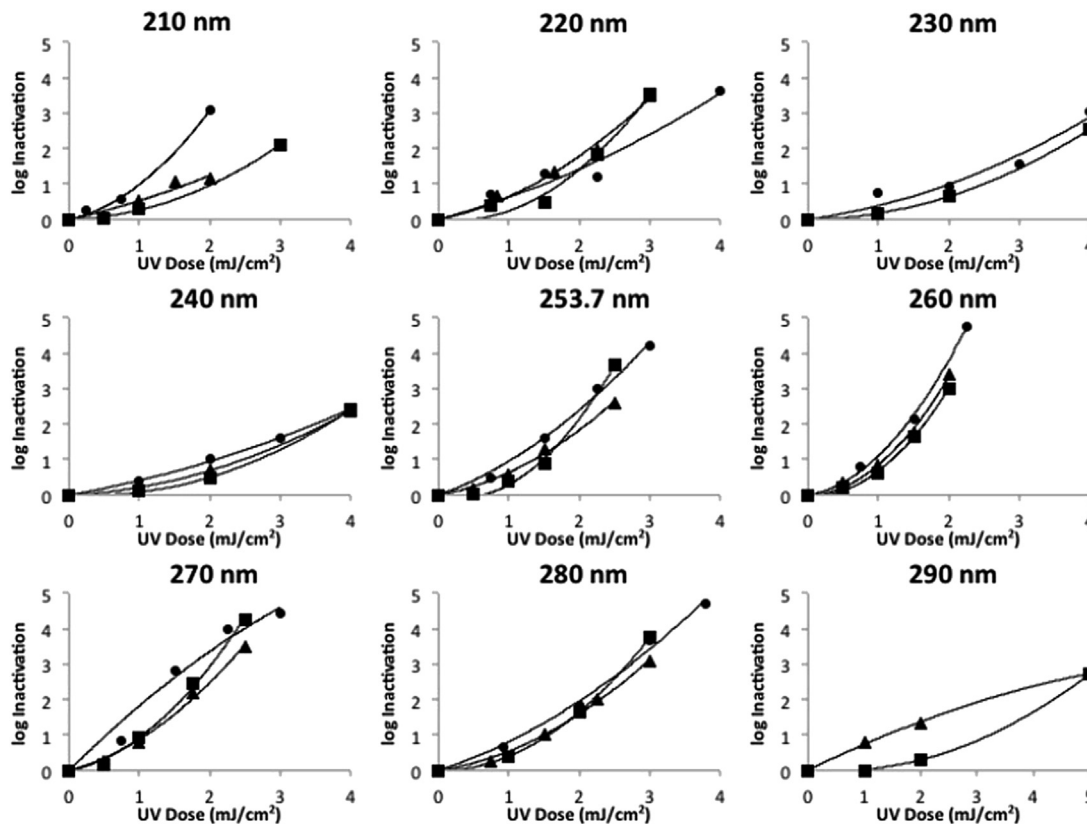


Fig. 1 – Dose response of *Cryptosporidium* to wavelength-specific UV light from the tunable laser. Symbols represent data from three independent trials: R1 (●), R2 (▲), and R3 (■). Average standard deviation between triplicate platings = 8.3 oocysts.

response exhibited a slight shoulder at each wavelength tested.

The dose response data was used to determine the spectral sensitivity or action spectrum of *C. parvum* relative to its response at 253.7 nm (Fig. 2, Table S1). This spectrum was compared to an action spectrum developed previously from a medium-pressure UV mercury vapor lamp and bandpass filters providing half-peak bandwidths of 9 nm–11 nm (Linden

et al., 2001). Above 240 nm, the two spectra were comparable. Below 240 nm, the spectrum developed in this study displayed a greater relative sensitivity of *C. parvum* to the lower UV wavelengths. This difference could be due to differences between the narrow-band light emitted from the monochromatic tunable laser used in this study and the broader output from the medium-pressure UV lamp with bandpass filters used previously. It could also be due to differences in the *C. parvum* assays. Nevertheless, unlike for some of the phage presented below, the lower wavelengths from polychromatic lamps do not provide increased inactivation of *C. parvum* relative to inactivation from LP UV light, which is consistent with the previous study (Linden et al., 2001).

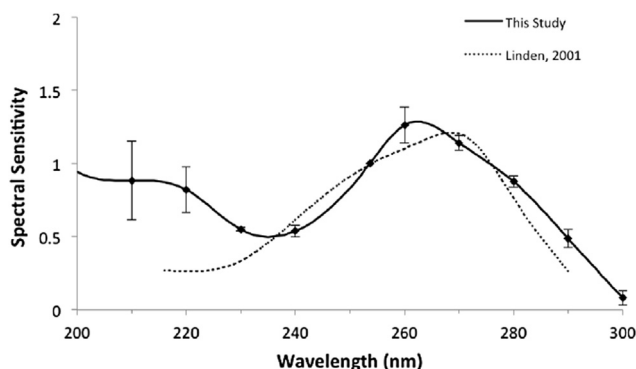


Fig. 2 – Relative spectral sensitivity of *Cryptosporidium parvum* to UV light compared to a previous research study (Linden et al., 2001). Error bars represent 1 standard deviation from the mean sensitivity value. $n = 3$ for all wavelengths except 230 nm and 290 nm, where $n = 2$.

3.2. Coliphage

Results from the four collimated beam trials with MS2 Coliphage are shown in Fig. 3. At the tunable laser emission of 253.7 nm, 2-log inactivation was attained at a UV dose between 29.4 mJ/cm² and 41.8 mJ/cm². This is consistent with the literature in which 2-log inactivation was reported at LP UV doses of 32.8 mJ/cm² and 35.1 mJ/cm² (Meng and Gerba, 1996; Park et al., 2011). Four of the six trials lay outside the National Water Research Institute (NWRI) bounds of 34 mJ/cm² to 54.5 mJ/cm² because the bounds assume a linear dose response where, in fact, the dose–response have curvature. The bounds were also developed using dose response data

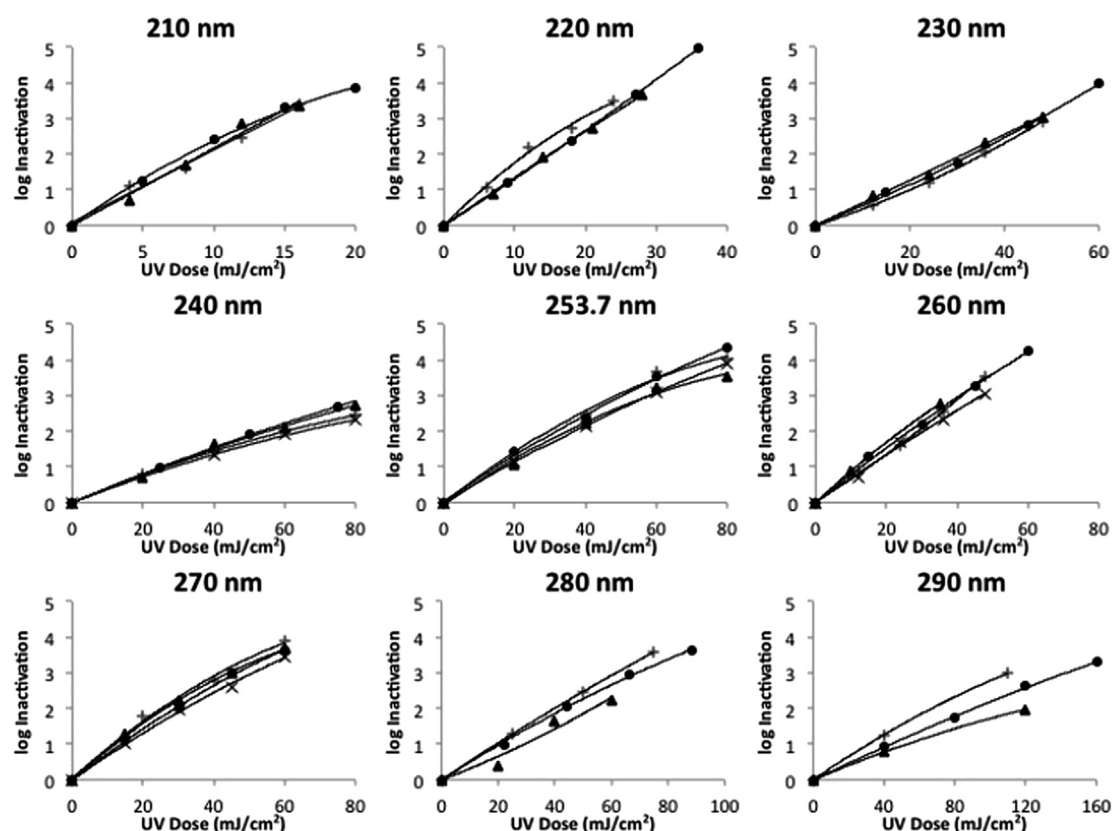


Fig. 3 – Dose response of MS2 Coliphage to wavelength-specific UV light from the tunable laser. Note different x-axis values. Symbols represent data from four separate trials: R1 (●) and R2 (▲) from one laboratory and R3 (+) and R4 (×) from two additional laboratories. Average standard deviation between replicate platings = 6.3 pfu/mL.

where the UV dose calculations did not account for divergence, reflection, or the non-uniformity of the light source across the Petri dish.

The dose response data was used to determine the spectral sensitivity or action spectrum of MS2 (Table S1), which is shown in Fig. 4, compared with two action spectra from the literature. The MS2 action spectrum was first determined in

1965 using a large diffraction grating monochromator, which dispersed monochromatic UV light approximately 1.2 nm in bandwidth (Rauth, 1965). In 2005, the action spectrum was measured with a monochromator with a maximum bandwidth of 10 nm (Mamane-Gravetz et al., 2005). The MS2 action spectrum from the present study agrees with the other two, with a relative maximum near 260 nm and an increased sensitivity below 240 nm.

Results from the collimated beam trials with T1UV, Q Beta, T7m, and T7 Coliphages are available in the [Supplementary Information](#). Table 2 contains the required UV doses for 1- to 4-log inactivation of each microorganism using the tunable laser emission of 253.7 nm. Also included are results for 2-log inactivation of each microorganism from exposure to low-pressure mercury vapor lamps, from this study and others, for comparison.

The action spectra for T1UV, Q Beta, T7m and T7 Coliphages are given in Table S1 and shown in Fig. 5 as compared with the MS2 Coliphage and *C. parvum* action spectra.

The T1UV Coliphage action spectrum agreed well with that developed previously using a medium-pressure mercury arc-lamp and bandpass filters (Stefan et al., 2007) at wavelengths above 240 nm, but differed below 240 nm. In both current and past studies, T1UV exhibited a relative peak sensitivity at 265 nm. Because T1UV has similar inactivation kinetics at 253.7 nm as the UV dose-requirements for *C. parvum* and *Giardia lamblia*, specified by the LT2ESWTR, it is used as a

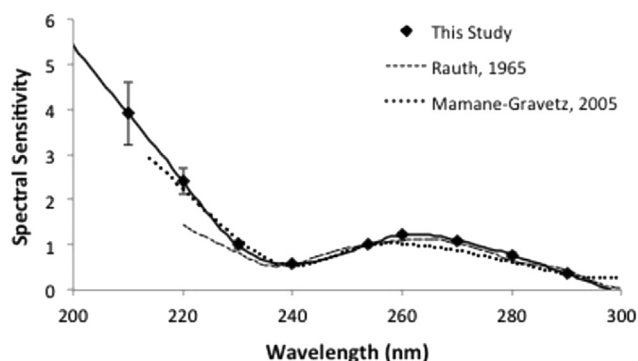


Fig. 4 – Relative spectral sensitivity of MS2 Coliphage to UV light as compared with previous studies (Rauth, 1965; Mamane-Gravetz, Linden et al. 2005). Error bars represent 1 standard deviation from the mean sensitivity value. $n = 4$ for 240 nm, 253.7 nm, 260 nm, and 270 nm and $n = 3$ for all other wavelengths tested.

Table 2 – UV dose response results for coliphage irradiated with the tunable laser at 253.7 nm and LPUV as compared with previous studies.

Coliphage		Tunable laser at 253.7 nm mJ/cm ² (±95% CI)					LPUV mJ/cm ² (±95% CI)		
		n	1-log	2-log	3-log	4-log	n	2-log This study	2-log Past studies
MS2	ssRNA	6	16.0 (±2.0)	33.6 (±3.8)	53.4 (±5.4)	77.6 (±6.4)	4	32.8 (±2.9)	32.8 ^a 35.1 ^b
T1UV	dsDNA	4	4.3 (±0.4)	8.5 (±0.9)	12.8 (±1.3)	17.0 (±1.8)	2	8.3 (±0.1)	9.1 ^c
Q Beta	ssRNA	1	10.8	22.0	33.8	46.2	1	19.8	23.9 ^c
T7	dsDNA	1	1.6	3.6	6.6	—	1	3.8	—
T7m	dsDNA	2	1.7 (±0.2)	3.8 (±0.4)	6.3 (±0.5)	10.6 (±0.2)	2	3.4 (±0.6)	—

^a Meng and Gerba (1996).^b Park et al. (2011).^c Stefan et al. (2007).

surrogate organism for those pathogens for UV reactor validation. However, differences in the action spectra of T1UV and *C. parvum* at wavelengths below 240 nm and above 253.7 nm may require application of an action spectra correction factor.

The action spectrum for **Q Beta Coliphage** also agreed well with the spectrum developed previously using an MP UV lamp and bandpass filters (Stefan, 2007). In both studies, Q Beta exhibited a peak relative sensitivity between 260 and 265 nm. Note that the action spectrum of Q Beta was very similar to that of MS2.

The action spectrum for **T7 Coliphage** matches that developed previously at 260 nm, where both exhibited a sensitivity of 1.3 (relative to 253.7 nm). However, the spectrum in the literature, obtained with a xenon lamp and a monochromator with 4 nm dispersion, decreased above 260 nm, whereas the T7 action spectrum developed in this study increases to a relative peak at 270 nm (Ronto, 1992). **T7m Coliphage** was slightly less sensitive than T7 with a relative peak of 1.1 at 260 nm and a decreasing sensitivity at higher wavelengths.

It was presumed that variations in the peaks of the coliphage action spectra were related, in part, to varying

compositions of nucleotide base pairs; however this does not appear to be the case. Adenine has a peak UV absorption near 260 nm. Uracil and thymine peak near 259 and 266 nm respectively. Cytosine has a relative peak near 268 nm; and guanine has two peaks: one near 246 nm and one near 277 nm (Jagger, 1967). Given the right shifting of the relative peaks of UV absorbance of T7 and T1UV, it was assumed that they had a higher guanine-cytosine (GC) content than the other phage. However, of the phage tested, MS2 had the highest GC content (52.1%), followed by T7m (49.9%), Q Beta (48.5%), and T7 (48.4%); the T1UV genome has not yet been sequenced.

The genome stranded state could have an effect on the sensitivity of phage to UV light and the shape of the action spectra. In the work of Rauth (1965), single-stranded nucleic acid was more sensitive to UV light at 253.7 nm than double-stranded. In this work, that was not the case. MS2 and Q Beta, both single-stranded RNA viruses, were more resistant to UV at 253.7 nm than T1UV, T7 or T7m. In Rauth's work, the single-stranded DNA and RNA viruses were more sensitive to the low wavelength light than double-stranded, possibly due to viral protein sensitizing the single-stranded nucleic acid. With the exception of T1UV (dsDNA), this work followed the same trend; MS2 and Q Beta were more sensitive at the lower wavelengths relative to their sensitivity at 253.7 nm, whereas T7m and T7 (both dsDNA phage) were less so. Protein-associated DNA has been shown to be more susceptible to UV-induced photoproducts than isolated DNA (Hegedus et al., 2003). Presumably, this would also be the case with protein-associated RNA.

Proteins also facilitate attachment to and infection of host cells. Amino acids, which compose proteins, absorb strongest below 220 nm (Saidel et al., 1952) and below 230 nm, the UV absorption of total proteins typically exceeds that of total DNA (Jagger, 1967). UV damage to phage proteins could affect attachment to the host cells and contribute to the loss of infectivity observed at those lower wavelengths.

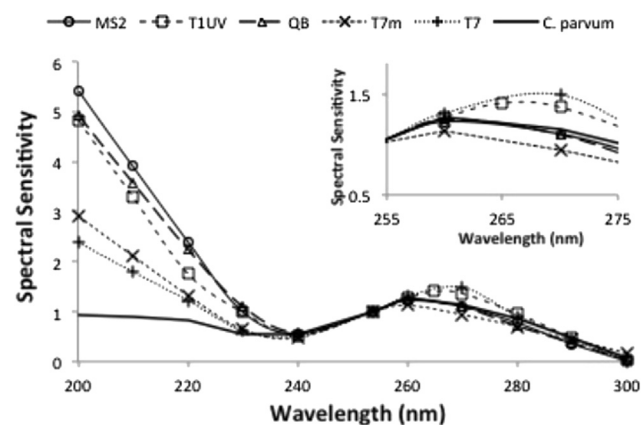


Fig. 5 – Relative spectral sensitivity of MS2, T1UV, Q Beta, T7m, and T7 Coliphages and *C. parvum* to UV light from the tunable laser. Note data points at 200 and 300 nm are extrapolated.

3.3. *Bacillus pumilus*

Results from the four collimated beam trials with *B. pumilus* spores are given in the [Supplementary Information](#). When irradiated with LP UV light, 2-log inactivation resulted from a

UV dose of 174.7 mJ/cm². This is a higher dose than that reported for 2-log LP UV inactivation of the same strain of *B. pumilus* spores (102 mJ/cm²), cultivated under the same conditions (0.1 mmol/L MnSO₄) for a shorter incubation time (5 days versus 6 days). A longer incubation time would result in increased accumulation of manganese, which is required for efficient sporulation and would increase the UV resistance.

The action spectrum for *B. pumilus* spore ASFUVRC (Fig. 6, Table S1) was consistent with that developed previously (Rochelle et al., 2010) for spores cultivated with a slightly different method (1 mmol/L MnSO₄ for 5 days). Although the UV resistance between the studies varied, as described above, the spectra were very similar. *B. pumilus* spores exhibited a relative peak in the 260–265 nm range. At 220 nm, the spores were 8–9 times more sensitive than at 253.7 nm. The increased effectiveness of this low wavelength light is presumed to be due to damage to the small acid-soluble proteins (SASP). SASPs bind to the spore DNA and mitigate DNA damage by forming thymidyl-thymidine spore photoproducts upon UV irradiation as opposed to thymine dimers (Setlow, 2006). These spore photoproducts can be repaired by the spore photoproduct (SP) lyase enzyme (Setlow, 2001). The 253.7 nm light wouldn't damage this SP repair mechanism; however, the lower UV wavelengths, near 220 nm, would damage the DNA binding proteins as well as the SP lyase enzyme that would otherwise repair the damage.

From 220 to 290 nm, the *B. pumilus* spore action spectrum is very similar to the action spectrum for adenovirus 2 (Fig. 6), which also was developed for this study and published previously (Beck et al., 2014). This similarity between *B. pumilus* spore and adenovirus 2 is consistent with other research (Rochelle et al., 2010). However, the relative spectral sensitivity of *B. pumilus* spore may decrease sharply below 220 nm given the absorbance of the spore homogenate (Rochelle et al., 2010). The adenovirus 2 action spectrum does not decrease sharply below 220 nm (Beck et al., 2014); adenovirus 2 is more sensitive than *B. pumilus* spore between 200 and 220 nm.

Fig. 7 shows a comparison of the dose response of MS2 to LP UV and MP UV light with the MP UV dose calculated two different ways. When the MP UV emission spectrum was weighted by the DNA absorption, the dose response of MS2

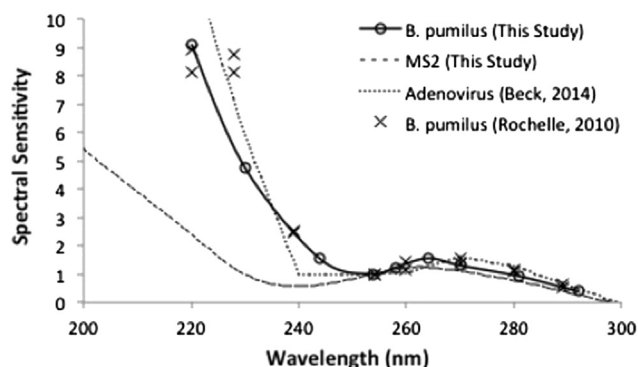


Fig. 6 – Relative spectral sensitivity of *Bacillus pumilus* to MP UV light with bandpass filters as compared with a *B. pumilus* spectrum from the literature (Rochelle et al., 2010) and the MS2 and adenovirus 2 (Beck et al., 2014) spectral sensitivity to UV light from the tunable laser.

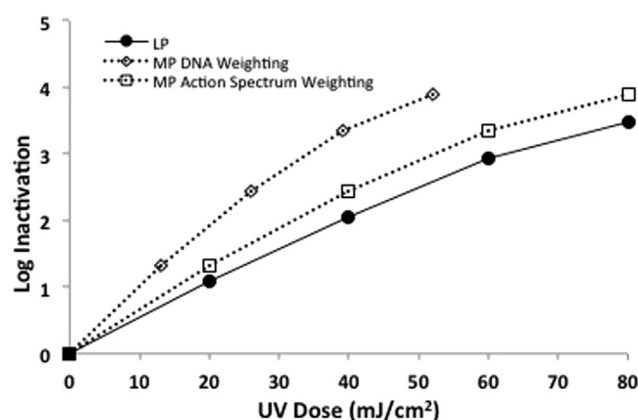


Fig. 7 – Dose response of MS2 coliphage to LP UV light and MP UV light with the MP UV dose determined by weighting the lamp emission with the DNA absorption or the MS2 action spectrum.

was significantly different than its response to LP UV light. Weighting the MP UV dose calculation by the action spectrum measured in this paper also yielded a statistically different response compared to the MS2 dose response to LP UV light. However, when the MP UV dose was weighted by the action spectrum measured in this paper, the dose response of MS2 was closer to its response to LP UV light, as illustrated in Fig. 7. Differences in the two dose responses could be due to experimental variability. Nevertheless, this theoretical comparison shows that there is better agreement when weighting with action spectrum than with a DNA absorption curve. It also suggests that the low wavelengths, which are less prominent in a DNA weighted UV dose, play a role in MS2 coliphage inactivation.

4. Conclusions

This research defined the UV action spectra of the pathogen *C. parvum*, and surrogates MS2, T1UV, Q Beta, T7, and T7m coliphages, as well as *B. pumilus*. These action spectra are essential for properly calculating action spectra correction factors for polychromatic UV systems such as during validation of MP UV systems and targeting UV sensors toward the most relevant wavelengths for dose monitoring.

Knowledge of action spectra for a suite of surrogates is important for matching appropriate challenge microorganisms to pathogens, and for improving UV dose monitoring. These action spectra can also serve as a guide for development of effective wavelength-targeted UV disinfection technologies such as excimer lamps, lasers and UV light emitting diodes (LEDs).

Acknowledgments

This research was funded by the Water Research Foundation Project 4376 and several organizations that provided cash or

in-kind contributions including: Los Angeles Department of Water and Power, Aquionics, Calgon Carbon Corporation, Atlantium Technologies, ITT Wedeco, ETS, Trojan Technologies, Tetra Tech Clancy Environmental Consultants, Hydroqual/HDR, CDM, Black & Veatch, CH2M Hill, Metropolitan Water District of Southern California, NY State Department of Health, CA DHS, WA DOH, and U.S. Environmental Protection Agency.

The authors would like to acknowledge Randi McQuin of Corona Environmental Consulting and Jeff Budzik of Analytical Services, Inc., for their microbiological expertise and work on the cell culture assays. We thank Paul Rochelle, Roberto Rodriguez, Brian Petri, and Joel Ducoste for their external review and comments during this research.

Disclaimer, The mention of certain commercial products in this paper is for information purposes only and does not constitute an endorsement of the product by the authors or their institutions.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2014.11.028>.

REFERENCES

- Beck, S.E., Rodriguez, R.A., Linden, K.G., Hargy, T.M., Larason, T.C., Wright, H.B., 2014. Wavelength dependent UV inactivation and DNA damage of adenovirus as measured by cell culture infectivity and long range quantitative PCR. *Environ. Sci. Technol.* 48, 591–598.
- Bohrerova, Z., Linden, K.G., 2006. Assessment of DNA damage and repair in *Mycobacterium terrae* after exposure to UV irradiation. *J. Appl. Microbiol.* 101 (5), 995–1001.
- Bolton, J.R., Linden, K.G., 2003. Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. *J. Environ. Eng.* 129 (3), 209–215.
- Coochill, T.P., 1991. Action spectra again? *J. Photochem. Photobiol.* 54 (5), 859–870.
- Eischeid, A.C., Meyer, J.N., Linden, K.G., 2009. UV disinfection of adenoviruses: molecular indications of DNA damage efficiency. *Appl. Environ. Microbiol.* 75 (1), 23–28.
- Fallon, K.S., Hargy, T.M., Mackey, E.D., Wright, H.B., Clancy, J.L., 2007. Development and characterization of nonpathogenic surrogates for UV reactor validation. *J. Amer. Water Works Assoc.* 99 (3), 73–82.
- Fayer, R., Morgan, U., Upton, S.J., 2000. Epidemiology of *Cryptosporidium*: transmission, detection and identification. *Intern. J. Parasitol.* 30 (12–13), 1305–1322.
- Guo, M.T., Hu, H.Y., Bolton, J.R., El-Din, M.G., 2009. Comparison of low- and medium-pressure ultraviolet lamps: photoreactivation of *Escherichia coli* and total coliforms in secondary effluents of municipal wastewater treatment plants. *Water Res.* 43 (3), 815–821.
- Harm, W., 1980. *Biological Effect of Ultraviolet Radiation*. Cambridge University Press, Cambridge, U.K.
- Hegedus, M., Modos, K., Ronto, G., Afeke, A., 2003. Validation of Phage T7 Biological Dosimeter by Quantitative Polymerase Chain Reaction Using Short and Long Segments of Phage T7 DNA.
- Hijnen, W.A.M., Beerendonk, E.F., Medema, G.J., 2006. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Res.* 40 (1), 3–22.
- Hijnen, W.A.M., Medema, G.J., 2010. *Elimination of Microorganisms by Water Treatment Processes*. IWA Publishing, London, UK.
- Jagger, J., 1967. *Introduction to Research in Ultra-violet Photobiology*. Prentice Hall, Englewood Cliffs, NJ.
- Johnson, A.M., Rochelle, P.A., DiGiovanni, G.D., 2010. Detection of Infectious *Cryptosporidium* in Conventionally Treated Drinking Water (WRF Final Report. Denver, CO).
- Lin, T.Y., Lo, Y.H., Tseng, P.W., Chang, S.F., Lin, Y.T., Chen, T.S., 2012. A T3 and T7 recombinant phage acquires efficient adsorption and a broader host range. *Plos One* 7 (2), e30954. <http://dx.doi.org/10.1371/journal.pone.0030954>.
- Linden, K.G., Darby, J.L., 1997. Estimating effective germicidal dose from medium pressure UV lamps. *J. Environ. Eng.* 123 (11), 1142–1149.
- Linden, K.G., Shin, G., Sobsey, M.D., 2001. Comparative effectiveness of UV wavelengths for the inactivation of *Cryptosporidium parvum* oocysts in water. *Water. Sci. Technol.* 43 (12), 171–174.
- Linden, K.G., Thurston, J., Schaefer, R., Malley, J.P., 2007. Enhanced UV inactivation of adenoviruses under polychromatic lamps. *Appl. Environ. Microbiol.* 73 (23), 7571–7574.
- Mackenzie, W.R., Hoxie, N.J., Proctor, M.E., Gradus, M.S., Blair, K.A., Peterson, D.E., Kazmierczak, J.J., Addiss, D.G., Fox, K.R., Rose, J.B., Davis, J.P., 1994. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *N. Engl. J. Med.* 331 (3), 161–167.
- Malley, J.P., Ballester, N.A., Margolin, A.B., Linden, K.G., Mofidi, A., Bolton, J.R., Crozes, G., Laine, J.M., Janex, M.L., 2004. Inactivation of Pathogens with Innovative UV Technologies. American Research Foundation and American Water Works Association.
- Mamane-Gravetz, H., Linden, K.G., Cabaj, A., Sommer, R., 2005. Spectral sensitivity of *Bacillus subtilis* spores and MS2 coliphage for validation testing of ultraviolet reactors for water disinfection. *Environ. Sci. Technol.* 39 (20), 7845–7852.
- McQuin, R.M., Clancy, J.L., 2003. Modifications to USEPA methods 1622 and 1623 for detection of *Cryptosporidium* oocysts and *Giardia* cysts in water. *Appl. Environ. Microbiol.* 69 (1), 267–274.
- Meng, Q.S., Gerba, C.P., 1996. Comparative inactivation of enteric adenoviruses, poliovirus and coliphages by ultraviolet irradiation. *Water Res.* 30 (11), 2665–2668.
- Park, G.W., Linden, K.G., Sobsey, M.D., 2011. Inactivation of murine norovirus, feline calicivirus and echovirus 12 as surrogates for human norovirus (NoV) and coliphage (F+) MS2 by ultraviolet light (254 nm) and the effect of cell association on UV inactivation. *Lett. Appl. Microbiol.* 52 (2), 162–167.
- Rauth, A., 1965. The physical state of viral nucleic acid and the sensitivity of viruses to ultraviolet light. *Biophys. J.* 5, 257–273.
- Rochelle, P.A., Blatchley III, E.R., Chan, P., Scheible, O.K., Shen, C.Y., 2010. Challenge Organisms for Inactivation of Viruses by Ultraviolet Treatment. Final Report. Water Research Foundation and US EPA, Denver, CO.
- Ronto, G., Gaspar, S., Berces, A., 1992. Phages T7 in biological UV dose measurement. *J. Photochem. Photobiol. B-Biol.* 12, 285–294.
- Saidel, L.J., Goldfar, A.R., Waldman, S., 1952. The absorption spectra of amino acids in the region two hundred to two hundred and thirty millimicrons. *J. Biol. Chem.* 197, 285–291.
- Setlow, P., 2001. Resistance of spores of *Bacillus* species to ultraviolet light. *Environ. Mol. Mutagen* 38 (2–3), 97–104.

- Setlow, P., 2006. Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat and chemicals. *J. Appl. Microbiol.* 101 (3), 514–525.
- Shin, G.A., Linden, K.G., Arrowood, M.J., Sobsey, M.D., 2001. Low-pressure UV inactivation and DNA repair potential of *Cryptosporidium parvum* oocysts. *Appl. Environ. Microbiol.* 67 (7), 3029–3032.
- Stefan, M., Odegaard, C., Petri, B., Rowntree, M., Sealey, L., 2007. Development, Characterization, and Application of a UV-sensitive Bacteriophage for UV Reactor Challenges. IUVA Congress, Los Angeles, CA.
- Sutherland, J.C., 2002. Biological effects of polychromatic light. *J. Photochem Photobiol.* 76 (2), 164–170.
- USEPA, 2003. Ultraviolet Disinfection Guidance Manual – Draft Version. US Environmental Protection Agency, Washington, D.C.
- USEPA, 2006a. Ultraviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule. US Environmental Protection Agency, Washington, D.C.
- USEPA, 2006b. Long Term 2 Enhanced Surface Water Treatment Rule. In: Federal Register, vol. 71. US Environmental Protection Agency, Washington, D.C. No. 3/Thursday, January 5, 2006.
- Wright, H.B., Mackey, E.D., Gaithuma, D., Fonseca, C., Baumberger, L., Drzurny, T., Clancy, J.L., Hargy, T.M., Fallon, K., Cabaj, A., Schmalweisser, A., Bierman, A., Gribbin, C., 2007. Optimization of UV Disinfection. Water Research Foundation, Denver, CO.