A Novel Dual-Collimation Batch Reactor for Determination of Ultraviolet Inactivation Rate Constants for Microorganisms in Aqueous Suspensions

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SUMMARY
We developed, characterized, and tested a new dual-collimation aqueous UV reactor to improve the accuracy and consistency of aqueous k-value determinations. This new system is unique because it collimates UV energy from a single lamp in two opposite directions. The design provides two distinct advantages over traditional single-collimation systems: 1) real-time UV dose (fluence) determination; and 2) simple actinometric determination of the Reactor Factor (RF = 1.06) that relates measured irradiance levels to actual irradiance levels experienced by the microbial suspension. This RF replaces three of the four typical correction factors required for single-collimation reactors. Using this dual-collimation reactor, *Bacillus subtilis* spores demonstrated inactivation following the classic multi-hit model with k=0.1395 cm^2/mJ. *Aspergillus niger* spores exhibited two-stage decay with a shoulder, with the resistant fraction of the population (f) of 0.251, k_1=0.1968 cm^2/mJ, k_2=0.0426 cm^2/mJ.

KEYWORDS
Collimated-beam reactor, Ultraviolet germicidal irradiation, Chemical actinometry

1 INTRODUCTION
Ultraviolet germicidal irradiation (UVGI) is used in schools, offices, healthcare settings, correctional facilities, social-assistance shelters, and homes to improve indoor air quality and reduce airborne disease transmission. While many successful UVGI systems have been installed in various settings, system design is often as much art as science. Accurate ultraviolet (UV) inactivation rate constants (k-values) for the microorganism(s) of interest are a key component of proper system design. A collimated-beam UV reactor is often used to determine k-values for microorganisms in aqueous suspensions. Measured k-values for many species of viruses, bacteria, and fungi have been published in the scientific literature (Kowalski, 2009). However, no standard methods exist for the determination of k-values, which makes reported values difficult to interpret and apply with certainty to system design. To help UVGI system designers make better decisions, improved standardized methods for k-value determination need to be developed. This work outlines a new dual-collimation UV batch reactor design that can be used to improve the accuracy of aqueous k-values.

UV exposure systems typically used for aqueous microbial inactivation studies collimate UV energy in only one direction, which results in two issues that may affect the accuracy of the results. First, the systems do not allow for UV irradiance measurements being made while

the microorganisms are being exposed. Instead, irradiance measurements are taken before and after microorganism exposure. The pre- and post-irradiance levels are then averaged, and the average value is assumed to be the irradiance applied to the microorganisms during an actual exposure test. Second, the systems require the determination and use of numerous correction factors (CFs) to relate the irradiance readings from a radiometer to the actual irradiance levels experienced by the microbial suspension. Four CFs are generally required for systems equipped with low-pressure mercury UV lamps (Bolton and Linden, 2003; Kuo et al., 2003): 1) Reflection Factor–the fraction of incident UV energy that enters the microbial suspension vs. the UV energy reported by the radiometer, 2) Petri Factor–the variation of irradiance over the surface area of the aqueous microbial suspension, 3) Divergence Factor–the divergence of the UV energy from a truly collimated beam, and 4) Water Factor–the UV energy absorbed as the beam passes through the microbial suspension itself. Including the four CFs in the UV dose calculation, while making the results more accurate, does not account for all aspects of system design or any lamp output variations that might occur during microbial exposures (when the radiometer sensor is not in place to record them). To address the above two issues with single-collimation reactors, and to ultimately improve the accuracy of microbial k-value determinations, we developed, characterized, and conducted microbial testing with a new dual-collimation UV batch reactor.

2 MATERIALS/METHODS

Dual-Collimation Reactor Design & Construction

The reactor design was conceived as an improvement to an earlier collimated-beam batch reactor fabricated at the Pennsylvania State University Department of Architectural Engineering using key references outlining reactor design and characterization (Blatchley, 1997; Bolton and Linden, 2003; Kuo et al., 2003). The new dual-collimation aqueous phase reactor, shown schematically in Figure 1, collimates UV energy in two directions, 180° apart. The reactor is built inside an enclosure with interior dimensions of 79.4 cm long × 27.3 cm wide × 57.5 cm high; however, the collimated beam portion within the enclosure has a height of 27.9 cm and is located between two aluminum shelves. The reactor contains two sockets for testing with either a Philips (Roosendaal, Netherlands) TUV PL-L 18W/4P (18 W lamp) or a TUV PL-L 35W/4P HO (35 W lamp) low-pressure mercury lamp positioned in the center of the reactor, and oriented perpendicular to the collimated-beam assembly.

The interior of the reactor is painted flat black to minimize unwanted UV reflections. The two aluminum shelves are 6.4 mm thick and incorporate 9.5 mm × 9.5mm × 1.6 mm wall thickness 6061 anodized aluminum channels. These channels are used to hold the collimating plates in place at four desired locations on either side of the lamp. The collimating plates are 27.3 cm wide × 27.3 cm tall × 6.3 mm thick unpainted, furniture-grade plywood with 3.8 cm high × 3.8 cm wide apertures. A Vincent Associates (Rochester, NY, USA) model CS65S3T1 electronic shutter is integrated into the first collimating plate on each side of the lamp to precisely control symmetric, incident UV exposure on an IL1700 research radiometer detector (International Light Technologies, Peabody, MA, USA) housed on the left side of the reactor and the quartz exposure cuvette holding the aqueous microorganism solution on the right side of the reactor. The front edge of the cuvette sample and the research radiometer detector head are each located 27.9 cm from the lamp center and they are centered in the collimating apertures. Reactor monitoring and control is achieved through the interface of sensor and control elements to National Instruments (Austin, TX, USA) data acquisition and control hardware modules and a custom-written LabVIEW software program.

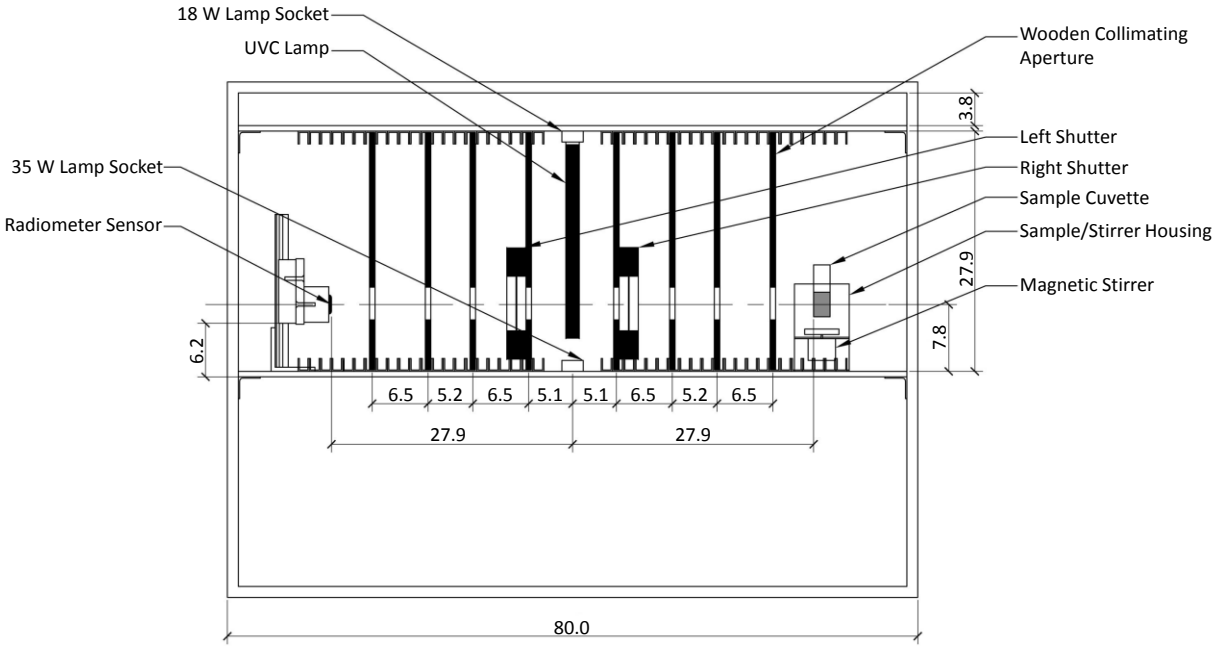

Figure 1. Schematic diagram of new dual-collimation UV batch reactor with key dimensions (in centimeters).

Actinometric Determination of Reactor Factor

Once the new dual-collimation reactor was designed and constructed, the Reactor Factor (RF) was determined using iodide-iodate chemical actinometry. The actinometric solution was prepared by vortexing 9.96 g of potassium iodide (KI), 2.14 g of potassium iodate (KIO_3), and 0.381 g of borax ($Na_2B_4O_7 \cdot 10H_2O$) in 100 mL of Type I distilled, deionized water for several minutes until the solids were dissolved (Rahn et al., 2006). To conduct a test, 5 mL of the actinometric solution was pipetted into quartz cuvettes measuring 1.4 cm square (internal) by 6 cm high with a wall thickness of 0.13 cm. A small 0.25 cm × 1.2 cm magnetic stir bar was added to the cuvette. The cuvette was then placed into a specially designed holder equipped with a custom-built magnetic stirrer. The holder served to position the cuvette in the center of the collimating apertures for UV exposure testing while ensuring that the actinometric solution was well-mixed.

Using the 18 W lamp, the cuvette containing the actinometric solution was then exposed to UV energy while the control program integrated radiometer sensor irradiance readings in real time to determine UV dose, with no preset correction factors (i.e., the dose was calculated solely from the sensor as it was calibrated by the manufacturer). UV exposure was stopped once the desired UV dose was reached. Actinometry tests were conducted in triplicate at 13 dose levels for initial determination of the RF. Those dose levels were 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, and 50 mJ/cm^2. A Thermo Scientific Genesys 10uv spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the absorbance of UV energy by the actinometric solution before and after UV exposure. Incident UV dose received by the actinometric solution is determined using the calculation procedure outlined by Rahn (1997). Once the RF was determined, a single actinometry test was conducted at each dose level to confirm reactor performance with the RF set in the LabVIEW software.

Microbiological Testing

Initial exposure tests in the aqueous phase reactor were conducted with individual, fresh suspensions of *Bacillus subtilis* spores and *Aspergillus niger* spores in Type I distilled, deionized water at the same 13 dose levels used for actinometric testing. Quartz cuvettes (described above) were filled with 5 mL of the appropriate microorganism suspension for each UV exposure test. Using the 18 W lamp, the cuvette containing the suspension was then exposed to UV energy until the control program, integrating real-time radiometer measurements with the RF set appropriately, terminated the exposure once the desired UV dose was reached. Although the Water Factor is still required for this new dual-collimation reactor design, it was determined to be 1.0 for all of the microbial suspensions used for testing. The microbial suspensions were continuously stirred during UV exposure using the miniature stir bars and magnetic stirrer described previously. Tests were conducted in duplicate for each microorganism at each of the dose levels.

Samples of the microbial suspensions prior to UV exposure were cultured in triplicate on nutrient agar plates using a Spiral Biotech (Bethesda, MD, USA) Autoplate Model 3000 spiral plating system. Tryptic Soy Agar (TSA) plates were used for culturing and enumeration of the *Bacillus subtilis* spores while Dichloran Glycerol Agar (DG-18) plates were used for the *Aspergillus niger*. For a given microbial test, the average number of colony forming units (CFU) from three pre-exposure replicates provided a measure of the number of viable organisms in the suspension prior to UV exposure (N_0). Similarly, samples of the suspension after exposure were cultured in triplicate and averaged to determine the number of organisms surviving the UV treatment (N). The survival fraction (S) was calculated as the ratio of the CFU post-exposure to the CFU prior to exposure (N/N_0). Once the microbiological data were obtained, MATLAB software (MathWorks, Inc., Natick, MA, USA) was used for non-linear curve fitting to determine the best dose response model for each organism from those typically reported in UV research.

3 RESULTS

Actinometric Determination of Reactor Factor

Comparing the UV dose determined by chemical actinometry in the quartz cuvettes to the UV dose determined from the radiometer readings allowed for an easy determination of the Reactor Factor. With this reactor design, the RF incorporates the reflection factor, petri factor, divergence factor, and any small differences attributable to the two opposite collimation paths into one distinct number. The water factor is still required. The results for the triplicate actinometry trials at each of the 13 doses are shown below in Figure 2A. The figure shows that the testing results are repeatable. The slope of the resulting line of 1.06 demonstrates that the samples inside the quartz cuvettes were actually being exposed to UV irradiance levels 1.06 times higher than the radiometer sensor was measuring. Thus, multiplying the radiometer sensor measurements by a RF of 1.06 would bring the radiometer measurements into agreement with the actual irradiance experienced by samples inside the quartz cuvettes. Figure 2B shows the results from a single set of actinometry tests conducted with the RF set to 1.06 in the LabVIEW control program. The resulting slope of 1.00 confirms that the radiometer was reading the same irradiance level and UV dose as the cuvette samples experienced.

Microbiological Testing

The results for duplicate tests with *Bacillus subtilis* spores, shown in Figure 3A, suggest that the *Bacillus* spores exhibited UV inactivation that followed the classic multihit target model, with k=0.1395 cm^2/mJ and a discrete number of hits required to inactivate the spores (n) of 6. At the highest dose of 50 mJ/cm^2, the *Bacillus* spores show between 99% and 99.9% inactivation.

The duplicate results for *Aspergillus niger* spores are shown in Figure 3B. The *Aspergillus* spores show roughly 90% inactivation at the 50 mJ/cm^2 UV dose, and they exhibited two-stage decay with a shoulder. The resistant fraction of the population (f) was 0.251. The inactivation rate constant for the susceptible fraction of the population (k_1) was 0.1968 cm^2/mJ, while the inactivation rate constant for the resistant fraction (k_2) was 0.0426 cm^2/mJ. As is common, the number of discrete hits required to inactivate the *Aspergillus* spores were assumed to be integers and equal, regardless of the population fraction, so $n_1 = n_2 = 4$.

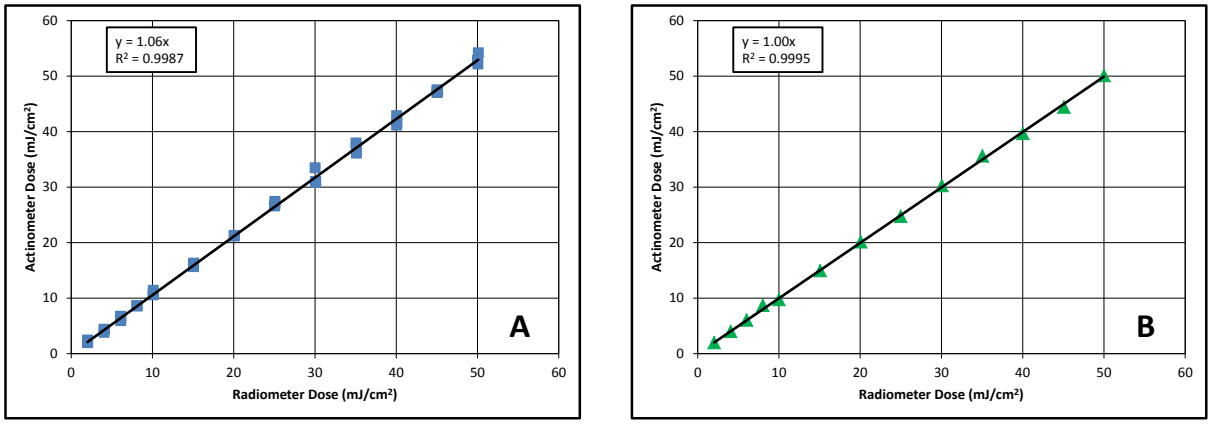

Figure 2. Chemical actinometry results: A) triplicate uncorrected trials showing a Reactor Factor (slope) of 1.06, B) single trial with Reactor Factor set to 1.06 confirming result (note that new slope equals 1.00).

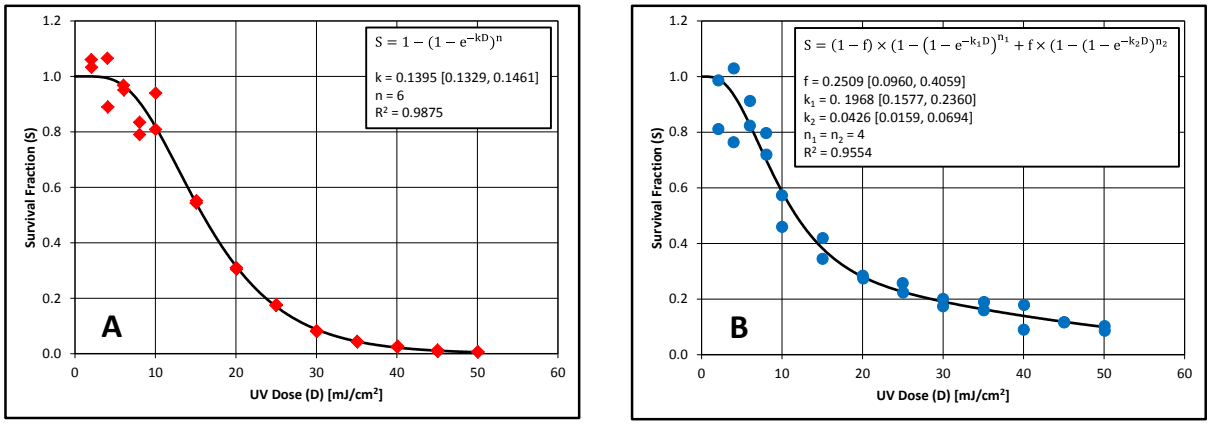

Figure 3. Replicate inactivation testing results and MATLAB non-linear curve fits showing equations for the best-fit inactivation model and values of key variables with 95% confidence intervals in brackets for: A) *Bacillus subtilis* spores and B) *Aspergillus niger* spores.

4 DISCUSSION

This new dual-collimation aqueous batch reactor design provides two distinct advantages over traditional single collimation systems. First, it allows for a real-time UV dose determination. Measuring the dose in real time will capture any UV lamp variations that occur during the exposure tests that would normally go unnoticed. Knowing the dose in real-time will also allow for microbial exposure tests to be terminated at desired dose levels instead of determining the actual dose after the test is completed (using an average irradiance level from measurements taken before and after the test). The ability to terminate the test at accurate dose levels will allow for more targeted testing of organisms with complex inactivation curves. For instance, shoulders or inflection points in the microbial inactivation curves can be pinpointed more accurately since tests can be terminated at more accurate dose levels around the phenomenon of interest. Secondly, this new dual-collimation design allows for a simple actinometric determination of an overall correction factor, the Reactor Factor, which relates irradiance levels measured by the radiometer sensor to the irradiance levels experienced by the microbial suspension. The RF replaces three of the four correction factors typically used in single collimation systems, with the Water Factor being the exception.

5 CONCLUSIONS

The new dual-collimation UV batch reactor system is the first system developed solely for UV inactivation studies of aqueous microorganisms that allows for real-time UV dose determination. The dual-collimation feature of the exposure system allows the radiometer sensor head to remain in place during actual microbial sample exposures. This provides distinct advantages over traditional single-collimation systems which are expected to improve the accuracy of overall k-value determinations for aqueous microorganisms. While this new reactor design, along with its simple characterization procedures, is one step toward more accurate k-values, additional steps are still needed to make reported k-values more useful for system design. For instance, standardized microbial procedures, including agar type(s), incubation temperature(s) and time(s), plating method(s), etc., need to be established for microorganisms so results from one study can be directly compared to results from another.

DISCLAIMER

The findings and conclusions in this conference paper are those of the author(s) and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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