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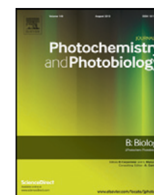

A new dual-collimation batch reactor for determination of ultraviolet inactivation rate constants for microorganisms in aqueous suspensions

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ABSTRACT

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 a reactor factor that relates measured irradiance levels to actual irradiance levels experienced by the microbial suspension. This reactor factor 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 $k = 0.1471$ cm^2/mJ (with 95% confidence bounds of 0.1426 to 0.1516).

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1. Introduction

Although this research focuses on ultraviolet germicidal irradiation (UVGI) systems designed to inactivate microorganisms in air, the roots of UVGI are steeped in the disinfection of water. The first documented use of UV energy to disinfect drinking water occurred in 1909 at Marseilles, France [1]. Seven years later, in 1916, the first UV system in the United States was installed to disinfect 1.136×10^7 L of water per day (3,000,000 gal/day) for the 17,000 residents of Henderson, Kentucky [2]. UV devices used for water treatment have been researched extensively, resulting in solid biodosimetry testing strategies, useful system design criteria, and regulatory oversight. The same cannot be said for UVGI air and surface disinfection systems.

UVGI air-disinfection systems are currently used in schools, offices, healthcare settings, correctional facilities, social-assistance shelters, and homes to improve indoor air quality, reduce airborne disease transmission, and disinfect surfaces. While many successful systems have been installed in various settings, air-disinfection system design is often as much art as science. Accurate ultraviolet (UV) inactivation rate constants (k-values) for microorganisms of interest are essential to proper system design. Inactivation rate constants are species-dependent and relate the susceptibility of a given microorganism population to UV radiation [3–6]. Ideally, k-values used for system design would be determined on the organism(s) of interest suspended in air. However, these results are very difficult to generate, so k-values determined using aqueous organism suspensions are generally used instead.

A collimated-beam UV reactor is often used to determine k-values for microorganisms in aqueous suspensions. While the term "collimated-beam" implies a beam of radiation with truly parallel rays, benchtop systems used for UV experiments do not meet that stringent criterion [7]. Regardless, the term is commonly used to describe UV exposure systems in the scientific literature. Measured k-values for many species of viruses, bacteria, and fungi have been published in the scientific literature [8]. 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 describes a new dual-collimation UV batch reactor design that can improve the accuracy of aqueous k-values over those determined using some classic single-collimation systems.

UV exposure systems used for aqueous microbial inactivation studies typically collimate UV energy in only one direction, which may affect

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the accuracy of the results in two ways. First, such systems do not allow UV irradiance measurements to be made while the microorganisms are being exposed. Instead, irradiance measurements are taken before and after microorganism exposure. The average of the pre- and post-irradiance levels is assumed to be the irradiance applied to the microorganisms during an actual exposure test. The impact this has on the accuracy of resulting k-value estimates depends on individual reactor design and operation. Second, single-collimation 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 [7,9]:

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 for lamp output variations that might occur during microbial exposures, when the radiometer sensor is not in place to record them. For instance, conducting tests before the lamp output stabilized, lamp output variations caused by control systems or dimmers, or performance issues with the lamp itself, can have an impact on measurement accuracy. To address the above two limitations of single-collimation reactors, and ultimately to 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

2.1. 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 [7,9–12]. The new dual-collimation aqueous phase reactor, shown in Fig. 1 and schematically in Fig. 2, collimates UV energy in two directions, 180° apart. The reactor is built inside an enclosure with interior dimensions of 79.4 cm length × 27.3 cm width × 57.5 cm height; however, the collimated-beam portion within the enclosure has a height of 27.9 cm and is located between two aluminum shelves that are each 0.6 cm thick. The bottom of the upper shelf and the top of the lower shelf have attached 1.0 cm × 1.0 cm × 0.2 cm wall thickness 6061 anodized aluminum U-channels. These channels hold the collimating plates in place at four desired locations on either side of the lamp. The interior of the reactor enclosure is painted flat black to minimize unwanted UV reflections.

The reactor contains two sockets for testing with either a Philips (Roosendaal, Netherlands) 18 W (TUV PL-L 18W/4P) or 35 W (TUV PL-L 35W/4P HO) low-pressure mercury lamp positioned in the center of the reactor, and oriented perpendicular to the collimated-beam assembly. While only one lamp is used at a time, the lamp sockets are positioned in the reactor such that the 18 W lamp extends upward from the lower shelf and the 35 W lamp extends downward from the top shelf. This makes switching between the lamps quick and easy.

To create a pseudo-collimated beam of UV-C radiation directed at both the sample cuvette and the radiometer detector, 27.3 cm wide × 27.3 cm tall × 0.6 cm thick unpainted, furniture-grade plywood collimating plates with 3.8 cm high × 3.8 cm wide apertures were used. The collimating plates are held in place by sliding them into the aluminum U-channels attached to the upper and lower shelves of the enclosure. There are 22 channels and 4 wooden slats on either side of the lamp. The extra channels allow for variation in the wooden slat positions, if desired.

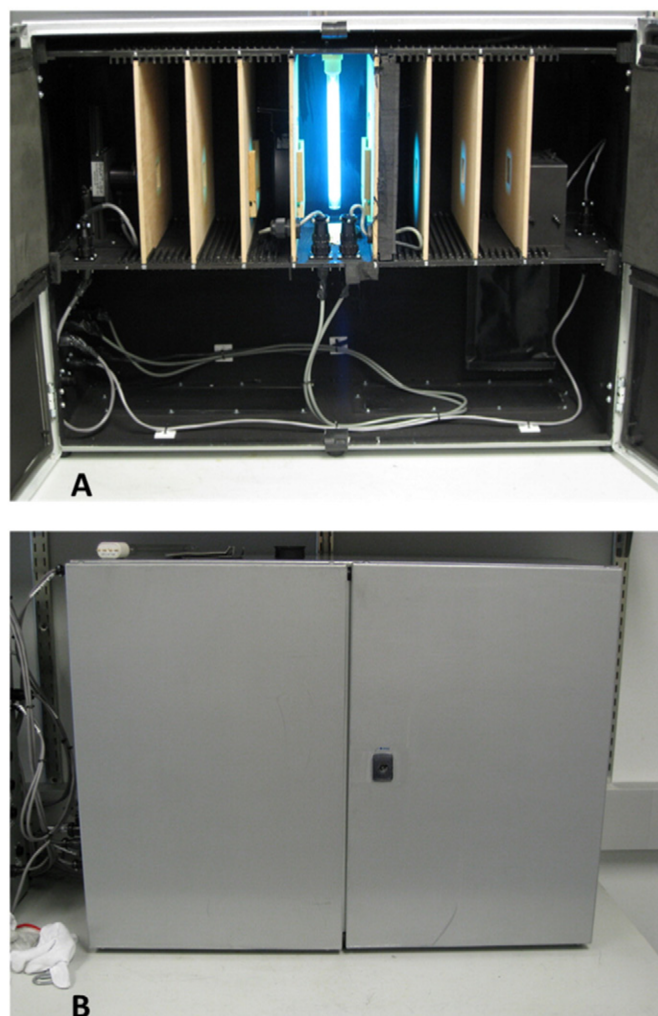

Fig. 1. Photographs of the new aqueous-phase, dual-collimated-beam UV reactor: A) Reactor with the doors open showing the lamp, dual collimating paths with collimating apertures, sample cuvette housing, and radiometer detector head; B) reactor with the door closed.

To precisely control the passage of UV radiation through both collimating paths, a combination of foam padding and shutters were used. Custom-fitted neoprene foam lining the reactor enclosure doors prevented light leakage around the wooden slats. Upon locking of the reactor enclosure doors, the foam is pressed against the wooden slats, creating the light seal. Vincent Associates (Rochester, NY, USA) model CS65S3T1 electronic shutters integrated into the first collimating plate on each side of the lamp precisely control the UV radiation through each of the two collimating paths. These 65 mm aperture iris shutters operate electromechanically to open in 29 ms and are driven by a Vincent Associates Uniblitz VMM-D3 Three Channel Shutter Driver, which was interfaced to the overall reactor control system.

The new dual-collimated-beam UV reactor was designed to expose microbial suspensions inside quartz cuvettes (Part # CQS114, VitroCom, Mountain Lakes, NJ). These cuvettes measured 1.4 cm square (internal) × 6.0 cm high with a wall thickness of 0.13 cm. Small 0.25 cm × 1.2 cm magnetic stir bars were placed inside the cuvettes before covering them with a small loose-fitting, non-reflective cap. To expose the microbial suspension, one cuvette at a time was placed into a housing station equipped with a magnetic stirrer that positioned the

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