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Bioassay and Dose Measurement in UV Disinfection

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A bioassay method was developed to measure the average intensity within a UV disinfection reactor. The survival of spores of *Bacillus subtilis* was determined as a function of UV dose to prepare a standard curve. Spores were added to unknown systems, and the survival rate was used to determine the average intensity. A modification was used for flow-through reactors by which spores were injected as a spike and collected at a known time after injection. A point source summation method for calculating intensity was verified by bioassay measurements in a simple cylinder. This calculation method was also applied to multiple-lamp reactors.

Environmental problems associated with chlorination have prompted research into alternatives for the disinfection of wastewater effluents. Residuals and by-products can be toxic to aquatic life in receiving waters (19). Chlorination by-products may be carcinogenic (12). One disinfection process that would not be expected to produce undesirable by-products is UV disinfection. In addition, UV irradiation may be more effective than chlorination in killing viruses, compared with the doses necessary to kill indicator bacteria (20).

Recent cost estimates from a full-scale wastewater plant showed UV disinfection to be the least expensive method for small systems. Treatment costs were only 38% higher than for chlorination in large systems (17). Very little research has been done on the efficiency of UV disinfection systems. With further development, UV may prove to be less expensive than chlorination even for large systems.

There have been several pilot and full-scale investigations of UV disinfection of wastewater (9, 13, 16, 17). Although these studies showed that UV disinfection was generally successful in meeting disinfection goals, comparison between these studies has been limited because there was no direct method of measuring UV doses nor any substantiated method for calculating doses in the complex geometries and absorbing solutions within practical reactors. In addition, lack of dose measurement methods has prevented the controlled evaluation of the effects produced by variables such as UV absorbance, filtration, reactor design, and the different sensitivity of various organisms.

There were several problems with the methods of UV dose estimation used in previous studies of UV disinfection. (i) UV radiometer detectors measure intensity on a planar surface and thus cannot be used near a long tubular lamp to measure the three-dimensional intensity to which a cell may be exposed (7, 13). (ii) A UV radiometer detector positioned in the wall of a disinfection reactor cannot be used to estimate the average intensity in an absorbing solution within the entire reactor (4, 6). (iii) Particles in wastewater scatter UV light so that spectrophotometers tend to overestimate the UV absorbance (9). (iv) Equations have been used that incorrectly calculate the intensity near a tubular lamp in an absorbing solution (5, 12, 17, 18). (v) In a flow-through reactor, distribution of exposure times is not simply related to volume and flow rate (13, 17).

It was the objective of this study to (i) develop a method for in situ measurement of intensity with a standardized bioassay, (ii) experimentally verify a method for calculating the intensity distribution around a tubular source, and (iii) separately evaluate the effects of intensity and the distribution of residence times in a flow-through system.

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MATERIALS AND METHODS

All measurements of intensity at 254 nm were made with an International Light IL-500 radiometer with an SEE-240 detector calibrated by reference to a National Bureau of Standards lamp. Output of the lamp at the 254-nm wavelength was measured by the following method (2). Intensity was measured at 10 angles from 0 to 90° in an arc around the lamp centroid in a plane with the lamp axis. The radiometer detector was

located at the end of the radius and moved around the arc, facing the centroid of the lamp. Intensity was measured far from the lamp centroid (190-cm radius) so that light rays from ends of the lamp were nearly perpendicular to the detector face. Output was calculated graphically by the following formula:

$$OPT = 4\pi r^2 \int_0^{\pi/2} I \sin\theta \, d\theta,$$

where θ is the angle measured from the lamp axis (θ = 0) to the radius to the detector, r is the radial distance from the lamp centroid to the detector, and I is the intensity at r and θ. Radiometer measurements perpendicular to the lamp centroid could then be used as an index to the output, since these measurements were proportional to the variations in output due to variations in applied voltage or temperature. Output was monitored during experiments.

Calculation of intensity. Common radiometer detectors cannot be used to measure intensity near a tubular lamp because they measure energy flux on the planar surface of the detector. Light received at angles other than 90° to the detector surface is attenuated, since the surface of the detector intercepts a smaller cross-section of the rays. Biological cells in motion in a solution, however, present a three-dimensional target and respond to the three-dimensional intensity from all angles within a disinfection reactor (10).

To calculate the UV intensity at a point near a tubular lamp in an absorbing solution, we used an equation from the nuclear engineering field which we call the point source summation (PSS) calculation (7, 10, 15). This equation assumes that the lamp is a line segment source and can be treated as the sum of a number of point sources. For a cylindrical reactor with a single central lamp surrounded by a quartz tube, a cylindrical coordinate system can be established (Fig. 1). The line source of UV output OPT is divided into N point sources, each of which has strength S (units in watts): $S = OPT/N$. The intensity at a point $I_{(R,Z_c)}$ due to one point source (Z_L) can then be treated as a product of the spherical spreading times the attenuation due to absorbance over a definite path length (P to P_1) between the point in solution (R_1Z_c) and the point source (Z_L). Applying Beer's law and simple geometry gives

$$I_{(Z_L),(R,\,Z_c)} = \frac{(S)}{4\pi\,[R^2 + Z^2_{LC}]} \exp\left(-a[R - R_1]\frac{P}{R}\right)$$

where a is the absorbance of the medium; the other geometry is shown in Fig. 1. The total intensity at point $I_{(R,Z_c)}$ is the sum of the contributions of each point source (at each Z_L) over the source length

$$I_{(R,\,Z_c)} = \sum_{Z_{L_n}}^{Z_{L_0}} I_{(Z_L),(R,\,Z_c)} \qquad (1)$$

The use of this calculation requires two measurements: the absorbance of the water, and the lamp UV output (9).

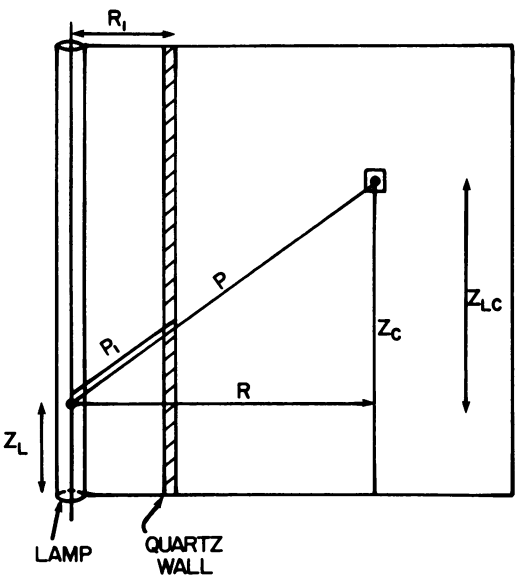

FIG. 1. Cylindrical reactor geometry for PSS calculation (modified from Jacob and Dranoff [7]).

From equation 1, the average intensity within a volume can be calculated by multiplying the intensity in each solution element [$I_{(R,Z)}$] by the volume of that element [$V_{(R,Z)}$] summed over each element of solution and divided by the total volume (V):

$$I_{ave} = \frac{\sum_{R_1,Z_{L_0}}^{R,Z_L} I_{(R,\,Z_c)}\, V_{(R,\,Z_c)}}{V} \qquad (2)$$

Bioassay method for measurement of intensity. A bioassay method was developed to measure average intensity in flow-through reactors as well as to verify a method of intensity calculation. Dose is defined as intensity times exposure time or, in units, mW s/cm^2 = (mW/cm^2) (s). The survival (N_s/N_0) of organisms is usually a function of dose: $N_s/N_0 = f$(dose), where N_0 and N_s are the density of organisms before and after irradiation, respectively. The dose and survival equations imply that intensity and exposure time may be varied reciprocally to obtain the same survival (the Bunsen-Roscoe Law [8]).

Bacillus subtilis (ATCC 6633) spores were used for the bioassays of UV dose. Preparation of spore stock is described elsewhere (9). Spores were suspended in buffered water (1). They were enumerated by pour-plating in triplicate on Thermoacidurans agar (Difco Laboratories).

The survival of spores of *B. subtilis* was determined as a function of the UV dose to standardize the sensitivity of the spores. Since intensity, as measured by a radiometer, was only applicable in a collimated beam, the spores were exposed for fixed periods of time to a beam of UV light collimated by a black tube (Fig. 2). The suspensions were kept in a stirred petri dish and contained either 5×10^4 or 5×10^5 viable spores per ml. The intensity at the surface of the

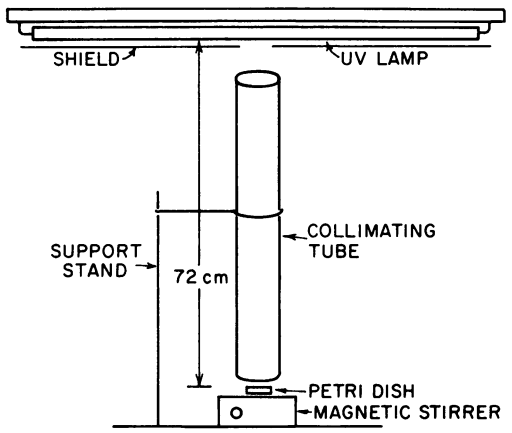

FIG. 2. Collimated beam apparatus.

suspension was measured with a radiometer. We assumed that 4% of the incident intensity was reflected at the water surface (8). Since fluid depth and absorbance were minimal, the dose could be calculated based on the measured intensity and the known exposure time. When absorbance was significant, the average intensity was calculated by an integration of Beer's law over the fluid depth (11). Standard curves of log survival versus dose were constructed (Fig. 3) and found to be reproducible over several months. The intensity could then be determined in an unknown system by (i) determining the survival (N_s/N_0), (ii) reading the dose corresponding to the observed survival on the standard curve (Fig. 3), and (iii) using the known exposure time (equation 2) to calculate average intensity.

Test of PSS calculation. To test the PSS calculation, we compared the calculated average intensity inside a cylinder to that measured by the spore bioassay. We used the PSS calculation in a computer program to average the intensity over the volume of a cylinder around a lamp. We did this for a series of cylinders of various radii and for fluids of different absorbances.

Suspensions of spores were exposed for a fixed time to UV light inside the cylindrical apparatus shown in Fig. 4. The apparatus had to meet several requirements: (i) it was necessary to use batch irradiation so that exposure time was accurately known; (ii) the lamp had to be equilibrated when exposure began so that output did not vary; and (iii) the solution had to be well mixed, with minimum interference from stirrers. A movable paper tube was located between the lamp and quartz sleeve so that the lamp could be warmed up and an exact exposure made. The suspensions were well stirred. Fulvic acid isolated by the method of Christman et al. (3) was added as a natural UV absorber. The survival of the spores was measured, and the assayed average intensity was determined as outlined above. Lamp output was checked between experiments in an identical setup, without the plexiglass cylinder, by an identical protocol. The temperature between the lamp and the quartz sleeve was monitored with a thermistor.

RESULTS AND DISCUSSION

The PSS calculations were generally verified by the bioassay measurements. Figure 5 shows a comparison between the calculated PSS curves (solid lines) and the bioassay data (data points). The correspondence was fairly good both for cylinders of different radii and for fluids of various absorbances. However, the calculated values tended to be a few percentage points higher than the bioassay measurements in the smaller cylinders, but only in one case was the difference more than 6%. The stirring device may have produced some shadowing loss in the small cylinders. These effects would be expected to be more important for small cylinders and low absorbances.

We also performed the same experiment with spores added to a secondary effluent. Particles in wastewater effluents may scatter as well as absorb UV light. Since the usual spectrophotometric measurements do not separate scattering and absorbance, we used special methods, detailed elsewhere (9), to determine the absorbance alone for the PSS calculations. The PSS calculation was within several percentage points of the mean bioassay intensity (1.53 mW/cm^2 calculated versus a mean of 1.41 mW/cm^2 assayed in two trials).

We also applied the calculation methods used in some previous studies (13, 17) to these cylinders, and those methods gave results that differed greatly from our bioassayed average intensities.

Jacob and Dranoff (7) used a specially designed detector to compare the PSS calculations with measurements of intensity profiles around a lamp at the axis of an annular photochemical reactor. The measurements of intensity deviated significantly from the PSS calculations. The measurements were made with a small photocell

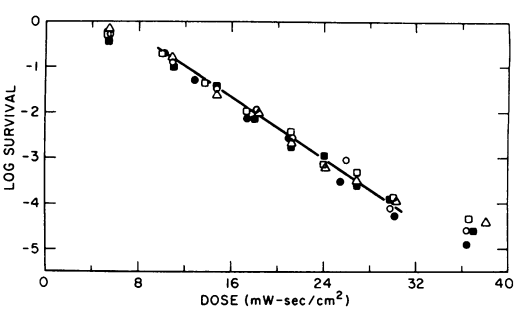

FIG. 3. Log survival of *B. subtilis* spores versus UV dose in a collimated beam of known intensity. Different symbols represent five different runs. Data from doses of 10 to 30.5 mW/cm^2 appeared to be linear and fit the regression line $Y = 0.167X + 1.01$ ($r = 0.98$).

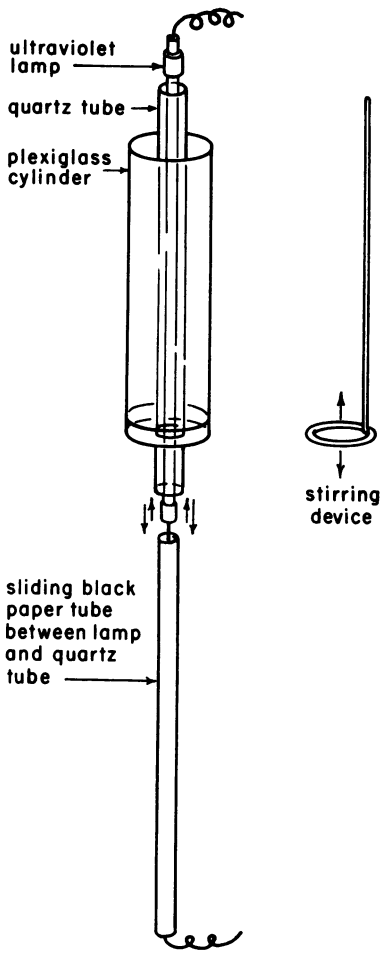

FIG. 4. Cylindrical batch irradiation apparatus.

mounted beneath a quartz window. The intensity measured by this probe was likely to be the planar intensity. We believe that those measurements were biased by the orientation of the planar surface. In fact, the pattern of deviation was what might be expected from such a bias.

Practical UV reactors are flow-through systems and have a distribution of exposure times. To use the bioassay of intensity in a flow-through system, we needed a way to determine a definite exposure time. To do this we used the spores in a manner analogous to that for a tracer injection study. To demonstrate this method, we used a flow-through tube surrounding a UV lamp. Spores were injected into the flowstream of water at the entrance to the tube, and the outflow fractions were collected in a rotating sampling tray as a function of time after injection. The injection was performed with the light on and repeated with the light off. The density of the unirradiated spores (N_0) is shown in Fig. 6a. The distribution of unirradiated spores reflects the residence time distribution (RTD). The density of surviving irradiated spores (N_s) is shown in Fig. 6b. The survival rate (N_s/N_0) was calculated for each flow fraction separately by comparing spore densities in the corresponding irradiated and unirradiated fractions at a given time after injection. The average intensity was then determined for each fraction by finding the corresponding dose from the standard curve and dividing by the time after injection. The assayed dose for each flow fraction was also plotted (Fig. 6b). The slope of the regression line of the assayed dose versus time after injection was equal to the average of the assayed intensities in the separate fractions. A modification of the spore injection bioassay may be used to measure average intensity in full-scale reactors.

The assayed average intensities within the flow-through tubes (Fig. 5, injection experiments) corresponded fairly well with the calculations of the PSS model (Fig. 5, lines) but were a little lower than the PSS calculations. The distribution of unirradiated and irradiated viable spores (Fig. 6) also showed that nearly all of the surviving spores emerged from the tube before the average retention time. This shows the important effect that flow dispersion can have on disinfection efficiency. As the equations for dose and survival rates show, survival depends on both exposure time and intensity. For non-ideal flow, the time for disinfection is not simply related to volume and flow rate, since there is a distribution of residence times.

Calculation of intensity in multiple-lamp reactors. To calculate average intensity in multiple-

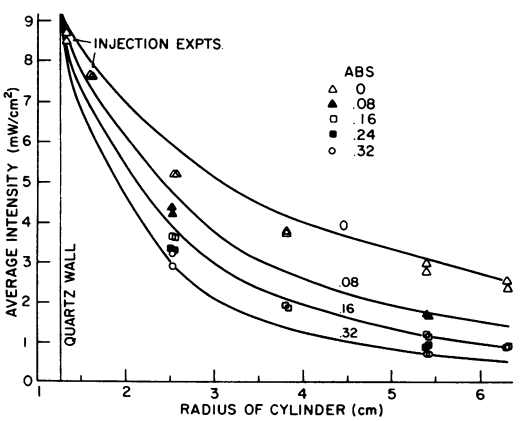

FIG. 5. Average intensity within a cylinder of radius R. The solid lines were calculated by PSS for several different absorbances (ABS). Data points represent bioassayed average intensity within the cylinders of various sizes. Data points for 1.32- and 1.59-cm radii were obtained from flow-through tubes rather than batch experiments.

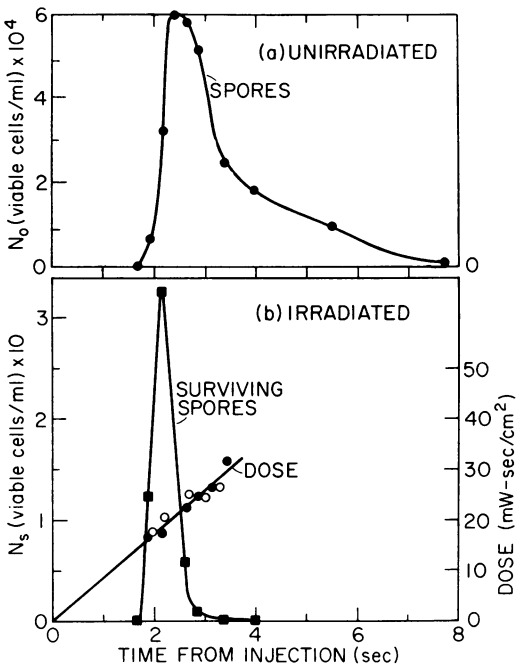

FIG. 6. Assay of average intensity in 1.32-cm radius flow-through tube by injection of spores and collection of separate fractions over time after injection. (a) Concentration of spores versus retention time (time after injection) with no irradiation. (b) Spore concentrations as a function of residence time when irradiated (at the same flow rate). Also shown is the assayed dose calculated from the N_0 and N_s of each fraction collected and the calibration curve. For clarity, the viable spore distribution curves are shown for only one experiment, but the assayed dose versus time shows data from two trials (●, ○). The assayed intensity for each point is (assayed dose)/(retention time), and the average corresponds to the slope of the regression line through all of the points.

lamp reactors, we used the following method. (i) Intensity at each point was considered to be the sum of contributions from each lamp calculated by the PSS model; (ii) intensity was mapped at each point on a grid of the cross-sections of the reactors; and (iii) intensities were averaged over the cross-sections and along the length of the reactor. The approach of mapping the intensity on a cross-section of the reactor was used by Petrasek et al. (13), who manually drew isopleths of intensity and averaged them with planimetry. Our calculations were performed with a computer program. Our calculations also made the simplifying assumptions that reflection from the reactor walls was negligible under actual operating conditions and that reflection and refraction by the quartz sleeves were negligible.

Applications. There are divergent views on the design of UV reactors. Some of these views are based on improper equations or conventional wisdom rather than on calculation or experimental measurement. This is because of the lack of adequate and comparable methods for measuring or calculating UV dose (see, e.g., reference 17). Our models can be useful in the research and development of reactor design. For example, we applied our calculations to contrast the efficiency of different schemes of lamp spacing in absorbing fluids (9).

The bioassay method employing the injection of spores can be used to measure the average intensity in full-scale flow-through UV reactors. Spores may be injected into the entrance of the reactor with a large syringe. A sample of the outflow may be collected with a syringe at a known time after injection. A number of such samples taken at different times after injection would provide data over the entire RTD curve. Accompanying samples with the lights off would provide the N_0 measurement. Thorough tracer studies should be done to establish the RTD in any flow-through reactor.

This method provides separate information on the average intensity and the RTD. The net effect on average survival of a non-ideal RTD and intensity is complex. Use of the bioassay technique to measure intensity and the RTD separately can help microbiologists more easily relate the results of batch experiments with pathogens (14, 20) to the actual disinfection expected in flow-through systems. In addition, it is essential to evaluate the intensity and RTD separately to approach the improvement of UV disinfection systems scientifically.

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