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UV Inactivation of Pathogenic and Indicator Microorganisms

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Survival was measured as a function of the dose of germicidal UV light for the bacteria *Escherichia coli*, *Salmonella typhi*, *Shigella sonnei*, *Streptococcus faecalis*, *Staphylococcus aureus*, and *Bacillus subtilis* spores, the enteric viruses poliovirus type 1 and simian rotavirus SA11, the cysts of the protozoan *Acanthamoeba castellanii*, as well as for total coliforms and standard plate count microorganisms from secondary effluent. The doses of UV light necessary for a 99.9% inactivation of the cultured vegetative bacteria, total coliforms, and standard plate count microorganisms were comparable. However, the viruses, the bacterial spores, and the amoebic cysts required about 3 to 4 times, 9 times, and 15 times, respectively, the dose required for *E. coli*. These ratios covered a narrower relative dose range than that previously reported for chlorine disinfection of *E. coli*, viruses, spores, and cysts.

Disinfection of water and wastewater with UV radiation appears to be a potential alternative to chlorine. Low concentrations of chlorine residuals are toxic to aquatic life (29), and some of the by-products of chlorination have been shown to be mutagenic (18). Germicidal UV radiation does not produce undesirable by-products and is effective in inactivating a variety of microorganisms (13, 30).

A number of pathogenic microorganisms, particularly viruses and cysts, are much more resistant to chlorine than is *E. coli* (18). Yip and Konasewich (30) suggested that the doses of UV light necessary to kill pathogens, including viruses, bacterial spores, and protozoa, are much more comparable to the doses of UV light necessary to kill indicator bacteria than is the case for chlorine. If so, the UV levels necessary to meet coliform standards may be relatively more effective than chlorination in killing pathogens. To date, much has been published on the inactivation of microorganisms by UV light. Because of the difficulties and subtleties of measuring UV dose (9), many of the dose values reported are not comparable. Dose is normally defined as intensity times exposure time. Sensitivity to UV disinfection can vary for a certain species of microorganism according to strain, growth medium, stage of the culture, and influences of the plating medium on repair of sublethal damage (9, 15). No comparison has included the coliform group from field samples which actually serves as the basis for present standards. Comparisons of UV resistance must be done by careful determination of UV dose and standardized growth conditions.

The objective of this study was to (i) accurately define the UV dose required to inactivate certain human pathogens, including bacteria, viruses, spores, and cysts, and (ii) evaluate the validity of using coliforms as indicators of UV disinfection efficiency.

MATERIALS AND METHODS

Bacteria. The bacteria used in this study were obtained from the American Type Culture Collection, Rockville, Md.: *Escherichia coli* (ATCC 11229), *Streptococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), *Shigella sonnei* (ATCC 9290), *Salmonella typhi* (ATCC 6539), and *Bacillus subtilis* (ATCC 6633). Except for the *B. subtilis* spores, bacteria were grown in nutrient broth until they were well into the stationary phase (20 to 24 h at 35°C). Samples of the broth culture were then filtered with a 0.45-μm-pore Millipore filter and rinsed with sterile buffered water. Cells were resuspended in sterile buffered water and filtered through a 1.0-μm Nucleopore polycarbonate membrane to remove aggregated groups of bacteria. The filtrate was used in the UV inactivation studies. The process of washing and irradiation was completed as quickly as possible to minimize the time in buffered water. Vegetative bacteria were enumerated on spread plates with nutrient agar (Difco Laboratories, Detroit, Mich.).

B. subtilis spores were produced in Schaeffer medium (10, 16). After sporulation, the suspension was heated to 80°C for 10 min and then sonicated for 2 min at 70 W in an ice bath. The spore suspension was washed by centrifugation, and the pellet was resuspended in distilled water. This procedure was repeated until microscopic examination showed individual spores free of cell debris. After the last centrifugation, the spores were again sonicated to ensure that there was no aggregation and stored in distilled water at 4°C. The viable spores were enumerated by using pour plates with Difco nutrient agar.

Viruses. Poliovirus type 1 (LSc2ab) and simian rotavirus (SA11) were used in this study. Poliovirus was propagated and assayed in MA104 cells, a continuous Rhesus monkey kidney cell line. SA11 was propagated in secondary African green monkey kidney cells and assayed in MA104 cells. Assays of both viruses were accomplished by cytopathic plaque formation in cell monolayers (6, 28). Monodispersed virus stocks were obtained by Freon extraction of the cell culture medium supernatant from 100% infected cultures, centrifugation at 105,000 × *g* for 2 h to pellet the virus, suspension of the virus pellet in phosphate-buffered saline, and filtration through a Tween 80-treated 0.08-μm-pore polycarbonate filter.

Amoebic cysts. A sample of *Acanthamoeba castellanii* (ATCC 30234) trophozoites was obtained from the American Type Culture Collection. The methods developed by Neff et al. (19) for production of amoebae and induction of synchro-

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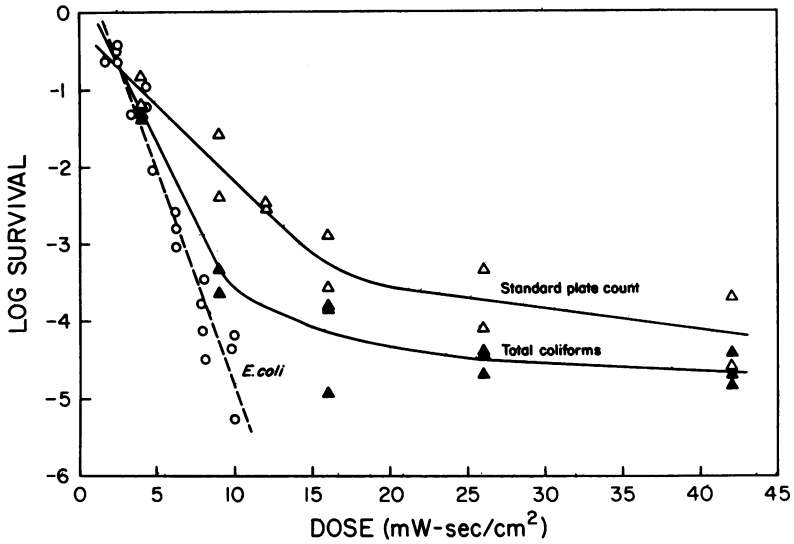

FIG. 1. Survival versus UV dose for cultured *E. coli*, filtered (10-μm pores) total coliforms, and microorganisms growing on standard plate count agar from a secondary effluent. Each datum point represents one irradiation and three replicate platings.

nous encystment were used. The amoebae were allowed to encyst for 1 week at 25°C with constant aeration. Encystment was greater than 90%.

A plaque assay developed by Rubin et al. (25) was used to enumerate amoebic cysts. A 0.1-ml sample of the cyst suspension together with 0.15 ml of a 24-h broth culture of *E. coli* were spread on Fulton plating medium agar. The plates were incubated at 30°C for 4 to 5 days, and the plaques on the bacterial lawn were counted.

Field samples. Samples of secondary effluent were collected from the Sandy Creek Wastewater Treatment Plant, Durham, N.C. A portion of the samples was gently filtered through a nylon screen with approximately 10-μm pores to remove most of the larger particles capable of harboring embedded bacteria. These samples were irradiated within 2 h. Coliform survivors were enumerated by the one-step total coliform membrane filtration procedure (1), since no significant difference was found for the survival of UV-irradiated coliforms as enumerated by either the standard membrane filtration or most-probable-number total and fecal coliform procedures (21). Standard plate count microorganisms were enumerated according to standard methods (1).

Irradiation. For accurate dose-survival data, samples were irradiated in an apparatus which provided a nearly collimated beam (22). Measurements of incident intensity (I_0) at the liquid surface 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. Stirred suspensions of 0.5-cm depth were irradiated in small petri dishes. The average intensity in the stirred suspension (I_{avg}) was calculated as follows (12):

$$I_{avg} = I_0(1 - e^{-A_eL})/A_eL$$

where A_e is the absorbance per centimeter (base e) and L is the path length. Spectrophotometric absorbance at 254 nm was measured with a Cary 219 spectrophotometer.

All dose-survival experiments with bacteria and cysts were done with six dose levels, two replicate irradiated suspensions, and three platings of each dilution. For viruses, two platings were used. Survival data were treated according to Chick's Law as log N_s/N_0 versus dose, where N_0 is the initial concentration of organisms and N_s is the density of survivors.

RESULTS AND DISCUSSION

Vegetative bacteria. Most of the vegetative bacteria (*E. coli*, *S. aureus*, *S. sonnei*, and *S. typhi*) exhibited similar resistance to UV light (Fig. 1 and 2) and required about the same dose for 3 log units (99.9%) of inactivation. The exception to this was *S. faecalis*, which required about a 1.4 times higher dose for three log units of inactivation. Several of the survival curves, including the one for *S. faecalis*, and especially those for the *B. subtilis* spores and the *A. castellanii* cysts (Fig. 3), exhibited an initial lag in slope or "shoulder." Such curves have elsewhere been attributed to multiple-hit kinetics or related phenomena (9). The multiple-

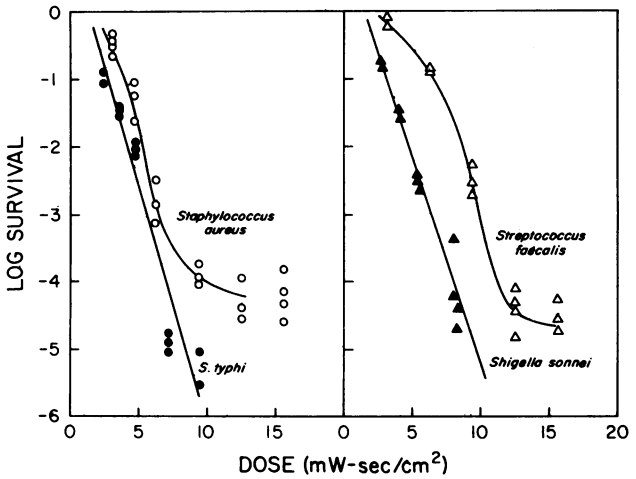

FIG. 2. Survival versus UV dose for *S. sonnei*, *S. typhi*, *S. faecalis*, and *S. aureus*. Each datum point represents one irradiation and three replicate platings.

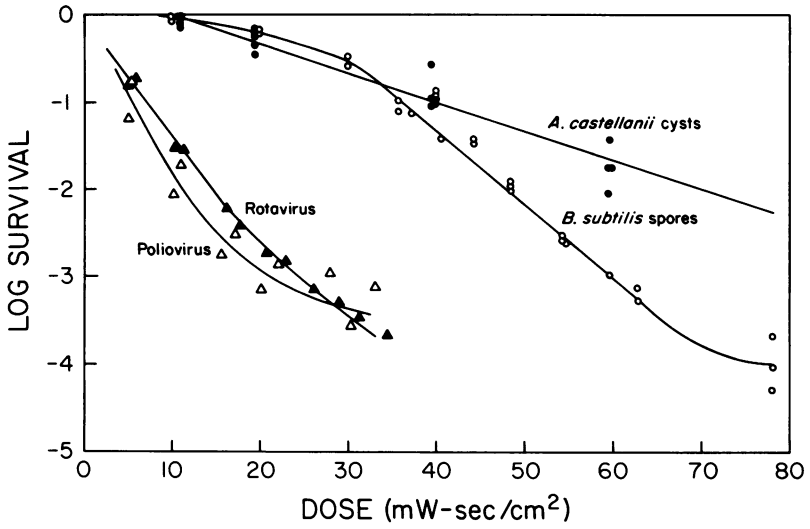

FIG. 3. Survival versus UV dose for poliovirus type 1, rotavirus SA11, *B. subtilis* spores, and *A. castellanii* cysts. Each datum point represents one irradiation and two (for viruses) or three replicate platings.

hit theories can apply both to aggregates of cells or single cells (9).

Our data agree well with the comparable survival data for *E. coli*, *S. typhi*, *Shigella paradysenteriae*, and *S. aureus* by Sharp (27), although his data did not extend below the −2 log survival level. The *Streptococcus* sp. used by Sharp appeared to be more sensitive than the one we used, however. Much of the early work has been summarized by Zelle and Hollaender (31), but the results are reported as the dose necessary for 90% inactivation, a dose level which may be unduly influenced by the initial lag in slope evident in much of the data (9). The most widely cited comparison of a wide variety of organisms is that of Nagy (17). Those data are difficult to compare with other data because the bacteria were irradiated on the surface of agar plates, and the data were summarized in terms of the dose necessary to completely destroy the microorganisms, a value dependent on the initial density of the organisms on the plate surface. A report of the sensitivity of *Legionella pneumophila* to UV radiation (2) indicates that it may be considerably less resistant than *E. coli*.

In comparing chlorine disinfection data, the concept of dose (14) is not as straightforward as it is for UV. However, a number of references have been summarized recently (18) in which comparisons are made on the basis of concentration (C) times time (t) for a survival level of 1%. The resistances to chlorine (HOCl) of several enteric bacteria are roughly comparable (18). At neutral pH, *S. typhi* strains are somewhat more resistant to HOCl than is *E. coli*. Thus, when UV light and chlorine disinfection are compared on the basis of a given level of inactivation of nonaggregated *E. coli*, the inactivation of vegetative enteric bacteria appears to be generally comparable.

Total coliforms and standard plate counts. The survival curve for the total coliforms in the filtered secondary effluent sample was relatively similar to that for the cultured *E. coli* at the higher levels of survival (Fig. 1), but the curves diverged at the lower levels of survival. Experiments involving the removal of particles both smaller and larger than 10 μm (20) and fractionation of particle sizes (S. F. Ossoff, Master's Technical Report, University of North Carolina at Chapel Hill, 1984) have shown that the tendency of wastewater coliform survival curves to level out at low levels of survival is caused by a small fraction of cells protected by their association with larger particles. Microscopic particle size measurements indicated that 10-μm filtration removed most, but not all, of the aggregates and particles capable of harboring protected coliform cells.

The total coliforms represent a group of bacteria, some unknown proportion of which may be *E. coli*. The fact that the bacteria were cultured did not appear to create dramatic differences between the survival curves of the cultured bacteria and those of the natural coliforms except at high levels of inactivation. These results suggest that the total coliforms can serve as an adequate indicator of disinfection for at least the vegetative bacteria used in this study. The microorganisms enumerated by the standard plate count represent a diverse assemblage. The survival curve for the standard plate count microorganisms (Fig. 1) is shifted toward somewhat higher doses than the curve for filtered total coliforms. The standard plate count and coliform survival curves suggest a relatively small range in UV resistance of the portion of the total microbial population enumerated by these methods. The standard plate count may be as good a measure of bacterial pathogen disinfection efficiency as the coliform tests.

Viruses. The survival curves of the two viruses studies are presented in Fig. 3. The curves for rotavirus and poliovirus are very similar, and these viruses exhibit three to four times more resistance to UV irradiation than do the vegetative bacteria (Fig. 4).

The viral disinfection data agree reasonably well with those of Hill et al. (8), who used eight enteric viruses. Estimating a corrected dose from their measurements of incident intensity, we found the dose necessary for −3 log survival ranged from about 28 to 42 mW−s/cm² (30 mW−s/cm² for poliovirus type 1). Morris (13) found the dose necessary for 98% inactivation of poliovirus to be about 2.5 times that for *E. coli*. The conclusion of Yip and Konasewich (30) that enteric viruses are somewhat more sensitive to UV light than *E. coli* was not supported by this study.

In terms of comparison of our data to those on chlorine, Clark et al. (4) found that *E. coli*, *S. typhi*, and adenovirus type 3 were all inactivated at approximately the same

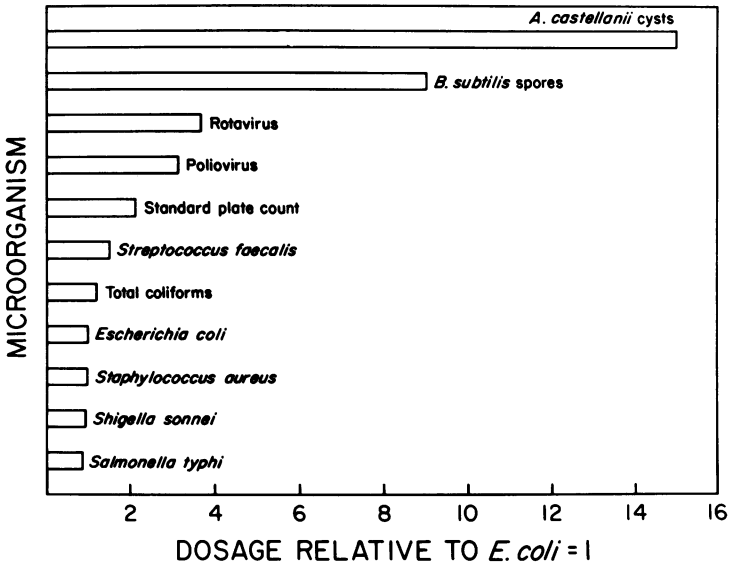

FIG. 4. Relative UV doses required for 99.9% inactivation of various microorganisms compared to that for *E. coli*.

concentration of free chlorine. This report of the low resistance of adenovirus type 3, however, appears anomalous among a number of reports concerning other enteric animal viruses (7, 18). In another study (5), the doses of free chlorine necessary to produce 99% inactivation at pH 6 and 5°C for six types of enteric animal viruses ranged from 3.5 to 43 times that necessary to inactivate *E. coli*. For poliovirus type 1, the dose was about 25 times that for *E. coli*. These relationships were different, however, at higher pH values. Scarpino et al. (26) found that poliovirus type 1 is about 40 times more resistant than *E. coli* at pH 6. However, Berman and Hoff (3) found that preparations of free simian rotavirus SA11 virions are completely inactivated in times comparable to those for *E. coli*.

Bacterial spores and protozoan cysts. The *B. subtilis* spores and the *A. castellanii* cysts were the most UV-resistant organisms (Fig. 3). The spores were about 9 times more resistant than the vegetative bacteria, and the cysts were about 15 times more resistant (Fig. 4). Although the UV sensitivity of spores prepared and plated on a particular medium was reproducible, the medium on which irradiated *B. subtilis* spores are plated can have an effect on the apparent survival. Spores produced with Schaeffer medium exhibited a different apparent survival when plated on Difco Thermoacidurans agar (21) than when plated on nutrient agar (see above).

Little data are available for UV disinfection of amoebic cysts. Rice and Hoff (23) found that cysts of *Giardia lamblia* are about 68 times more UV resistant than *E. coli* at the −0.6 log survival level. The *A. castellani* cysts used in this study were considerably more sensitive (Fig. 4). Although the UV dose necessary to cause a 99% inactivation of the cysts used in this study was within the operating range of many UV disinfection systems, it was beyond the usual operating dose.

Morris (14) calculated that bacterial spores were approximately 400 times more resistant to chlorine than enteric bacteria. McGrath and Johnson (11), with the same preparation of *Bacillus* spores used in this study, found a resistance to HOCl that was similar to that summarized by Morris (14), considering temperature differences. The data for protozoan cysts are generally difficult to compare with data for *E. coli* because experiments with cysts are generally performed at higher temperatures. From data summarized by the National Research Council (18), the $C \cdot t$ product at 30°C for *Entamoeba histolytica* is approximately 500 times that of *E. coli* at 5°C, so at similar temperatures the difference would be far greater. For *Naegleria gruberi*, a species morphologically similar to *A. castellani* the $C \cdot t$ product at 25°C (25) was about 200 times that for *E. coli* at 5°C. The experiments of Rice et al. (24) with *G. lamblia* were performed at 5°C. Estimating the $C \cdot t$ product from the most sensitive strain used in their experiments, they found that the *G. lamblia* cysts appeared to be 1,000 to 2,000 times more resistant than *E. coli*.

Implications for disinfection. The vegetative bacteria we studied exhibited similar resistance to UV disinfection. The viruses were 3 to 4 times more resistant, the spores were about 9 times more resistant, and the cysts were 15 times more resistant than the vegetative bacteria. Given problems in relating relative resistances to chlorine of *E. coli* and other organisms, probably only large differences should be considered significant in comparing ranges of dose necessary for UV and chlorine disinfection. Still, the difference in dose necessary to inactivate *E. coli*, bacterial spores, and protozoan cysts seem to be much greater for chlorine disinfection than for UV disinfection. With some exceptions, this also appears to be true for enteric viruses, especially for poliovirus, for which the most data are available. The UV resistance of viruses and *E. coli* are not as comparable as implied by Yip and Konasewich (30).

The range of UV dose necessary to disinfect pathogens is narrower than it is for chlorine disinfection. While the minimum dose to effectively eliminate all pathogens is the limiting factor for ideal disinfection, the economic comparisons and routine evaluations of different disinfectants have been made based on coliform or *E. coli* disinfection data. Neither *E. coli* nor coliforms can serve as a quantitative model for disinfection of viruses, spores, or cysts. Also, as Rice et al. (23) point out, the extreme resistance of cysts like *G. lamblia* makes it unlikely that either normal chlorination or UV irradiation procedures would be sufficient to destroy the cysts. It has been noted that organisms used to indicate fecal contamination of water supplies should be distin-

guished from organisms used as models of disinfection (18). As is the case with chlorine disinfection, the survival of coliform bacteria in UV-irradiated effluents does not directly indicate the same level of disinfection for the more resistant organisms.

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