Check for updates

Bacterial responses to ultraviolet irradiation

E.R. Blatchley III[*], N. Dumoutier[**], T.N. Halaby[*], Y. Levi[***] and J.M. Laîné[**]

[*]Environmental and Hydraulic Engineering Group, School of Civil Engineering, Purdue University, West Lafayette, IN 47907-1284 USA

[**]CIRSEE, Lyonnaise des Eaux, 38 Rue du Président Wilson, 78230 Le Pecq, France

[***]Université Paris Sud, Faculté de Pharmacie, Laboratoire Sante Publique – Environnement, 5 Rue J.B. Clément, 92296 Chatenay-Malabry Cedex, France

Abstract The UV dose-response behavior of laboratory cultures of waterborne bacteria were examined for UV doses ranging from *ca.* 0 –100 mW•s/cm^2 using a collimated-beam reactor. Specific physiological responses measured in these tests included viability (ability to reproduce) and respiration (oxygen uptake rate). The results of these exposures indicated that resistance to UV-imposed loss of viability in *E. coli* cultures can be partially attributed to agglomeration during the irradiation process. From these results, it is conjectured that a bacterial population may be comprised of two sub-populations: one with low resistance (discrete or paired cells) and a second with high resistance (bacterial aggregates). A small fraction of the high-resistance portion of the population appears to be essentially unaffected by UV irradiation, thereby causing a discontinuity in the measured dose-response behavior. Moreover, the dose-response behavior of the highly resistant fraction is variable and difficult to describe quantitatively. The basis of these statements and most information in the literature is microbial viability as quantified by the membrane filtration assay. In contrast to these findings, the results of analyses for bacterial activity (respiration) suggest that comparatively little change in the population can be found to result from UV irradiation. This suggests that UV radiation accomplishes inactivation of the bacteria, but does not "kill" the bacterial cells *per se*, thereby highlighting the importance of considering bacterial repair processes in the design of UV disinfection systems.

Keywords Bacteria; respiration; UV disinfection; viability

Water Science and Technology **Vol 43 No10 pp 179–186** © IWA Publishing 2001

Introduction

Of the process components that define the response of a UV system, microbial dose-response behavior is perhaps the simplest to measure experimentally. For example, a properly designed collimated-beam system will provide radiation of essentially uniform incident intensity to a reactor vessel for which the period of exposure, and consequently the UV dose, can be accurately controlled and measured. These systems have been used extensively for measurement of microbial dose-response behavior. Knowledge of microbial responses to disinfectant exposure is critical for predictions of process behavior. However, natural variations in microbial physiology and chemistry dictate that all organisms will respond differently to UV irradiation. Moreover, the assays used to characterize microbial responses provide indirect, and often times incomplete information regarding the condition of a microbial population. As such, these assays must be recognized as surrogate measures of the true risks to human health posed by waterborne microorganisms.

A comprehensive understanding of microbial UV dose-response behavior requires the selection of an appropriate microbial response and an assay for its measurement. Bacterial viability, as it relates to disinfection processes, is normally defined as the ability of bacteria to reproduce. Conventional membrane filtration procedures provide a measure of bacterial viability under nearly optimal growth conditions. A motivation for the use of viability assays is that they provide an indication of the ability of a given organism to reproduce under a pre-defined set of (near-optimal) growth conditions. As such, it has been argued

Downloaded from http://iwaponline.com/wst/article-pdf/43/10/179/428679/179.pdf
by guest

that they provide a measure of the ability of an organism to cause infection in a host. However, other assays exist for characterization of the physiological condition of bacteria or bacterial populations. For example, 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) has been applied for visualization and quantification of respiring bacteria (Rodriguez *et al.*, 1992). CTC, a colorless, soluble dye is reduced in bacterial electron transport processes to insoluble, fluorescent CTC-formazan which accumulates within bacterial cells. CTC reduction is believed to take place in parallel with oxygen reduction as part of the bacterial electron transport process; therefore, it is assumed that the ability to reduce CTC to CTC-formazan within an individual bacterial cell indicates its ability to respire. By counter-staining bacteria with a DNA-specific fluorochrome, such as 4'-6-diamidino-2-phenylindole (DAPI), it is possible to quantify total and actively-respiring bacteria within a population simultaneously using epifluorescence microscopy (Schaule *et al.*, 1993). It may be possible for bacterial cells to acquire damage sufficient to eliminate their ability to reproduce (i.e. viability), but allow them to retain the ability to respire, a form of bacterial activity. Though the CTC assay has the benefit of providing information regarding the respiratory activity of individual bacterial cells, it is nonetheless an indirect measure of respiration. Direct measurements of O_2 uptake, as are possible using the methods of respirometry, can be used to augment CTC assay results, and to provide quantitative information regarding a bacterial population. The use of the CTC assay in conjunction with respirometry can provide a more comprehensive assessment of respiration than is possible with either method alone.

Measurements of bacterial responses other than viability are particularly germane to UV disinfection because microbial damage resulting from UV irradiation is thought to be largely in the form of dimerization of nucleic acids. This is in direct contrast to some chemical disinfectants (e.g. chlorine or ozone), wherein damage may also be in the form of metabolic interference or cell lysis. Therefore, it is conceivable that nucleic acid damage within bacteria could be sufficient to prevent reproduction, yet still allow the bacteria to perform some functions that are commonly associated with "live" cells. Indeed, differentiation of "live" and "dead" bacterial cells can be ambiguous. The ability of bacteria to repair sub-lethal damage imposed by UV radiation makes the distinction between "live" and "dead" bacteria a potentially important one in the design and implementation of UV disinfection systems, especially those where product water is likely to be used for direct human consumption.

With these facts in mind, the goal of the research described herein was to characterize the dose-response behavior of important waterborne bacteria to UV radiation. Cultures of pure bacteria were exposed to a broad range of UV doses using a collimated-beam reactor system. Bacterial responses were characterized on the basis of conventional membrane filtration assays. In parallel with these measurements, subsamples of the irradiated and non-irradiated bacterial cultures were assayed for respiration, either by CTC or respirometry. The results of these assays are discussed in the context of UV disinfection systems that are used in wastewater treatment and in the production of potable water.

Materials and methods

A low-pressure mercury arc lamp (λ = 253.7 nm) was used as the source of radiation. Radiation from the lamp was imposed on a shallow (liquid depth = 1.0 cm), well mixed batch reactor using a flat-plate collimator. Mixing was accomplished by the use of a small magnetic stir bar. Numerical analysis of the intensity field around the lamp indicated that the collimator provided an essentially uniform incident intensity field across the surface of the reactor. Details of the intensity field analysis for the flat-plate collimator as well as its application in the measurement of photochemical kinetics are provided elsewhere (Blatchley, 1997). Incident radiation intensity was measured periodically during the course

Downloaded from http://iwaponline.com/wst/article-pdf/43/10/179/428679/179.pdf
by guest

of the microbial exposures using a radiometer (International Light Model 1700, Newburyport, MA). Microorganisms were exposed to UV radiation at an incident intensity of approximately 0.1 mW/cm^2. The period of exposure, and therefore the UV dose, were controlled by the use of an opaque shutter placed between the end of the collimator and the reactor surface. UV dose was calculated as the product of exposure time and the depth-integrated intensity.

Transmittance ($\lambda = 253.7$ nm, $L = 1.0$ cm) was measured using a Beckman DU-64 spectrophotometer. The optical density in these experiments was typically 0.7 or lower. For these conditions, the mimimum intensity within the reactor (at the bottom of the reactor) was roughly 50% of the incident intensity. Given that the batch reactor was well mixed and the periods of exposure ranged from 30 seconds to 30 minutes, it is evident that this system of exposure delivered an essentially uniform UV dose to the entire sample in the reactor. Immediately after exposure, samples were placed in an opaque enclosure and transferred to the laboratory for assaying. In all cases, the time interval between UV exposure and initiation of microbial assays was kept to a minimum, usually less than 30 minutes. These conditions were included in the experimental protocol so as to minimize the effects of photoreactivation and dark repair.

Several strains of bacteria were investigated in this research. In all cases, bacterial cultures were developed from catalogued bacterial collections, using sterile laboratory techniques. The specific bacteria and their conditions of incubation and assay are listed on Table 1.

Respiration and aggregation of *E. coli* were evaluated by epifluorescence microscopy. Suspensions of *E. coli* collected before (dose = 0) and after UV irradiation (doses of *ca*. 15 and 100 mW•s/cm^2) in the collimated-beam reactor were co-stained with CTC and DAPI, incubated for four hours, collected on black membrane filters (nominal pore size = 0.2 µm) followed by examination by epifluorescence microscopy.

Respiratory activity among cultures of bacteria was also measured using a Gilson Oxygraph respirometer. Bacterial suspensions at equilibrium with ambient air (dissolved oxygen ≈ 9 mg/L) were exposed to a range of UV doses using the collimated-beam reactor. Subsamples (2 mL) of the irradiated suspensions were placed in the respirometer cell to observe background dissolved oxygen concentration. After establishment of a stable background signal, the subsample was dosed with 150 µL of growth medium (see Table 1) to promote oxygen uptake. Dissolved oxygen concentrations in the respirometer cell were observed for 10 –60 minutes; during this time, dissolved oxygen concentration in the cell was reduced to 3 –5 mg/L. Oxygen uptake rate for each sample was reported as the slope of the dissolved oxygen profile in units of mg/L•min.

Results

For purposes of this paper, the term "viability" will be used in reference to organisms that display the ability to replicate. In contrast, the term "activity" will be used in reference to

Table 1 Summary of bacteria used, conditions used for development of cultures, and assays conducted

Bacterium	Source / Strain	Growth Medium	Incubation Conditions	Assays Conducted
Escherichia coli	CIP 5530	Nutrient Agar	37°C, 18 –24 hr	Membrane Filtration CTC / DAPI
Escherichia coli	ATCC 11229	Tryptone Yeast Extract	37°C, 24 hr	Membrane Filtration Respirometry
Pseudomonas aeruginosa	ATCC 15442	Tryptic Soy Broth	37°C, 24 hr	Membrane Filtration Respirometry
Streptococcus faecalis	ATCC 6569	Tryptic Soy Broth	37°C, 24 hr	Membrane Filtration Respirometry

Downloaded from http://iwaponline.com/wst/article-pdf/43/10/179/428679/179.pdf
by guest

microorganisms that retain (some) functions necessary for life in an individual organism. Therefore, it is possible for bacterial cells to be "non-viable" yet "active," whereas the converse (i.e. inactive yet viable) cannot be true.

Bacterial viability was examined for doses up to approximately 100 mW•s/ cm^2. These tests were repeated several times to provide an indication of variability in the response behavior. A graphical summary of these measured dose-response relationships is provided in Figure 1. The dose-response behavior of *E. coli* showed some variation between samples, but in general the relationship was characterized by behavior that was consistent with the series-event model (see Severin *et al.*, 1983) for doses up to approximately 10 mW•s/cm^2. Above this dose, the bacteria displayed tailing.

As a test of the possibility that UV-induced genetic flaws could result in population heterogeneity and high resistance to UV irradiation among a small fraction of bacteria in a population, a single colony from a sample exposed to a UV dose of approximately 100 mW•s/cm^2 was harvested from its membrane filter and used to develop a new bacterial culture. The resulting culture was subjected to the same range of UV doses as used in other tests. The dose-response behavior of the parent and daughter cultures are summarized in Figure 2. No discernable difference in the dose-response behavior of the parent or daughter cultures was evident over the dose range 0 –100 mW•s/cm^2.

The second measure of bacterial response was the ability to respire, as indicated by epifluorescence microscopy. Unirradiated and irradiated bacteria (from the concentrated cultures) were co-stained with DAPI and CTC. Microscopic evaluations of the stained bacteria revealed that the fraction of bacteria retaining respiratory function was essentially unchanged as a result of irradiation; approximately 10% of the bacteria demonstrated evidence of respiratory function, regardless of UV dose.

Some physical characteristics of the bacterial culture were also made evident by the epifluorescence microscopic analyses. In the unirradiated culture, all bacteria observed under the microscope existed as discrete cells or cell pairs. In contrast, a substantial fraction of the bacteria subjected to UV doses in the range 15 –100 mW•s/cm^2 (*i.e.*, doses above which

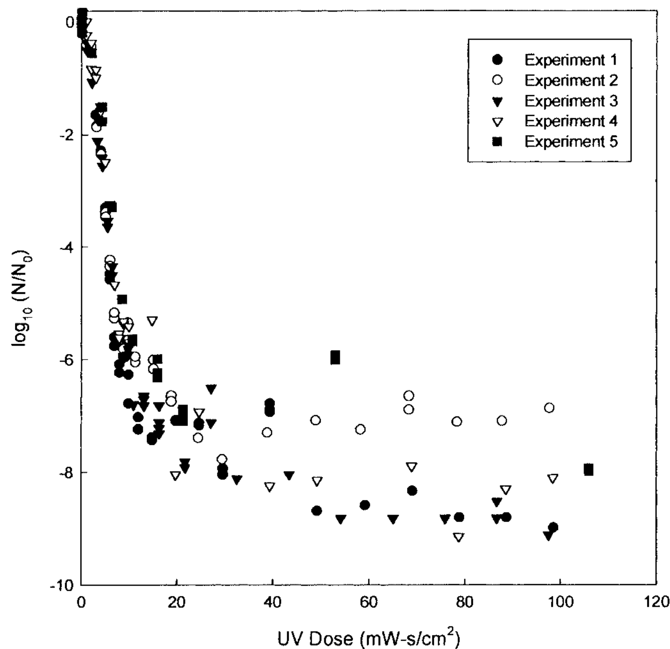

Figure 1 Viability dose-response relationship for *E. coli* (CIP 5530)

Downloaded from http://iwaponline.com/wst/article-pdf/43/10/179/428679/179.pdf
by guest

further inactivation was not observed) were found to exist in aggregates, with clumps consisting of as many as 50 –100 bacteria, and having dimensions as large as approximately 10 μm.

The results of the CTC testing suggested that respiratory activity among *E. coli* was largely unaffected by exposure to doses of UV radiation that were capable of inactivating the vast majority of the population. Respirometric measurements were used to test further the validity of this hypothesis. Table 2 provides a summary of the normalized viability and oxygen uptake rates for suspensions of *E. coli*, *P. aeruginosa* and *S. faecalis*. Bacteria followed a regular pattern of inactivation that was consistent with the series-event model up to the point at which tailing was evident. Measured inactivation responses were typically on the order of 6 –7 $\log_{10}$ units for the dose ranges used. Over this same range of UV doses, oxygen uptake was observed to decline by as much as 75% ($[R/R_0]=10^{-0.12}$), thereby suggesting that the damage to respiratory function over this dose range was much smaller than the loss of viability. It is hypothesized that the majority of bacteria retained their ability to respire (and metabolize substrate) over this range of doses, though stress caused by exposure to UV radiation may have reduced this ability somewhat within individual bacterial cells.

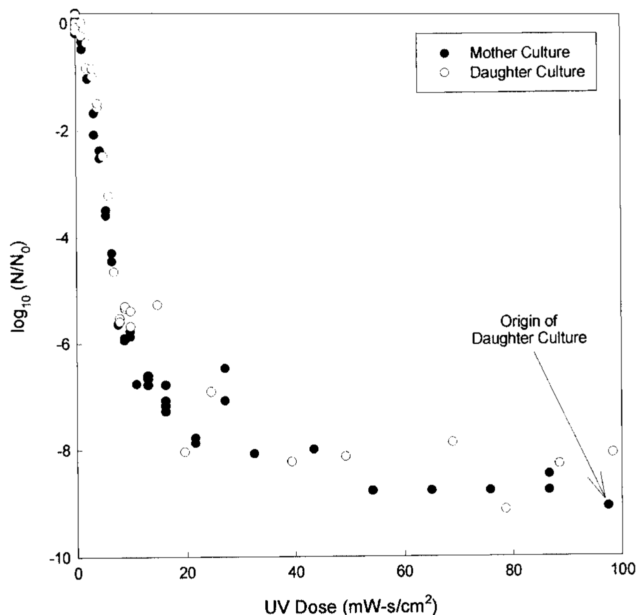

Figure 2 Viability dose-response relationship for 'mother' and 'daughter' cultures of *E. coli* (CIP 5530)

Table 2 Summary of viability and respiration responses for bacterial cultures. Reported viability and respiration rate responses are normalized with respect to their values for non-irradiated cultures (N_0 and R_0, respectively)

Bacterium	UV Dose (mW•s/cm^2)	N/N_0	R/R_0
E. coli	100	$10^{-6.8}$	0.23
P. aeruginosa	50	Na	0.26
S. faecalis	70	$10^{-6.0}$	0.25

Downloaded from http://iwaponline.com/wst/article-pdf/43/10/179/428679/179.pdf
by guest

Discussion

The existence of a shoulder in bacterial inactivation curves has been reported previously (Severin *et al.*, 1983; Chiu *et al.*, 1999), though the magnitude of the shoulder appears to be a function of the bacterial strain and the aquatic chemical matrix. Non-linear regression of the data presented in Figure 1 for UV doses less than 10 mW•s/cm^2 was found to be well described by the series-event model. Beyond this dose range, the data were not consistent with any existing model for description of microbial dose-response behavior.

Microscopic analyses of the *E. coli* cultures prior to UV exposure indicated that the bacteria existed as single or paired cells. Therefore, it will be assumed that the response behavior corresponding to doses of 0–10 mW•s/cm^2 was representative of non-aggregated bacteria. At doses above approximately 10 mW•s/cm^2, a small fraction of the bacterial population was able to retain viability. The fraction of the bacterial population able to reproduce at elevated UV doses represented between 10^{-9} and 10^{-7} of the original bacterial concentration, and was quite variable between experiments. Within in any given experiment, the fraction of bacteria able to retain viability was essentially constant for doses of 10 –100 mW•s/cm^2; these bacteria appeared to be completely unaffected by the process of UV irradiation. This suggests that a small fraction of the bacteria within these bacterial aggregates receives essentially complete protection from the imposed radiation.

It is possible that the factors governing viability among coliform bacteria in these cultures at high UV doses were not well controlled in these experiments. In particular, it is possible that variations in culture incubation time (18–24 hours) allowed the cultures to advance to different stages of physiological development along the stationary phase segment of the bacterial growth curve. This is germane to disinfection systems for potable water and wastewater wherein the bacterial populations are likely to experience low nutrient concentrations, and are therefore likely to exist in the stationary phase of growth, though the status of a bacterial population along the spectrum of conditions within the stationary phase may vary between systems; similar variations may also be expected over time within any particular system as a result of natural changes in water chemistry. Alternatively, it is possible that this erratic behavior was attributable to a natural chaotic response of the bacteria, which cannot be predicted using conventional (*i.e.*, deterministic) mathematical models.

As described previously, a number of hypotheses have been put forward to account for tailing in microbial dose-response relationships, including: particles, genetic/cultural variability, and resistance induced by disinfectant exposure. Given that the responses of the parent and daughter cultures were indistinguishable (Figure 2), it appears that the resistance was not attributable to natural or induced genetic characteristics. Although the mechanism of bacterial inactivation by UV irradiation is dimerization within cellular nucleic acids, the ability to resist this damage is evidently not passed on to successive generations.

Microscopic examination of the bacterial culture before and after UV irradiation indicated that bacterial aggregation (i.e. particle formation) resulted from irradiation. The fact that these aggregates could not be found in the unirradiated sample suggests that aggregation was attributable to the irradiation process. It is hypothesized that these bacterial aggregates provided shelter to some organisms from the UV radiation, thereby allowing the bacteria to demonstrate a viable response in the membrane filtration assay. Therefore, aggregation by the bacteria could be viewed as a natural defense mechanism for protection from radiation. This observation is consistent with bacterial responses to other forms of stress, many of which also result in aggregation or particle association (e.g. Fletcher and Marshall, 1982; Bossier and Verstraete, 1996).

The results of the epifluorescence microscopic analyses indicated essentially no change in the fraction of the population able to respire attributable to UV exposure, even for doses

Downloaded from http://iwaponline.com/wst/article-pdf/43/10/179/428679/179.pdf
by guest

as high as 100 mW•s/cm^2. In these experiments, approximately 10% of the bacterial population displayed respiratory function, regardless of UV dose. This value is in agreement with literature values for non-disinfected bacterial cultures (Rodriguez *et al.*, 1993; Schaule *et al.*, 1993). Therefore, it appears that bacterial cells can lose viability but retain (at least some forms of) activity following UV irradiation. In particular, the bacterial electron transport process does not appear to be significantly affected by the process of UV irradiation. This hypothesis is supported by the data from the respirometry experiments wherein it was demonstrated that oxygen uptake rates among UV-irradiated bacterial cultures were only marginally affected by UV radiation doses that were capable of causing almost complete inactivation of the population.

The fact that bacteria "survive" UV exposure but are unable to reproduce has several important implications in terms of disinfection operations.

1. Active, non-viable bacteria may be able to regain viability through dark-repair or photoreactivation. The fraction of non-viable bacteria able to regain viability through these processes is largely dependent on the extent of UV-imposed damage (Lindenauer and Darby, 1994). UV applications where water is produced for human contact or consumption should therefore ensure that UV doses are high enough to limit reactivation to acceptable levels.
2. Active, non-viable bacteria are not likely to cause severe illness in humans because they are not able to multiply. However, these bacteria may retain their metabolic functions, which may allow for low-level infections in human hosts if ingested in sufficient numbers.
3. In terms of human disease transmission, it is unclear if microbial "viability" or "activity" will determine pathogenicity. However, a large body of empirical evidence suggests that wastewaters and drinking waters subjected to UV irradiation under conditions commonly used in disinfection practice do not contain viable *or* active microbial pathogens at concentrations that result in communicable disease transmission.

Conclusions

The results of this research point out several important limitations of currently available theories regarding microbial responses to UV radiation. Engineers and scientists involved in the design and implementation of UV disinfection systems for disinfection of potable water or wastewater should be aware of the deviations from simple first-order disinfection kinetics that are commonly assumed. Furthermore, the implications of microbial testing (i.e., viability vs. activity) should also be incorporated into the decision-making process regarding these systems.

Acknowledgements

Much of this work was performed when the first author was on sabbatical leave with Lyonnaise des Eaux, CIRSEE, Le Pecq, France. The authors are grateful for the contributions of Nathalie Castle, Cyril Strec, Philippe Savoye, and Cécile Hamard to this work. Portions of the work were also performed at Purdue University under a grant issued by the National Science Foundation.

References

Blatchley III, E.R. (1997). Numerical Modelling of UV Intensity: Application to Collimated-Beam Reactors and Continuous-Flow Systems, *Wat. Res.*, **31**(9), 2205 –2218.

Bossier, P. and Verstraete, W. (1996). Triggers for Microbial Aggregation in Activated Sludge, *Appl. Environ. Microbiol.*, **45**, 1 –6.

Downloaded from http://iwaponline.com/wst/article-pdf/43/10/179/428679/179.pdf
by guest

Chiu, K., Lyn, D.A., Savoye, P., Blatchley III, E.R. (1999). Integrated Model of UV Disinfection Based on Particle Tracking, *J. Env. Engr, ASCE*, **125**, 1, 7 –16.

Fletcher, M., Marshall, K.C. (1982). Are Solid Surfaces of Ecological Significance to Aquatic Bacteria?, *Appl. Microbial Ecol.*, **6**, 199 –236.

Lindenauer, K.G. and Darby, J.L. (1994). Ultraviolet Disinfection of Wastewater: Effect of Dose on Subsequent Reactivation, *Wat. Res.*, **28**, 805 –817.

Rodriguez, G.G., Phipps, D., Ishiguro, K., Ridgway, H.F. (1992). Use of Fluorescent Redox Probe for Direct Visualization of Actively Respiring Bacteria, *Appl. Environ. Microbiol.*, **58**, 1801–1808.

Shaule, G., Flemming, H.-C., Ridgway, H.F. (1993). Use of 5-Cyano-2,3-Ditolyl Tetrazolium Chloride for Quantifying Planktonic and Sessile Respiring Bacteria in Drinking Water, *Appl. Environ. Microbiol.*, **59**, 3850 –3857.

Severin, B.F., Suidan, M.T., Engelbrecht, R.S. (1983). Kinetic Modeling of U.V. Disinfection of Water, *Wat. Res.*, **17**, 1669 –1678.

Downloaded from http://iwaponline.com/wst/article-pdf/43/10/179/428679/179.pdf
by guest