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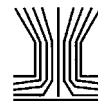
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Effects of Relative Humidity on the Ultraviolet Induced Inactivation of Airborne Bacteria

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Ultraviolet germicidal irradiation (UVGI) as an engineering control against infectious bioaerosols necessitates a clear understanding of environmental effects on inactivation rates. The response of aerosolized *Serratia marcescens*, *Bacillus subtilis*, and *Mycobacterium parafortuitum* to ultraviolet irradiation was assessed at different relative humidity (RH) levels in a 0.8 m³ completely-mixed chamber. Bioaerosol response was characterized by physical factors including median cell aerodynamic diameter and cell water sorption capacity and by natural decay and UV-induced inactivation rate as determined by direct microscopic counts and standard plate counts. All organisms tested sorbed water from the atmosphere at RH levels between 20% and 95% (up to 70% of dry cell mass at 95% RH); however, no concomitant change in median aerodynamic diameter in this same RH range was observed. Variations in ultraviolet spherical irradiance were minor and not statistically significant in the 20–95% RH range. Cell water sorption and inactivation response was similar for each of the pure cultures tested: when RH exceeded approximately 50%, sorption increased markedly and a sharp concurrent drop in UV-induced inactivation rate was observed.

INTRODUCTION

Ultraviolet germicidal irradiation (UVGI) at wavelengths near 260 nm can inhibit the replication of microorganisms collected from UV irradiated airstreams and water (Collins 1971; Gates 1929; Hollander 1942; Riley et al. 1976; Wells and Wells 1936). Successful UVGI applications reported for the disinfection of drinking water and wastewater suggest that UVGI

may be useful in the inactivation of bioaerosols in contaminated indoor air (Qualls and Johnson 1983; Chang et al. 1985; Montgomery Consulting Engineers 1985). Biological aerosol disinfection practice, however, is poorly developed. Previous bioaerosol UVGI investigations (Miller and Macher 2000; Riley et al. 1962, 1971; Macher et al. 1992) coupled with anecdotal (Stead et al. 1996) and epidemiological evidence (Wells et al. 1942) suggest the potential utility of UVGI to control infectious airborne disease and have been useful in defining important environmental and operational parameters. Proper, full-scale bioaerosol disinfection (inactivation) system design requires a comprehensive understanding of the environmental factors that influence the inactivation rates of airborne bacteria.

Relative humidity (RH) may be an important consideration in the UV-inactivation of room air; the literature, however, contains little information regarding microorganism-specific inactivation rates that consider RH as a sensitive variable. Previous studies conducted in a plug flow aerosol chamber (Riley and Kaufman 1972, p. 93) reported significant decreases in the UV-inactivation rate of airborne *Serratia marcescens* at RH levels above 60%. Experiments with *Mycobacterium smegmatis* (Gillis 1974) and *E. coli* (Wells and Wells 1936) also produced decreases in UV-inactivation rates with increases in RH. While the literature generally supports this conclusion, other studies performed with *E. coli* (Rentschler et al. 1941) demonstrate no specific relationship between UV-inactivation rate and RH. Further, an increase in the lethal effect of sunlight with increasing RH has been observed for *Streptococcus pyogenes*, *Staphylococcus aureus*, and a general mix of naturally occurring airborne flora (Lidwell and Lowbury 1950). Indeed, the literature in this area is tenuous.

The mechanism(s) responsible for the dependence of UV-inactivation on RH are poorly understood. The existing bioaerosol literature in this area is dated, somewhat anecdotal, and describes only empirical UV-inactivation-RH observations; the mechanistic basis for this relationship has not been demonstrated. UV irradiance, and thus bioaerosol dose, has been implicated as labile to attenuation by vapor at higher RH levels. Water sorption onto airborne cells may also provide protection

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against UV-induced DNA damage as RH increases. Riley and coworkers (Riley and Kaufman 1972) reported an increase in the fraction of cells observed on stage five (aerodynamic diameter [d_{50}] 1.0–2.0 μm) of an Andersen sampler relative to stage six (<1.0 μm) as the RH increased (from 20% to 90%) while UV-inactivation rate decreased. However, this research did not report specific impactor stage recoveries or aerodynamic diameter analysis for the multiple RH levels tested. Small increases in the aerodynamic diameter of fungal spores ($\cong 10\%$ from 30% to 90%) have been observed in response to increases in RH, but were only significant ($\cong 20\%$ increase) at RH levels above 90% (Madelin and Johnson 1992; Reponen et al. 1996). While the aerodynamic diameter of airborne microorganisms does not appear to respond to RH, the mass of some airborne bacteria does. Water sorption studies with *Serratia marcescens* and *Bacillus subtilis* revealed that these bacteria can sorb up to 60% of their dry weight in water when RH was increased from 20% to 99% (Bateman et al. 1962; Rubel 1997).

Germane to bioaerosol inactivation, water sorption may also produce changes on biopolymers interior to the cell. Relationships reported between RH and cellular water uptake suggest that biological aerosols can act as a condensation site for water vapor. Sorption of water from the atmosphere hydrates nucleic acids, proteins, and cell wall components and may change the character of the cell wall or other important biopolymers in a manner that protects the cell from desiccation and/or attenuates incident UV irradiation. Cell-water vapor interactions may be organism dependant given the variable physiology of bacteria.

In response to the limited data available regarding the relationship between RH and UV-inactivation rates of airborne bacteria, we determined the effects of RH on the following factors: UV-inactivation rate, natural decay rate, UV spherical irradiance, aerodynamic diameter, and cell water sorption capacity. These parameters were measured for three pure bacterial cultures in the RH range between 20% and 95%. Experiments were executed in a 0.8 m^3 completely-mixed bioaerosol chamber with a well-characterized UV spherical irradiance field.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

All cultures used in this study were supplied by the American Type Culture Collection (ATCC) (Manassas, VA). *Bacillus subtilis* (ATCC #090287) is a Gram stain positive rod 2–3 μm in length with a red or brown pigment (Claus and Berkeley 1984). Only vegetative cells were used; no experiments were performed with *B. subtilis* spores. *Mycobacterium parafortuitum* (ATCC #19689) is a Gram stain positive rod 2–4 μm in length, yields pale yellow colonies, and is considered a rapid growing *Mycobacterium* (Wayne and Kubica 1984). *Serratia marcescens* (ATCC #13880) is a Gram stain negative rod with pigment that is influenced by culture conditions (Grimont and Grimont 1984). Each strain was grown on Soybean-Casein Digest Agar (SCDA) (Difco Laboratories, Detroit, MI) at 37°C. *S. marcescens* exhibits

a light brown pigment under these conditions. Cultures were removed from agar plate surfaces by scraping and were suspended in sterile deionized water. Immediately before aerosolization, cell concentrations were diluted to a concentration of approximately 1×10^9 CFU/ml. Microscopic examination of bacterial suspensions (via wet mount) was used to confirm the dispersed state of cells prior to their aerosolization.

Bacterial Enumeration

Both culturable and total bacteria were quantified in the aerosolization stock and from impinger-recovered air samples. A modification of the standard plate count method was used to enumerate culturable bacteria. Within 2 h after collection, samples from liquid impingers were diluted (usually 1:10) in 50 mM phosphate-buffer saline (PBS) (150 mM NaCl, pH 7.2) solution and plated using a spiral plater method (Spiral Biotech, Inc., Bethesda, MD) according to the manufacturer's recommendations. At least three replicates of each sample were plated. *B. subtilis* and *S. marcescens* were incubated at 37°C for 24 h and *M. parafortuitum* was incubated at 37°C for 3 days. A comparison of spiral plater counts with standard spread plate counts showed no difference between methods (based on an independent *t*-test, $\alpha = 0.05$) and that the spiral plater method variability was lower than that of the spread plate method (coefficient of variance (CV) was 5% lower for the spiral plating method, $n = 10$). All plating was performed in indirect dimmed light, and incubations were carried out in the dark to control for photoreactivation.

Epi-fluorescent microscopy was used to enumerate total bacteria (culturable and nonculturable). All cells were stained with 4'-6-diamidino-2-phenylindole (DAPI) (Sigma Chemicals, St. Louis, MO), a DNA binding fluorescent stain (Ward et al. 1965) in accordance with previously described methods (Hernandez et al. 1999). Samples from total cell counts were stained at a final concentration of 0.5–1.0 μg DAPI/ml, incubated for 1 min at room temperature, and filtered through 25 mm diameter, 0.2 μm , black polycarbonate filters (Poretics, Inc., Livermore, CA) as previously described (Hobbie et al. 1977). At least 5–10 random fields and >200 total cells were counted per slide. All direct counts were reported as the average of 5–10 fields. Direct counts from aliquots having CVs >30% were discarded and new sample aliquots were stained and counted until a uniform distribution was observed (Hernandez et al. 1999). The average CV for all direct counts was 13.3%. Mounted filters were examined under 1100 \times magnification using a Nikon Eclipse E400 epi-fluorescent microscope fitted with a mercury lamp and polarizing filters (HBO-100 W mercury lamp; D360/40 excitation filter; 420 emission filter; 400DCLP beamsplitter; ChromaTechnology Corp., Brattleboro, VT).

Aerosol Chamber

A pilot scale chamber (0.8 m^3) was constructed (Figure 1) to determine bacterial bioaerosol inactivation rates from UV irradiation under sustained RH levels and well-mixed conditions. The chamber was cubic and constructed of 1.27 cm thick clear

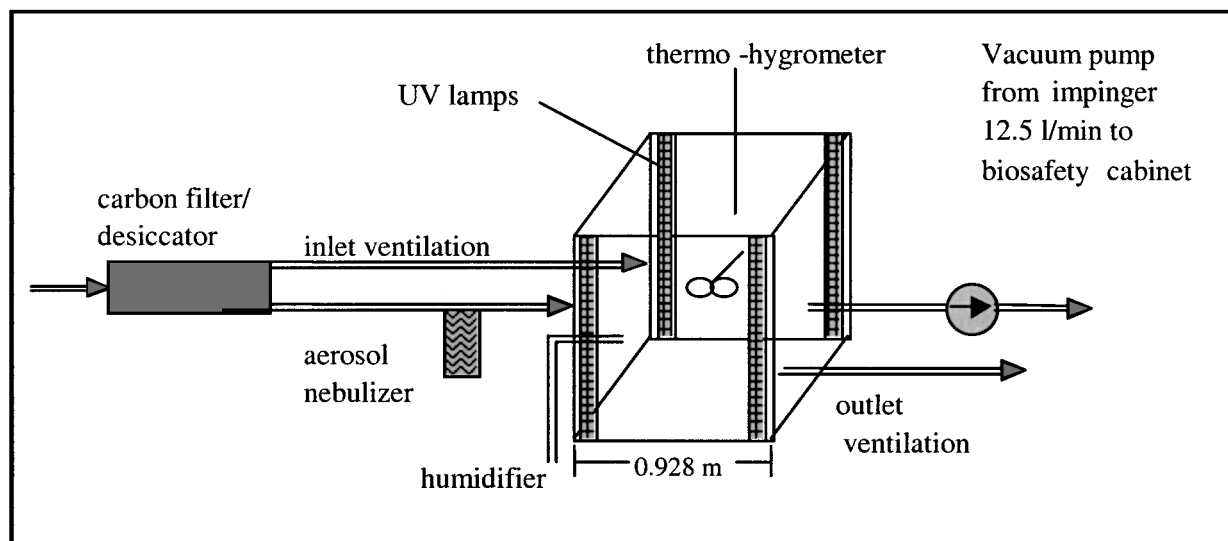


Figure 1. Schematic of 0.8 m³ aerosol chamber used in this study. The chamber was cubic and constructed of Lucite.

Lucite plastic. The chamber could be operated in completely-mixed flow-through reactor mode (CMFR) or batch (no flow) mode. Experiments were conducted to most accurately determine UV effects in realistic settings; thus UV experiments were performed in CMFR mode rather than batch mode.

Calibration and Flow Regime. Air was supplied to the chamber using compressed outdoor air. Supply air was filtered through a 20 cm × 3.8 cm (I.D.) cylindrical PVC filter containing equal volumes of size 6–14 mesh activated carbon (Fisher Scientific, Pittsburgh, PA) and Drierite indicating desiccant (W.A. Hamond Drierite Company Ltd., Xenia, OH). Two Nalgene (0.63 cm I.D.) tube lines equipped with inline 0.2 μm particle filters (Bacterial Air Vent, Gelman Sciences, Port Washington, NY) supplied inlet air that was metered by two 50 l/min capacity flow meters (Gilmont Instruments, Barrington, IL). The chamber was mixed by three 1.5 watt fans (Caframo Model 727TT, Wiarton, Ontario, Canada). Flow meter chamber calibration and mixing efficiency were determined using a nonreactive tracer gas, sulfur hexafluoride (SF₆). A mixture of 82.43% He and 17.57% SF₆ was injected into the chamber and allowed to mix for 1 min. The chamber was operated in CMFR mode at given flow meter settings and samples were taken at 5 symmetrically distributed chamber locations including the outlet. Flow meter settings were calibrated to air exchange rates using average SF₆ exponential decay observed at the 5 sampling points distributed in the chamber. Tracer gas concentrations were measured by gas chromatography (Model 101, Lagus Applied Technology Inc., San Diego, CA). Air exchange rates for each flow meter setting were determined three separate times. The chamber was calibrated to air exchange rates between 1.0 h⁻¹ and 7.35 h⁻¹.

Chamber ventilation effectiveness (ε_c) was calculated using four sample locations symmetrically distributed in the chamber according to Equation (1) and previously described methods

(Brauns 1991).

$$\frac{C_e - C_o}{C_r - C_o} = \varepsilon_c, \quad [1]$$

where C_e (μl/m³) is the concentration in outlet air, C_o (μl/m³) is the concentration outside of the reactor, and C_r (μl/m³) is the concentration at location of interest in the reactor (μl/m³). An ε_c of 1 corresponds to a completely-mixed chamber. Chamber ventilation effectiveness for four sample points throughout the chamber were (± std. err, $n = 12$) 1.02 ± 0.004, 0.98 ± 0.007, 1.02 ± 0.012, and 1.0 ± 0.014 for air exchange rates of 1.0 h⁻¹, 2.8 h⁻¹, 4.6 h⁻¹, and 7.3 h⁻¹ respectively. The chamber behaved as a nearly ideal completely-mixed system at all exchange rates used in this study.

UV Lamps and Irradiance Quantitation. Thirty watt low-pressure, mercury vapor, (G30T8, Osram-Sylvania, Hanover, MA) UV lamps were installed to irradiate the contents of the aerosol chamber. To provide a uniform line source, lamps were installed in each corner and extended the full vertical length of the chamber (Figure 1). Lamps were wrapped in 8 layers of aluminum air-conditioning filter mesh (Research Products Corporation, Madison, WI) to attain the desired experimental UV irradiance levels. The screens attenuated original irradiation by approximately two orders of magnitude. Screens were placed at a 1 cm gap from the bulb surface to ensure the proper operating temperature of the lights. Real-time monitoring of irradiation using a radiometer with a narrow-band 254 nm filter (International Light, Newburyport, MA) revealed that lamps converged to a constant irradiance within a 15 min warm-up time at a chamber air exchange rate of 3.5 h⁻¹ (normal operating conditions). The spectral power distribution (SPD) for the brand and model of the UV lamps used showed that 95% of power was emitted

at 253.7 nm after 100 h of operation (Levin 1999). Lamps were operated for 100 h before their use in these experiments.

Chemical actinometry was used to quantify UV irradiance according to previously described methods (Rahn 1997; Rahn et al. 1999). A 0.6 M solution of KI with 0.1 M KIO₃ in a 0.01 M borate buffer, pH 9.25, was loaded into 0.5 cm³ spherical quartz actinometry cells. This solution is transparent to wavelengths >330 nm and opaque to wavelengths <290 nm. The spherical geometry of the cells simulates the radial irradiation to which airborne bacterial cells are subjected, hence this irradiance measurement was termed spherical irradiance (Rahn et al. 1999). In this chemical actinometry, triiodide is formed in the presence of UV irradiation. Triiodide was measured spectrophotometrically at 352 nm with a UV 160U Shimadzu recording spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD), and the dose was calculated according to published calibration data (Rahn 1997; Rahn et al. 1999). Actinometry solutions were prepared immediately before each experiment.

Actinometry was used to measure spherical irradiance at multiple locations in the chamber through the range of RH levels tested. This method measures the irradiation incident upon a spherical object and was used to more accurately estimate irradiance which bacteria (nearly spherical) were exposed to in this study. For each experiment, actinometry cells were hung by thin threads to form a symmetrical 25 point sampling grid in the chamber. Spherical irradiance at three equally spaced levels representing the top, middle, and bottom of the aerosol chamber was measured. Five RH levels, 20%, 40%, 60%, 80%, and 95%, were tested in this manner. RH was controlled in the chamber using an ultrasonic humidifier filled with sterile deionized water (Holmes Products Inc., Milford, MA). Two wall-mounted digital hygrometers/thermometers that were calibrated for use between 25% and 95% (Fisher Scientific, Pittsburgh, PA) were used to monitor RH. Three 1.5 W fans ran continuously inside the chamber during the experiments to ensure an even distribution of water vapor and aerosolized bacteria. The UV lamps were turned on to achieve steady-state operating temperature, and actinometry cells were subsequently exposed to UV irradiation for 30 min. The actinometry cells were monitored for condensation at RH levels >60%. Average UV spherical irradiance was calculated using the 25 grid data at each height level and RH.

Aerosolization and Collection. Bioaerosol was generated using a Collision six-jet nebulizer (BGI Inc., Waltham, MA) operated at 20 psi. Air supplying the nebulizer was carbon filtered and desiccated through a filter apparatus (Model 3074, TSI, Inc., St. Paul, MN) and particle filtered by a 0.2 μm particle filter (Bacterial Air Vent, Gelman Sciences, Port Washington, NY) immediately before introduction into the nebulizer. The nebulizer was connected to the front of the chamber through a 5/8" Swagelok compression fitting (Denver Valve and Fittings Co., Denver, CO). One 1.5 W fan was directed toward the effluent nebulizer stream to ensure immediate mixing upon entering the chamber.

Aerosols were collected by glass impingers (AGI-30, Ace Glass Inc., Vineland, NJ) and 6 stage impactors (Andersen Instruments, Smyrna, GA). Six impinger sampling ports, each connected to flow meters with control valves (model 32460-48, Cole-Parmer, Vernon Hills, IL), were connected to a rotary vane-type vacuum pump (Gast Model 0523-V4A-G180DX, Gast Inc., Benton Harbor, MI) and collected air at 12.5 l/min. Before each experiment, impingers were washed with deionized water, autoclaved, and wrapped with aluminum foil to shield collected bacteria from further UV irradiation. Sterile 50 mM PBS was used as the impinger collection media.

Andersen 6 stage impactors were used for determining geometric mean aerodynamic diameter and associated geometric standard deviation (ACGIH 1999) at different RH levels within the chamber. The impactors had the following six size ranges of aerodynamic diameters defined by stage cut-off diameter d_{50} : (6) 0.65–1.0 μm; (5) 1.1–2.0 μm; (4) 2.1–3.2 μm; (3) 3.3–4.6 μm; (2) 4.7–6.9 μm; and (1) ≥ 7 μm. The impactor was connected to a vacuum pump (model 10 709, Anderson Samplers Inc., Atlanta, GA) which collected air at 28.3 l/min. Agar plates loaded into the impactors were prepared according to the manufacturer's recommendations using Soybean-Casein Digest Agar (Difco Laboratories, Detroit, MI). Bacteria were collected from the chamber air after equilibration with atmospheres containing 20%, 40%, 50%, 60%, 80%, and 95% RH; these experiments were performed in triplicate. Impinger and impactor pumps were calibrated before each experiment using a bubble generator (Gillian Instrument Corp., Clearwater, FL).

Chamber Operation and Experimental Protocol. UV-inactivation experiments were performed in the bioaerosol chamber after UV lamps were warmed to achieve operating temperature. Lamps were turned off and bacteria were aerosolized into the chamber for 5 min. During the first 3 min of aerosolization, chamber RH levels were adjusted to a predetermined level and maintained throughout the experiment. Once initial airborne bacteria concentrations and chamber RH was achieved, ventilation was initiated at 3.5 h⁻¹ and a "zero time" sample was collected to determine the initial airborne bacteria concentration. The UV lamps were then turned on and five sequential air samples were taken at predetermined time intervals. The time intervals were set such that concentrations could be measured through at least three effective air changes. After the final sample was collected, the chamber was evacuated at a rate of 8 h⁻¹ and impinger samples were analyzed according to the methods described above. All chamber experiments and sample handling were performed in the dark to control for photoreactivation effects. As in the ventilation effectiveness testing, the chamber was well mixed by three 1.5 W fans.

Reported UV-Inactivation-Z Value: Normalizing Chamber Air Exchange Rates to UV Dose. All chamber experiments yielded total and culturable cell counts as a function of time. First order rate coefficients were used to describe the exponential decay of cells within the chamber, and hence equivalent air exchange rates were determined according to a completely-mixed

flow chamber model for total and culturable bacteria:

$$\frac{dN_t}{dt} = -(\lambda_{vent.} + EAC_{dep.+other})N_t, \quad [2]$$

$$\frac{dN_c}{dt} = -(EAC_{natural} + EAC_{UV})N_c, \quad [3]$$

where $\lambda_{vent.}$ (h^{-1}) is the loss rate of total bacteria due to ventilation only (approximated by SF₆ decay); $EAC_{dep.+other}$ (h^{-1}) is the loss rate of total bacteria (expressed as Equivalent Air Changes) due to deposition and other physical removal processes expressed as equivalent air exchange rate (total count derived rate $-\lambda_{vent.}$); $EAC_{natural}$ (h^{-1}) is the loss rate of culturable bacteria due to natural decay expressed as equivalent air exchange rate (total count derived rate–UV off plate count derived rate); EAC_{UV} (h^{-1}) is the loss rate of culturable bacteria due to UV-inactivation as equivalent air exchange rate (UV off plate count derived rate–UV on plate count derived rate); N_t (#cells/m³) is the concentration of total (culturable and nonculturable) airborne bacteria determined by direct microscopy; N_c (#CFU/m³) is the concentration of culturable airborne bacteria determined by plating.

Assuming no other unaccounted removal mechanisms are relevant in the chamber, deposition can be reported as an “inclusive” deposition velocity (Nazaroff et al. 1993) (v_d):

$$v_d = EAC_{dep.+other} \cdot \left(\frac{V}{S}\right), \quad [4]$$

where V (m³) is the chamber volume and S (m²) is the chamber internal surface area.

For this chamber configuration, effective air exchange rates were normalized to average spherical irradiance and reported as Z values [cm²/μW · s] (Kethley 1973; Riley et al. 1971):

$$Z = \frac{\ln N_o/N_{UV}}{UVdose} = \frac{EAC}{I}, \quad [5]$$

where N_o (#CFU/m³) is the airborne bacteria culturable concentration with no UV; N_{UV} (#CFU/m³) is the airborne bacteria culturable concentration with UV; and I (μW/cm²) is the average chamber spherical irradiance.

To estimate variability and determine an average Z value for each aerosolized bacterial culture under the RH condition tested, UV irradiation and natural decay experiments were executed in three independent trials. Replicate UV-on and UV-off EAC rates for each RH were randomly paired, and averages and standard errors were calculated from the three trials.

Cellular Sorption of Water Vapor

The amount of water sorbed from water vapor at RH levels between 20% and 95% was experimentally determined for *S. marcescens*, *B. subtilis*, and *M. parafortuitum*. Sorption experiments were performed in the 0.8 m³ aerosol chamber, and RH and mixing was controlled as described above. Aluminum

weighing tins (Environmental Express, Mt. Pleasant, SC) holding thin films of desiccated cells were placed on an analytical balance (Mettler Toledo AB54, Mettler, Switzerland) contained in the chamber. Desiccated bacterial films were prepared by drying known quantities of cells suspended in deionized water at <10% RH for 24 h at room temperature. To determine the quantity of excess water left on the cells after the initial desiccation at room temperature, and to close a comparative water mass balance, duplicate tins were immediately weighed, then placed in a 105°C oven overnight, then re-weighed.

The analytical balance was tarred, and cells were allowed to equilibrate with the vapor in the chamber, which was initially maintained near 20% RH. The RH was increased incrementally and the cells' weights recorded. Weights were recorded only after sorptive equilibrium was reached between the immobilized cells and water vapor (ca. 15–45 min for RH < 55% and 45–65 min for RH > 55%). To calculate the weight of water per total dry weight of cells, dry cell weight was determined by drying the hydrated samples in a 105°C oven overnight. Three independent experiments were performed for each pure culture tested. Desiccated aluminum weighing tins containing no bacteria served as controls.

RESULTS

Spherical Irradiance

The average spherical irradiance did not change significantly in response to increases in chamber RH. These results suggest no attenuation of UV spherical irradiance within the RH range tested. A one way analysis of variance (Berthouex and Brown 1994; ANOVA; $\alpha = 0.05$) applied to the average spherical irradiances for all RH levels tested showed that RH had no effect on spherical irradiance. The average spherical irradiance measured in the chamber was 7.53 μW/cm² (± standard error 0.13 μW/cm²) ($n = 15$). Average spherical irradiance for the chamber with two lights operating (all other measurements were for 4 lamps) was 3.7 μW/cm².

Spatial distribution of spherical irradiance in the chamber is summarized in Figure 2. The mean spatial coefficient of variance for 25 sample points in all chamber experiments was 37.5% ± 1.3%. The average spherical irradiance at the bottom level of the chamber (23.25 cm from the floor) was 7.0 μW/cm² ± 0.1 μW/cm². The middle level (46.5 cm from the chamber floor) had an average spherical irradiance of 7.8 μW/cm² ± 0.02 μW/cm², and the top level (69.75 cm from the chamber floor) had an average spherical irradiance of 7.7 μW/cm² ± 0.02 μW/cm².

Aerodynamic Diameter

Andersen six stage impactors were used to determine median aerodynamic diameter of *Bacillus subtilis*, *Serratia marcescens*, and *Mycobacterium parafortuitum*. No trend of increased cell impaction on higher aerodynamic diameter cut-off impactor stages was observed with increasing RH. As judged by the

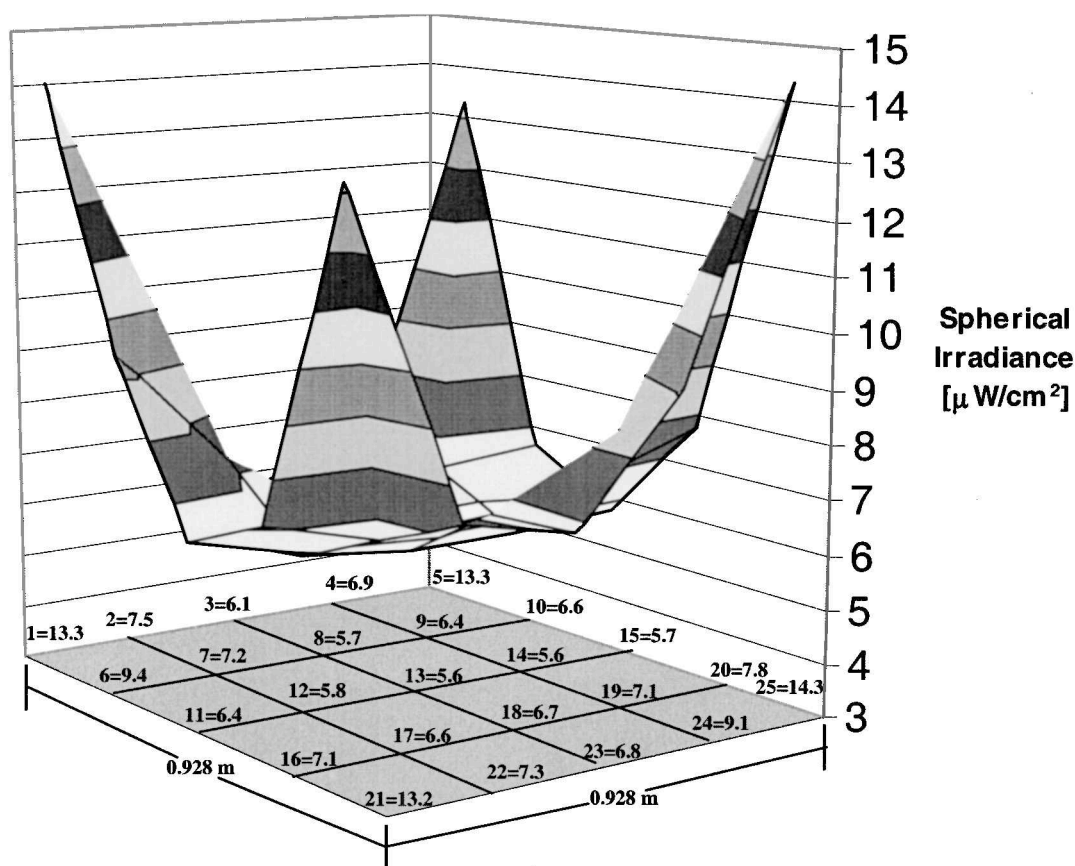


Figure 2. Spatial distribution of spherical UV irradiance in aerosol chamber using one lamp (line source) in each corner. Characteristic plot shown is for the middle tier of the reactor at 60% RH. The irradiance observation from the 25 point sampling grid is demonstrated below the plot along with the spherical irradiance measured at each point in $\mu\text{W}/\text{cm}^2$. Average spatial spherical irradiance for this plot is $7.94 \mu\text{W}/\text{cm}^2$; average for 15 independent determinations (5 RHs at 3 reactor levels) of average spatial spherical irradiance was $7.53 \mu\text{W}/\text{cm}^2 \pm 0.16 \mu\text{W}/\text{cm}^2$. Grid points are spaced at 23.2 cm.

Anderson impactor, airborne bacteria cells size did not change in response to increasing RH. At 95% confidence, an ANOVA concluded that the variance between triplicate median aerodynamic diameter determinations for each RH level tested was greater than the variance between average median aero-

dynamic diameters for all RH levels tested. The average standard error for triplicate median aerodynamic determinations at a single RH level was $0.14 \mu\text{m}$. Average median aerodynamic diameter for each pure culture tested are reported in Table 1.

Table 1

Average median aerodynamic diameter and average deposition velocity for *S. marcescens*, *B. subtilis*, and *M. parafortuitum* for 20%–95% RH and water vapor first order exponential sorption coefficients

Organism	Aerodynamic diameter		k (%RH ⁻¹) ± standard error (n = 3)*	Average deposition velocity (m/h), V/S = 0.16 ± standard error (n = 5)
	GM (μm)	GSD		
<i>S. marcescens</i>	2.8	1.3	0.024 ± 0.004	0.06 ± 0.03
<i>B. subtilis</i>	2.7	1.3	0.029 ± 0.003	0.06 ± 0.02
<i>M. parafortuitum</i>	2.0	1.3	0.032 ± 0.003	0.1 ± 0.1

*First order model = $dw/dRH = k (\% \text{RH})$, where w = equilibrium weight of water/weight of dry cell mass.

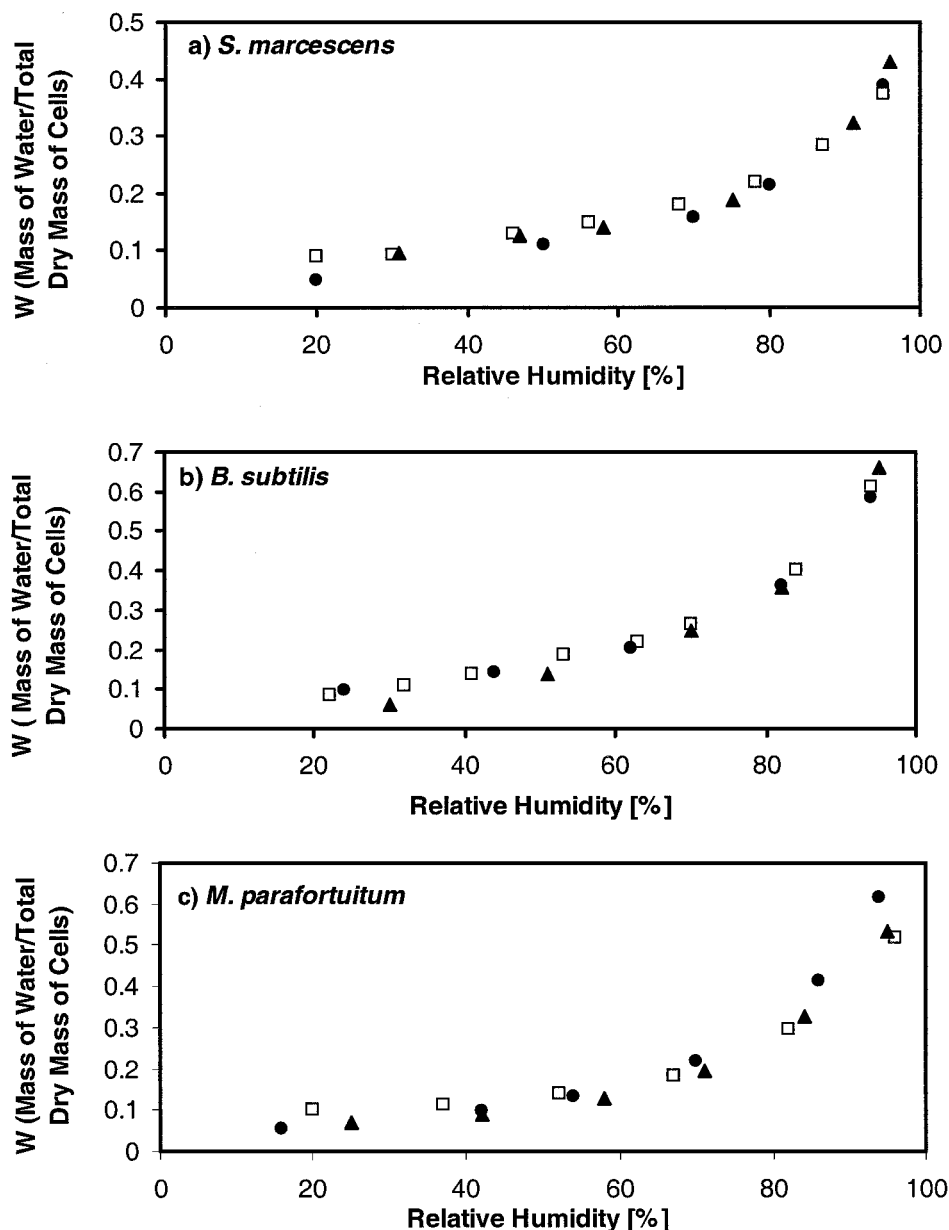


Figure 3. Water sorption isotherms for (a) *Serratia marcescens*, (b) *Bacillus subtilis*, and (c) *Mycobacterium parafortuitum*. Symbols represent replicate isotherm experiments.

Sorption of Water Vapor onto Cells

Figure 3 presents water sorption isotherms for the three species used in this study. Only a small increase in cellular water sorption was observed until RH approached 65%. Throughout the entire range tested, specific cellular water vapor sorption data were unsuccessfully fit to Langmuir and BET isotherm sorption models that describe single and multilayer adsorption, respectively (Sawyer et al. 1994). To compare extent and rate between pure cultures, the sorption data was successfully fit (all r^2 values were > 0.98) with a first order exponential model. Empirical sorption coefficients are presented in Table 1.

Z Values and Natural Decay Rates and Deposition Velocity

Z values and natural decay rate coefficients for *S. marcescens*, *B. subtilis*, and *M. parafortuitum* at different RH levels are presented in Figure 4. "Natural decay" is defined here as an endogenous loss of culturability under the environmental conditions tested—not including UV-inactivation. Equivalent air exchange rates for natural decay could not be accurately measured to a resolution of less than a 1 h^{-1} air exchange rate. A hypothesis stating that the average value for natural decay rates at all RH levels tested were equal was credited through an ANOVA,

$\alpha = 0.05$, for *B. subtilis* and *S. marcescens* and discredited for *M. parafortuitum*. A single natural decay rate observation for *M. parafortuitum* at 60% RH was responsible (average at 60% = 2.8 h^{-1} compared to 0.8 h^{-1} average for all other RH levels). A similar ANOVA was performed for deposition velocities for

all RH levels tested. No measured dependence of deposition on RH could be observed at 95% confidence. Average deposition velocities for each pure culture used are presented in Table 1. No condensation was observed in the chamber at any RH level studied.

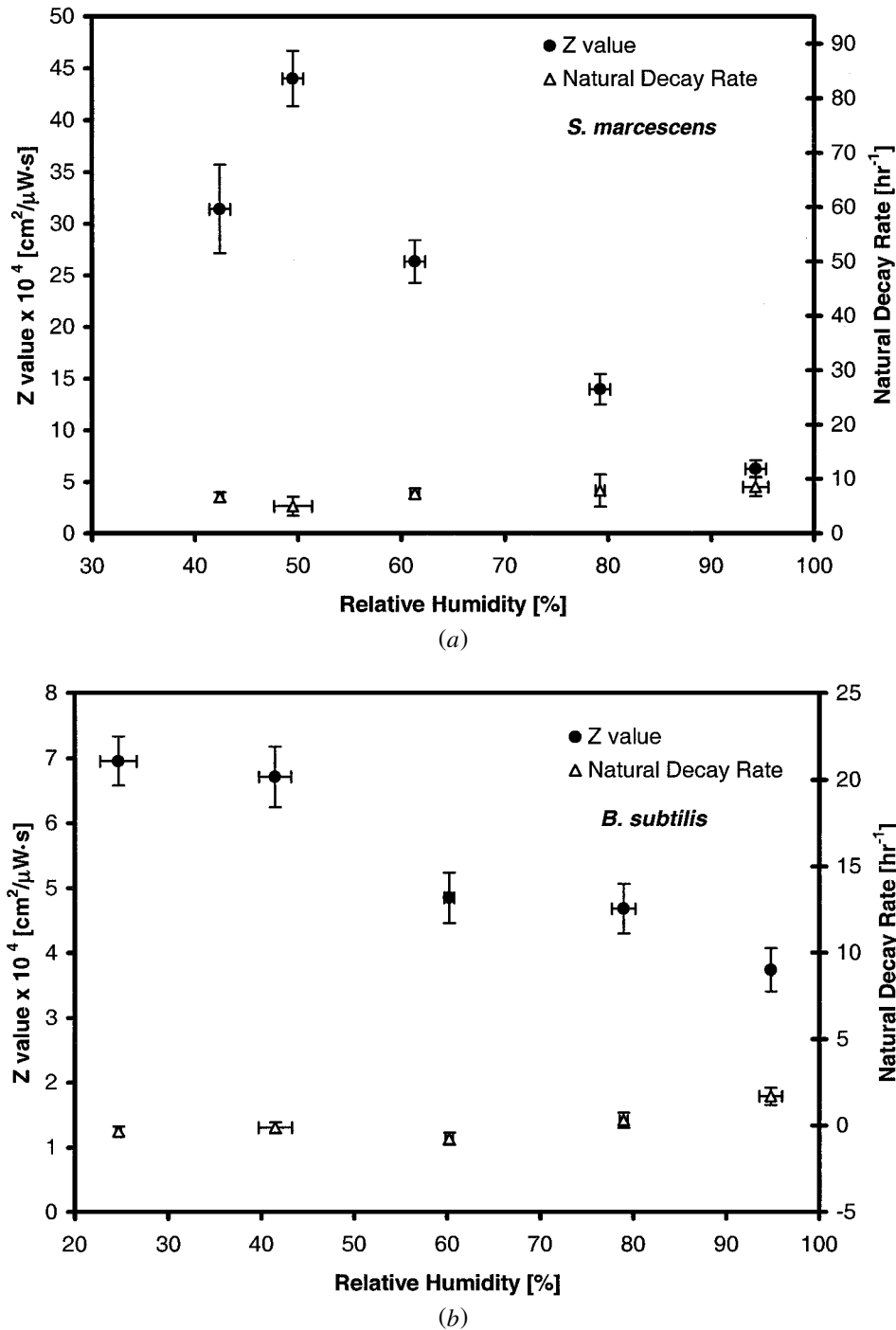


Figure 4. Z values for aerosolized (a) *Serratia marcescens* (●), (b) *Bacillus subtilis* (●), and (c) *Mycobacterium parafortuitum* (● – 4 lamps, ○ – 2 lamps) at different RH levels. Natural Decay as equivalent (□) air exchange rates are presented on the right abscissa. These axes are scaled to reflect the maximum UV induced equivalent air exchange rate but correspond accurately to only the natural decay rates. (Continued)

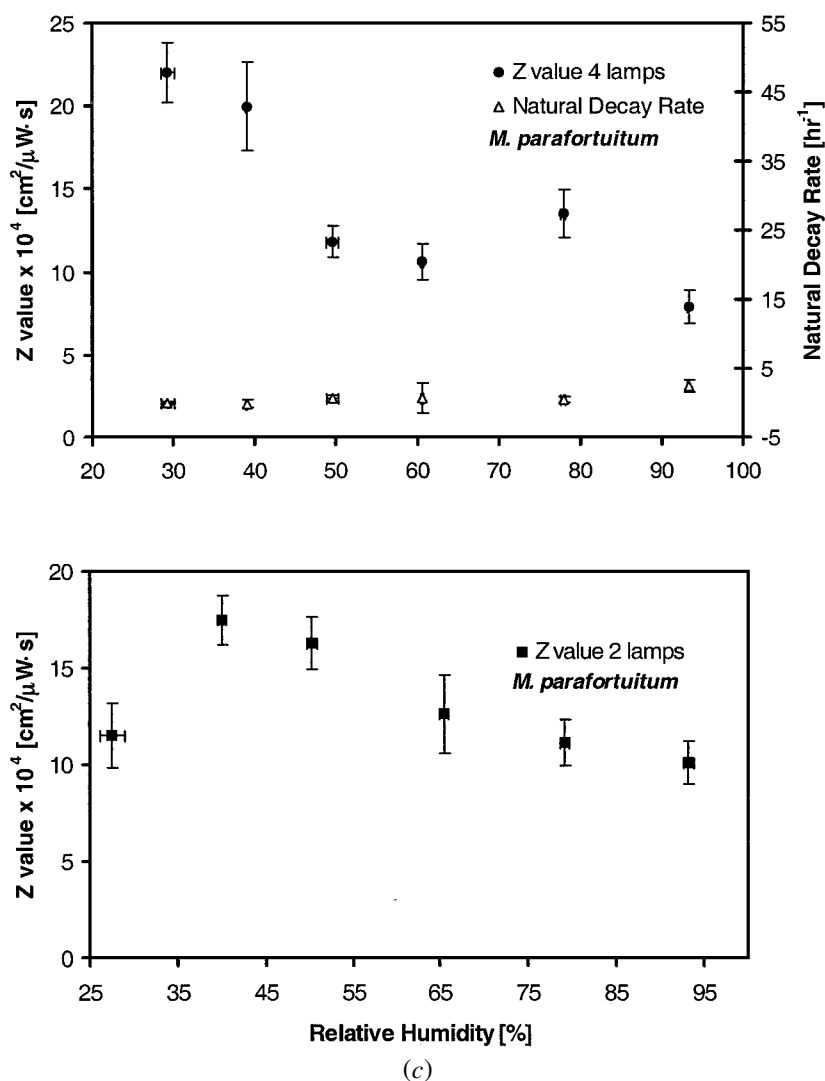


Figure 4. (Continued)

Control for Equivalent Bioaerosol Loading

Total microscopic counts were used as a control to ensure that UV-on and UV-off experiments were conducted at approximately the same concentrations and under the same collection, airflow, and mixing conditions. A comparison of these total count derived rates, using an independent *t*-test ($\alpha = 0.05$), indicated that the difference between the UV-off and UV-on direct count measured air exchange rates for each experiment was insignificant; therefore the ventilation and deposition decay, and other physical removal mechanisms, were the same for all experiments. Representative results for each pure culture at 60% RH are presented in Figure 5.

DISCUSSION

Our results, and those previously reported (Gillis 1974; Riley and Kaufman 1972; Wells and Wells 1936), support the con-

clusion that there is a significant decrease in airborne bacteria inactivation rates induced by UV irradiation at RH levels in excess of 50%. Above 50%, Z values in this experiment decrease, apparently asymptotically, to a final value that is greater than natural decay. This Z value trend was observed in each pure culture regardless of cell wall physiology or Gram stain type. The maximum change in Z value, from a maximum near 50% to a minimum at 95%, however, was different for each pure culture tested. *S. marcescens* Z values decreased by a factor of 6.5, *B. subtilis* by a factor of 1.5, and *M. parafortuitum* by a factor of 3.2 over this RH range. While Gram stain positive cells (cell wall) appear to be less susceptible to the RH effect, water sorption rate and extent measured in this study were independent of Gram stain type.

Table 2 lists Z values determined in this investigation as well as values previously published. Z values determined for *Mycobacterium parafortuitum* in this study were slightly lower

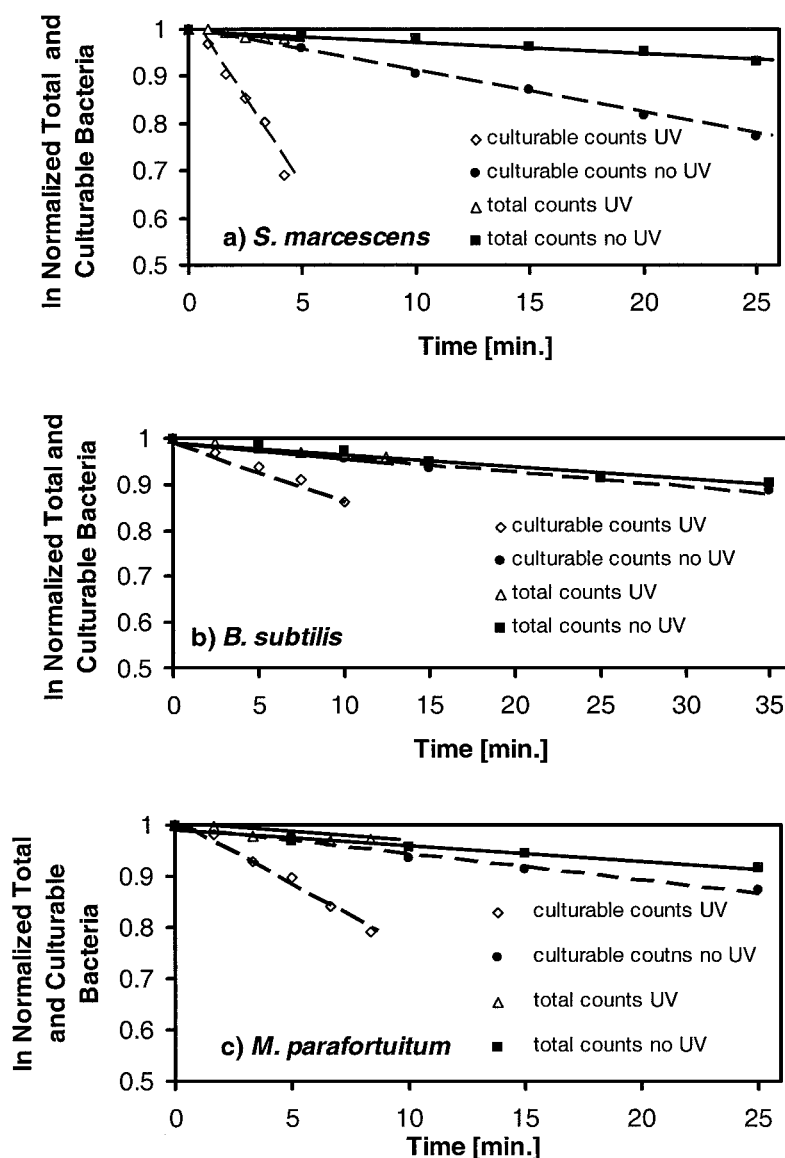


Figure 5. Summary of a typical Z value experiment. Plots report the normalized, natural log direct ($\#/m^3$) and culturable counts ($\#/CFU/m^3$) of (a) *Serratia Marcescens*, (b) *Bacillus subtilis*, and (c) *Mycobacterium parafortuitum* at 60% RH. Lines are linear curve fits of the normalized natural log of culturable counts (---) and total counts (—). Z values reported were determined from three such experiments performed for each RH. All experiments were performed at a chamber UV spherical irradiance of $7.53 \mu W/cm^2 \pm 0.13 \mu W/cm^2$.

than values previously published for *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis* strains and greater than those values reported for *Mycobacterium phlei* (Riley et al. 1976). The response (as judged by Z value) of airborne *S. marcescens* determined in our studies was markedly lower than that previously reported. This difference, and other possible differences reported, for *Mycobacterium* species associated Z values may be due to the underestimation of UV irradiance (and therefore overestimation of Z values) inherent in the radiometer measurements used in previous studies. Radiometers measure UV at flat surfaces and cannot account for the spherical geometry

and total radial irradiance incident on airborne bacterial cells (Rahn et al. 1999). Additionally, methods and airflow velocity measurements, and hence dose, reported in previous plug flow-based UV-irradiation experiments (Riley and Kaufman 1972; Riley et al. 1976) were not described and therefore the results are difficult to interpret. Our experiments used liquid impinger samplers instead of the previously used agar impactors. Although similar collection efficiencies for Andersen six stage impactors and AGI-30 impingers have been observed (Jensen et al. 1992), cell desiccation and impact stress caused by impactors (Stewart et al. 1995), and the instantaneous hydration

Table 2
Reported *Z* values at 50% RH

Organism	<i>Z</i> value × 10 ⁴ at (cm ² /μW · s)	Reference
<i>Mycobacterium tuberculosis</i>	(23–42) Erdman strain	Riley et al. 1976
	(44–55) 199RB	Riley et al. 1976
<i>Mycobacterium bovis</i> BCG	(33–39) culture #1	Riley et al. 1976
	(23–28) culture #2	Riley et al. 1976
<i>Mycobacterium phlei</i>	(2.0–5.3)	Riley et al. 1976
	(10)	Kethley 1973
	(14) Gordon 644-5	Gillis 1973
<i>Mycobacterium smegmatis</i>	(19) ATCC 607	Gillis 1973
<i>Mycobacterium parafortuitum</i>	(12–15)	This study
	(20–22)*	This study
<i>Bacillus subtilis</i>	(6.3–6.6)*	This study
<i>Serratia marcescens</i>	(183–245)	Riley et al. 1976
	(35–45)**	This study

*Results from 20% and 40% RH, where RH has no observable effect on *Z*.

**Results from 40–50% RH with four lamps.

that occurs in impinger reservoirs (Terzieva et al. 1996), suggest that UV-inactivation rates determined by culturability may be influenced by the type of sampler used. It appears there is no data readily available that compares the effects of liquid impingement and agar impaction on the measurement of *Z* value. Well-mixed full-scale room air UV-inactivation rates of *M. bovis* BCG (Riley et al. 1976) would be useful to compare with the *M. parafortuitum* *Z* values reported herein as the reactor configurations (CMFR) are similar; however, UV irradiance values were not reported in this BCG study. Nicas and Miller (1999) estimated UV irradiance values in Riley's full-scale room experiment and calculated a *Z* value of 5.3 cm²/μW · s; however, the experiments were performed under winter conditions and the mixing between the upper and lower regions of the room was not characterized. Given these differences in experimental design, comparisons of *Z* value magnitude between *M. parafortuitum* and other *M. tuberculosis* surrogates should remain tentative until comparative experiments are performed in the same type of well-mixed chamber (i.e., plug flow or CMFR).

Under identical low RH (RH < 40%) conditions, the *Z* value response of *M. parafortuitum* was different when the UV dose was decreased (two lamps instead of four lamps). While *Z* values for the two and four lamp systems are similar above 40%, there is a twofold difference at RH = 30%. Also, a similar decrease in *Z* value was observed in this study for *S. marcescens* below 50% RH. These decreases in *Z* values at low RH levels are corroborated (in behavior but not magnitude) by Riley and Kaufman (1972), where they observed for lower UV doses a similar decrease in inactivation potential with decreasing RH at levels below 50%. Similarities in *Z* value for the two and four lamp experiments above 40% RH and differences below

40% RH suggest that *Z* value may not be independent of dose at low RH levels.

Although previous research reported no UV-inactivation of airborne bacteria above 90% RH, our data supports the conclusion that UV-inactivation rates of airborne bacteria are measurable above 95% RH, and such rates approach those of cells in liquid suspension or immobilized on agar. The following are estimates for the energy required to inactivate 90% of active cultures in liquid are similar to that of *S. marcescens* determined herein—3217 μW · s/cm² ± 90 μW · s/cm² at 95% RH: *S. marcescens* —2,500 μW · s/cm² (Sharpe 1938); *E. coli* —2,400 μW · s/cm² (David 1973); and *E. coli* —3,000 μW · s/cm² (Hollander 1942). Similarly, the UV-associated energy required for 90% inactivation of *M. tuberculosis* reported in liquid suspension 2,800 μW · s/cm² (David 1973) is comparable to the 90% reduction observed for *M. parafortuitum* in this study at 95% RH—2916 μW · s/cm² ± 290 μW · s/cm². Finally, estimates for inactivation of 63% of *B. subtilis* vegetative cells in liquid suspension, 2,000 μW · s/cm² (Munakata and Rupert 1974), correlated well with the 63% inactivation of aerosolized *B. subtilis* vegetative cells at 95% RH—2,260 μW · s/cm² ± 80 μW · s/cm².

Natural decay rates measured via liquid impingers in this study did not change with RH levels. The bioaerosol literature provides both similar and contrasting results based on impactor derived rates. While observed natural decay rates of *S. marcescens* (Lighthart et al. 1971) demonstrated a decrease with increasing RH, observed natural decay rates for *E. coli* showed little variation with RH when impactor collection is normalized to one constant RH (Cox 1966). RH has been shown to affect bacterial survival during impaction (recovery is 2–4 times higher at 90% RH than at 30% RH; Cox 1966; Thompson et al.

1994). The observed influence of RH, coupled with the inability to perform total cell enumerations on impactor samples, limits the usefulness of agar impactors to determine natural decay rates, and perhaps Z values, through a broad RH range. While decreasing natural decay rates with increasing RH may suggest possible mechanisms for UV-inactivation dependence on RH, the limitations of past impactor studies and the data presented herein suggest that the actual mechanisms underlying the RH influences cannot be ascertained by classical microbiological approaches.

Understanding the mechanisms that cause RH-dependant shifts in UV-inactivation rate is necessary to extend empirical data to the prediction of inactivation rates for other airborne microorganisms and for the design of engineering controls to mitigate infectious bioaerosols. Results presented herein are useful in discrediting the following mechanism: as judged by actinometry, increases in RH to 95% did not attenuate UV spherical irradiance.

Airborne bacterial cells sorb water as RH increases. While small increases in aerodynamic diameters with increasing RH have been reported (Reponen et al. 1996; Riley and Kaufman 1972), we did not observe this phenomenon. In contrast to aerodynamic diameter measurements, investigations that describe atmospheric water sorption onto cells, on a gravimetric basis, appear to yield useful quantitative information. Water sorption data presented herein, and by others (Bateman et al. 1962), with *Serratia marcescens* reveal that these bacteria sorb up to 60% of their dry weight from water vapor at 95% RH. This weight increase corresponds to a twofold volume increase (Bateman et al. 1962) and, for *S. marcescens*, only a 25% increase in diameter in response to a RH change from 20% to 95%. The increase in cellular diameter due to water sorption ($<0.5 \mu\text{m}$ for most bacterial cells) should not present a significant barrier to incident UV irradiation. The attenuation of UV through a water layer of this thickness is negligible (Koller 1952). Our impactor results indicated that cell size did not increase over the range of RHs tested. However, because the stages of the Andersen impactor cannot accurately distinguish aerodynamic diameter changes below $0.45 \mu\text{m}$ (average standard error for triplicate median aerodynamic determinations at a single RH level was $0.14 \mu\text{m}$), the use of common impactors to quantitatively track aerodynamic diameter increases due to water sorption was indeterminate; the detection limit, however, suggests that UV attenuation due to water layers alone is negligible.

The sorption isotherms presented in this investigation correspond with previous work performed gravimetrically with *S. marcescens* (Bateman et al. 1962) and using single particle levitation with *B. subtilis* (Rubel 1997) and extend observations on the water sorption behavior of bacterial cells to include *M. parafortuitum*. The similar behavior of each species suggests that bacterial cells incorporate water vapor in a manner that is not dependent on Gram stain type. Bateman and coworkers (Bateman et al. 1962) found that cellular water sorption progressed through different stages as RH levels increased. The formation

of a water monolayer was followed by multilayer sorption that terminates at approximately 90% RH. Above 90%, gross condensation ends and intermolecular structure is thought to limit the expansion of cells. The close fit of a BET isotherm below 90% and poor fit above 90% (based on r^2 values from linearized isotherm models) corroborates this sorption mechanism.

Finally, other water-mediated mechanisms may be important in understanding the UV-inactivation-RH relationship. Bacterial cells in a dehydrated state might be expected to respond differently to UV-inactivation for the following reasons: (i) hydration and rehydration could change protein structures and hence affect DNA repair enzymes, and (ii) nucleic acid and cell wall biopolymer hydration, and thus conformation, may control the magnitude of UV-inactivation for various RH levels.

CONCLUSIONS

We determined irradiance normalized inactivation rates (Z values) at multiple RH levels for three bacteria relevant to the study of UV-inactivation of bioaerosols. Inactivation rates were sensitive to changes in RH and were a minimum near saturation (95% RH). UV spherical irradiation was well characterized and was not affected by changes in RH ranges between 20% and 95%. Because our chamber was well mixed and UV lamps extend the entire length, Z values presented are independent of variability in room mixing regimes encountered in full-scale observations. Bacteria can sorb significant amounts of water from air with respect to their dry weight; their mean aerodynamic diameter, however, does not change significantly. Based on the data presented, energy requirements determined in liquid suspension should be applied to airborne scenarios where RH is $>95\%$.

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