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UV-Induced Inactivation Rates for Airborne *Mycobacterium bovis* BCG

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Engineering ultraviolet irradiation systems as a control against infectious airborne diseases requires a knowledge of intrinsic ultraviolet (UV) inactivation rates of airborne bacteria. Ultraviolet inactivation rates for airborne Mycobacterium bovis *bacillus Calmette-Guérin (BCG) were determined at 50% and 95% relative humidity (RH) in a 0.8 m^3 bioaerosol reactor. Ultraviolet inactivation response of waterborne* M. bovis *BCG pure cultures was also determined. At 50% RH the airborne UV inactivation rates observed were two times greater than those observed in saturated air (RH = 95%), and rates at 95% RH were similar to those observed in otherwise identical cultures suspended in water. Intrinsic UV inactivation rates for* M. bovis *BCG were statistically similar to rates observed for* Mycobacterium parafortuitum *at 50% and 95% RH, indicating that* M. parafortuitum *is a valid surrogate for studying airborne UV responses of* M. bovis *BCG and* Mycobacterium tuberculosis. *Results also confirm that UV inactivation responses for bacteria suspended in water cannot be used to estimate UV dose response in unsaturated air.*

Keywords airborne bacteria, inactivation rate, *Mycobacterium bovis* BCG, relative humidity, UV radiation

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INTRODUCTION

Inactivation of airborne bacteria by placing germicidal ultraviolet (UV) lamps in the upper levels of rooms or in ventilation ducts has been proposed as a method for controlling airborne infectious disease.[1–9] The Centers for Disease Control and Prevention (CDC) has recommended UV radiation as a supplemental control for tuberculosis (TB) transmission in hospital isolation rooms.[8,10] While UV systems have been implemented successfully in water and wastewater treatment industries, paralleled UV applications for reducing airborne infection risks have achieved limited successes and acceptance. A lack of engineering design parameters available for aerosol UV irradiation systems has been suggested as a reason for widely varying efficacy observed in full and pilot scale applications.[9] Fundamental to the design of effective aerosol control systems is an understanding of intrinsic UV inactivation rates that can be used to model the airborne fate of pathogenic bacteria under a broad range of environmental conditions and controls.

There is a particular need to measure inactivation rates for the causative organisms of tuberculosis, *M. tuberculosis,* and *M. bovis*. *M. bovis* bacillus Calmette-Guérin (BCG) has been previously established as an acceptable surrogate of assessing *M. tuberculosis* UV inactivation.[10–13] Experiments on hydrated agar plates[13] and in air at 50% relative humidity (RH) have demonstrated similar inactivation rates for the two species. Both bacteria are members of the *Mycobacterium* complex and share similar physiological and genetic characteristics.[14]

However, difficulties in culturing the currently accepted surrogate, *M. bovis* BCG, and associated safety concerns in aerosolizing these organisms has limited their study. While the paucity of previous airborne UV inactivation studies with *M. bovis* BCG[10–12] clearly shows that UV effectively reduces the viable airborne concentration under a variety of room configurations and mixing conditions, this empirical information is not broadly applicable for design. Intrinsic UV inactivation rate coefficients needed to model and design systems for inactivation of airborne *M. bovis* BCG or *M. tuberculosis* at multiple RH levels have not been determined, and there is uncertainty in interpreting rate coefficient information derived from untested surrogate organisms such as *Mycobacterium parafortuitum* and *Bacillus subtilis* spores. Critical intrinsic responses of known pathogens such as airborne UV inactivation rates are needed to validate the use of surrogates for extensive study of their environmental behavior.

Ultraviolet inactivation rates for airborne (50% and 95% relative humidity levels) and waterborne *M. bovis* BCG were compared with past UV inactivation observations for air and waterborne *M. parafortuitum* to determine suitability of this organism and the usefulness in using waterborne UV inactivation rates for the accurate design of UV systems to inactivate airborne bacteria in variably saturated air.

METHODS AND MATERIALS

Bacterial Cultures and Growth Conditions

M. bovis BCG was grown in Proskauer-Beck broth[15] amended with 0.5% Tween 80 (Sigma Chemical, St. Louis, Mo.). *M. bovis* BCG is a slow-growing member of the *Mycobacterium* complex[14] and is phenotypically similar to *M. tuberculosis*. *M. bovis* BCG has therefore been used as a surrogate in UV inactivation studies targeting *M. tuberculosis*.[10–13]

Bacterial Enumeration

Culturable bacteria were quantified from impinger-recovered air samples and liquid batch UV experiments. The standard plate count method was used to enumerate culturable bacteria.[16] Within 2 hours after collection, samples were diluted (usually 1:10) in sterile 50 mM PBS (150 mM NaCl, pH 7.2). At least three replicates of each sample were plated. *M. bovis* BCG cells were incubated at 37°C for 21days on 7H11 agar media.[17] All culturing was performed in indirect, dimmed light and incubations were completely protected from light to control for photoreactivation.

UV Reactors

A bench-scale reactor was constructed to perform experiments that determine bacterial bioaerosol inactivation response from UV irradiation under sustained RH and temperature levels. The reactor was 0.8 m^3, constructed of 1.27 cm thick clear Lucite® plastic and was operated in a completely mixed flow through mode (CMFR). The reactor was mixed (ventilation effectiveness $[\varepsilon_c] = 0.98$ to 1.02, where $\varepsilon_c = 1$ is completely mixed) by three 1.5 W fans (Caframo model 727TT; Wiarton, Ontario, Canada) and capable of sustaining air exchange rates between 0 to 7.3 hr^{-1}, as determined by SF_6 tracer gas analysis. The reactor was operated under negative pressure with respect to the surrounding laboratory and vented to a certified Class II biological safety cabinet. The most recent *Biosafety in Microbiological and Biomedical Laboratories* guide[18]was published after the completion of this work and includes *M. bovis* BCG along with *M. tuberculosis* and *M. bovis* for Biosafety Level 3 work practices, containment equipment, and facilities.

Temperature and RH in the reactor could be maintained at stable levels at any air exchange rate used. Low-pressure, 30 W, mercury vapor ultraviolet lamps (G30T8; Osram-Sylvania Hanover, Mass.) were installed to irradiate the contents of the aerosol reactor. To provide a uniform line source, lamps were placed in each corner and extended the full height of the reactor. Lamps were wrapped in eight layers of aluminum filter mesh (Research Products Corp., Madison, Wis.) to attain the desired experimental UV radiance levels. The spectral power distribution (SPD) measured for the UV lamps with screens showed that 95% of power was emitted at 253.7 nm after 100 hours of operation. Chemical actinometry served to quantify UV irradiance in the reactor at 25 spatially distributed locations and through an RH range between 20% to 95%. The average UV spherical radiance in the aerosol reactor was 7.53 ± 0.13 (standard error [SD]) $\mu W/cm^2$. A comprehensive description of the reactor has been previously published.[19,20]

Liquid UV inactivation experiments were performed in a sterile 100 mm × 15 mm plastic, uncovered petri dish (Fisher Scientific, Pittsburgh, Pa.). The test suspensions (diluted in sterile deionized water) were continuously mixed on a magnetic stir plate at approximately 100 rpm by a 1-cm Teflon®-coated stir bar immersed in the petri dish. UV lamps (G30T8; Osram-Sylvania) and the liquid reactor were positioned in the Class II biosafety cabinet (Labconco Inc., Kansas City, Mo.) approximately 0.6 m apart. The UV lamps were wrapped with aluminum screen coverings (Research Products, Madison, Wis.) to control the UV flux delivered to the suspended bacteria.[20] The UV dose delivered to the bacterial suspensions was measured directly by quartz actinometry spheres that which were permanently mounted on the bottom of the petri dishes and submerged in the bacterial suspensions; this provided an absolute UV radiation measurement integrated over the depth of the suspensions. The average UV spherical radiance in the liquid was 7.5 ± 0.03 (SD) $\mu W/cm^2$.

UV Inactivation Experimental Protocols

All UV inactivation experiments were performed after UV lamps were warmed to achieve operating temperature. During aerosol experiments, lamps were turned off and bacteria were aerosolized into the reactor for 5 min. Aerosols were generated using an air-jet nebulizer (6 Jet Collison; BGI Inc., Waltham, Mass.) operated at 20 psi according to manufacturer's recommendations. For airborne UV inactivation experiments, *M. bovis* BCG was aerosolized directly from Proskauer-Beck broth into the aerosol reactor. Microscopic examination of bacterial suspensions (via wet mount) confirmed the dispersed state of cells prior to their aerosolization and use in liquid experiments.

During the first 3 min of aerosolization, reactor RH was adjusted to a predetermined level and maintained throughout the experiment. Once aerosolization of bacteria was complete, and reactor RH was stable, ventilation was initiated at 3.5 h^{-1} air exchange rate, 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 effectively through a 95% UV-induced reduction of culturable bacteria. Glass impingers (AGI-30; Ace Glass Inc., Vineland, N.J.) collected bacteria from reactor air in accordance with accepted methods.[21]

After the final sample was collected, the reactor air was evacuated with filter sterilized air at a rate of 8 h^{-1}air changes for 30 min in the presence of UV radiation. Impingers were retrieved and their contents analyzed for culturable bacteria. Experiments were performed in duplicate for the following reactor scenarios: (1) UV lamps on at 50% and 95% RH levels, and (2) UV lamps off at 50% and 95% RH levels.

Liquid-based UV inactivation rates were determined as follows: Bacteria were suspended in sterile deionized water at initial culturable concentration of approximately 10^6 CFU/mL of *M. bovis* BCG cells. Bacteria were UV irradiated in the dark and samples taken until a two-order of magnitude reduction in culturability was observed. The UV lamps were then turned off. All samples were stored in the dark at 4°C for less than two hours and analyzed for total and culturable bacteria as previously described.

Samples taken represented a time-series of culturable cell concentrations during all aerosol and liquid experiments. First order rate coefficients were used to estimate the culturable decay of cells within the reactor according to a previously described completely-mixed flow reactor model that accounted for deposition, natural decay, and ventilation losses.[19] For aerosol and liquid experiments, first order reaction rate coefficients were normalized by average spherical irradiance and reported as Z values [$cm^2/\mu W{\cdot}s$] in accordance with accepted convention for UV inactivation in air:[22,23]

$$Z \text{ value} = \frac{K_{UV}}{I} \quad \textbf{(1)}$$

where

Z ($cm^2/\mu W \cdot s$) = Z value response
k_{UV} (1/sec) = first order loss rate coefficient
I ($\mu W/cm^2$) = average reactor spherical irradiance.

Because Z value is directly proportional to a first order rate coefficient, a higher Z value indicates a lower resistance of the organism to UV radiation (greater inactivation), while a lower Z value indicates a higher resistance.

All decay experiments were executed in at least two independent trials. All bacterial concentration data for each treatment scenario were log transformed (natural log base) and pooled, and the rate coefficients estimated using a least squares method for determining best data fit. The LINEST function in Microsoft® Excel[24] was used to estimate the standard error for the reported rate coefficients. To assess the differences between rate coefficients estimated for different experimental treatments, a method of dummy variables was used.[25] Standard errors were propagated for Z value response calculations through accepted methods of random error propagation by linear combinations and multiplicative expressions.[26]

RESULTS

Z Value Response of *M. bovis* BCG.

Results for airborne Z value responses at 50% RH and 95% RH are presented in Figure 1. Differences between Z value response at 50% RH and 95% RH were statistically significant (90% confidence) for aerosolized *M. bovis* BCG. Z values decreased with increasing RH. Natural decay rates (no UV radiation) at 50% RH were less than 5% of UV-induced inactivation rates (data not shown). The Z value response[19,20] of a newly proposed *Mycobacterium* complex surrogate, *M. parafortuitum*, was compared with the Z value response of *M. bovis* BCG. No difference in Z value response (independent t-test, $\alpha = 0.05$) was observed between the aerosolized *M. parafortuitum* and BCG at 50% RH and 95% RH.

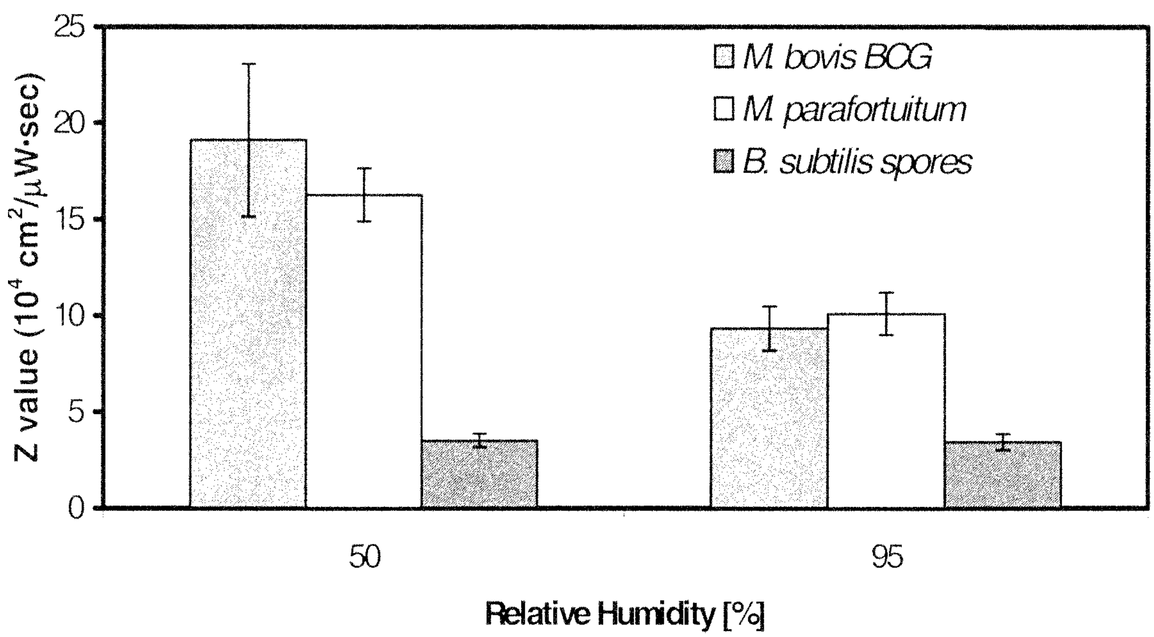

FIGURE 1. *Z* value response of *M. bovis* BCG compared with previously described *Z* values of *M. parafortuitum* and *B. subtilis* spores. Error bars represent one standard error.

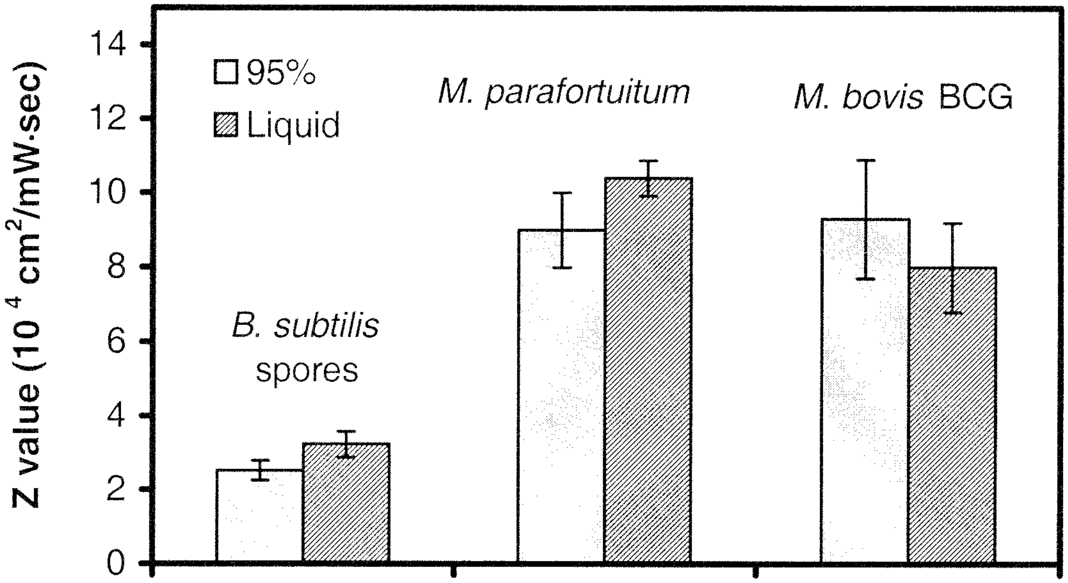

FIGURE 2. *Z* value response observed for bacteria suspended at 95% RH and in liquid suspension. Error bars represent one standard error.

Bacillus subtilis spores are commonly regarded as highly resistant to UV radiation, and doses required for their inactivation represent a conservative estimate for other bacteria. The Z value response of *M. bovis* BCG was significantly greater than the response of *B. subtilis* spores[20] at all RH levels tested, indicating that system design using rates derived from *B. subtilis* spores would be sufficient for *M. tuberculosis* inactivation at all RH levels.

Inactivation Rate in Liquid Suspension

Test organisms were also UV irradiated in liquid suspensions and their *Z* value response compared to those same bacterial cultures suspended in air. Figure 2 presents the *Z* value response derived from liquid pure culture suspensions and associated *Z* values from the same pure cultures suspended in nearly saturated air (95% RH). These values have been compared with values for *M. parafortuitum* and *B. subtilis* spores irradiated under identical conditions.[20] Differences in *Z* value response derived from liquid and 95% RH aerosol suspension were not statistically significant for *B. subtilis* spores, *M. parafortuitum*, and *M. bovis* BCG; however, inactivation rates observed at 50% RH compared with those in liquid for *M. parafortuitum* and *M. bovis* BCG were significantly different ($\alpha = 0.05$).

DISCUSSION

Table I lists *Z* value responses determined in this investigation, along with airborne experimental values previously published. At 50% RH, the *Z* value responses determined for *M. bovis* BCG were slightly lower in this study than values previously published for *M. bovis* BCG and *M. tuberculosis* strains, and greater than those values reported for *Mycobacterium phlei*.[11] Critical differences between the available UV measurement technology at the time those experiments were executed, as well as differences in experimental protocols and conditions, make meaningful comparisons between these Z values and the *Z* values presented here difficult. Data from Table I, do however, clearly indicate that *Serratia marcescens* is more susceptible to UV radiation than *Mycobacterium* species under all RH conditions and that *B. subtilis* spores are more resistant than *Mycobacterium* under all conditions. This conclusion for *B. subtilis* spores is also supported for full-scale efficacy studies.[10]

Results from the bench-scale aerosol reactor demonstrated no significant differences between *Z* value responses for the two *Mycobacterium* species (*M. parafortuitum* and *M. bovis* BCG). These experiments were performed at 50% and 95% RH and in liquid suspension. The UV susceptibility of hydrated (95% RH or liquid suspension) *M. parafortuitum* and *M. bovis* BCG (Figure 2) were also statistically similar. Because these comparisons were made in the same reactor under identical conditions and the uncertainty between measurements could be characterized, these similarities in *Z* value responses clearly demonstrate that *M. parafortuitum* could be considered as an acceptable surrogate for UV inactivation behavior of *M. tuberculosis* (*M. bovis* BCG is considered an acceptable surrogate). This similar UV response in air is also corroborated in a recently published study by Xu and co-workers[10] in which UV efficacy (determined by percent reduction) in a full-scale upper level irradiation experiment was statistically similar for *M. parafortuitum* and *M. bovis* BCG.

Comparing the UV susceptibility of *M. parafortuitum* with slow-growing, nonpigmented *Mycobacterium* species (*M. tuberculosis* and *M. bovis* BCG) is significant because it allows for more rapid and safer study for engineering controls of these

TABLE I. Reported *Z* Value Response Bacteria Maintained in Air at 50% RH, in Liquid Suspension, and on Agar Plates

Organism	Airborne 50% *Z* Value Response $\times 10^4$ (cm^2/μW·sec)	Reference No.	Hydrated *Z* Value Response $\times 10^4$(cm^2/μW·sec)	Reference No.
Mycobacterium	(23–42) Erdman strain	(11)	$(7.7)^A$	(13)
tuberculosis	(44–55) 199RB	(11)		
Mycobacterium	(33–39) Culture #1	(11)		
bovis BCG	(23–28) Culture #2	(11)		
	$(5.3)^B$	(11)		
	(19.1)	This study	$(10.4)^C$	This study
	12.0)	(10)		
Mycobacterium phlei	(2.0–5.3)	(11)	$(3.8)^A$	(13)
	(14) Gordon 644-5	(27)		
Mycobacterium smegmatis	(19) ATCC 607	(27)	$(3.6)^A$	(13)
Mycobacterium	(12–16.3)	(19)	$(8)^C$	(19)
parafortuitum	(10)			(10)
Bacillus subtilis veg.	$(6.3–6.6)^D$	(19)		
Bacillus subtilis spores	(3)	(20)	$(3.2)^C$	(29)
Serratia marcescens	(183–245)	(22)	(7.4)	(28)
	$(35–45)^E$	(19)	$(21)^C$	(29)

^{A}Results from irradiation on agar plate surfaces.
^{B}Rates derived in full-scale room using one upper level UVGI lamp, irradiance estimated by Nicas and Miller.[30]
^{C}Results from irradiation in liquid suspension.
^{D} Results from 20% and 40% RH, this RH range has no observable effect on *Z* value response-four lamps.
^{E}Results from 40%–50% RH-four lamps.

microorganisms. In contrast to *M. tuberculosis* and *M. bovis* BCG, *M. parafortuitum* can be aerosolized safely and grows to useful assay levels at a substantially faster rate (ca. 2.5 days versus 21 days). *M. parafortuitum* is not as difficult to disperse in solution as some of its *Mycobacterium* relatives. This is a benefit to disinfection studies where the dispersed nature of cells is important. The *M. parafortuitum* cell wall has a lower percent of mycolic than *M. bovis* BCG and *M. tuberculosis*.[14] The similarities of their air- and waterborne UV inactivation behavior and *Z* value response to RH suggest that (as does previously proposed nucleic acid hydration mechanisms that describe the RH dependence on UV inactivation rates[20]) cell wall hydrophobicity does not substantially influence the UV inactivation-RH relationship for these species.

Z value responses of different bacterial pure cultures suspended in water and in nearly saturated air (95% RH) are similar (Figure 2). UV inactivation rates of airborne bacteria at 95% RH are the same as the inactivation rates of cells in liquid suspension or immobilized on agar surfaces (i.e.,cells in a fully hydrated state). On the basis of *Z* value response, the rates estimated for *M. tuberculosis* inactivation when UV irradiated on agar plates, and for *M. parafortuitum* and *M. bovis* BCG in liquid suspension, are comparable with the Z value response of *M. parafortuitum* and *M. bovis* BCG in air at 95% RH (Table I). The similarities have also been observed in *B. subtilis* spores (Figure 2).

In vegetative bacteria, liquid-based UV inactivation rate is a poor indicator of the UV inactivation potential in air when RH levels are below 95%. This finding is empirically supported by UV inactivation data from the literature.[5,22] Additionally, the hydration state of DNA and the subsequent different types of DNA damage retained by airborne bacterial cells at different RH values have been presented as a fundamental mechanism for the increase in *Z* value with decreasing RH.[18] The results presented here corroborate previous empirical data, and the fundamental biochemical mechanisms described in the literature suggesting that accurate UV inactivation rates for airborne vegetative bacteria must be derived from airborne studies and not liquid-based UV inactivation experiments.

The general trends observed from *Z* value response-RH relationships dictate that environments in which aerosol UV disinfection systems are operated should be maintained in the range between 40% and 60% RH for maximum effectiveness, or their designs expanded to deliver higher UV doses at higher RH levels. Above and below this range, *Z* value responses decrease, or at best, remain constant. An advantage of using liquid-derived UV inactivation rates or rates from a surrogate such as *B. subtilis* spores for the design of air disinfection systems is the (yet to be defined) safety factor such a practice provides; liquid-derived *Z* value responses are a similar or conservative estimate of airborne derived *Z* value response, depending on the RH level. Such a factor may not be warranted in situations where high UV intensities produce human exposures above thresholds set by the American Conference of Governmental and Industrial Hygienists (ACGIH®).[31]

Additionally, high UV irradiation may increase power costs unnecessarily while providing only limited returns in UV inactivation efficacy. The rates measured here are intrinsic UV inactivation rates and are useful to the practitioner in two ways. The first use is to develop an understanding of how relative humidity affects the UV inactivation of *M. bovis* BCG and to provide information on the relative susceptibility *M. bovis* BCG compared with other microorganisms. The second use is to provide rate coefficients for input into a predictive efficacy model that accounts for room mixing, ventilation, and the specific UV field.

CONCLUSIONS

This work presented intrinsic cell/UV inactivation rates in air at markedly different RH levels. Based on these results, the following conclusions and applications to the practice of UV radiation in air are suggested:

- *M. parafortuitum* and *M. bovis* BCG responses to UV radiation, 50% RH, 95% RH, and liquid cultures were similar. Use of *M. parafortuitum* as a *M. tuberculosis* /*M. bovis* surrogate is reasonable for disinfection studies and system design.
- UV inactivation rates of *Mycobacteria* in nearly saturated air (95% RH) are similar to rates in liquid-based experiments.
- UV inactivation rates in air at 50% RH are substantially higher than rates derived from liquid-based cultures. RH must be considered as a critical design parameter in the engineering of UV aerosol disinfection systems.
- Use UV inactivation rates for *B. subtilis* spores in system design provides a conservative estimate for predicting inactivation of *Mycobacterium* species.

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