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Efficacy of ultraviolet germicidal irradiation of upper-room air in inactivating airborne bacterial spores and mycobacteria in full-scale studies

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Abstract

The efficacy of ultraviolet germicidal irradiation (UVGI) for inactivating airborne bacterial spores and vegetative mycobacteria cells was evaluated under full-scale conditions. Airborne bacteria inactivation experiments were conducted in a test room ($87\,m^3$), fitted with a modern UVGI system (216 W all lamps operating, average upper zone UV irradiance $42 \pm 19\,\mu W\,cm^{-2}$) and maintained at 25°C and 50% relative humidity, at two ventilation rates (0 and 6 air changes per hour). *Bacillus subtilis* (spores), *Mycobacterium parafortuitum,* and *Mycobacterium bovis* BCG cells were aerosolized continuously into the room such that their numbers and physiologic state were comparable both with and without the UVGI and ventilation system operating. Air samples were collected using glass impingers (9 breathing-zone locations) and multi-stage impactors, and collected bacteria were quantified using direct microscopy and standard culturing assays. UVGI reduced the room-average concentration of culturable airborne bacteria between 46% and 80% for *B. subtilis* spores, between 83% and 98% for *M. parafortuitum*, and 96–97% for *M. bovis* BCG cells, depending on the ventilation rate. An additional set of experiments, in which *M. parafortuitum* was aerosolized into the test room and then allowed to decay under varying UVGI and ventilation rates, yielded an inactivation rate of $16 \pm 1.2\,h^{-1}$ for the UVGI system, all lamps operating. The Z value (inactivation rate normalized to UVGI irradiance) was estimated to be $1.2 \pm 0.15 \times 10^{-3}\,cm^2\,\mu W^{-1}\,s^{-1}$ for aerosolized *M. parafortuitum* at 50% relative humidity.

Keywords: Bioaerosols; Air disinfection; Air cleaning; UV lamps; Bacteria

Introduction

Airborne transmission of *Mycobacterium tuberculosis* and other infectious agents within indoor environments has been a recognized hazard for decades (Blumberg et al., 1995; Center for Disease Control and Prevention, 1994; Ikeda et al., 1995; Kearns et al., 2000; Riley et al., 1962). Recent outbreaks of multidrug-resistant strains of *M. tuberculosis* in healthcare and correctional facilities have heightened the concern and need to prevent transmission (Fackelmann, 1998; Kearns et al., 2000; Nivin et al., 1998). Although resurgence of tuberculosis (TB) in the United States has halted and the incidence is once again falling, there is a global epidemic of TB fueled in part by the epidemic of HIV-AIDS (Frankel, 1995; Morbidity and Mortality Weekly Report, 2002;

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World Health Organization, 2000). The World Health Organization estimates that between 2000 and 2020, nearly one billion people will be newly infected with TB, 200 million people will get sick, and 35 million will die from TB if control is not further strengthened (World Health Organization, 2000).

For control of TB transmission in hospital isolation rooms, the Centers for Disease Control and Prevention (CDC) recommends ventilating the room with clean, outside air at a rate of 6 and 12 air changes per hour (ACH, h^{-1}) and maintaining the room under negative pressure relative to adjacent areas (Centers for Disease Control and Prevention, 1994). Many isolation rooms currently receive less than the recommended 6 ACH (Blumberg et al., 1995; Ikeda et al., 1995), and renovating facilities to achieve ventilation rates between 6 and 12 ACH can be expensive. Thus, there has been renewed interest in auxiliary means to remove airborne infectious agents from room air, such as the use of high-efficiency-particle-air (HEPA) filters or the application of ultraviolet germicidal irradiation (UVGI) (Centers for Disease Control and Prevention, 1994; Nagin et al., 1994; Nardell, 1995).

UVGI is mainly used for disinfecting air in two configurations: ventilation duct irradiation and upper-room air irradiation (Nagin et al., 1994). Animal studies compellingly demonstrated that ventilation duct irradiation prevented the transmission of TB from hospitalized tuberculosis patients to guinea pig colonies (Riley et al., 1957, 1962). Initial observations of the efficacy of upper-room air irradiation to control airborne infection were reported in the early 1900s by Wells (Wells and Fair, 1935; Wells, 1955). For upper-room air irradiation, germicidal lamps are suspended from the ceiling or attached to the walls; the bottom of the lamp is usually shielded or louvered to direct radiation upward above a predetermined height. The objective of this configuration is to inactivate airborne infectious agents in the upper part of the room, while minimizing radiation exposure to persons in the lower part of the room. *Inactivation* in this context means the loss of the ability to replicate and form colonies. Commercially available germicidal lamps contain mercury vapors under low pressure that emit energy in the UV-C wavelength range, 100–290 nm, with about 90% of the total spectral power emitted at 254 nm.

To expand the limited experimental data on the effect on airborne bacteria of irradiating room air, a modern UVGI system was investigated in realistic physical scenarios under carefully controlled laboratory conditions. This study was based on a previously developed and tested design for evaluating UVGI effects (Hernandez et al., 1999; Miller and Macher, 2000). The UVGI response of two mycobacterium species was investigated—*Mycobacterium parafortuitum* and *Mycobacterium bovis* BCG, a *M. tuberculosis* surrogate—and compared to that of airborne *Bacillus subtilis* spores. Airborne bacteria concentrations were generated that allowed for concurrent enumeration using direct epi-fluorescent microscopy techniques and standard plate counts. Air samples were collected at a large number of locations within a full-scale test room with replicates. The UV irradiance distribution within the room was characterized with radiometry and a newly developed actinometry technique. Experiments using two methodologies were conducted, constant generation and decay. In the constant generation method, UVGI efficacy was determined by comparing measurements of the concentration of culturable airborne bacteria with and without exposure to UVGI. In the decay method, UVGI efficacy was determined by the rate at which airborne microorganisms were inactivated.

2. Materials and methods

2.1. Culture preparations

All cultures used in this study were supplied by the American Type Culture Collection (ATCC). Three bacteria were aerosolized during the experiments: *B. subtilis* (ATCC #090287), *M. parafortuitum* (ATCC # 19689), a rapid-growing, rod-shaped mycobacterium that yields pale yellow colonies, and *M. bovis* Bacillus Calmette-Guérin (BCG), Pasteur strain, ATCC #35734 (Trudeau Mycobacteriology Collection #1011), an attenuated strain of *M. bovis* used in human tuberculosis vaccination. *M. bovis* BCG is closely related phenotypically and genotypically to *M. tuberculosis* and has, therefore, been used as its surrogate in bioaerosol studies (Riley et al., 1976). *M. parafortuitum* was chosen because both it and *M. bovis* BCG are related mycobacteria and *M. bovis* BCG has been reported to be sensitive to UVGI (David, 1973; Hollander, 1942; Riley et al., 1976). Compared with *M. bovis* BCG, *M. parafortuitum* is easier to work with because it grows faster (3 days compared to $\sim$4 weeks) and is considered a lower-level hazard by the CDC (CDC and NIH, 1999).

These three bacteria were selected to represent a range of UVGI susceptibilities (Collins, 1971; David, 1973; Hollander, 1942; Miller and Macher, 2000; Riley et al., 1976; Wells, 1955). Wells (1955) determined the lethal power of UVGI on select aerosolized bacteria. The vulnerability of *B. subtilis* (spore) was 0.22 and *M. tuberculosis* was 0.84, relative to *E. coli* (*E. coli* — 1.0). David (1973) found that the relative UV sensitivity of liquid suspensions of *M. tuberculosis* was 0.40 relative to *E. coli* (1.0). David (1973) also reports that the differences in response to ultraviolet light of members of the mycobacteria are small and are correlated with the size of the bacterial genome, the cellular concentration of carotenoids, and the repair efficiency. UV

susceptibility was shown to be similar for *M. tuberculosis* and *M. bovis* BCG (Collins, 1971 (agar plate surfaces), Riley et al., 1976 (airborne bacteria)). Collins (1971) reports that compared with mycobacteria, all of the gram-negative bacteria (including *E. coli* and *S. marcescens)* tested were twice as sensitive to UV inactivation. Peccia and colleagues (Peccia, 2000; Peccia and Hernandez, 2002) measured in a small-scale aerosol reactor statistically similar UV inactivation rates for *M. parafortuitum* and *M. bovis* BCG.

M. parafortuitum was grown on Soybean-Casein Digest Agar (SCDA) (Difco Laboratories, Detroit, MI) at 37°C and incubated for 60 h. *M. bovis* BCG was grown in Proskauer-Beck broth (Difco Laboratories, Detroit, MI) amended with 0.5% Tween 80 (Sigma Chemical, St. Louis, MO). *M. bovis* BCG cultures were grown for 21 days at 37°C under constant mixing using a Teflon®-coated magnetic stir bar and magnetic stir plate. Sporulating *B. subtilis* cultures were grown on agar plates ($8.5\,g\,l^{-1}$ nutrient agar with 0.002% $MnCl_2 \cdot 6H_2O$) at 37°C.

After 5 days, *B. subtilis* spores and cells were removed from the agar surface by scraping the plates with a sterile glass rod, and then suspending in sterile deionized water. Spores were separated from remaining vegetative cells by repeated centrifugation and decanting vegetative cell-containing supernatant. Using Shaeffer-Fulton stains *B. subtilis* spore purity was confirmed in the range 94–100% immediately prior to their aerosolization. Spores were stored at 4°C in sterile deionized water for up to 24 h prior to use, and were not pasteurized as this step was found to increase the germination potential of spores before UV exposure and during impinger collection (Peccia, 2000).

Fresh *M. parafortuitum* cultures were removed from agar plate surfaces by scraping, and were suspended in sterile deionized water immediately prior to aerosolization. *M. bovis* BCG was aerosolized directly from its liquid broth culture (Proskauer-Beck). Immediately before aerosolization, cell concentrations (except *M. bovis* BCG) and spore solutions were diluted to a concentration of approximately $1 \times 10^9\,cells\,ml^{-1}$ as determined by direct microscopy.

Stock cultures of *B. subtilis* vegetative cells and *M. parafortuitum* were maintained on SCDA at 4°C for short-term storage (<4 weeks). For storage longer than 4 weeks, all cultures, including *M. bovis* BCG, were stored at −20°C in sterile deionizing water containing 40% (vol/vol) glycerol.

2.2. Test facility

A simulated, health-care room was established at the Joint Center for Energy Management's Larson Building Systems Laboratory, University of Colorado at Boulder. An 87-m^3 room housed inside the laboratory was used for testing. The room has an infiltration rate between 0.1–0.3 ACH. The floor-to-ceiling height is 2.5 m and contains $35\,m^2$ of clear floor area. The room has insulated walls, raised floor, plenum ceiling, one door and no windows.

The test room is equipped with a computer-controlled ventilation system that delivers from 2 to 8 ACH of HEPA-filtered outside air through two circular diffusers located in the ceiling. Air is exhausted through two outlets in the ceiling. To minimize airborne bacteria exiting the test room, HEPA filters were installed within the exhaust ducting. The ventilation system was operated during testing to maintain a slight negative pressure (12 Pa) within the room relative to the surrounding laboratory (Centers for Disease Control and Prevention, 1994). The ventilation rate was measured and controlled by airflow sensors (Eliminator 3000, Ebtron Inc., CA) installed in the ducts. This rate was verified with tracer gas (72% SF_6 and 18% H_2) tests. The room air was mixed with two box fans (48-cm diameter, MODEL 3723, Lasko Inc., TX) operated on both sides of the room. The room air mixing was characterized using the age-of-air method both by using a gas and particle tracer (Xu and Miller, 1999). The room air was well mixed for particles, both with and without the box fans operating, provided the supply air was at the same temperature as the room air. Mixing was somewhat better when the ventilation was at 0 or 3 ACH compared to 6 ACH.

The temperature and relative humidity in the room are controlled by two feedback-control loops linked with cooling coil and steam-injection humidifiers. The room is capable of maintaining a temperature in the range between 15°C and 35°C and a relative humidity in the range between 50% and 100%. The temperature and relative humidity for all the experiments were maintained at 24 ± 1°C, and 50 ± 5%.

The UVGI system (Lumalier, Memphis, TN) consists of five luminaries, four mounted in each of the corners of the room and one hung from the center of the ceiling (Fig. 1). The manufacturer rated the lamps within the luminaries at 18 W. The center luminary is rated at a total of 72 W, consisting of four lamps. The corner luminaries are rated at 36 W each containing two lamps installed with parabolic aluminum reflectors on the back of the luminaries. Each luminary is equipped with concentric black louvers of 1.9-cm spacing. The height of the luminaries is approximately 25 cm and they were installed so that the lower edge was located 2.1 m above the floor and the top was 10 cm below the ceiling. This placement created a band of UVGI in the upper level of the room, with an average depth of approximately 30 cm. The depth of the band varied throughout the room, with the narrowest dimensions being right next to the lamps at 18 cm and the widest dimensions being in between luminaries.

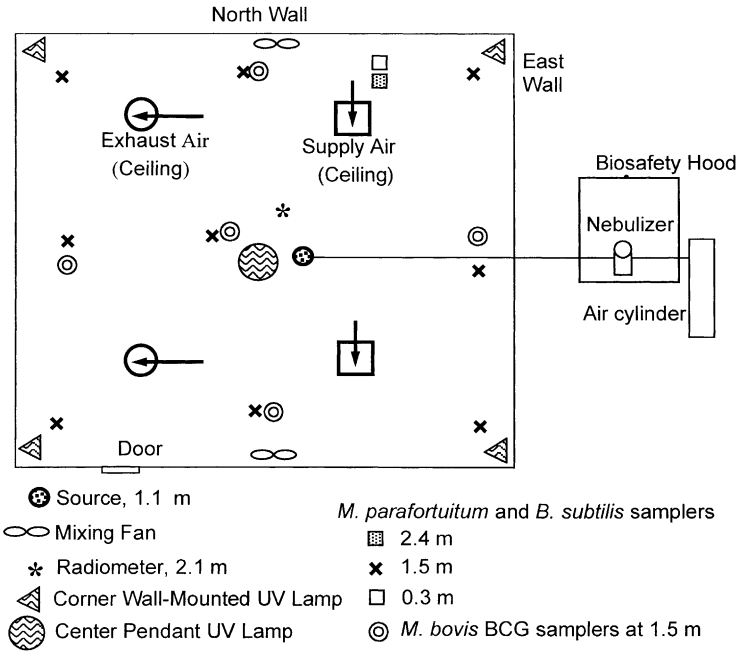

Fig. 1. Configuration of 87-m^3 test room (plan view). Heights above the floor are given. Biosafety hood contains the bioaerosol generation system. Test room was sealed during experiments.

The UVGI system was operated for 100 h before experiments were conducted. The UV luminaries were rated "ozone free" and negligible ozone levels were verified in the test chamber during UVGI operation using a portable ozone monitor (Black et al., 2000).

2.3. Airborne bacteria generation and collection

The test bioaerosols were generated using a six-jet Collison nebulizer with a large reservoir (CN 25, BGI Inc., MA). The nebulizer was located outside of the test room, within a biosafety cabinet (Nuaire Inc., MN) in an adjacent room housed within the laboratory. The bioaerosol was delivered from the CN discharge port into the test room through 3 m of plastic tubing (1.9-cm inner diameter). The nebulizer was operated at 137 kPa (20 psia), generated by a compressed air cylinder in series with an air supply system that included a dehumidifier, a HEPA filter, and a pressure regulator (Model 3074, TSI, Inc. MN). To ensure a stable particle size distribution, and a consistent airborne bacteria concentration over the course of the experiments, the cell suspension was replenished every 30 min. The test bioaerosol was released near a heated mannequin's head, approximately 1.0 m above the floor. The heated mannequin, equipped with 108 W heating tape, was seated in a chair in the middle of the room to simulate a person. The mannequin generated a thermal plume similar to that of a human body, which influenced air mixing near the bioaerosol source. The mannequin and bioaerosol source were located in the room between the ventilation exhaust and supply (Fig. 1), in accordance with the CDC's recommendation that clean air first flow to less contaminated areas, then flow across the infectious source and into the exhaust (Centers for Disease Control and Prevention, 1994; Miller-Leiden et al., 1996).

Airborne microorganisms were sampled using 33 AGI-30 impingers (AGI-30, ACE Glass Inc., NJ) clustered in triplicate at 11 room locations for *M. parafortuitum* and *B. subtilis* spore tests. Nine of the sampling locations were positioned in the breathing zone, 1.5 m above the floor. One was located near the ceiling (2.4 m above the floor) and one near the floor (30 cm above the floor). Five breathing-zone locations were sampled in triplicate, also using AGI-30 impingers, during the *M. bovis* BCG tests. Impingers were positioned at each sampling location such that their intake nozzles were at 60° angles to each other, thereby eliminating flow bias to any single impinger.

Airborne bacteria was collected for 30 min at 12 l min^{-1} through tubing connected to a manifold in series with a high-flow sampling pump (Model 1023-1 01 Q, Gast Manufacturing Inc., MI) regulated by rotameters (Model 7400, King Instrument Inc., CA). The impingers concentrated the bacteria into 30 ml of sterile, phosphate buffer (150 mM NaCl, pH 7.2) with minimal damage to the microorganisms. Before each experiment, the pump and rotameters were calibrated using a bubble meter (Gillibrator, Scientific Industries, FL).

2.4. Enumeration by culturing and epifluorescent microscopy

Following collection, the impingers' contents were mixed and divided into equal aliquots for two types of analysis: culturing of colony-forming units (CFU) and direct counting using epifluorescent microscopy. 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) solution (150 mM NaCl, pH 7.2) and cultured using a spiral plating method (Spiral Biotech, Inc., MD) according to manufacturer's recommendations. At least three replicates of each sample were plated. *B. subtilis* spores were incubated at 37°C for 24 h, *M. parafortuitum* was incubated for 60 h, and *M. bovis* BCG was incubated for 21–35 days. All organisms were plated on SCDA agar except *M. bovis* BCG, which was plated on 7H11 media (Dubos and Middlebrook, 1947). A comparison of cultured bacteria counts using the spiral plater with standard spread plate methods showed no significant differences (independent *t*-test, $\alpha = 0.05$). The variability of the spiral plater method was lower than that of the spread plate method (coefficient of variance 5% lower for the spiral plating method, $n = 10$). All plating was performed in indirect dimmed light, all experiments and incubations were carried out in the dark to control for photoreactivation.

Epifluorescent microscopy was used to directly measure the total number of airborne microorganisms. Total numbers were used to judge whether the number of organisms aerosolized was identical between UV-on and UV-off experiments. Vegetative cells were stained with 4′,6-diamidino-2-phynylindole (DAPI) (Sigma Chemicals, MO), a DNA-binding fluorescent stain (Ward et al., 1965), and spores were stained with a nonspecific fluorescein derivative (5-[4,6-dichlorotriazin-2-yl] aminofluorescein (DTAF), in accordance with previously described methods (Hernandez et al., 1999). Samples were stained at a final concentration of 0.5–1.0 μg DAPI ml^{-1} and 200 μg DTAF ml^{-1} at pH 9.0, respectively. Vegetative cells were incubated in DAPI for 1 min at room temperature and spores in DTAF solution were incubated for 30 min at 37°C in the dark. Vegetative cell and spores were then filtered through 25-mm diameter, 0.2 μm, black polycarbonate filters (Poretics Inc., CA) with the exception that DTAF-stained spores were rinsed with 50 mM sodium carbonate buffer at pH 9.0. Mounted filters were examined under 1100 × magnification using a Nikon Eclipse E400 epifluorescent microscope fitted with a mercury lamp and polarizing filters (Chroma Tech. Corp., VT). At least 10 random fields, and greater than 200 total cells, were counted per slide. All direct counts were reported as the average of 10 fields. Direct counts from aliquots having coefficient of variance of greater than 30% were discarded and new sample aliquots were stained and counted until a uniform distribution was observed (Hernandez et al., 1999).

In addition, the particle size distribution of culturable airborne bacteria was measured using a six-stage, multiple-hole impactor (Grasby Andersen, Andersen Instruments GA). This impactor sampled at 28.3 l min^{-1} and had d_{50} (diameter with 50% collection efficiency) cut points of 7, 4.7, 3.3, 2.1, 1.l, and 0.65 μm for the six stages. A modification to the standard impactor operation was implemented allowing for longer, more accurate, sample collection without concerns about overloading the plates. Plain gelatin with glycerin and water was used as the impactor plate media for *B. subtilis* spores and *M. parafortuitum* (Blomquist et al., 1984), and 7H11 agar media was used or *M. bovis* BCG, instead of the usual agar medium.

2.5. UV irradiance measurement

Spherical actinometers were used to characterize the UVGI distribution in the room according to Rahn et al. (1999). These spherical actinometers measured three-dimensional irradiance, which is suitable for this study since multiple UVGI luminaries were installed at different locations within the room. Spherical glass irradiation cells (1-cm diameter) were filled with an actinometric solution of 0.6 M KI and 0.1 M KIO_3 in 0.01 M borate, pH 9.25. The cells were suspended at 20 evenly spaced locations in the upper zone of the room, approximately 2.3 m from the floor and exposed to UVGI for 30 and 90 min. The UVGI lamps were warmed up for 30 min before measurement. The absorptivities of the solutions were measured by a spectrophotometer before and after UVGI exposure. The actinometric method was also used to measure UVGI in the breathing zone (1.5 m above the floor) by filling the glass cells with the actinometric solution, hanging them at 20 locations, and exposing them for 12 h. The lamps were operated very limited hours during the experiments and the UVGI output remained steady during the study.

Actinometer irradiance measurements were made for 100% (216 W), 75% (162 W), 50% (108 W), and 25% (54 W) of the UVGI lamps operating. These measurements followed the same procedure as for the full UVGI measurements. For 50% UVGI, one lamp in each of the corner fixture and two lamps in the center fixture were turned off. For 75% UVGI, since there were 12 lamps in five UVGI fixtures in the room, three lamps were turned off. Two of them were in the ceiling fixture and the other was in the northeast corner fixture. For 25% UVGI, only three lamps were turned on. To have a more uniform distribution of UV irradiance, a lamp in each of three corner fixtures was turned on.

A factory calibrated radiometer (model 1400 International Light Inc., Newburyport, MA) was also used to measure UV irradiance. In the upper zone of the room, along a 0.6-m × 0.6-m grid, the UV irradiance at 113 points was measured approximately 2.3 m above the floor (measurements nearest lamps were made 4 ft away). Since airborne bacteria were exposed to UVGI from all directions, at each point in the room, the UV irradiance was measured in six directions. Assuming that the point of measurement is a cube, these directions are normal to the front, back, top, bottom, left, and right faces of the cube. These directions were oriented within the room along the room's wall surfaces, that is, towards the N, W, E and S walls and also the ceiling and floor. The sum of the irradiance measured in these six directions was a reasonable estimation of UV irradiance at that point (Rahn et al., 1999). The radiometer was also used to track the UVGI irradiance between experiments to make sure it did not vary between runs.

2.6. Constant generation method protocol

UVGI efficacy can be characterized in terms of the percentage of airborne bacteria that cannot be cultured when exposed to UVGI during constant generation, or continuous aerosolization of bacteria. Ten experiments were conducted (Table 1), each two times to estimate repeatability, using the following constant generation protocol.

After cleaning the test room by supplying HEPA-filtered outside air via the ventilation system, the negative pressure in the room and the ventilation rate were established. The box fans were turned on to ensure room air mixing. The test room door was closed and sealed to reduce infiltration. All luminaries (both UV and overhead fluorescent) were turned off for all experiments, except during the UV on experiment, in which only the UVGI system was operated. Bacteria aerosolization was started and then maintained for 90 min. During the last 30 min of generation, samples were collected. When the experiment was completed, the test room was cleared of all airborne bacteria by operating the ventilation system at 8 ACH for at least 1 h.

Experiments were performed at two ventilation rates, 0 ACH and 6 ACH. Negative pressure was maintained so that there was some leakage, and it was determined that this resulted in an air-exchange rate of approximately 0.2 ACH.

UVGI effectiveness, E, quantifies the impact of UVGI on room bioaerosol concentrations and is estimated by comparing the measured culturable airborne bacteria concentration with the UVGI system on, $C_{\mathrm{UV\ on}}$ (CFU m^{-3}) to the airborne culturable bacteria concentration without UVGI, $C_{\mathrm{UV\ off}}$ (CFU m^{-3}). Although

Table 1
Experimental scenarios[a]

Test organism	Ventilation configuration	Methodology	UVGI configuration
B. subtilis spores	0 ACH	Constant generation	100%
B. subtilis spores	0 ACH	Constant generation	Off
B. subtilis spores	6 ACH	Constant generation	100%
B. subtilis spores	6 ACH	Constant generation	Off
M. parafortuitum	0 ACH	Constant generation	100%
M. parafortuitum	0 ACH	Constant generation	Off
M. parafortuitum	6 ACH	Constant generation	100%
M. parafortuitum	6 ACH	Constant generation	Off
M. bovis BCG	0 ACH	Constant generation	100%
M. bovis BCG	0 ACH	Constant generation	Off
M. parafortuitum	0 ACH	Decay	100%
M. parafortuitum	0 ACH	Decay	50%
M. parafortuitum	0 ACH	Decay	Off
M. parafortuitum	3 ACH	Decay	100%
M. parafortuitum	3 ACH	Decay	75%
M. parafortuitum	3 ACH	Decay	50%
M. parafortuitum	3 ACH	Decay	25%
M. parafortuitum	3 ACH	Decay	Off
M. parafortuitum	6 ACH	Decay	100%
M. parafortuitum	6 ACH	Decay	50%
M. parafortuitum	6 ACH	Decay	Off

[a] All experiments were duplicated.

the aim was to reproduce the same generation rate of airborne bacteria for both UV on and UV off experiments, there was some variability in total bacteria concentration in the room. Therefore, in calculating effectiveness, the culturable airborne bacteria concentrations were normalized by the total bacteria concentration as determined by total direct counts (Hernandez et al., 1999). The effectiveness of the UVGI system is given by

$$E = 1 - \frac{C_{\text{UV on}}}{C_{\text{UV off}}}\frac{D_{\text{UV off}}}{D_{\text{UV on}}}, \quad (1)$$

where $D_{\text{UV off}}$ and $D_{\text{UV on}}$ (# m^{-3}) are the total airborne bacteria concentrations measured by direct counts for UVGI off and on.

2.7. Decay method protocol

UVGI efficacy can also be characterized in terms of the rate in which it inactivates microorganisms. Eleven experiments using *M. parafortuitum* were conducted, each two times to estimate repeatability, by the following decay method protocol (Table 1).

After cleaning the test room by supplying HEPA-filtered outside air, airborne bacteria were generated continuously to raise the concentration in the room to a suitable level for detection. No ventilation was provided during this period, the box fans were turned on to ensure mixing, and the lights including the UVGI system were off. After 30 min, aerosolization was stopped and the ventilation rate and negative pressure was established and the UVGI system was turned on. Airborne bacteria were sampled five times at approximately 3–4-min intervals as the concentration decayed over 15–20 min with duplicate impingers. Thus in total, 10 samples were collected over the decay period. The impingers were located underneath the ventilation exhaust at 1.5 m above the floor. Additional decay experiments were conducted without the UVGI system operating to measure removal by ventilation and natural die-off.

Experiments were performed at three ventilation rates: 0, 3, and 6 ACH. Two to four UV irradiance levels were tested to determine the relationship between the inactivation rate of UVGI and UV irradiance, in terms of Z value. Z value is the UVGI inactivation rate expressed in s^{-1} normalized to UV irradiance. It is an index used to express the dose–response relationship of different organisms and is solely dependent on the microorganism physiology. The higher the Z value, the more quickly the targeted microorganism is inactivated by UVGI. The UV irradiance was varied by switching on/off some of the UVGI lamps.

A completely mixed room (CMR) model is needed to evaluate UVGI inactivation rates. The model is based on the assumption of complete mixing, which results in uniform airborne bacteria concentrations throughout the volume of the room. A material balance is applied to airborne culturable bacteria within a room with volume V (m^3). Culturable bacteria are emitted from a source at a generation rate G (CFU h^{-1}). Ventilation air flows through the room removing airborne bacteria, reducing the indoor culturable bacteria concentration. The ventilation rate is expressed as the volumetric airflow rate through the room divided by the volume of the room (ACH, h^{-1}). In addition, the UVGI system inactivates airborne bacteria when operated. This inactivation rate can be approximated with a first order model, denoted ACH_{UV} (h^{-1}). Gravitational settling, natural die-off, and other natural decay mechanisms for bacteria are denoted ACH_{N} (h^{-1}).

The rate of change of the indoor airborne culturable bacteria concentration with the UVGI system on, $C_{\text{UV on}}(t)$ (CFU m^{-3}), is given by

$$\frac{\text{d}C_{\text{UV on}}(t)}{\text{d}t} = \frac{G}{V} - (\text{ACH} + \text{ACH}_{\text{UV}} + \text{ACH}_{\text{N}})C_{\text{UV on}}(t). \quad (2)$$

Rates for UVGI inactivation can be determined by interpreting the airborne bacteria concentration data collected during decay method experiments using the CMR model. From Eqn. (2), the rate of change of the culturable airborne bacteria concentration with time during the decay period is given by the relationship:

$$C_{\text{UV on}}(t) = C_{\text{o}}e^{-(\text{ACH}+\text{ACH}_{\text{UV}}+\text{ACH}_{\text{N}})t}. \quad (3)$$

A similar expression can be written for the culturable airborne bacteria concentration without UVGI, $C_{\text{UV on}}(t)$.

To derive the overall removal rate, $\text{ACH} + \text{ACH}_{\text{UV}} + \text{ACH}_{\text{N}}$, the natural-log form of Eqn. (3) is linearly fit to data (natural-log transformed) collected during experiments with the UVGI system operating using a least-squares method. The slope of the line is equal to the overall removal rate. Similarly, data from experiments without the UVGI system operating is linearly fit to derive the removal rate due to ventilation and natural decay only, $\text{ACH} + \text{ACH}_{\text{N}}$. The UVGI inactivation rate, ACH_{UV}, is ultimately determined by calculating the difference between these two rates.

3. Results

3.1. UV irradiance

The results of the actinometer measurements for 100% of the UVGI lamps operating (216 W) are presented in Fig. 2. The UV spherical irradiance peaked in the region closest to the luminaries, and the area along the diagonals of the room. For the 30-min exposure test, the spatial average value $\pm$ standard deviation was $42 \pm 19\ \mu\text{W cm}^{-2}$. The maximum value

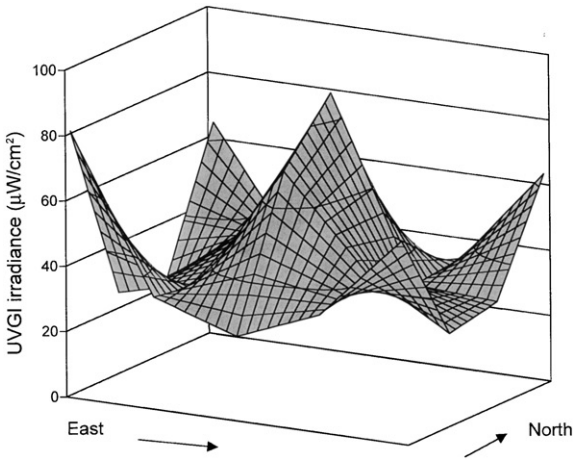

Fig. 2. Spatial distribution of ultraviolet irradiance measured using actinometry in the upper-room zone with 100% UVGI (216 W, all UV lamps operating).

was $81\,\mu W\,cm^{-2}$, and the minimum was $25\,\mu W\,cm^{-2}$. For the 90-min exposure test, the spatial average value was $44 \pm 20\,\mu W\,cm^{-2}$. The maximum and minimum values were 87 and $23\,\mu W\,m^{-2}$, respectively. The results from 30- and 90-min tests were statistically equivalent (independent t-test, $\alpha = 0.01$), which indicated that the response of the system was linear over the dose range and the test was repeatable. The average UV spherical irradiance at a height of 1.5 m was $0.08 \pm 0.02\,\mu W\,cm^{-2}$.

The average UV spherical irradiance with partial UVGI lamps operating was also measured. For 25% (54 W), 50% (108 W), and 75% (162 W) of the UVGI lamps operating, the UV irradiance was 12 ± 8.8, 20 ± 8.1, and $33 \pm 18\,\mu W\,cm^{-2}$, respectively.

The spatial average value in the upper zone measured by radiometry was $54 \pm 20\,\mu W\,cm^{-2}$. The maximum and minimum values were 131 and $22\,\mu W\,cm^{-2}$. These radiometer measurements in the upper zone agreed with those from actinometry (independent t-test, $\alpha = 0.05$). The average UV irradiance at the height of 1.5 m as measured by radiometry was $0.2 \pm 0.04\,\mu W\,cm^{-2}$.

3.2. Cell concentration and particle size

The airborne bacteria total and culturable concentrations measured in the breathing zone for each constant generation method test are listed in Table 2. *B. subtilis* spores and *M. parafortuitum* were tested at both 0 and 6 ACH. *M bovis* BCG was tested at 0 ACH successfully. At 6 ACH, however, its culturability was below the detection limit. During the 0 ACH experiments, airborne bacteria concentrations continuously changed with time. During the 6 ACH experiments, concentrations approached equilibrium after 30 min.

The total number of bacteria aerosolized was statistically equivalent (independent t-test, $\alpha = 0.05$) during all experiments comparing UVGI on and off. The concentration of culturable airborne bacteria was less than the concentration of total airborne bacteria by up to an order of magnitude, depending on the type of microorganism, suggesting that the bacteria were stressed through the aerosolization process or lost culturability while airborne.

Based on culturable concentrations recovered from the impingers, the vertical and horizontal spatial concentration distribution within the room was relatively uniform. There was no statistically significant difference in the concentrations of airborne bacteria collected at all 11 locations (9 in the breathing zone, 1 at the ceiling, 1 at the floor; Fig. 1) as judged by independent t-tests ($\alpha = 0.05$). This result supports the conclusion that the room air was mixed.

Impactor data demonstrated that most of the collected airborne bacteria aerodynamic diameters were between 0.65 and 2.1 μm (d_{50}'s for impactor stages 4 through 6), with a geometric mean of 1.6 μm (geometric standard deviation = 1.2). No significant difference in size distribution was observed among the different microorganisms aerosolized (independent t-test, $\alpha = 0.05$).

3.3. Effectiveness of UVGI

The full UVGI system (all lamps operating) reduced the room-average breathing-zone concentration of culturable airborne bacteria, between 46% and 80% for *B. subtilis* spores, between 83% and 98% for *M. parafortuitum,* and 96% and 97% for *M. bovis* BCG at 50% relative humidity, depending on the ventilation rate (Eqn (1); Fig. 3). *B. subtilis* spores were more resistant to inactivation by UVGI than either mycobacteria cultures tested. The effectiveness was not statistically different for *M. parafortuitum* and *M. bovis* BCG at 0 ACH ventilation rate (independent t-test, $\alpha = 0.05$). While increasing the ventilation rate from 0 to 6 ACH appeared to decrease the effectiveness of UVGI-induced microbial inactivation for *M. parafortuitum* and *B. sublilis* spores, these decreases were not statistically significant (independent t-test, $\alpha = 0.05$).

3.4. UVGI inactivation rate (ACH_{UV})

Decay experiment data used to determine inactivation rates induced by UVGI are presented in Figs. 4–6. Standard deviations of the regression lines are reported and propagation of errors was applied to determine the uncertainty in the inactivation rates. All slopes were statistically significant (F tests, $\alpha = 0.05$).

At 0 ACH ventilation rate, the inactivation rate ($\pm$standard deviation) with all UV lamps operating was

Table 2
Total and culturable count average (standard deviation) concentrations for constant generation method experiments[a]

	Ventilation configuration	UVGI configuration	Total count concentration ($\#\,m^{-3}$)	Culturable count concentration ($CFU\,m^{-3}$)
B. subtilis spores	0 ACH	100%	$1.06\ (0.13) \times 10^8$	$1.12\ (0.32) \times 10^5$
		off	$8.59\ (0.18) \times 10^7$	$4.64\ (0.59) \times 10^5$
	0 ACH	100%	$4.39\ (0.19) \times 10^7$	$3.38\ (0.72) \times 10^6$
		off	$4.13\ (0.31) \times 10^7$	$1.41\ (0.08) \times 10^7$
	6 ACH	100%	$4.93\ (0.35) \times 10^6$	$4.78\ (2.10) \times 10^5$
		off	$5.56\ (0.63) \times 10^6$	$1.38\ (0.02) \times 10^6$
	6 ACH	100%	$8.66\ (1.72) \times 10^6$	$5.51\ (0.50) \times 10^5$
		off	$7.36\ (1.54) \times 10^6$	$8.73\ (1.37) \times 10^5$
	0 ACH	100%	$2.10\ (0.43) \times 10^8$	$6.12\ (5.80) \times 10^5$
		off	$1.60\ (0.54) \times 10^8$	$2.20\ (0.62) \times 10^7$
	0 ACH[b]	100%	N/A	$1.33\ (1.47) \times 10^4$
		off	N/A	$1.92\ (0.85) \times 10^5$
M. parafortuitum	6 ACH	100%	$1.58\ (0.21) \times 10^8$	$1.31\ (0.29) \times 10^7$
		off	$1.51\ (0.22) \times 10^8$	$2.57\ (0.23) \times 10^8$
	6 ACH	100%	$7.45\ (0.77) \times 10^7$	$2.34\ (0.35) \times 10^7$
		off	$7.84\ (0.18) \times 10^7$	$1.44\ (0.20) \times 10^8$
	0 ACH	100%	$6.80\ (0.51) \times 10^5$	$1.01\ (0.12) \times 10^3$
M. bovis BCG		off	$6.64\ (0.48) \times 10^5$	$3.71\ (0.22) \times 10^4$
	0 ACH	100%	$6.14\ (1.50) \times 10^5$	$5.51\ (0.76) \times 10^3$
		off	$3.49\ (1.60) \times 10^5$	$7.67\ (4.43) \times 10^4$

[a] Average (standard deviation) concentration was calculated using measurements from 9 breathing zone locations within the room.
[b] Average (standard deviation) efficacy was calculated using culturable counts only; total counts were not available.

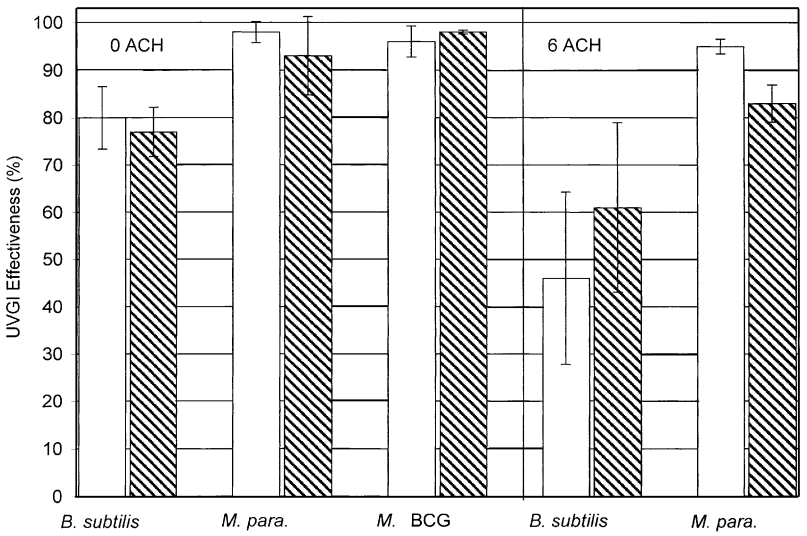

Fig. 3. Effectiveness of UVGI-induced microbial inactivation for constant-generation method experiments with 0 or 6 air changes per hour (ACH) ventilation rate for *B. subtilis* spores, *M. parafortuitum,* and *M. bovis* BCG. Height of the bar represents the average of breathing-zone measurements at 9 room locations; error bars represent the standard deviation. Experiments were repeated: ☐ indicates experiment 1, ▨ indicates experiment 2.

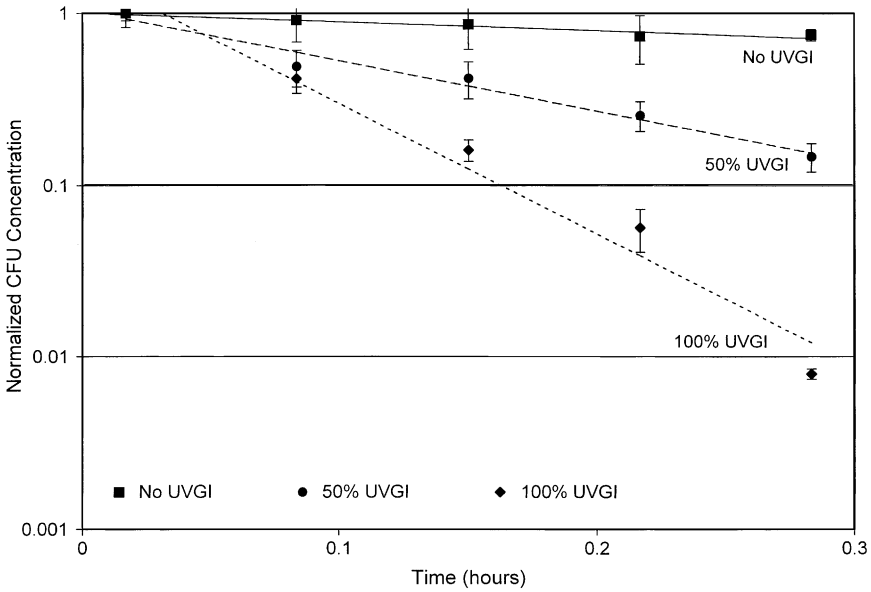

Fig. 4. Data from decay method experiments used to determine inactivation rates for *M. parafortuitum* induced by UVGI with ventilation rate of 0 air changes per hour and UVGI at 100%, 50% and off. UVGI inactivation rates are the difference between the slopes of the lines with UVGI on and off. Slopes of lines ± standard deviation of the lines are—no UV: $-1.2 \pm 0.21\,h^{-1}$, 50% UV: $-6.7 \pm 0.66\,h^{-1}$, 100% UV: $-17.5 \pm 1.8\,h^{-1}$. All slopes were statistically significant (F tests, $\alpha = 0.05$).

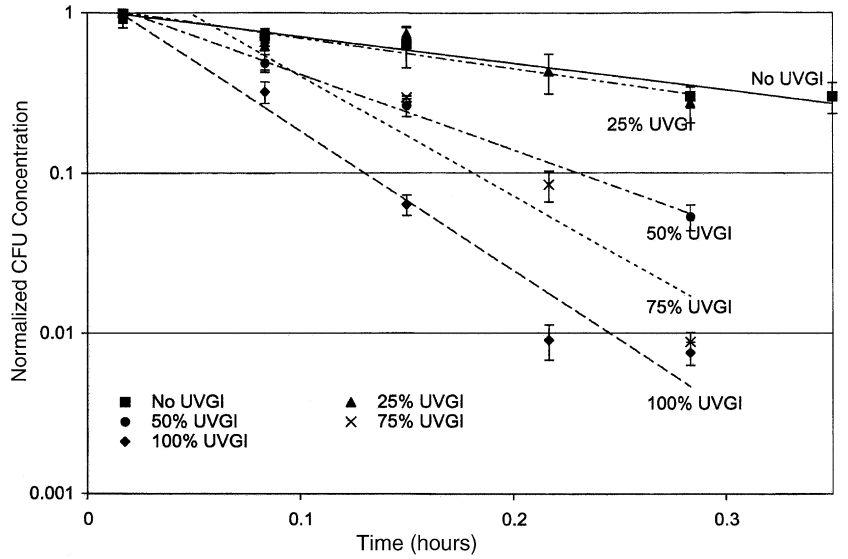

Fig. 5. Data from decay method experiments used to determine inactivation rates for *M. parafortuitum* induced by UVGI with ventilation rate of 3 air changes per hour and UVGI at 100%, 75%, 50%, 25%, and off. UVGI inactivation rates are the difference between the slopes of the lines with UVGI on and off. Slopes of lines ± standard deviation of the lines are — no UV: $-3.2 \pm 0.40\,h^{-1}$, 25%UV: $-4.4 \pm 1.0\,h^{-1}$, 50%UV: $-10.9 \pm 0.40\,h^{-1}$, 75%UV: $-17.3 \pm 3.1\,h^{-1}$, 100%UV: $-20.0 \pm 2.4\,h^{-1}$. All slopes were statistically significant (F tests, $\alpha = 0.05$).

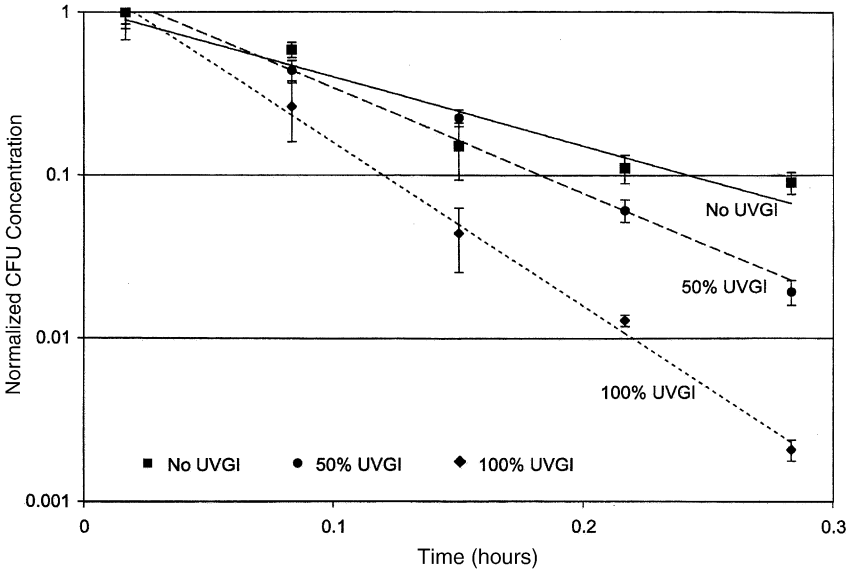

Fig. 6. Data from decay method experiments used to determine inactivation rates for *M. parafortuitum* induced by UVGI with ventilation rate of 6 air changes per hour and UVGI at 100%, 50% and off. UVGI inactivation rates are the difference between the slopes of the lines with UVGI on and off. Slopes of lines ± standard deviation of the lines are — no UV: $-9.7 \pm 1.8\,h^{-1}$, 50% UV: $-14.8 \pm 1.1\,h^{-1}$, 100% UV: $-23.1 \pm 0.78\,h^{-1}$. All slopes were statistically significant (F tests, $\alpha = 0.05$).

$16 \pm 1.8\,h^{-1}$ (Fig. 4). When the UVGI irradiance was cut by 50%, *M. parafortuitum* responded to the UVGI system with an inactivation rate of $5.6 \pm 0.69\,h^{-1}$. Experiments conducted at 3 ACH ventilation rate, and for 25%, 50%, 75% and 100% of the UVGI lamps operating, resulted in ACH_{UV} values of 1.2 ± 1.1, 7.7 ± 0.57, 14 ± 3.1, and $17 \pm 2.4\,h^{-1}$, respectively (Fig. 5). Experiments conducted at 6 ACH ventilation rate for 50% and 100% UV lamps operating resulted in ACH_{UV} values of 5.1 ± 2.1 and $13 \pm 1.9\,h^{-1}$, respectively (Fig. 6). The average inactivation rate of the UVGI system from experiments under different ventilation rates in our study for *M. parafortuitum* was $16 \pm 1.2\,h^{-1}$ for 100% UV, and $6.1 \pm 0.76\,h^{-1}$ for 50% UV.

3.5. Z value

The response of *M. parafortuitum* as judged by inactivation rates was nearly linear as the UVGI system was increased from 25% to 100% capacity, during the 3 ACH ventilation rate experiments. To estimate Z value, UV irradiance levels measured by actinometry were first converted to effective UV irradiance by dividing by the ratio of upper-room UV volume to total room volume (0.3/2.5 m). Fig. 7 shows the linear relationship between inactivation rate versus effective UV irradiance. The least-squares method was used to fit a line to the data; the slope of the line (± standard deviation of the regression line), which is the Z value, is $1.2 \pm 0.15 \times 10^{-3}\,cm^2\,\mu W^{-1}\,s^{-1}$.

4. Discussion

Overall, our data demonstrate that the upper room-air UVGI system tested was able to inactivate airborne bacteria spores and mycobacteria and significantly decrease their culturable airborne cell concentrations. Factors influencing the efficacy of upper-room air UVGI include room ventilation rates, UV irradiance levels, bacterial physiology and species, airflow patterns, relative humidity (RH), and photoreactivation. While this paper addresses effects of ventilation rates, UV irradiance levels, and bacteria species, we have also studied the effects of airflow patterns, RH, and photoreactivation (Peccia et al., 2001; Peccia and Hernandez, 2001; Xu and Miller, 1999).

In our study, we used two indices to describe the impact of UVGI on airborne bacteria. One is the *effectiveness,* E (Eqn. (1)). The other is the *inactivation rate,* ACH_{UV} (Eqn. (3)). The effectiveness provides a clear picture of the decrease in culturable airborne bacteria concentrations that can be achieved under given environmental conditions. Also, it is not based on assuming the room air is well mixed. The disadvantage of effectiveness is that it is not an intrinsic parameter of UVGI itself; it is a relative parameter depending on

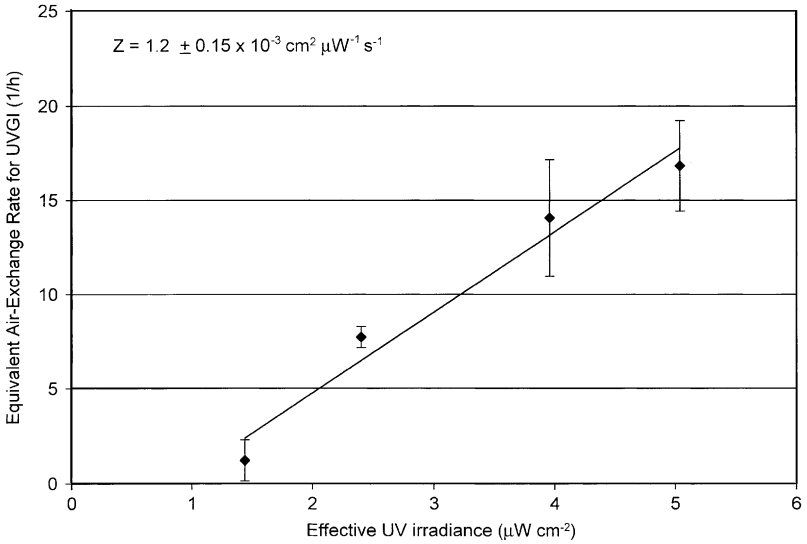

Fig. 7. UVGI inactivation rate as a function of effective UVGI spherical irradiance for *M. parafortuitum.* Effective UVGI spherical irradiance is the irradiance measured by actinometry in the upper-room zone only normalized to the fraction of room volume irradiated by UV (0.3/2.5 m).

factors such as the ventilation rate, air mixing conditions, and room configuration.

In our data we observed that increasing the ventilation rate from 0 to 6 ACH decreased the effectiveness for *B. subtilis* spores. This decrease was not statistically significant as judged by an independent *t*-test at the $\alpha = 0.05$ level, although it was significant at the $\alpha = 0.09$ level. Increasing the ventilation rate did not statistically decrease the effectiveness for *M. parafortuitum* ($\alpha = 0.2$). Our data suggests that modifying the ventilation rate in an environment in which UVGI is applied may change its effectiveness, however our data is by no means conclusive. Theoretically, one can argue that increasing the ventilation rate will result in a higher overall removal of airborne contaminants, but it will lessen the relative effectiveness of the UVGI system since the average residence time of the bacteria within the room will be reduced and the bacteria will not receive as much UV exposure. More studies on the impact of ventilation on effectiveness need to be conducted to conclusively determine if ventilation reduces the relative influence of UVGI and at what level.

The inactivation rate is unrelated to the ventilation rate and provides a direct measure of the removal rate of airborne bacteria due to a given level of UVGI. It is, however, based on the assumption of complete mixing. If mixing is not consistent, the ACH_{UV} can not be interpreted nor will it be reproducible. Inactivation rates can only be derived accurately with time-dependent data.

Our results demonstrate that the UVGI system plus ventilation achieved reductions of culturable airborne bacteria with effectiveness ranging 46–98%. Previous researchers have also found UVGI effectiveness to be near this range. Goldner et al., measured approximately half the concentration of culturable, ambient airborne bacteria in two operating rooms when ceiling-mounted UVGI lamps were operated (Goldner et al., 1980). An investigation of a hospital waiting room in which UVGI luminaries were installed demonstrated effectiveness ranging between 14–19% for ambient airborne bacteria (Macher et al., 1992, 1994). Miller and Macher determined in a series of 36-m^3 room experiments that the effectiveness of one wall-mounted germicidal luminary (15 W, unlouvered) and 2 ACH ventilation was approximately 50% for *B. subtilis* spores and *Micrococcus luteus.* For *Escherichia coli,* effectiveness was close to 100% (Miller and Macher, 2000). Ko et al., (2002) found for ceiling- and wall-mounted fixtures (59 W total), effectiveness under mixed conditions ranged between 53% and 88% with 6 ACH ventilation and 62% with 2 ACH ventilation. Note that our calculations of effectiveness include normalization by total bacterial counts, to account for the possible variability in bioaerosol generated between experiments (Eqn. (1)). The other experiments cited above did not account for how much bacteria was aerosolized, and instead assumed that it was the same, which could lead to errors in the estimation of effectiveness if the amount of bioaerosol released was different between experiments.

Previous research has suggested that *M. bovis* BCG has similar responses to irradiation as virulent forms of *M. tb* (Riley et al., 1976). We observed similar UVGI effectiveness between *M. parafortuitum* and *M. bovis* BCG, suggesting that under certain full-scale environmental conditions, *M. parafortuitum* behaves similarly to *M. bovis* BCG in terms of its inactivation response as judged by effectiveness.

With the room air well mixed, the UVGI system (all lamps operating, 216 W) provided an average $16\,h^{-1}$ as an inactivation rate for *M. parafortuitum*. Riley et al. (1976) reports inactivation rates of $10–33\,h^{-1}$ depending on the total power of the UVGI lamps, for *M. bovis* BCG. This study was conducted in a smaller room ($64\,m^3$ with $18.4\,m^2$ floor area) using 17 and 46 W ceiling mounted unlouvered lamps. No UV irradiance measurements were made. Miller and Macher (2000) measured in a 36-m^3 room ($14.7\,m^2$ floor area) with one unlouvered 15-W wall lamp an inactivation rate for *B. subtilis* spores of $4\,h^{-1}$.

A linear relationship was observed between the UVGI inactivation rate, ACH_{UV}, and level of UV irradiance. This implies that higher irradiance from a UVGI system provides better inactivation of airborne bacteria. Due to experimental limitations, it is not known whether this linear relationship can be extended to UV irradiance levels higher than the levels provided by the tested system. There is most likely an upper threshold at which adding more UVGI will not achieve similar additional inactivation. However, because the UVGI irradiance of all previously published studies were below this threshold (Miller and Macher, 2000; Riley et al., 1976), there is no scientific evidence yet to support this hypothesis. We found that at UV irradiance levels below an average of $12\,\mu W\,cm^{-2}$ in the upper-room zone, minimal inactivation effects were observed against *M. parafortuitum*. Such low upper-room UV irradiance levels only provided $1.2\,h^{-1}$ inactivation under well-mixed conditions.

Using this linear relationship between UVGI inactivation rates and irradiance, we derived a Z value of $1.2 \pm 0.15 \times 10^{-3}\,cm^2\,\mu W^{-1}\,s^{-1}$ for aerosolized *M. parafortuitum*. In our opinion, the Z value is one of the best methods to parameterize the impact of UVGI on microorganisms, because it is only dependent on microorganism physiology. Only a handful of studies have derived Z values for airborne bacteria. A separate study was carried out in a small-scale chamber ($0.8\,m^3$) at the University of Colorado parallel to the studies reported here (Peccia, 2000; Peccia et al., 2001; Peccia and Hernandez, 2001). The entire chamber was irradiated and the UV irradiance was scalable to our tests. Under the same environmental conditions, Z values determined for aerosolized mycobacteria were $1.9 \times 10^{-3}\,cm^2\ \mu W^{-1}\,s^{-1}$ for *M. bovis* BCG and $1.6 \times 10^{-3}\,cm^2\,\mu W^{-1}\,s^{-1}$ for *M. parafortuitum,* which agrees well with our value for *M. parafortuitum*. The Z values determined by Riley et al., (1976) are $2.3–3.9 \times 10^{-3}$ for aerosolized *M. bovis* BCG and $2.3–4.2 \times 10^{-3}$ for aerosolized *M. tuberculosis* (Erdman strain).

The UVGI luminaries installed in the test room were positioned at different locations around the room. Consequently the irradiation at a given point in the room was from all directions. To characterize the distribution of UV irradiance, three-dimensional measurements were needed. The actinometric measurement technique was useful because the spherical actinometers that were employed are able to evaluate a three-dimensional UV irradiance field. Traditional radiometer measurements detect surface UV irrradiance from one direction. Any UV measurement technique should be altered to account for UV irradiance from many directions, as was done in our evaluation. We found that a three-dimensional measurement could be approximated adequately using a radiometer by measuring the irradiance in six different directions (up, down, and all four horizontal directions) and summing (Rahn et al., 1999).

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