

Method for Evaluating Germicidal Ultraviolet Inactivation of Biocontaminated Surfaces

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Safety issues related to work-site conditions often deal with potential worker exposure to infectious airborne microorganisms due to their dissemination in indoor air and contamination of surfaces. Germicidal ultraviolet (GUV) radiation is used in health-care settings and other occupational environments for microbial inactivation. In this study, a new methodology for determining the efficiency of GUV microbial inactivation of surfaces was developed and evaluated. The method utilizes identical chambers in which test microorganisms are irradiated on agar surfaces at different humidity and irradiation intensity levels. The effects of GUV intensity and exposure time on microbial inactivation were examined for *Micrococcus luteus* and *Serratia marcescens*. It was found that at low humidity levels (20-25%) both organisms can be inactivated with at least 95% efficiency if the GUV intensity exceeds $50 \mu\text{W}/\text{cm}^2$ for at least 3-5 min (corresponding to a dose of $\sim 10 \text{ mJ}/\text{cm}^2$). The radiation dose needed for effective inactivation of *S. marcescens*, as measured by a UV meter near the microbial sample, was found not to be affected by the humidity level, whereas

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that of *M. luteus* increased at higher humidities. The findings of this study can be used to determine sufficient GUV inactivation doses for occupational environments with various microbial contaminations.

UV inactivation microorganisms agar colony chamber

1. INTRODUCTION

Biocontamination of occupational environments is of increasing public concern as it is associated with potential health hazards caused by airborne microorganisms. Biological aerosols are considered a significant source for the spread of viral infections and the increase of allergic reactions (Husman, 1996). These health effects depend upon several factors, including the microbial concentration in air (Fernandez-Caldas, Trudeau, & Ledford, 1994).

Germicidal ultraviolet (GUV) radiation is often recommended as an engineering control or preventive measure for inactivation of airborne bacteria in biocontaminated occupational environments. Several GUV applications for bioaerosol disinfection, such as duct irradiation techniques (Iseman, 1992; Nagin, Pavelchak, London, DePriss, & Melius, 1994), self-contained air cleaning units (Nagin et al., 1994), and upper-room air irradiation devices (Nardell, 1993) have been used in hospitals and other health-care facilities, drug-treatment centers, correctional facilities, as well as in some industrial environments.

GUV is ultraviolet radiation from the UV-C region of the electromagnetic spectrum (wavelength $\lambda = 200\text{--}260$ nm). Most of the commercially available GUV sources operate at $\lambda = 254$ nm and are known to be an effective germicidal agent (Riley, Knight, & Middlebrook, 1976; Salie, Scarpino, Clark, & Willeke, 1995). A microorganism's susceptibility to GUV varies by species and depends on the air humidity level and the state of irradiated organisms (e.g., airborne vs. attached to a surface).

Irradiation of airborne organisms has been the primary focus of many studies (see, e.g., the review by Riley & Nardell, 1989). However, bioaerosol particles may not remain airborne, as they can deposit onto indoor surfaces due to gravity, air turbulence, and other effects. Deposited organisms may reproduce and become airborne again, thus potentially increasing the bioaerosol concentration. Effective GUV irradiation of surfaces containing viable microorganisms may, therefore, be desirable in many situations in workplaces.

In this study, a methodology was developed for determining the efficiency of microbial inactivation on surfaces exposed to germicidal ultraviolet radiation. The test microorganisms were irradiated on agar surfaces in specially designed chambers at different humidity levels. As microorganisms have the greatest opportunity to recover after UV irradiation while they are attached to a nutrient agar, microbial irradiation on agar surfaces gives the lowest achievable and hence most conservative decontamination efficiency. Irradiation of bacteria on agar plates was first used by Gates (1928) and since then by several other researchers, for example, by Collins (1971). The two microorganisms used for testing this method and system, *Micrococcus luteus* and *Serratia marcescens*, are rated as biosafety level 1 (Murray, 1995). They represent significantly different susceptibility levels to GUV irradiation and have been used in several previous experimental studies on GUV irradiation, for example, by Riley and Kaufman (1972) and Salie et al. (1995). Among the many bacteria of interest, the inactivation of *Mycobacterium tuberculosis* (*Mtb*, biosafety level 3) is of particular interest, because of the increasing spread of tuberculosis in occupational environments and the prevalence of drug and multi-drug resistant strains of *Mtb*. However, very limited data are available on *Mtb* inactivation caused by GUV irradiation (Riley & Nardell, 1989). *M. luteus* and *S. marcescens* were chosen to represent GUV susceptibility levels that are believed to be lower and higher, respectively, than those of *Mtb*, thus bracketing the unknown *Mtb* dose response (Riley & Nardell, 1989; Salie et al., 1995).

2. MATERIALS AND METHODS

2.1. Description of the New Method

The new method and test system have been developed in this study to provide a tool for evaluating the GUV inactivation efficiency of biocontaminated surfaces. This system consists of four identical test chambers. In each chamber, the test microorganisms were deposited onto an agar surface and were then irradiated by a 9-watt, low pressure, mercury GUV lamp (model PL-S 9W/TUV, Phillips Lighting Company, Somerset, NJ). While employing GUV sources of the same power, the irradiation intensity sensed by the microorganisms was set to a different level in each chamber through an adjustable aperture installed under each lamp.

Thus, four UV intensity levels, 25, 50, 75, and 100 $\mu\text{W}/\text{cm}^2$, were tested simultaneously in every experiment.

Figure 1 schematically shows the design of one of the identical test chambers. Each chamber was made of 1/2-inch-thick (1.27 cm) Plexiglas. Temperature and humidity controlled air was supplied from the top of the chamber at 5 L/min and exhausted at the bottom. The UV lamp was permanently installed at the top of the chamber and was centered over an adjustable aperture so that only UV passing through the aperture would expose the agar surface. The periphery of the agar plate was covered by opaque material, thus limiting the UV irradiation and subsequent colony enumeration to a circular area of 5 cm^2 . Spectral irradiance measurements, performed with the spectroradiometer system (model OL754, Optronic Laboratories Inc., Orlando, FL) from $\lambda = 200$ to 800 nm at 1 nm intervals, confirmed that 85% of the intensity from the UV-C region was produced at $\lambda_{\text{mean}} = 254$ nm. Some of the lamp's

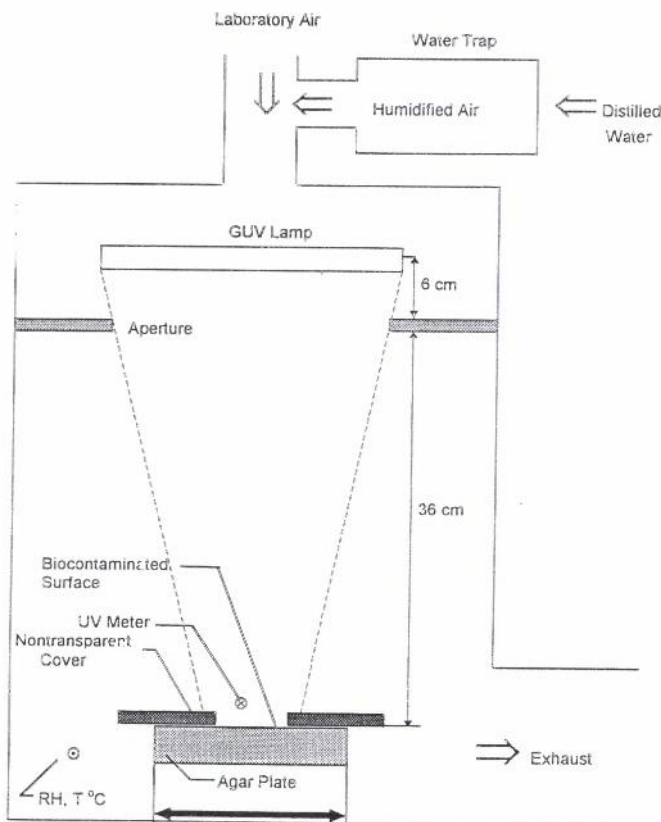


Figure 1. Test system for evaluating germicidal ultraviolet (GUV) inactivation of biocontaminated surfaces.

optical energy was emitted at wavelengths up to 800 nm. As the lamps were entirely surrounded by Plexiglas, potential occupational exposure situations were entirely avoided.

A standard petri dish (Fisher Scientific, Pittsburgh, PA) containing 20–30 ml of agar was inoculated with the test bacteria prior to each experiment (the culture preparation is described below). The agar plate was centered on the movable stand in the chamber at the distance of 42 cm from the UV source. The UV photodetector (model 1400, International Light, Inc., Newbury Port, MA) that measured the UV intensity near the irradiated surface was also attached to this stand. The stand could slide in and out of the chamber through an access door so that the inoculated plates could be subjected to the irradiating environment for the test time interval without having the lamp turned on and off. This assured that the approximately 5 min warm-up time needed for the stable operation of the GUV lamp did not affect the experimental data. Prior to each test, the spatial orientation of each agar plate was checked relative to the lamp. The duration of each test was measured with a timer (Gralab, model 171, Dimco-Gray Co., Centerville, OH).

Four agar plates were irradiated simultaneously during the exposure phase (one per chamber). In addition, one control sample was set aside for the same time interval as used during irradiation testing. After the exposure, the plates were incubated (procedure described later) and the colony enumeration was performed on the irradiated area of each plate (a circle was drawn on each test and control plate prior to testing). Microbial survival was defined as the ratio of colony forming units (CFU) developed and counted on the irradiated sample to the CFU's on the control (non-irradiated) sample. Microbial inactivation was defined as the difference between unity and microbial survival, and has been recorded as a percentage. This percentage was determined for each chamber at different GUV intensity levels, exposure times, and relative humidities.

In order to vary the relative humidity, a 1/4-inch (0.64 cm) brass hose inlet was built into the top of each chamber for introducing humidified air. The desired humidity level was achieved by supplying distilled water through the water trap filled with dry rite. The water trap allowed to collect excess condensation. The humid air was mixed with the laboratory air at a certain dilution ratio to set up specific relative humidity. The enclosures were placed at both openings of the chamber during the test to maintain this humidity level. The relative humidity

and temperature was measured over time, using a thermohygrometer (Hygrotest 6400, Testoterm Inc., Mount Freedom, NJ).

2.2. Laboratory Testing of the Method

2.2.1. Culture preparation

The test microorganisms, *Micrococcus luteus* ATCC 4698, and *Serratia marcescens* ATCC 14756, were obtained from the American Type Culture Collection (Rockville, MD). These organisms were incubated in Trypticase soy broth (Becton Dickinson Microbiology Systems, Inc., Cockeysville, MD) at 37 °C for 48 and 24 hrs, respectively, in an IEC Centra shaker/incubator at 110 rpm (model 3528, Labline, Melrose Park, IL). The bacterial concentration in the suspension after incubation was approximately 10^8 CFU/ml for each microorganism. Four trials were performed to confirm that this concentration level was consistent. To attain a desired bacterial concentration per plate, 1:10 dilutions were performed by mixing 1 ml aliquot into 9 ml of potassium phosphate buffer (PB10). A 100 μ l aliquot of the final dilution was then placed on a Trypticase soy agar plate resulting in a concentration of 10^3 CFU per plate. Each plate was spread in the same manner to ensure similar surface distributions of the organisms. The spreader was placed against the edge of the petri dish and the plate was rotated five times in a clockwise direction (American Public Health Association [APHA], 1995).

2.2.2. Test conditions

The maximum GUV irradiance level of 100 μ W/cm² was chosen because this level always resulted in a 100% microbial inactivation so that the results became redundant at higher levels. The minimum irradiance level of 25 μ W/cm² was selected because it was expected to be insufficient for microbial inactivation. The irradiation intensities of 50 and 75 μ W/cm² were selected to see the transition between the two extremes.

The chosen GUV intensity range and the distance from the source to the biocontaminated surfaces corresponds to other related studies. For instance, Salie et al. (1995) used a UV source having irradiance of 35 μ W/cm² at 1 m (these tests were conducted with airborne *E. coli*, *M. luteus*, and *B. subtilis*). Our study involves bacteria on agar surfaces that are assumed to be less susceptible to GUV irradiation than airborne

bacteria. The exposure time in our tests ranged from 1 to 9 min, resulting in doses of 1.5 to 54 mJ/cm² for the tested irradiance levels.

The tests were conducted at different humidity levels ranging from 20 to 95%. The reported data represent two extremes, low RH = 20–25% and high RH = 90–95%. The longitudinal humidity gradient, measured at four positions in the chamber (at the GUV lamp's level, and at 15, 30, and 45 cm downstream from the lamp) was found to be insignificant at RH \lesssim 80–85%: the difference in RH between the meters positioned at the two farthest points did not exceed 5%. However, in preliminary studies, the actual GUV irradiance measured near the test agar surface was found to decrease with humidity at RH \gtrsim 80%. This decrease became more pronounced with time. Water droplets were observed in the chamber after about 1–1.5 hrs of continuous testing at RH = 90%. Therefore, continuous testing at high humidities did not exceed 1 hr and the referred RH value was determined by the humidity meter nearest to the agar plate (shown in Figure 1). Every new test required opening the access door twice, at the beginning and at the end, in order to pull in or out the movable stand with the agar plate. This was found to disturb the humidity equilibrium in the chamber when RH was above 90%. A time of about 1 to 5 min (depending on RH) was needed to reach equilibrium after the access door was closed and irradiation began. Therefore, the tests at RH = 90–95% were conducted only for exposure times that exceeded 5 min. The control plates were placed in the chamber for the same time duration as the test plates, but with the lamp off. No significant difference was observed in the CFU count on non-irradiated plates exposed to the laboratory air environment (RH \approx 50%) versus the dry or humid air environments in the chamber.

At least three trials were performed for each set of the test conditions. On average, the tests were conducted with six repeats.

2.2.3. Colony enumeration

The CFU enumeration on the test and control plates was performed after the designated incubation times: 48 hrs for *M. luteus* and 24 hrs for *S. marcescens* (Murray, 1995). An optical microscope (model 104 SM2-U, New Brunswick Scientific, Inc., Edison, NJ) was used at 10X and 50X. The number of countable colonies in most of the tests varied from 30 to 300 CFU per designated circular area of the plate, thus meeting the colony enumeration criteria (APHA, 1995; Chang, Hwang,

Grinshpun, Macher, & Willeke, 1994; Chang et al., 1995). When the GUV dose was sufficiently high to ensure a 100% level of microbial inactivation, no colonies were observed on the counting area. After enumeration, all the plates were put into the incubator for another approximately three days, and were then counted again to ensure that there was no bias due to microbial injury and further recovery (no change in CFU number was noticed after this prolonged incubation).

3. RESULTS AND DISCUSSION

This section presents some test results that illustrate the performance of the method and system introduced in the previous section. The standard deviation (*SD*) of microbial inactivation, calculated for each set of the test conditions based on at least three repeated measurements, was as low as 3% when the test time exceeded $t = 3\text{--}5$ min and the GUV intensity was $50 \mu\text{W}/\text{cm}^2$ or higher. At $25 \mu\text{W}/\text{cm}^2$, the microbial inactivation data had reduced precision and reproducibility for different exposure time intervals, which was attributed to microbial recovery occurring at insufficient irradiating doses. Therefore, further discussion is focused only on data obtained at GUV irradiation levels of 50, 75, and $100 \mu\text{W}/\text{cm}^2$. For the shorter time intervals, the *SD* was relatively high for all irradiation levels: about 30–40% for *S. marcescens* at $t = 1$ min. The time needed to move the agar plates in and out during every test was about 5–10 s. This may have significantly affected the accuracy of microbial inactivation for the tests with an exposure time of 1 min.

Figure 2 shows the microbial inactivation for the two microorganisms on agar as a function of exposure time at GUV intensities of 50, 75, and $100 \mu\text{W}/\text{cm}^2$ and $\text{RH} = 20\text{--}25\%$. At all three intensities, the microbial inactivation percentages reached a plateau of $\geq 95\%$ after exposure times from 3 to 5 min. The irradiating dose needed to assure a 95% or higher efficiency of microbial inactivation at $\text{RH} = 20\text{--}25\%$ was found to be on the order of $10 \text{ mJ}/\text{cm}^2$ for both microorganisms: approximately $5\text{--}10 \text{ mJ}/\text{cm}^2$ for *S. marcescens* and $10\text{--}20 \text{ mJ}/\text{cm}^2$ for *M. luteus*. Whereas 95% was considered as the microbial inactivation criterion, it should be noted that the microbial inactivation level was 99% or higher in most tests after 5 min of exposure to GUV radiation.

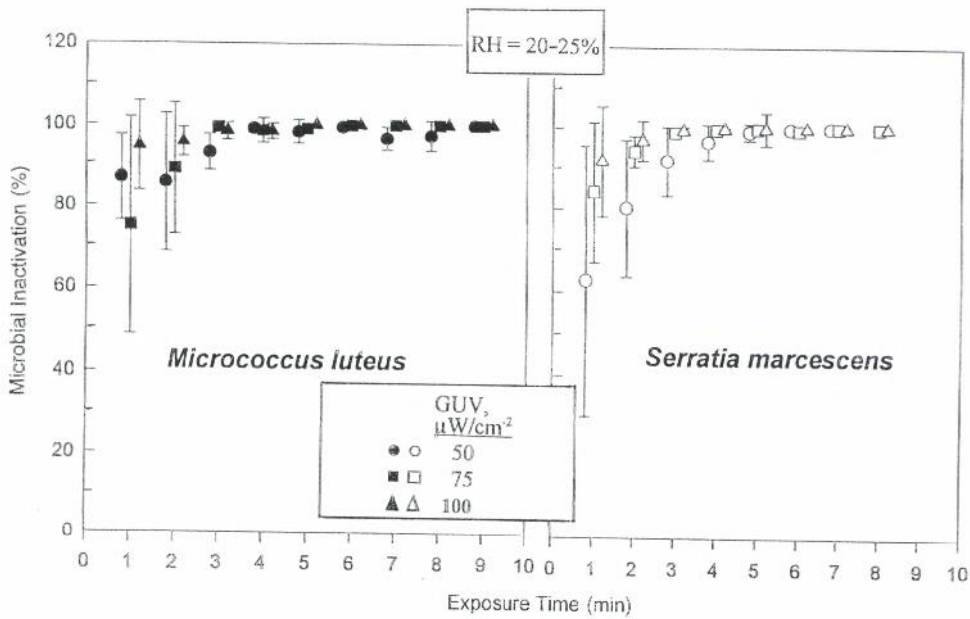


Figure 2. Effect of exposure time and germicidal ultraviolet (GUV) intensity on microbial inactivation at low humidity (RH = 20–25%).

At higher humidity levels, a significant difference was observed between the GUV inactivation of *M. luteus* and *S. marcescens*. Table 1 shows the microbial inactivation values measured at RH = 90–95% and GUV irradiance of $50 \pm 5 \mu\text{W}/\text{cm}^2$. The accuracy of GUV intensity measured near the microbial surface was lower at the higher humidities due to absorption or scattering of GUV light, caused by water condensation in the chamber. The indicated exposure times of 5 to 9 min resulted in doses of 15 to 27 mJ/cm^2 . These doses appeared to be sufficient for complete inactivation of *S. marcescens*, but were insufficient

TABLE 1. Effect of Exposure Time on Microbial Inactivation at High Humidity (RH = 90–95%) and Germicidal Ultraviolet (GUV) Intensity of $50 \pm 5 \mu\text{W}/\text{cm}^2$

Exposure Time (min)	Dose (mJ/cm^2)	Microbial Inactivation % (mean \pm SD)	
		<i>M. luteus</i>	<i>S. marcescens</i>
5	15	54 \pm 20	100
6	15	67 \pm 24	100
7	21	74 \pm 25	100
8	24	87 \pm 15	100
9	27	93 \pm 10	100

for *M. luteus*. The inactivation of the latter was found to increase with exposure time. This difference in inactivation of *M. luteus* versus *S. marcescens* in humid air is attributed to their different sensitivity to the microbial stress caused by GUV irradiation.

Occupational environments are characterized by a variety of microbial species (ranging from sensitive bacterial vegetative cells to hardy bacterial or fungal spores). There are also a variety of environmental conditions: for example, RH ranges from about 30% (health-care units) to 100% (some industrial environments). Therefore, the GUV inactivation dose may considerably vary from one workplace to another. This suggests that existing and newly-developed GUV irradiation facilities must be evaluated in a laboratory at different humidities with specific microorganisms prior to their field testing. The method and test system described in this paper can accommodate this laboratory evaluation.

4. CONCLUSIONS

The new method and test system, developed for evaluating GUV inactivation of biocontaminated surfaces in occupational environments, was shown to work well for the irradiation of bacteria on agar media at different UV intensities, exposure time intervals and humidity levels. The microorganisms are irradiated on agar plates in order to test at the highest microbial survival rate. The new methodology was tested with two bacteria, *M. luteus* and *S. marcescens*. The irradiating dose needed for effective inactivation of *S. marcescens* was found not to be affected by the humidity level. In contrast, *M. luteus* was found to be inactivated less efficiently at higher humidities. The humidity effect observed for *M. luteus* alters the effectiveness of the ultraviolet radiation as the germicidal agent. The physical and microbiological mechanisms that can adequately explain this effect need to be further studied. The findings of this study can be used to assess the inactivation of *Mtb* in the presence of GUV radiation, as the two test organisms bracket the *Mtb* susceptibility to GUV. The newly-developed method and test system was proven to be effective for evaluating GUV inactivation of biocontaminated surfaces. The findings of this study can be used to determine sufficient GUV inactivation doses for occupational environments with various microbial contaminations.

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