ELSEVIER



Chemical Engineering Journal

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

The effectiveness of UV irradiation on vegetative bacteria and fungi surface contamination

M.Y. Menetrez^{a,*}, K.K. Foarde^{b,1}, T.R. Dean^a, D.A. Betancourt^{a,2}

 ^a U.S. Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, Air Pollution Prevention and Control Division, 109 TW Alexander Drive, Research Triangle Park, NC 27711, USA
 ^b Research Triangle Institute, Microbiology Department, Research Triangle Park, NC 27709, USA

ARTICLE INFO

Article history: Received 29 April 2009 Received in revised form 3 December 2009 Accepted 4 December 2009

Keywords: UV Antimicrobial Germicidal Antimicrobial Efficacy HVAC Biocontaminant

1. Introduction

Ultraviolet (UV) irradiation has been used for the disinfection of air streams for many years [1,2]. The range of UV wavelengths found to be most effective was 220–300 nm, and the peak effectiveness was determined to be 265 nm [3]. The production of UV light employs an electrical discharge through low-pressure mercury vapor enclosed in a quartz glass tube [3]. This technique produces a tube type bulb with a primary wavelength of 253.7 nm, and is within the "C" band of UV (UVC) [3]. The UVC or UV germicidal (UVGI) form of irradiation has been demonstrated to deactivate bacteria, fungi, viruses, and mycoplasmas [4–8].

Among the applications of UVGI irradiation on indoor biocontaminants is controlling the transmission of infectious diseases (mainly tuberculosis) in medical facilities [5–7], or bioterrorism countermeasures in public buildings [9]. However, a growing trend in UVGI applications is the use of heating, ventilating, and airconditioning (HVAC) duct-mounted UVGI treatment of indoor air [10,11].

ABSTRACT

Ultraviolet irradiation has been used in the indoor environment to eliminate or control infectious diseases in medical care facilities. Heating, ventilating, and air-conditioning (HVAC) system components such as duct-liners, cooling coils, drip pans, interior insulation and areas subjected to high levels of moisture can create an environment which is prone to biological contamination on surfaces. The movement of indoor air being dominated by HVAC system operation can carry biological contaminants which can expose large numbers of building occupants to bioaerosols. The use of germicidal ultraviolet lamps (UVGI) in commercial and residential HVAC systems has increased. UVGI treatment has focused on HVAC component internal surfaces and airflow. A method to determine the antimicrobial efficacy of UVGI irradiation was developed and tested on the surface of agar plates with four species of vegetative bacteria and seven species of fungi. The percent kill and the kinetics of the rate of killing, *k* value, were calculated for each organism.

© 2010 Published by Elsevier B.V.

HVAC systems in constant use can show adherence of dirt and possible hazardous substances to the surface of the components and duct lines [7,8,12]. The degree of surface dirt and debris, as well as the environmental conditions involving HVAC system operation and maintenance which can introduce moisture, may initiate the active growth of microorganisms [8,13]. Surface contaminants can then become airborne and transported throughout the building interior. Exposure to these microorganisms and their metabolites can cause significant health consequences, especially to individuals with pulmonary disease [13–17]. HVAC system intakes import large quantities of outside air (OA) and distribute a mixture of OA and recycled air throughout the building interior. Both OA and recycled air can carry biological contaminants which can expose large numbers of building occupants to toxic and pathogenic microorganisms [4,17,18].

The use of UV lamps in HVAC system ducts for the control of biocontaminants has increased and with it the need for the proper design of UV applications to maximize UVGI antimicrobial efficacy. A variety of research approaches have focused on either biocontaminants in air flowing through ducts, or on surfaces. The research presented by the Air-Conditioning and Refrigeration Technology Institute (ARTI) was limited to in-duct use of UV lamps intended to disinfect flowing air [3]. UV applications can involve the disinfection of air or surfaces. HVAC surfaces in contact with condensate flow (such as cooling coils or drip pan) provide a dark and moist environment conducive to the growth of biological contaminants. Once the contaminants are established, these surfaces can act as

^{*} Corresponding author. Tel.: +1 919 541 7981; fax: +1 919 541 2157. *E-mail addresses:* menetrez.marc@epa.gov (M.Y. Menetrez), kkf@rti.org (K.K. Foarde).

¹ Tel.: +1 919 541 8018; fax: +1 919 541 6936.

² Tel.: +1 919 541 7981; fax: +1 919 541 2157.

^{1385-8947/\$ –} see front matter © 2010 Published by Elsevier B.V. doi:10.1016/j.cej.2009.12.004



Fig. 1. UVGI exposure chamber.

a source for the distribution of biological pathogens and toxins throughout the building.

For constant and uniform irradiance, the disinfection effect of UVC on a population of microorganisms can be expressed by the following set of equations [19]:

$$\frac{N_t}{N_0} = \exp(-k \times \text{dose}) \tag{1}$$

where N_t = the number of microorganisms at any time t, N_0 = the number of microorganisms at start, before exposure begins, N_t/N_0 = the fraction of microorganisms surviving, k = a microorganism-dependent rate constant, cm²/µWs, dose = the product of $E_{\rm eff} \Delta t$, in µWs/cm², $E_{\rm eff}$ = the effective irradiance (constant) received by the microorganism, in µW/cm², and, Δt = exposure duration, in s, between the start and time t.

The concentration-time product calculated in Eq. (1) depicts the exponential decay of a population of viable microorganisms with constant exposure to UVGI. The fractional kill after time *t* is $(1 - N_t/N_0)$. Eq. (1) can also indicate the ability of populations of microorganisms to withstand low to high doses of UVGI irradiation.

Increasing use of UV lamps in building HVAC systems underscores the need for understanding the physical factors affecting UVGI antimicrobial efficacy. Menetrez et al. [20] examined the antimicrobial efficacy of UVGI bulbs on *Bacillus* bacteria. The larger question of how to optimize the application of UVGI design, with criteria specifying bulb irradiation intensity, positioning and the effectiveness in eliminating all common forms of biological contaminants on surfaces, should be developed for and made available to the UVGI manufacturing and installation industries. This study addresses another part of this much needed UVGI application research. We have measured the *k* value kinetic responses of common forms of biological contamination (vegetative bacteria and fungi) to UVGI exposure.

The objective of this research is to determine the antimicrobial efficacy of UVGI irradiation on surfaces and the potential of this technology to kill or inactivate vegetative bacterial and fungal contaminants by:

- identifying the variable physical factors affecting UVGI irradiance dose from light placement and time of exposure with regard to target surfaces, and
- 2. calculating the percent reduction in microorganism populations after exposure to UVGI.

To accomplish these objectives the measurement of populations of microorganisms receiving various levels of UV dosages was performed and compared using Eq. (1), which calculates the UVGI dose received. Dose, a function of distance from source, was kept constant and time of exposure was varied; dose was calculated for the irradiance which was measured in a test apparatus described below.

Representative microorganisms from three different groups were used in these experiments. The first group was the vegetative bacteria which would be the most readily killed by UVGI. Next were the yeasts, which would be somewhat more resistant. The last group was the fungal spores which are the most resistant to UVGI inactivation.

2. Materials and methods

Tests were conducted on a variety of bacteria and fungi which were representative of their microorganism group. In addition, these microorganisms chosen for testing can form a biofilm, or are human pathogen, or have innate cellular resistance to UV irradiation.

2.1. Microorganisms

Four species of vegetative bacteria (*Deinococcus radiodurans*, *Pseudomonas fluorescens*, *Serratia marcescens*, and *Staphylococcus epidermidis*), three species of yeast (*Candida albicans*, *Geotrichum candidum*, and *Rhodotorula mucilaginosa*), and four species of mold (*Aspergillus versicolor*, *A. fumigatus*, *Penicillium chrysogenum*, and *Cladosporium cladosporioides*) were exposed to UVGI irradiation. Most of these microorganisms have been identified as being present in HVAC air-handling units [10], and some may also be responsible for airborne disease [9].

D. radiodurans, a bacterium is one of the most radiation-resistant microorganisms that has been discovered. In the exponential phase of growth, *D. radiodurans* is 33 times more resistant to UV than *E. coli*, predominantly due to cellular mechanisms allowing the bacteria to rapidly repair double stranded DNA breaks [21]. *P. fluorescens*, a Gram-negative bacterium, is well known for forming biofilms and has been extensively studied to elucidate the conditions which support biofilm formation [22].

S. marcescens, a Gram-negative bacterium and opportunistic pathogen, can cause infections in the urinary tract, respiratory infections and infections in the blood stream, as well as ocular infections. The ability of *S. marcescens* to form biofilms may give this microorganism a competitive advantage and allow it to become infective [23]. *S. epidermidis* are Gram-positive cocci which have been found to cause infections following surgical implantation of medical devices. These infections are caused in part by the ability of *S. epidermidis* to form biofilms and can result in high morbidity and mortality due to the increasing prevalence of antibiotic-resistant microorganisms [24].

C. albicans is a yeast which forms biofilms and is frequently discovered in waterlines in medical and dental centers [25]. *G.*

candidum, another yeast that is isolated from medical water lines [25], is also an opportunistic pathogen attacking immunocompromised patients [26]. *R. mucilaginosa* is a yeast emerging as an opportunistic pathogen. Ailments attributed to this microorganism include endocarditis, meningitis, and peritonitis. This microorganism is now resistant to fluconazol, and studies are now being aimed at searching for optimal treatment strategies [27].

2.2. Test procedure

The UVGI light was placed inside a square HVAC duct mock-up behind a removable guillotine (Fig. 1, UVGI Exposure Chamber). The duct mock-up was lined with galvanized steel. The duct-mounted guillotine was constructed of galvanized steel and was used to separate the UVGI light source from the test microorganism to ensure exact exposure times. Before beginning each experiment, the UVGI light was turned on and allowed to warm up for approximately 15 min. The guillotine was also checked to insure that it was in the proper position (down) and adjusted if necessary. The exposure chamber was maintained at room temperature which was $22 \pm 0.55 \circ C$ (71.6 $\pm 1.0 \circ F$).

The inoculated plates were placed on a stand in the duct mockup and the door closed. The exposure time was initiated when the guillotine was very quickly lifted allowing the UV light to travel down the duct to impact the plates. At the end of the exposure time, the guillotine was quickly lowered back into the duct mock-up. In all experiments, the plates were kept the same distance from the light (144.0 cm, or 56 in.). As listed in Eq. (1), dose is a function of distance and time of exposure. With distance and irradiance constant, time of exposure is the controlling variable.

For each microorganism, a 10% transmittance suspension was prepared in sterile water. The suspension was diluted as necessary in sterile buffer to obtain the desired number of colony forming units (CFU). Once the proper dilution was obtained, 0.1 mL was spread onto a Petri dish filled with Saboraud Dextrose Agar (SDA) for the fungi and tryptic soy agar for the bacteria. The plates were pre-labeled with the microorganism name, length of UV exposure, and the date. A line was drawn across the middle of the plate to separate the half of the plate to be exposed to the light from the half not to be exposed. The target for the inoculum was 100–200 CFU per half of the plate. Both exposed and unexposed sides of plates were incubated and counted to check for even distribution on both sides. To minimize error, three plates were inoculated with the microorganism for each exposure time. Only the plates for one exposure duration were spread at one time.

Once the plates for one exposure time were inoculated, they were taken to the chamber. Before exposure, the plate lids were removed and the plates were covered halfway with aluminum foil to block exposure to the light. With the guillotine down, the plates were placed inside the chamber and set on their side, facing the light. A timer was set for the proper exposure time and started when the guillotine was fully lifted. When the exposure time had expired, the guillotine was replaced, the plates removed from the chamber, and then a new set of plates could be inoculated to use for the next exposure time. The newly inoculated plates were then exposed to the UVGI light in the same manner, with a total of three or four exposure times and a zero exposure time as a control. Upon completion of the experiment, all of the plates were placed into an incubator at the appropriate temperature [15]. Two control plates inoculated with the microorganism were also placed in the incubator to represent the zero exposure time.

The plates were incubated until moderate growth was visible, then the CFUs were counted. The percentage killed was obtained

Table 1

Exposure time and dose received (146 $\mu W/cm^2$).

Exposure to UV (s)	Dose (µWs/cm ²)		
5	730		
10	1460		
30	4380		
60	8760		
90	13,140		
120	17,520		
300	43,800		
480	61,320		
540	78,840		
660	96,360		
900	131,400		

by the following equation (2):

$$%kill = 100 \times \left\{ 1 - \left(\frac{C_{\rm E}}{C_{\rm NE}}\right) \right\}$$
(2)

where C_E is the number of colonies on the side of the plate exposed to UV and C_{NE} is the number of colonies on the side of the plate not exposed to UV. Irradiance measurements were conducted using an International Light (IL) IL 1700 Research Radiometer fitted with a factory NIST-traceable calibrated IL SED240 detector, QNDS2 filter, and W quartz diffuser. The detector has a 185–310 nm wavelength spectral response. For every exposure, irradiance intensity was measured at the position of the surface of the exposed agar Plates 146 μ W/cm². This position was maintained throughout the series of experiments allowing dose to be calculated as a function of time of exposure. The dosage is defined as the UVGI irradiance received by a microorganism during the duration of exposure. For an example of the many different durations of exposure, the dose was calculated and is listed in Table 1.

Ozone is produced by a variety of UV bulbs in the 185 nm emission line for mercury in low-pressure discharge lamps. However, the majority of low-pressure UVGI lamps used for air treatment are produced with a quartz glass that does not pass the 185 nm wavelength, effectively reducing or eliminating ozone discharge [3]. Measured ozone concentrations in the exposure chamber were found to be at background levels.

Like all gaseous discharge lamps, UVGI lamp output diminishes as air temperature increases or decreases from the optimal. Air distribution ducts are by their nature either in the heating or cooling mode of operation. As an example, a Phillips Lighting manufactured lamp will have a UVGI output at 10 °C (50 °F) that is 88% of that at 20 °C (68 °F). In addition, air velocity will further influence output by cooling the lamp. The operating life of the lamp can also be reduced by operating at low temperatures. However, high output UVGI emitter lamps manufactured by Westinghouse and Phillips Lighting minimize the temperature effects [3]. The tests used a standard UV lamp bulb with a 1.6 cm (5/8 in.) diameter, single-ended, 61 cm (24 in.) length, and with a heavy preheat, self-start ballast and the bulb was mounted perpendicular to the platform holding the inoculated plate during exposure. As stated, the closed exposure chamber was kept at a constant 22 °C (71.6 °F), and eliminated the effects of air flowing past the UVGI lamp.

3. Results and discussion

Tests were conducted on four species of bacteria (*S. epidermidis, S. marcescens, P. fluorescens,* and *D. radiodurans*), three species of yeast (*R. mucilaginosa, G. candidum,* and *C. albicans*), and four species of mold (*C. cladosporioides, P. chrysogenum, A. versicolor* and *A. fumigatus*) to compare the concentrations of microorganism populations between UVGI irradiation-exposed and non-exposed. When the colonies were mature but not overgrown the CFUs were counted from the 391 exposed plates and 16 unexposed plates (con-

Table 2a

Mean bacteria percent kill by UV period of exposure.

Deinococcus radiodurans							
Exposure (s)	300	420	540	600	660	900	
n	9	6	6	3	6	3	
Irradiance ($\mu W/cm^2$)	$1.46 imes 10^2$	$1.46 imes 10^2$	1.46×10^2	$1.46 imes 10^2$	1.46×10^2	1.46×10^{2}	
Dose (μ Ws/cm ²)	4.38×10^4	$6.13 imes 10^4$	$7.88 imes 10^4$	$8.76 imes 10^4$	$9.64 imes 10^4$	$1.31 imes 10^5$	
% Kill	37%	68%	92%	98%	98%	99.9%	
Pseudomonas fluorescens							
Exposure (s)	10	20	30	40	60	90	
n	6	6	12	6	3	3	
Irradiance ($\mu W/cm^2$)	$1.46 imes 10^2$	$1.46 imes 10^2$	1.46×10^2	$1.46 imes 10^2$	1.46×10^2	1.46×10^{2}	
Dose (μ Ws/cm ²)	$1.46 imes 10^3$	2.92×10^3	$4.38 imes 10^3$	$5.84 imes 10^3$	8.76×10^3	$1.31 imes 10^4$	
% Kill	46%	86%	97%	99.6%	99.8%	99.8%	
Serratia marcescens							
Exposure (s)	10	20	30	40	60	90	120
n	6	6	12	6	3	3	3
Irradiance (µW/cm ²)	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$	1.46×10^2	$1.46 imes 10^2$
Dose (μ Ws/cm ²)	$1.46 imes 10^3$	2.92×10^3	4.38×10^3	$5.84 imes10^3$	8.76×10^3	$1.31 imes 10^4$	$1.75 imes 10^4$
% Kill	42%	74%	89%	98%	99.8%	99.8%	99.8%
Staphylococcus epidermidis							
Exposure (s)	5	10	15	20	30		
n	6	6	6	6	3		
Irradiance (µW/cm ²)	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$		
Dose (μ Ws/cm ²)	$7.30 imes 10^3$	1.46×10^3	$2.19 imes 10^3$	$2.92 imes 10^3$	$4.38 imes 10^3$		
% Kill	46%	80%	94%	99%	99.6%		

trol plates). The CFUs from the exposed side and non-exposed side were used to calculate the percentage killed by use of Eq. (2). The length of exposure was varied to allow for a wide array of percent kill (up to 100%), which increased with increasing exposure. The longest exposure periods yielded kill percent values which averaged greater than 90%, except for *R. mucilaginosa* which averaged 74% for a 420 s exposure.

The exposure period (s), number of exposed plates for sample period (n), and mean percentage of kill for *S. epidermidis*, *S. marcescens*, *P. fluorescens*, and *D. radiodurans* is listed in Table 2a (bacteria); for *R. mucilaginosa*, *G. candidum*, and *C. albicans* is listed in Table 2b (yeast); and for *C. cladosporioides*, *P. chrysogenum*, *A. versicolor* and *A. fumigatus* is listed in Table 2c (mold). Equation 1 was used to calculate the k factor from the dose received (Table 1) and measured percent kill for each plate counted (see Tables 2a–2c). The k values are used to compare the susceptibility of different microorganisms to UVGI and are often used to design UVGI systems. The k values for each of the four vegetative bacteria, three yeasts and four molds and the statistical analysis (mean, standard deviation, relative standard deviation, and sample size) are listed in Table 3. A comparison of the statistical analysis of the k values for 391 indi-

Table 2b

Mean yeast percent kill by UV period of exposure.

vidual plate exposures is listed in Fig. 2. All 16 unexposed plates had approximately 200 CFUs per plate.

From the statistical analysis listed in Table 3 and depicted in Fig. 2, the standard deviation and relative standard deviation varied for each of the eleven species of microorganisms tested, as did the mean k value. Error bars are used to depict one standard deviation in Fig. 2. The k value results listed in Table 3 showed variability for the three normally susceptible vegetative bacteria from 1.25×10^{-03} cm²/ μ Ws to 2.88×10^{-05} cm²/ μ Ws (with a range of relative standard deviation from 28% to 53%); yeast varied from $1.09 \times 10^{-04} \text{ cm}^2/\mu\text{Ws}$ to $1.73 \times 10^{-05} \text{ cm}^2/\mu\text{Ws}$ (with a range of relative standard deviations from 68% to 83%); and mold varied from 2.78×10^{-05} cm²/ μ Ws to 7.36×10^{-06} cm²/ μ Ws (with a range of relative standard deviation from 56% to 132%). Although highly variable the *k* values are both reproducible and similar for all species tested. The sample size and similarity in results are additional assurances of accuracy for a treatment exposure with inherent microorganism variability. These results were similar to that found by Menetrez et al. [20], which measured the k values for *Bacillus* bacteria ranging from a high of 7.46×10^{-5} cm²/ μ Ws (for B. s. orange) to a low of 3.23×10^{-5} cm²/ μ Ws (for B. megaterium).

Candida albicans							
Exposure (s)	30	60	90	120			
n	6	6	6	6			
Irradiance (µW/cm ²)	$1.46 imes 10^2$	1.46×10^2	$1.46 imes 10^2$	1.46×10^2			
Dose (µWs/cm ²)	$4.38 imes 10^3$	$8.76 imes 10^3$	$1.31 imes 10^4$	$1.75 imes 10^4$			
% Kill	6%	34%	82%	98%			
Geotrichum candidum							
Exposure (s)	30	60	75	90	120		
n	12	12	6	9	3		
Irradiance (µW/cm ²)	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$		
Dose (µWs/cm ²)	$4.38 imes 10^3$	8.76×10^3	$1.10 imes 10^4$	$1.31 imes 10^4$	$1.75 imes 10^4$		
% Kill	5%	68%	84%	95%	83%		
Rhodotorula mucilaginosa							
Exposure (s)	30	240	300	360	420		
n	3	6	6	6	3		
Irradiance (µW/cm ²)	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$		
Dose (µWs/cm ²)	$4.38 imes 10^3$	$3.50 imes 10^4$	4.38×10^4	$4.38 imes 10^4$	$6.13 imes 10^4$		
% Kill	1%	40%	56%	63%	74%		

Table	2c	
N /		 1.111

Mean mold percent kill by UV period of exposure.

Aspergillus versicolor						
Exposure (s)	120	180	240	300		
n	12	12	12	12		
Irradiance (µW/cm ²)	$1.46 imes 10^2$	1.46×10^2	1.46×10^2	1.46×10^2		
Dose (μ Ws/cm ²)	$1.75 imes 10^4$	$2.63 imes 10^4$	$3.50 imes 10^4$	$4.38 imes 10^4$		
% Kill	36%	59%	74%	90%		
Aspergillus fumigatus						
Exposure (s)	120	180	240	300		
n	3	3	3	3		
Irradiance (µW/cm ²)	1.46×10^2	1.46×10^2	1.46×10^2	1.46×10^2		
Dose (μ Ws/cm ²)	$1.75 imes 10^4$	$2.63 imes 10^4$	$3.50 imes 10^4$	$4.38 imes10^4$		
% Kill	21%	46%	78%	91%		
Penicillium chrysogenum						
Exposure (s)	120	180	240	300	360	420
n	11	11	14	14	3	3
Irradiance (µW/cm ²)	1.46×10^2	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$
Dose (µWs/cm ²)	$1.75 imes 10^4$	$2.63 imes 10^4$	$3.50 imes 10^4$	$4.38 imes10^4$	$5.26 imes 10^4$	$6.13 imes 10^4$
% Kill	-2%	23%	56%	72%	96%	97%
Cladosporium cladosporoides						
Exposure (s)	120	180	240	300		
n	11	11	14	14		
Irradiance (µW/cm ²)	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$	$1.46 imes 10^2$		
Dose (μ Ws/cm ²)	$1.75 imes 10^4$	$2.63 imes 10^4$	$3.50 imes 10^4$	4.38×10^4		
% Kill	8%	12%	11%	23%		

The *k* values are as expected for the different microorganism groups and illustrate how important it is to take in the natural variability of a population of even a single species.

These values are also within the range of one order of magnitude of agreement with ARTI published literature [3]. However, in the ARTI experiments k values were generated for an airborne mixture of vegetative bacteria, mold spores and bacteria inside a duct with a significant flow rate of air using three UVC bulbs. Any further comparison between these experiments would be difficult and beyond the scope of this study.

Menetrez et al. [20] found significant kill of *Bacillus* spores was achieved with increasing periods of exposure (up to a high kill rate of 81% for a 120 s exposure). Spores exposed to a similar dose of UV irradiation for an extended period (as would be found on surfaces such as cooling coil fins within an air-handler) would be expected to be relatively free of *Bacillus*.

In addition, 156 samples of the mold *Stachybotrys chartarum* were exposed to UVGI irradiation. However, the maximum exposure period of 300 s failed to produce significant kill data that could be used for this evaluation. This identifies the fact that exposure of *S. chartarum* would need to exceed 300 s to produce significant reductions in concentrations.

Fig. 3 depicts the percent kill as a function of the period (s) of UV exposure. *D. radiodurans* (bacteria), *R. mucilaginosa* (yeast), and *C. cladosporioides* (mold) were the most difficult microorganisms to destroy in their groups. As anticipated, the four species of mold required a longer period of exposure to exhibit significant die-off than the vegetative bacteria. It is difficult to infer further UV exposure effects between microorganisms as comparatively diverse as fungi and bacteria.

Microbial growth in moisture-damaged buildings has been associated with respiratory health effects, and the spores of the mycotoxin-producing fungus *A. versicolor* are frequently present in the indoor air [27]. The spores of *A. versicolor* have been shown to cause acute inflammation in mouse lungs, which indicates the potential to provoke adverse health effects in the occupants of moisture-damaged buildings [28]. *A. fumigatus* is one of the most common *Aspergillus* spp. to cause disease in immunocompromised individuals [29]. Capable of growth at 37 °C (human body temperature), spores are common inhalation pollutants. Typically, however, these microorganisms are quickly eliminated by the immune system in healthy individuals [29]. *A. fumigatus* is common in the natural environment and can also be found in the upper respiratory tracts of healthy individuals. Exposure to *A. fumigatus* in immuno-

Table 3

Mean k values (cm^2/μ Ws), statistical analysis, standard deviation (sd), relative standard deviation (% rsd), sample size (n).

	k value, m (cm ² / μ Ws)	Standard deviation	Relative standard deviation	Number of samples		
Bacteria						
Deinococcus radiodurans	$2.88 imes 10^{-5}$	1.54×10^{-5}	53%	33		
Pseudomonas fluorescens	$7.42 imes 10^{-4}$	$2.48 imes 10^{-4}$	33%	36		
Serratia marcescens	5.34 X 10 ⁻⁴	1.65 X 10 ⁻⁴	31%	39		
Staphylococci epidermidis	$1.25 imes 10^{-3}$	$3.52 imes 10^{-4}$	28%	27		
Yeast						
Candida albicans	$1.09 imes 10^{-4}$	9.06×10^{-5}	83%	24		
Geotrichum candidum	1.33×10^{-4}	1.04×10^{-4}	78%	42		
Rhodotorula mucilaginosa	$1.73 imes 10^{-5}$	1.17×10^{-5}	68%	24		
Molds						
Aspergillus versicolor	4.17×10^{-5}	2.34×10^{-5}	56%	48		
Aspergillus fumigatus	$3.78 imes 10^{-5}$	2.56×10^{-5}	68%	12		
Penicillium chrysogenum	$2.78 imes 10^{-5}$	2.85×10^{-5}	102%	56		
Cladosporium cladosporoides	7.36×10^{-6}	9.69×10^{-6}	132%	50		





Fig. 2. Comparison of $k (cm^2/\mu Ws)$ value mean and standard deviation.

compromised individuals can lead to aspergillosis, a pulmonary infection [29,30]. *P. chrysogenum* (also known as *P. notatum*) is the mold which is the source for penicillin, the first antibiotic. In *P. chrysogenum* the conidia are blue to blue-green and typically are carried by air currents to new colonization sites [31–33]. *C. cla-dosporioides*, a dematiaceous (pigmented) mold widely distributed in air and rotten organic material is frequently isolated as a contaminant on foods [34,35]. Some species are predominant in tropical and subtropical regions. *Cladosporium* spp. are causative agents of skin lesions, keratitis, onychomycosis, sinusitis and pulmonary infections [32–35].



Fig. 3. Comparison of percent kill by UV period of exposure.

Table 4 k Values (cm²/ μ Ws).

Genus and species	k value	Source
Bacteria		
Bacillus anthracis	$5.09 imes 10^{-4}$	Sharp [42]
Bacillus anthracis Sterne	4.53×10^{-5}	Menetrez et al. [20]
Bacillus cereus	5.61×10^{-5}	Menetrez et al. [20]
Bacillus cream	6.30×10^{-5}	Menetrez et al. [20]
Bacillus megaterium	4.52×10^{-5}	Menetrez et al. [20]
Bacillus orange	7.72×10^{-5}	Menetrez et al [20]
Bacillus numilus	5.23×10^{-5}	Menetrez et al [20]
Geobacillus stearothermonhilis	5.15×10^{-5}	Menetrez et al [20]
Bacillus subtilis	2.62×10^{-4}	VanOsdell and Foarde [3]
Bacillus thuringiensis	5.12×10^{-5}	Menetrez et al [20]
Deinococcus radiodurans	3.60×10^{-5}	Table 3
Pseudomonas fluorescens	1.05×10^{-3}	Table 3
Pseudomonas fluorescens	0.35×10^{-3}	VanOsdell and Foarde [3]
Decudomonas garuginosa	3.55×10^{-3}	Table 2
Pseudomonas geruginosa	2.03×10^{-4}	Abshire and Dupton [27]
Pseudomonas deruginosa	0.40×10	Colling [20]
Pseudomonas deruginosa	2.38×10^{-4}	Collins [39]
	7.50×10^{-3}	Idule 5
Serratia marcescens	7.48×10^{-3}	Vallosdell and Foarde [3]
Serratia marcescens	1.05×10^{-3}	Sharp [42]
Serratia marcescens	4.45×10^{-3}	Sharp [43]
Serratia marcescens	2.21×10^{-3}	Collins [39]
Staphylococcus aureus	8.86×10^{-4}	Sharp [42]
Staphylococcus aureus	3.48×10^{-3}	Sharp [43]
Staphylococcus aureus	1.18×10^{-3}	Gates [40]
Staphylococcus aureus	4.19×10^{-4}	Abshire and Dunton [37]
Staphylococcus aureus	9.60×10^{-3}	Luckiesh [41]
Staphylococci epidermidis	2.09×10^{-3}	Table 3
Staphylococci epidermidis	1.72×10^{-3}	VanOsdell and Foarde [3]
Yeasts		
Candida albicans	$1.83 imes 10^{-4}$	Table 3
Geotrichum candidum	2.59×10^{-4}	Table 3
Rhodotorula mucilaginosa	1.73×10^{-5}	Table 3
Molds	0 1 1 1 0 F	
Aspergillus amstelodami	3.44×10^{-5}	Luckiesh [41]
Aspergillus fumigatus	3.78×10^{-5}	Table 3
Aspergillus versicolor	4.17×10^{-5}	Table 3
Aspergillus versicolor	2.35×10^{-4}	VanOsdell and Foarde [3]
Cladosporium cladosporoides	7.39×10^{-6}	Table 3
Cladosporium herbarum	3.70×10^{-5}	Luckiesh [41]
Mucor mucedo	3.99×10^{-5}	Luckiesh [41]
Penicillium chrysogenum	$2.78 imes 10^{-5}$	Table 3
Penicillium chrysogenum	$4.34 imes 10^{-5}$	Luckiesh [41]
Penicillium digitatum	$7.28 imes 10^{-5}$	Asthana and Tuveson [38]
Penicillium italicum	$1.26 imes 10^{-4}$	Asthana and Tuveson [38]
Rhizopus nigricans	$8.61 imes 10^{-5}$	Luckiesh [41]
Scopulariopsis brevicaulis	$3.44 imes 10^{-5}$	Luckiesh [41]

Numerous studies have measured the quantitative effects of UV irradiation on microorganisms by various techniques and methodologies [1-11,20,37-45]. The *k* values listed in Table 3 were combined with *k* values from past relevant research as calculated by Kowalski (2000) [45]. Table 4 lists the combined current and past *k* values for comparison and reference purposes. The results from this study compare favorably with other research involving the exposure of microorganism to UV irradiation.

Most UV studies have addressed specific applications such as water disinfection, medical germicide, food sanitation, and pest control. In this study, and Menetrez et al. [20], as well as a number of previous studies an agar plate technique and exposure apparatus was used to quantify the relationship between UV dosage over time to microorganisms for the purpose of providing guidance for UV applications in air conveyance systems.

4. Conclusions

This research demonstrated that UVGI lamp irradiation inactivated four species of vegetative bacteria, three species of yeast, and four species of mold to a reproducible degree under conditions of fixed dose. The dose received and percent kill of microorganisms was measured, and the k value and kill rate kinetics was calculated with Eqs. (1) and (2). The microorganisms were tested on the surface of an agar plate to simulate the surface of an HVAC air-handler and cooling coils. An agar plate may not completely represent the inside of an HVAC air-handler, however, the difficulty associated with monitoring actual cooling coil fins or drip pan surfaces was not consistent with the given experimental plan. As with the surface of the cooling coil, the agar plates provided a moist nutrient surface on which to grow. During the cooling season microorganisms are able to grow on surfaces within an air-handler due to the near saturation RH. Two types of measurements are commonly used to evaluate material surface moisture: most engineers think in terms of moisture content (MC) and microbiologists utilize water activity (a_W) [36]. The a_W is defined as the equilibrium RH (ERH) above a sample of a material, divided by 100. Therefore, the RH of the air of the HVAC system is not the critical moisture measurement, the moisture available at the surface is the critical measurement. The surface of the agar plate is at or near 100% as is the surface of a cooling coil.

The results of UVGI damage to microorganisms did vary as expected (see Fig. 2). The experiment of UVGI irradiation-exposed 391 plates and developed both a method of measurement and an extensive data set on eleven species of microorganisms.

A UVGI irradiation system design for use in a HVAC application is based on the expected operating conditions and antimicrobial efficacy of UVGI lamp performance. Efficacy information on kill rates and k values for UVGI irradiation exposure of bacteria, yeast and mold spores can be used in Eqs. (1) and (2) to develop guidance for HVAC applications. Manufacturer output information is branded on UVGI lamp bulbs. Unbranded lamp bulbs must have their irradiance output measured during operating conditions.

The ability to eliminate microorganism contaminants within HVAC and HAC system surfaces should contribute toward reducing indoor occupant exposure and the opportunity for the spread of these microorganisms based on infectious diseases. Additionally, HVAC operational efficiency and the resultant energy savings are possible, as well as reductions in airborne microorganism viability can be achieved with UVGI use, however addressing these issues were beyond the scope of this study.

4.1. Recommendations for future research

Although many UVGI lamps are designed to account for use in a cooling environment such as an air-handler, an examination of the temperature effects on UV radiance output would be an important contribution. Additionally, this information should be developed into a HVAC design guidance document for applying UV irradiation.

Investigating the antimicrobial efficacy of UV irradiation on porous surfaces and hard to reach surfaces (where indirect reflectance is the only source of irradiation) would allow for additional knowledge to be generated on conditions which are closer to real world applications of this technology.

References

- I.L. Shechmeister, Sterilization by ultraviolet radiation, in: S.S. Block (Ed.), Disinfection, Sterilization, and Preservation, Lea & Febiger, Philadelphia, 1991, pp. 553–565.
- [2] E.C. Cole, K.K. Foarde, Biocides and antimicrobial agents. bioaerosols: assessment and control, in: J. Macher (Ed.), American Conference of Governmental Industrial Hygienists, vol. 16, Cincinnati, OH, 1999, pp. 1–9.
- [3] D. VanOsdell, K.K. Foarde, Defining the Effectiveness of UV Lamps Installed in Circulating Air Ductwork, 2002, ARTI-21CR/610-40030-01.
- [4] P.R. Morey, Microorganisms in buildings and HVAC systems. A summary of environmental studies, in: Proceedings of IAQ'88–Engineering Solutions to Indoor Air Problems, Atlanta, GA, April 11–13, American Society of Heating, Refrigerating and Air-Conditioning Engineers, Atlanta, GA, 1988, pp. 10–25.

- [5] O.M. Lidwell, Ultraviolet radiation and the control of airborne contamination in the operating room, J. Hosp. Inf. 28 (1994) 245–248.
- [6] M.A. Calvo, M. Agut, R.M. Calvo, J. Larrondo, Effect of ultraviolet light irradiation and nitrosoguanidine on viability of 46 strains of Arthinium and their antibiotic production, Microbios 98 (1999) 179–187.
- [7] T.A. Slieman, W.L. Nicholson, Artificial and solar UV radiation induces strand breaks and Cyclobutane pyrimidine dimers in Bacillus subtilus spore DNA, Applied and Environmental Microbiology 66 (1) (2000) 199–205.
- [8] J. Peccia, H.M. Werth, S. Miller, M. Hernandez, Effects of relative humidity on the ultraviolet induced inactivation of airborne bacteria, Aerosol Science and Technology 35 (2001) 728–740.
- [9] P.W. Brickner, R.L. Vincent, M. First, E. Nardell, M. Murray, W. Kaufman, The application of ultraviolet germicidal irradiation to control transmission of airborne disease: bioterrorism countermeasure, Public Health Reports 118 (2003) 99–114.
- [10] E. Levetin, R. Shaughnessy, C.A. Rogers, R. Scheir, Effectiveness of germicidal UV radiation for reducing fungal contamination within air-handling units, Applied and Environmental Microbiology 67 (8) (2001) 3712–3715.
- [11] C.F. Green, V.S. Pasquale, The use of ultraviolet germicidal irradiation (UVGI) in disinfection of airborne bacteria, Environmental Engineering Policy 3 (2002) 101–107.
- [12] I. Ezeonu, J. Nobel, R. Simmons, Effect of relative humidity on fungal colonization of fiberglass insulation, Applied and Environmental Microbiology 60 (6) (1944) 2149–2151.
- [13] J.D. Miller, Fungi as contaminants in indoor air, Atmospheric Environment 26 (12) (1992) 2163–2172.
- [14] K.K. Foarde, D. VanOsdell, K.E. Leese, E.A. Myers, P.D. Dulaney, Field moisture measurement and indoor mold growth, in: Proceedings of the IAQ 96, Paths to Better Building Environments, 1996, pp. 19–25.
- [15] K.K. Foarde, E. Cole, D. VanOsdell, J. Chang, Static chamber method for evaluating the ability of indoor materials to support microbial growth, ASTM STP 1287, Characterizing Sources of Indoor Air Pollution and Related Sink Effects, 1996, pp. 87–97.
- [16] K.K. Foarde, D. VanOsdell, M. Owen, J. Chang, Fungal emission rates and their impact on indoor air, in: Proceedings of the Air and Waste Management Association Specialty Conference, Engineering Solutions to Indoor Air Quality Problems, VIP-75, 1997, pp. 581–592.
- [17] P.R. Morey, C.M. Williams, Is porous insulation inside an HVAC system compatible with a healthy building? in: Proceedings of IAQ 1991, Healthy Buildings, Washington, DC, September 4–8, American Society of Heating, Refrigerating and Air-Conditioning Engineers, Atlanta, GA, 1991, pp. 128–135.
- [18] K.K. Foarde, D. VanOsdell, M.Y. Menetrez, Investigation of the potential antimicrobial efficacy of sealants used in HVAC systems Journal of Air and Waste Management Association 51 (2001) 1219–1226.
- [19] Phillips Lighting Division, Disinfection by UV-radiation, Booklet 3222 C34 00671, 1992.
- [20] M.Y. Menetrez, K.K. Foarde, T.D. Webber, T.R. Dean, D.A. Betancourt, Efficacy of UV irradiation on eight species of *Bacillus*, Journal of Environmental Engineering Sciences 5 (4) (2006) 329–334.
- [21] K.S. Makarova, L. Aravind, Y.I. Wolf, R.L. Tatusov, K.W. Minton, E.V. Koonin, M.J. Daly, Genome of the extremely radiation-resistant bacterium *Deinococcus* radiodurans viewed from the perspective of comparative genomics, Microbiology and Molecular Biology Review 65 (2001) 44–79.
- [22] M. Simoes, M.O. Pereira, S. Sillankorva, J. Azeredo, M.J. Vieira, The effect of hydrodynamic conditions on the phenotype of *Pseudomonas fluorescens* biofilms, Biofouling 23 (2007) 249–258.
- [23] R.M.Q. Shanks, N.A. Stella, E.J. Kalivoda, M.R. Doe, D.M. O'Dee, K.L. Lathrop, F.L. Guo, G.J. Nau, A Serratia marcescens OxyR homolog mediates surface attachment and biofilm formation, Journal of Bacteriology 189 (20) (2007) 7262–7272.

- [24] J.P. O'Gara, H. Humphreys, *Staphylococcus epidermidis* biofilms: importance and implications, Journal of Medicinal Microbiology 50 (2001) 582–587.
- [25] J. Szymanska, Antifungal efficacy of hydrogen peroxide in dental unit waterline disinfection, Annals of Agricultural and Environmental Medicine 13 (2006) 313–317.
- [26] G. Samonis, D. Bafaloukos, Fungal infections in cancer patients: an escalating problem, In Vivo 6 (1992) 183–193.
- [27] J. Jussila, H. Komulainen, V.M. Kosma, A. Nevalainen, J. Pelkonen, M.R. Hirvonen, Spores of Aspergillus versicolor isolated from indoor air of a moisture damaged building provoke acute inflammation in mouse lungs, Inhalation Toxicology 14 (12) (2002) 1261–1277.
- [28] F.F. Tuon, G.M. Almeida, S.F. Costa, Central venous catheter-associated fungemia due to Rhodotorula spp. A systematic review, Medicinal Mycology 45 (2007) 441–447.
- [29] J.A. Washington, Principles of diagnosis, in: S. Barron, et al. (Eds.), Barron's Medical Microbiology, 4th ed., Univ of Texas Medical Branch (via NCBI Bookshelf), 1996, ISBN 0-9631172r-r1-1.
- [30] C.B. Cui, H. Kakeya, H. Osada, Spirotryprostatin B, a novel mammalian cell cycle inhibitor produced by *Aspergillus fumigatus*, Journal of Antibiotics 49 (1996) 832–835.
- [31] R.A. Samson, R. Hadlok, A.C. Stolk, A taxonomic study of the Penicillium chrysogenum series, Antonie van Leeuwenhoek 43 (2) (1977) 169–175.
- [32] G.S. De Hoog, F. Queiroz-Telles, G. Haase, G. Fernandez-Zeppenfeldt, D.A. Angelis, A. van den Ende, T. Matos, H. Peltroche-Llacsahuanga, A.A. Pizzirani-Kleiner, J. Rainer, N. Richard-Yegres, V. Vicente, F. Yegres, Black fungi: clinical and pathogenic approaches, Medicinal Mycology 38 (2000) 243–250.
- [33] G.S. De Hoog, J. Guarro, J. Gené, F. Figueras, Atlas of Clinical Fungi, 2nd ed., Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, 2000.
- [34] L. Collier, A. Balows, M. Sussman, Topley and Wilson's Microbiology and Microbial Infections, vol. 4, 9th ed, Arnold, London, Sydney, Auckland, New York, 1998, Chapters 6 and 7.
- [35] D.M. Dixon, A. Polak-Wyss, The medically important *dematiaceous* fungi and their identification, Mycoses 34 (1991) 1–18.
- [36] K.K. Foarde, D.W. VanOsdell, J.C.S. Chang, Evaluation of fungal growth on fiberglass duct materials for various moisture, soil, use, and temperature conditions, Indoor Air 6 (1996) 83–92.
- [37] R.L. Abshire, H. Dunton, Resistance of selected strains of *Pseudomonas* aeruginosa to low-intensity ultraviolet radiation, Applied and Environmental Microbiology 41 (1981) 1419–1423.
- [38] A. Asthana, R.W. Tuveson, Effects of UV and phototoxins on selected fungal pathogens of citrus, International Journal of Plant Science 153 (1992) 442-452.
- [39] F.M. Collins, Relative susceptibility of acid-fast and non-acid-fast bacteria to ultraviolet light, Applied Microbiology 21 (1971) 411–413.
- [40] F.L. Gates, A study of the bactericidal action of ultraviolet light, Journal of General Physiology 13 (1929) 231–260.
- [41] M. Luckiesh, Applications of Germicidal, Erythemal and Infrared Energy, D. Van Nostrand Co., New York, 1946.
- [42] G. Sharp, The effects of ultraviolet light on bacteria suspended in air, Journal of Bacteriology 37 (1939) 447–459.
- [43] G. Sharp, The lethal action of short ultraviolet rays on several common pathogenic bacteria, Journal of Bacteriology 38 (1940) 535–547.
- [44] G.D. Harris, V.D. Adams, D.L. Sorensen, M.S. Curtis, Ultraviolet inactivation of selected bacteria and viruses with photoreactivation of the bacteria, Journal of Water Resources 26 (6) (1987) 687–692.
- [45] W.J. Kowalski, Mathematical Modeling of Ultraviolet Germicidal Irradiation for Air Disinfection, Quantitative Microbiology 2 (2000) 249–270.