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Bioaerosol size as a potential determinant of airborne *E. coli* viability under ultraviolet germicidal irradiation and ozone disinfection

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Abstract

Ultraviolet germicidal irradiation (UVGI) and ozone disinfection are crucial methods for mitigating the airborne transmission of pathogenic microorganisms in high-risk settings, particularly with the emergence of respiratory viral pathogens such as SARS-CoV-2 and avian influenza viruses. This study quantitatively investigates the influence of UVGI and ozone on the viability of *E. coli* in bioaerosols, with a particular focus on how *E. coli* viability depends on the size of the bioaerosols, a critical factor that determines deposition patterns within the human respiratory system and the evolution of bioaerosols in indoor environments. This study used a controlled small-scale laboratory chamber where *E. coli* suspensions were aerosolized and subjected to varying levels of UVGI and ozone levels throughout the exposure time (2–6 s). The normalized viability of *E. coli* was found to be significantly reduced by UVGI (60–240 μW s cm^{-2}) as the exposure time increased from 2 to 6 s, and the most substantial reduction of *E. coli* normalized viability was observed when UVGI and ozone (65–131 ppb) were used in combination. We also found that UVGI reduced the normalized viability of *E. coli* in bioaerosols more significantly with smaller sizes (0.25–0.5 μm) than with larger sizes (0.5–2.5 μm). However, when combining UVGI and ozone, the normalized viability was higher for smaller particle sizes than for the larger ones. The findings provide insights into the development of effective UVGI disinfection engineering methods to control the spread of pathogenic microorganisms in high-risk environments. By understanding the influence of the viability of microorganisms in various bioaerosol sizes, we can optimize UVGI and ozone techniques to reduce the potential risk of airborne transmission of pathogens.

Keywords: UVGI disinfection, ozone, impactor, bioaerosol, size-dependent, viability

1. Introduction

Bioaerosols are mixtures of viable and nonviable biological particles suspended in the air (Peccia and Hernandez 2006, Lee 2011, Xu *et al* 2011). Inhalation of these particles can pose potential health risks to humans which include respiratory infections, allergies, and infectious diseases (Ostro *et al* 2001, Fabian *et al* 2008, Xu *et al* 2011). The COVID-19 pandemic has led to increased attention towards bioaerosols and airborne transmission in indoor environments (Morawska

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et al 2020). To mitigate infection risk, it is essential to implement appropriate and effective engineering control techniques that can address airborne transmission of bioaerosols and maintain indoor air quality. Some techniques are developed to reduce the load and viability of pathogens in bioaerosols through physical and mechanical means, such as air filtration, ultraviolet (UV) radiation, non-thermal plasma, and electrostatic precipitation (Hao *et al* 2020, 2021 and 2022, Kujundzic *et al* 2006, Su and Lau 2011, Wu *et al* 2015, Xia *et al* 2019, Zhang *et al* 2023). These methods explore factors that influence the survival and spread of airborne microorganisms in various environments, which pave the way to develop strategies that mitigate infection and disease transmission risks. However, it is noted that while these techniques effectively reduce bioaerosol viability, they may not completely eliminate microorganisms of varying sizes. Furthermore, the effectiveness of each method may differ depending on the specific microorganisms present in the bioaerosols along with the environmental conditions (Crook and Sherwood-Higham 1997, Ghosh *et al* 2015).

Ultraviolet germicidal irradiation (UVGI) has proven effective in disinfecting indoor air in various settings, such as hospitals, laboratories, and HVAC systems (Ko *et al* 2000, Brickner *et al* 2003, Kujundzic *et al* 2006, Nunayon *et al* 2020, Zhang *et al* 2020, Peng *et al* 2023). This technique employs ultraviolet light within the 220–300 nm wavelength range, which effectively penetrates cell walls and interacts with genetic material. This interaction can cause damage or alterations to the genetic structure, which ultimately leads to the death of exposed microorganisms (Brock *et al* 2003, Walker and Ko 2007). The effect of UVGI dose (related to intensity and duration) on bioaerosol survival has been studied by many researchers. (Peccia *et al* 2001, Lin and Li 2002, Xu *et al* 2003, Beggs *et al* 2006, Kujundzic *et al* 2006, Beggs and Avital 2020). Li *et al* (2003) demonstrated that a UVGI dose ranging from 289 to 860 μW s cm^{-2} was necessary to achieve a 5-log reduction in the concentration of *Legionella pneumophila*. Besides intensity and duration, UV disinfection depends on factors such as relative humidity (RH), microorganism type, and bioaerosol size (Tseng and Li 2005).

As a possible byproduct of UV light, ozone (O_3) is also a potent oxidant capable of disinfecting airborne bioaerosols effectively (Zoutman *et al* 2011, Lara-F *et al* 2020). The primary mechanism of ozone inactivation involves the direct destruction of genetic material (Lee *et al* 2021). It primarily targets unsaturated fatty acids, lipid fatty acids, glycoproteins, glycolipids, amino acids, and sulfhydryl groups of specific enzymes (Rojas-Valencia 2011). However, a potential limitation of using ozone is its potential toxicity to humans when inhaled at high concentrations. The toxicity can lead to breathing difficulties, coughing, shortness of breath, and asthma attacks (Bromberg 2016).

Although the effects of UVGI and ozone on pathogen disinfection have been widely studied, there remain gaps in understanding how the size of bioaerosols influences the effectiveness of disinfection. The sizes of bioaerosol strongly determine their deposition pattern in the human respiratory system and their transport dynamics in indoor environments, such as residence time in the air, gravitational settling, and wall deposition (Morawska 2005, Guzman 2021). At the beginning of the COVID-19 pandemic, the U.S. Centers for Disease Control and Prevention (CDC) (Control and Prevention 2020) and the World Health Organization (WHO) (WHO 2020) recommended a size of 5 μm to differentiate aerosols and droplets. Their recommendations identified that the transmission of SARS-CoV-2 mainly occurs through the droplet mode, where virus-containing particles would settle down to the ground within 6 feet (2 m). Although later research pointed out that 100 μm is a better size to separate aerosols and droplets (Wang *et al* 2020, Prather *et al* 2020, Wang *et al* 2021), understanding how microorganisms of different sizes behave in the air and their viability is crucial for studying disease transmission mechanisms in order to develop adequate disinfection measures.

Numerous studies have extensively used cascade impactors to study the size-dependent behavior of bioaerosols in various infectious disease transmission environments (Demokritou *et al* 2002, Fabian *et al* 2009, Lednicky and Loeb 2013, Guzman 2021, Shankar *et al* 2022) because they can collect bioaerosols as a function of aerodynamic sizes. The Sioutas Cascade Impactor was used in a study that detected SARS-CoV-2 viruses in submicron bioaerosols in hospitals (Liu *et al* 2020). The collection and analysis of bioaerosols using filters facilitates the study of the size, concentration and viability of the collected particles, and contributes to understanding the size-dependent survival and behavior of bioaerosols in different size ranges. Until now, however, no studies have examined the size-dependent viability of pathogens in bioaerosols under UVGI and ozone treatment.

In this study, we conducted experiments to quantify the effects of UVGI and ozone on the size-dependent viability of *E. coli* in bioaerosols under laboratory settings. Using culturing and quantitative polymerase chain reaction (qPCR) techniques, we evaluated the effects of varying UVGI and ozone exposure on the size-dependent viability of *E. coli*-containing bioaerosols. Further, we provide in-depth discussions of the fundamentals and performance evaluation of UVGI and ozone systems on size-dependent viability. This provides insights into their practical applications for controlling airborne transmission of bioaerosols.

2. Methods

2.1. Test microorganisms

Escherichia coli (ATCC 15597) was selected as the test species for this study. *E. coli*, a Gram-negative rod-shaped bacterium, has a nominal size of 1.5 μm in length and 0.5 μm in width, which can vary depending on the growth phase and nutrient conditions (Pierucci 1978). *E. coli* is widely employed in bioaerosol research and recommended as a standard test bacterium due to its well-characterized genetics and ease of laboratory cultivation (Zhen *et al* 2014). Its

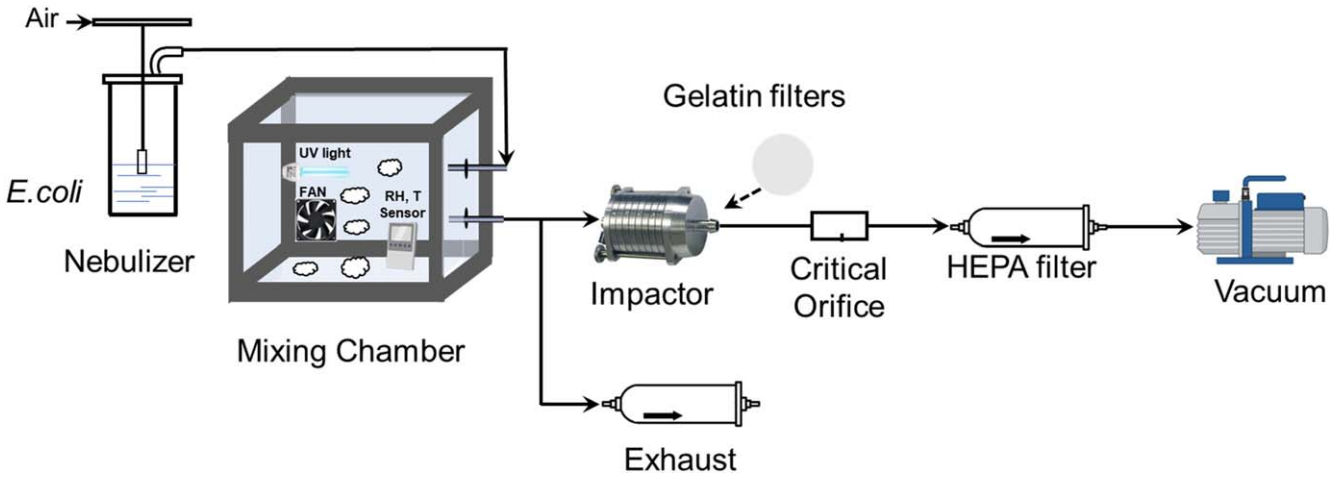

Figure 1. Schematic diagram of *E. coli* bioaerosol generation and sampling in a small-scale controlled laboratory chamber using the Sioutas Cascade Impactor.

physical and biological resemblance to other bioaerosol species make *E. coli* a suitable surrogate for studying airborne microorganism behavior (An *et al* 2006, Hospodsky *et al* 2010, Chang and Chou 2011).

Fresh *E. coli* bacterial suspensions were prepared for each experiment. *E. coli* suspensions in 30 ml of Luria-Bertani (LB) broth were pre-cultured overnight in a 37 °C incubation shaker. Turbidity measurements were performed to confirm the concentration of the bacterial solution after overnight incubation in order to obtain approximately the same concentration of 2.76×10^9 cells ml^{-1} for each experiment. For calibration purposes, the number of colony-forming units (CFU) on each plate was counted. Appropriate sterilization techniques were implemented during the entire process to ensure the accuracy and reliability of the results. Triplicate experiments on different days were conducted throughout the study.

2.2. Experimental setup and measurement

2.2.1. Bioaerosol generation. Figure 1 shows a schematic diagram illustrating the experimental setup used for generating and sampling *E. coli* bioaerosols. The *E. coli* stock solution was aerosolized using a six-jet Collison nebulizer (CH Technologies Inc., Westwood, NJ, USA) which operated at a flow rate (Q) of 12 l min^{-1} under a pressure of 20 psi. Airborne *E. coli* aerosols and droplets were then carried into a small-scale chamber (0.5 m × 0.5 m × 0.5 m). *E. coli*-containing bioaerosols were exposed to UVGI with and without ozone before being sampled using a Sioutas cascade impactor (SKC Inc., Eighty Four, PA, USA) connected to a vacuum pump. The tubes were made of antistatic material, and their length was minimized to reduce particle loss. The entire operation followed the Missouri Institutional Biosafety Committee Guidelines and conducted in a certified biosafety cabinet.

The aerosolization process lasted 5 min to achieve a stable bacteria concentration at the sampling point. There was no noticeable accumulation of droplets on the walls and floors of the chamber. Figure 2 shows the size distribution by number concentration of *E. coli* bioaerosol as a function of particle size, which was measured in the chamber using an Aerodynamic Particle Sizer (APS, Model 3321, TSI Inc., Shoreview, MN, USA). Immediately following UVGI and ozone exposure lasting between 2 and 6 s, as the intense radiation in the small chamber significantly reduced the survival of *E. coli* in the bioaerosols, bioaerosol samples were collected for five minutes continuously. Using a temperature and RH sensor (Model GPS-6, Elitech Inc., San Jose, CA), we monitored the environmental conditions of the experiments, which were in the range of 23 ± 2 °C and 50 ± 5% RH, as shown in table 1, which also summarizes the tasks and experimental conditions for the generation and collection of *E. coli* bioaerosols under UVGI and ozone disinfection.

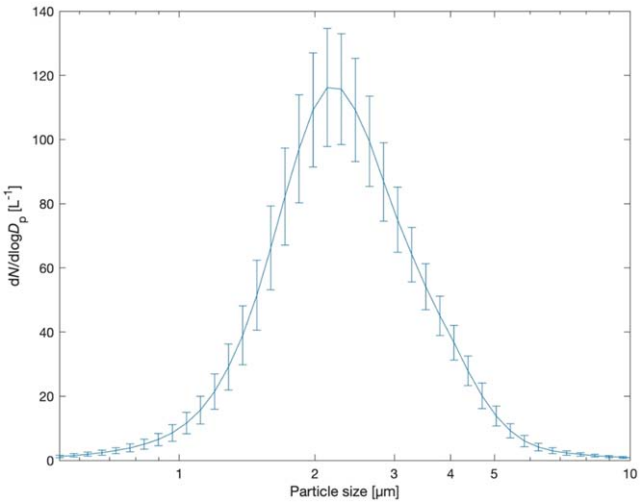

Figure 2. Size distribution by number concentration of *E. coli* bioaerosol as a function of particle size was measured in the chamber using APS (aerodynamic particle sizer). The average of the three measurements is shown.

2.2.2. Bioaerosol collection. Aerosolized bacteria within the chamber were collected using a Sioutas Cascade Impactor

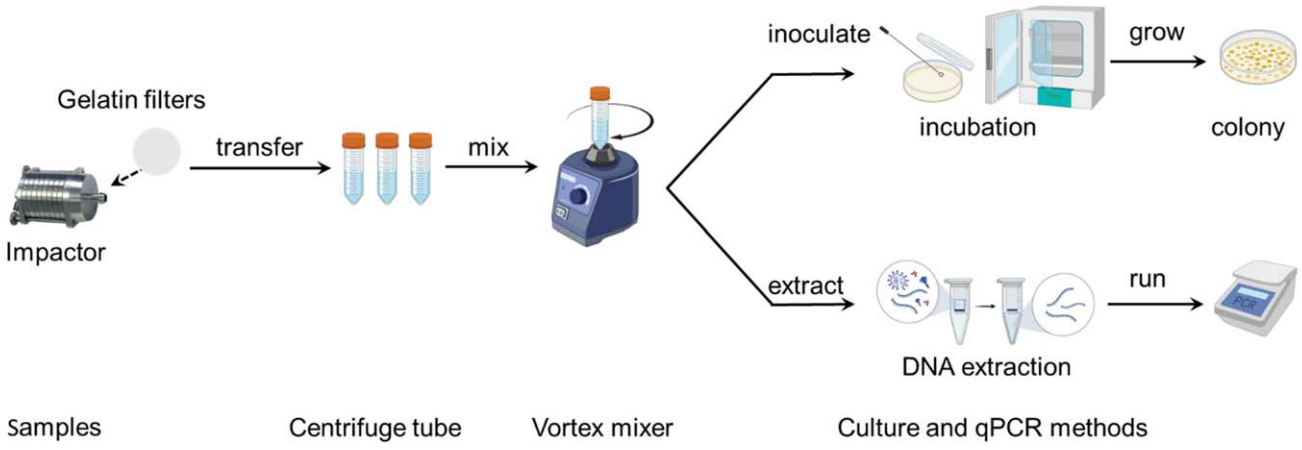

Figure 3. Bioaerosol quantification of the viable and total number of collected bacteria in the *E. coli* bioaerosol with culture and qPCR methods. Figure components are adapted from BioRender.com.

Table 1. Experimental conditions for generating and collecting *E. coli* bioaerosols under two UVGI and ozone disinfection tasks. Task 1: ozone-free UVGI disinfection; task 2: ozone-producing UVGI disinfection.

Task	Bacteria conc. (cells/ml)	Aerosolized time (min)	Sampling time (min)	Exposure time (s)	UVGI dose (μW s/cm^2)	Ozone level (ppb)	Temperature (°C)	Relative humidity (%)
1	2.76×10^9	5	5	0	0	45	23 ± 2	50 ± 5
				2	60	45		
				4	148	45		
				6	240	45		
2	2.76×10^9	5	5	0	0	45	23 ± 2	50 ± 5
				2	60	105		
				4	152	120		
				6	252	131		

equipped with gelatin filters (SKC Inc., Eighty Four, PA, USA), which could provide collection efficiency, pathogen viability, and solubility upon digestion. As depicted in figure 3, the filter was then removed from the impactor, and the samples were eluted from the filter using aseptic techniques and placed in 50 ml centrifuge tubes containing 10 ml of LB Broth buffer for further analyzes. The filters were dissolved directly in the LB Broth buffer with rapid vortexing at 2000 RPM for 2 min. This ensured proper mixing and extraction of collected microbes into the solution. All samples were stored appropriately in the laboratory at 4 °C, and microbiological analysis was performed within 24 h. Replicates and blanks were used to control contamination and verify the accuracy of the method.

The impactor consists of four impaction stages and an after-filter, which was used in conjunction with a vacuum pump operating at a flow rate of 9 l min^{-1}. The impactor collects five size ranges of aerodynamic diameters: (1) >2.5 μm, (2) 1–2.5 μm, (3) 0.5–1 μm, (4) 0.25–0.5 μm, and (5) <0.25 μm. In this study, we targeted particle sizes of 0.25–2.5 μm, which is the typical size range for *E. coli*. Due to the extremely low concentration of bacteria with aerodynamic diameters greater than 2.5 μm, we excluded these results from the analysis. Note that the main objective of this study was to investigate the *E. coli*-containing bioaerosol size and its ability to survive after UV radiation and ozone exposure rather than to suggest the microorganism size. Before each experiment, the flow rate through the cascade impactor was calibrated within 5% of the 9 l min^{-1} using a bubble flow meter (Gilibrator-2 Calibrator, Sensodyne, LP, Clearwater, FL, USA). The flow rate was verified again after each sampling experiment.

2.2.3. Bioaerosol measurement. Data analysis employed agar plate colony counts and quantitative polymerase chain reaction (qPCR) to determine *E. coli* bioaerosol viability. The culture method determined the number of viable bacteria using countable agar plates, while the qPCR method detected the total number of DNA gene copies of collected viable and nonviable bacteria by targeting 16S ribosomal DNA.

In the culture method, we plated 100 μl samples onto agar plates in triplicates. Bacteria were uniformly distributed across the agar surface using a disposable plastic inoculation loop. Subsequently, the agar plates were incubated at 37 °C in a laboratory incubator overnight. After 16–24 h, we enumerated the number of colonies present on each plate. In the qPCR method, DNA was isolated from the samples. The DNA extraction process included cell lysis and nucleic acid purification using a commercial QIAamp DNA Mini Kit

(Qiagen Inc., Hilden, Germany) according to the manufacturer's instructions. Lysis refers to the disruption of cell, spore walls, or virus coatings and the subsequent release of genomic DNA. The purification process aimed to avoid interference with nucleic acid measurement and prevent enzymatic inhibition of the PCR reaction. After the DNA extraction, the forward primer 5'-TCCTACGGGAGGCAGCAGT-3', the reverse primer, 5'-GGACTACCAGGGTATCTAATCCTGTT-3', and the probe, (6-FAM)−5'-CGTATTACCGCGGCTGCTGGCAC-3'-(TAMRA) were used. Amplification and detection of DNA by real-time PCR were performed with the ABI-PRISM 7700 Sequence Detection System (Applied Biosystems) using optical grade 96-well plates. Triplicate samples were routinely used to determine DNA for real-time PCR, and the mean values were calculated. The qPCR reaction was performed in a total volume of 20 μl which contained the TaqMan Universal PCR Master Mix (Applied Biosystems), 100 nM of each universal forward, reverse primer, and the fluorogenic probes. The reaction conditions for the amplification of DNA were 50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. DNA standards were used for determining bacterial numbers by real-time PCR.

2.3. Experimental plan

We tested two different UVGI bulbs manufactured by Black Magic 3D Inc.—one without ozone generation (ozone-free 254 nm UVGI) and the other with ozone generation (ozone-producing 185 nm/254 nm UVGI), as shown in table 1. Note that our experimental setup was unable to test the ozone alone inactivation efficiency. In our setting, ozone production is intrinsically linked to UV light source irradiation. This limitation will be addressed in the future study.

Illustrated in figure 4, UVGI and ozone were calibrated using an ozone meter detector (VISLONE Inc.) and a digital UV meter (General Tools Inc.) before the experiments. The UVGI dose was determined by multiplying the irradiance reading from the UV meter and the exposure time (2, 4, and 6 s), which is controlled by switching the bulbs on and off. The results showed that both bulbs emitted comparable radiation intensities, falling within the effective UVGI range of 30–50 μW cm^{-2} (light-blue shading) at the time of testing (Dose 2019). Incorporating the exposure time, the UVGI doses were 60, 148, and 240 μW s cm^{-2} without ozone (with an ambient ozone level of 45 ppb) and 60, 152, and 252 μW s cm^{-2} when UVGI was combined with ozone (with ozone levels of 105, 120, and 131 ppb) for exposure of 2, 4, and 6 s, respectively. Note that only ozone-producing UVGI bulb produce significant amounts of ozone (figure 4), rising from a baseline of 45 ppb (with minimal fluctuations) to approximately 130 ppb within a 6 s exposure time, exceeding the indoor ozone limit of 100 ppb (red dashed line). In this study, the brief aerosolization period and regular chamber cleaning ensured no significant impact on UV irradiance due to droplet accumulation on the light bulb. Before starting the experiments of two tasks, all parts of the nebulizer, exposure chamber and instruments were either cleaned with 70% isopropyl alcohol, autoclaved, or sterilized. These measures were taken to eliminate potential sources of contamination that could interfere with the experimental results.

2.4. Normalized viability calculation

As described in equation (1), the viability of microorganisms (f_v) under each condition was determined by calculating the ratio of colony-forming units per cubic meter (N_{CFU}, with a unit of CFU/m^3) obtained by incubation methods to the number of 16S rDNA copies per cubic meter measured by qPCR (N_{qPCR}, with a unit of copies/m^3). By analyzing bioaerosol samples collected from different stages of the cascade impactor, we could determine the viability of *E. coli* (f_v) in relation to the size of the bioaerosols:

$$f_v = N_{CFU}/N_{qPCR}. \quad (1)$$

It should be noted that f_v for control groups (without UVGI or ozone exposure) is far from 100%, because not all the *E. coli* in the bacterial solution were viable. Moreover, during the aerosolization process, shear force and impingement of bacteria-containing droplets further inactivate the *E. coli* (Thomas *et al* 2011, Zhen *et al* 2014). To focus on the influence of UVGI and ozone on *E. coli* viability, we normalized the obtained viability in experimental groups ($f_{v,exp}$) against that in the control group ($f_{v,ctrl}$). The normalized viability ($f_{v,n}$) is calculated using equation (2), where

$$f_{v,n} = f_{v,exp}/f_{v,ctrl}. \quad (2)$$

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was conducted to examine the effects of varying ozone-free UVGI and ozone-producing UVGI exposure times and different bioaerosol size ranges on the normalized viability. This method was chosen to statistically evaluate whether there were significant differences in the mean normalized viabilities across different exposure time points for each size range. Analyzes were performed using the Python statistical package. The significance level (α) is set at 0.05.

3. Results and discussion

3.1. Effect of ozone-free UVGI on the viability of E. coli in bioaerosols of different sizes

Figure 5 shows the size-dependent viability of *E. coli* bioaerosol under ozone-free UVGI disinfection. Size-fractionated bacteria-containing bioaerosols were collected at different impactor stages in relation to their aerodynamic particle size. The majority of bacteria were collected in the particle size ranges of 0.25–2.5 μm, while sizes above 2.5 μm were below the detection limit. Therefore, we only reported between 0.25 and 2.5 μm. Although the physical nominal size of *E. coli* bacteria is around 1.5 μm in length and 0.5 μm in width, collection for cascade impactors is based upon the

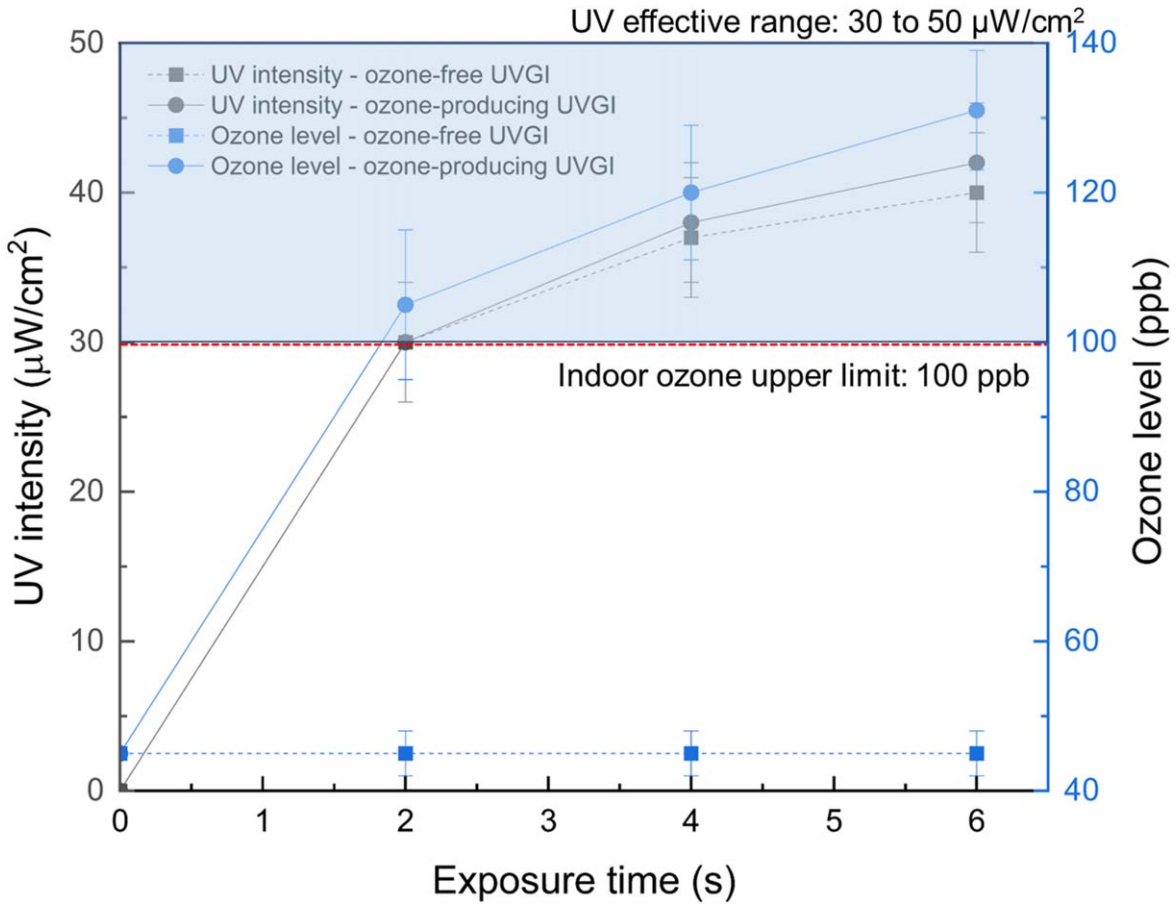

Figure 4. Calibration of UVGI intensity and ozone level before experiments. The standard effective UVGI range is 30–50 μW cm^{-2} (light-blue shading). The indoor ozone upper limit is 100 ppb (red dashed line).

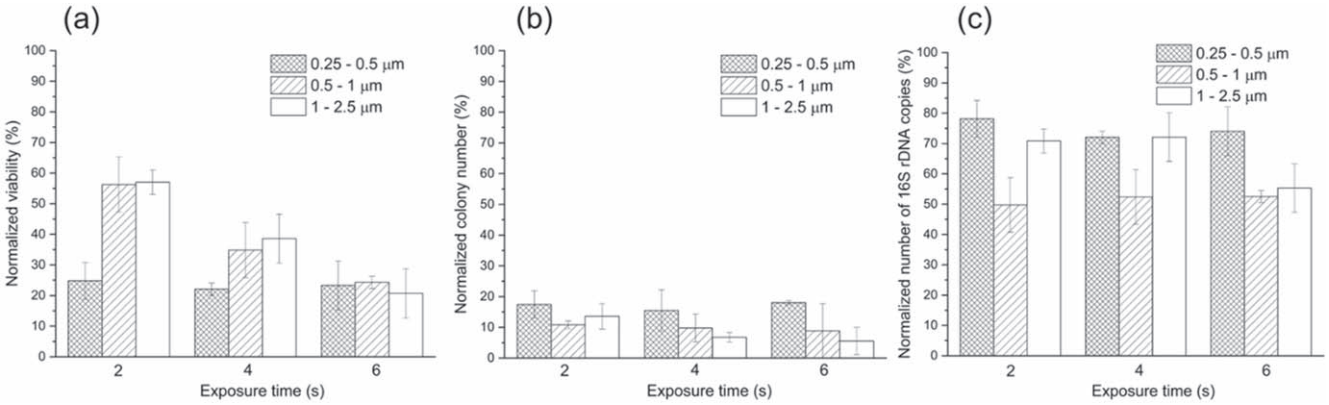

Figure 5. Normalized size-dependent viability, colony number, and 16S rDNA copies of *E. coli* bioaerosol under ozone-free UVGI disinfection. (a) normalized size-dependent viability, (b) normalized size-dependent colony number, and (c) normalized size-dependent number of 16S rDNA copies.

aerodynamic diameter, which can be influenced by particle shape, size, density, and aerodynamic behavior. Moreover, microorganisms grown at different stages may have sizes smaller than their nominal sizes. For example, with a nominal size of 6 μm, *Saccharomyces kudriavzevii* can be observed in the aerodynamic size ranges of 1.5–4 μm (Chen *et al* 2022). Previous research has also reported the aerodynamic size range of airborne bacterial fragments between 0.37 and 0.523 μm (Zhen *et al* 2014). These fragments could comprise the cell wall, membrane, or other cellular components resulting from cell damage or lysis. Since they are not complete cells, they are considered nonviable and cannot reproduce or perform typical cellular functions. Nevertheless, the DNA within these bacterial fragments may remain intact and be detected using qPCR. It is worth noting that the size of bioaerosol particles does not depend solely on the microorganisms they carry. Factors such as water, organic and inorganic matter, and other substances that may be attached to

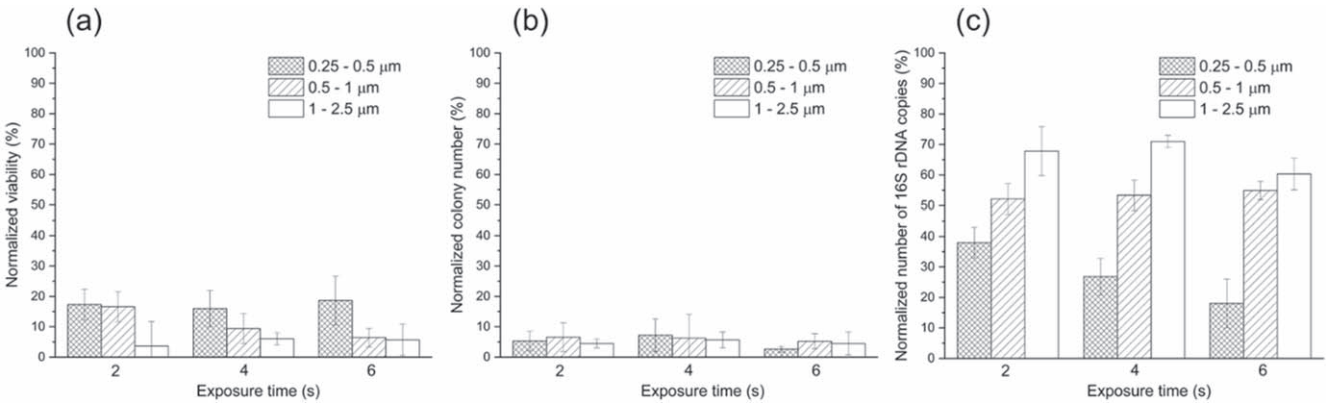

Figure 6. Normalized size-dependent viability, colony number, and 16S rDNA copies of *E. coli* bioaerosol under ozone-producing UVGI disinfection. (a) normalized size-dependent viability, (b) normalized size-dependent colony number, and (c) normalized size-dependent number of 16S rDNA copies.

the microorganisms can also have an effect. Therefore, a larger bioaerosol does not necessarily mean it contains more or larger microorganisms. Similarly, the concentration of bioaerosols in the air is not directly related to the concentration or size of these microorganisms. However, we need to be aware that many pathogens are found primarily in bioaerosols suspended in the air. The size of bioaerosols determines the transport of aerosols in the environment (e.g. dispersion and settling) and the deposition pattern in the human respiratory system. The non-biological materials in bioaerosols can also shield pathogens from various environmental impacts. Given the importance of bioaerosols as vectors for the survival and spread of pathogens, focusing on the size of the bioaerosols in this study becomes critical. Understanding the size distribution of these bioaerosols can help us delineate their behavior, deposition patterns in the respiratory system, and potential health effects. Hence, this study investigated the *E. coli*-containing bioaerosol size and its ability to survive after UV radiation and ozone exposure.

Figure 5(a) illustrates the effect of ozone-free UVGI on size-dependent normalized viability, while also showing that smaller particles (0.25–0.5 μm) have lower normalized viability ($f_{v,n}$) than larger particles (0.5–2.5 μm). Figure 5(a) also shows the significant change in viability with exposure time for larger particle sizes ($p < 0.05$) but not for smaller particle sizes ($p > 0.05$). This unchanged viability for smaller particle sizes can be attributed to the more substantial impact of UVGI on smaller particles that do not shield the bacteria well, which inactivated the bacteria even within the shortest exposure time tested. For larger particle sizes, the viability decreases over time, which shows that longer exposures yield lower viability. Figures 5(b) and (c) represent the normalized colony number and the number of 16S rDNA copies of *E. coli* bioaerosols in different size ranges under ozone-free UVGI exposure conditions, respectively, showing that while the colony number of larger particles decreases over time, the number of 16S rDNA copies does not demonstrate any significant changes during the exposure period.

Since airborne *E. coli* bacteria are not the only constituent in the bioaerosols, water sorption onto the bacterial surface can absorb UV radiation and protect against UV-induced DNA damage. Thus, if bacteria-containing aerosols lose water vapor and approach the size of the bacteria itself, their resistance to UVGI may decrease due to the reduced water shielding (Peccia *et al* 2001), suggesting that smaller particles with reduced water content are more susceptible to UVGI, leading to a reduction in their viability, as shown in figure 5(a). Another consideration of the effect on viability is the surface area to volume ratio. Smaller bioaerosols in the size range of 0.25–1 μm have a higher surface area to volume ratio than their larger counterparts (1–2.5 μm). This higher ratio means that a larger proportion of the bacterial surface is exposed to UVGI, resulting in more efficient killing of the microorganisms. The increased surface area also results in smaller particles losing their water content more quickly than larger particles based on the Kelvin equation (Friedlander 2000). While UVGI can impact the cells of microorganisms, different microorganisms may respond differently to UVGI due to their unique nature. The finding suggests that using ozone-free UVGI significantly impacts the viability of *E. coli* contained in smaller bioaerosols. This information is critical for developing effective UVGI disinfection techniques to mitigate the spread of pathogenic microorganisms in high-risk settings.

3.2. Effect of ozone-producing UVGI on the viability of E. coli in bioaerosols of different sizes

Certain UVGI can generate ozone as a byproduct, which exhibits different mechanisms and effects on the viability of bioaerosols. Figure 6 shows the combined effects of UVGI and ozone exposure on the viability of *E. coli* in bioaerosols of different sizes. The normalized viability at smaller particle sizes is higher than that at larger particle sizes (figure 6(a)). This counters what is observed for ozone-free UVGI in section 3.1. This difference may be elucidated by considering figures 6(b) and (c). Figure 6(b) shows that the normalized colony number did not exhibit noticeable changes across size-dependent *E. coli* bioaerosols, nor did it vary significantly with the tested exposure time. Similar to the ozone-free UVGI condition in section 3.1, we observed a significant number of bacteria collected in the smaller particle size ranges of

0.25–0.5 μm. However, the colony number is significantly lower than ozone-free UVGI. Figure 6(c) illustrates that the normalized number of 16S rDNA copies is significantly size-dependent, while the influence of increased exposure time is relatively less significant. In qPCR detection, we found that larger particles (1–2.5 μm) had a higher number of 16S rDNA copies than smaller particles (0.25–0.5 μm). This observation suggests that upon reaction with ozone, the number of detectable DNA copies is reduced in smaller particles at the size range of 0.25 to 0.5 μm. This reduction is likely due to ozone penetration, as ozone is a strong oxidizing agent that may penetrate smaller bioaerosols more effectively than larger ones due to their increased surface-area-to-volume ratio. This enhanced penetration may lead to more efficient degradation of the genetic material in smaller bioaerosols, resulting in fewer detectable DNA copies during qPCR analysis. Larger bioaerosols might also have a shielding effect that protects the bacteria's genetic material from ozone exposure. This shielding can be due to the presence of other water or salts, which can react with ozone and contribute to its antimicrobial effect. As a result, more DNA copies may be detected in larger bioaerosols after ozone disinfection.

The normalized viability at smaller particle sizes is higher than that at larger particle sizes due to fewer collected DNA copies but similar colony numbers. Therefore, the method of using normalized viability has its limitations in that the genetic materials may be damaged due to UVGI and ozone processing. Future studies may consider employing multiple molecular (e.g. protein quantification) and culture techniques simultaneously to address potential underestimation. These approaches might help avoid potential pitfalls associated with DNA degradation. The efficacy of ozone in killing bioaerosols can depend on various factors, such as ozone concentration, humidity level, temperature, and duration of exposure. Additionally, different strains and types of bacteria may have different sensitivities to ozone. The specific properties of the *E. coli* strain being targeted may therefore also affect its susceptibility to ozone. These findings emphasize the size-dependent nature of *E. coli* bioaerosol response to combined UVGI with ozone exposure, with a more pronounced effect on the detectable DNA copies in smaller particles. This information is valuable for understanding the efficacy of combined UVGI and ozone treatment, which may guide future research and applications to optimize disinfection strategies for airborne bacteria in various environments.

3.3. Compare size-dependent viability in UVGI and ozone disinfection

We compared the total normalized viability of three collected particle size ranges and the normalized viability for each particle size fraction under ozone-free UVGI and ozone-producing UVGI exposure conditions as a function of increasing UVGI exposure time from 2 to 6 s (figure 7). To achieve this normalization, we first aggregated the raw data of colony numbers and qPCR 16S rDNA copies of each particle size in each treatment group. These aggregated counts were then paired with the total counts for all particle sizes and divided by the control groups to produce the normalized results. This approach ensures that misinterpretations are minimized and an accurate depiction of the particle size distribution.

The total normalized viability at all sizes decreased from 28% to 13% with increasing exposure time under ozone-free UVGI. On the other hand, the ozone-producing UVGI condition maintained a relatively unchanged total normalized viability at approximately 8% over exposure time. The ozone-producing UVGI condition consequently exhibited a 1–4 fold greater reduction in viability than the ozone-free UVGI condition, and highlights the significant impact of adding ozone to the disinfection process.

We further observed that the normalized viability for ozone-free UVGI exposure was approximately 8% at the longest exposure time of 6 s, compared to 3% at the shorter exposure times of 2 and 4 s for particle sizes ranging from 1 to 2.5 μm. These results suggest that an increase in survival was observed over time in this particle size range. In contrast, the viability decreased from around 16% and 9% at the shortest exposure time of 2 s to 2% and 3% at the longest exposure time of 6 s for particle sizes ranging from 0.25 to 0.5 and 0.5 to 1 μm, respectively. This observation is likely attributable to bacteria being shielded by salt particles and water, which provided protection against increasing UVGI exposure time. As discussed in section 3.1, it is essential to consider that airborne *E. coli* bacteria are not the only constituent in bioaerosols. Under ozone-producing UVGI exposure conditions, we observed a relatively larger decrease in viability over time for particles within the size of 0.5–1 μm. However, we did not observe such a trend for the smallest particle size range of 0.25–0.5 μm, likely due to the smaller fraction of viability for the total particle sizes, leading to larger variances. In addition, it is possible that these particles have low initial viability but quickly reach a stable or equilibrium state. This means that subsequent exposure may not result in further significant decreases in the range we tested.

These results also suggest that both UVGI and ozone disinfection effectively inactivate airborne bacteria quickly with size-dependent susceptibilities. Combining UVGI with ozone can enhance the inactivation of airborne bacteria compared to ozone-free UVGI exposure, particularly for smaller particle sizes between 0.25 and 1 μm. This indicates that smaller microorganisms exhibit lower viability to disinfection than their larger counterparts. Our findings demonstrated that a combined approach utilizing UVGI and ozone proved more effective to inactivate bioaerosol microorganisms and reduce their concentration in the air compared to UVGI alone. This synergistic strategy involving UVGI and ozone significantly reduced viable microorganisms in bioaerosols to prevent disease transmission. These findings have important implications for developing more effective disinfection strategies in various settings, including healthcare facilities and public spaces.

This study addresses the unexplored influence of bioaerosol size on the viability of pathogens in bioaerosols when exposed to UV irradiation and ozone treatment.

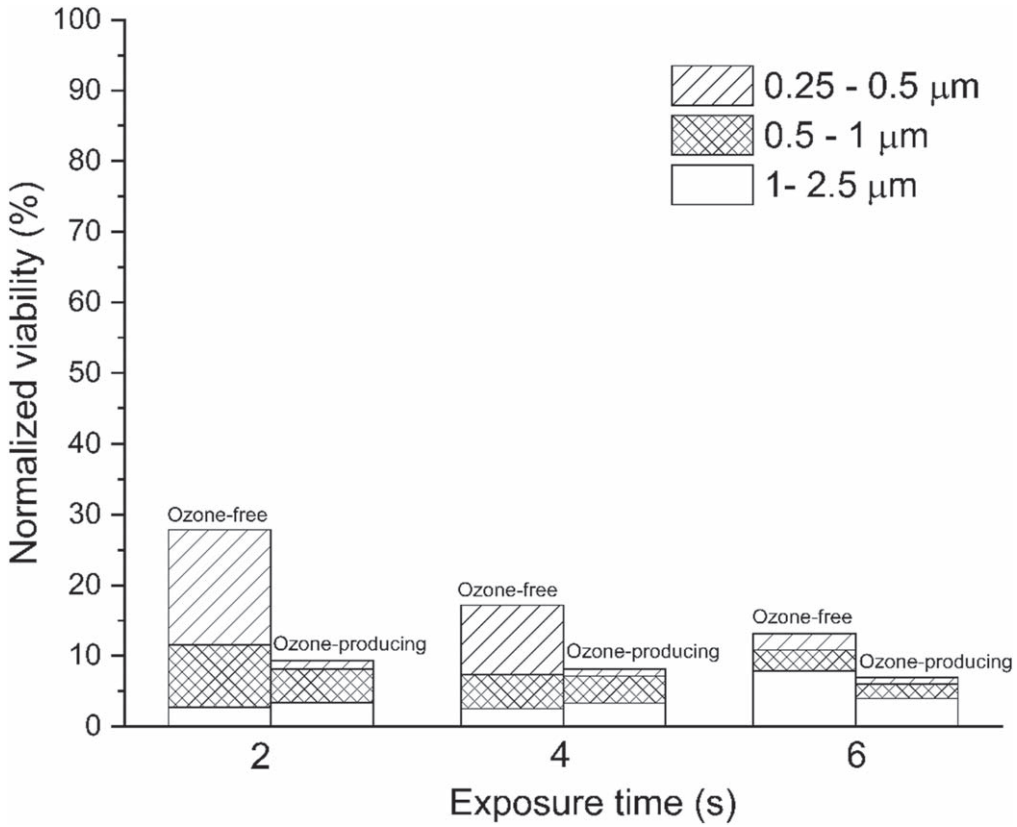

Figure 7. Comparison of the size-dependent normalized viability of three collected particle size ranges under ozone-free UVGI and ozone-producing UVGI disinfection at exposure times of 2 s, 4 s and 6 s.

Previous studies have explored the effects of bioaerosol size on its impact on disease transmission (Prather *et al* 2020, Wang *et al* 2020, Wang *et al* 2021), but the size-dependent effect on disinfection efficiency remains unclear. Our experiments uniquely focused on the survival of *E. coli* in bioaerosols of different sizes and their response to UV irradiation and ozone. By employing both cell culture and qPCR methods, we reveal the size-dependent behaviors of *E. coli* bioaerosols upon disinfection treatment. With this study, we advance the knowledge that may lead to better designs and implementations of more effective disinfection strategies for a variety of indoor environments.

Further investigation of the effect of environmental factors such as temperature and humidity on UVGI effectiveness and airborne bacterial survival is necessary. Although our primary objective was to determine viability under different engineering control methods, it is essential to assess the bacterial concentration thresholds for various sizes that may result in infection. Combining concentration-viability-infection data enables a more comprehensive understanding of bioaerosol infection control. In real-world disinfection scenarios, substantial reductions, such as 4-log reductions, may be necessary to prevent airborne transmission (Rutala and Weber 2008). Importantly, further investigation into the effects of UVGI light and ozone bulb placement to help measure load concentrations and improve disinfection strategies. We also need to compare the collection efficiencies between different instruments such as filter holders and SKC BioSamplers. In doing so, we can enhance our ability to control the spread of pathogenic microorganisms in high-risk settings and protect public health.

3.4. Nanotechnology approaches for the disinfection of airborne pathogens

UVGI and ozone disinfection have significant potential in mitigation, and their increased performance is consistent with the focus on transmission and mitigation of airborne pathogens. The unique properties of nanoparticles, primarily their high surface-to-volume ratio, are particularly beneficial when used in conjunction with UVGI and ozone disinfection mechanisms.

In our experiments using the controlled small-scale laboratory chamber, *E. coli* suspensions were atomized and exposed to different UVGI doses and ozone concentrations. Our findings showed that the survival of *E. coli* decreases as UVGI exposure time increases. Differences in microbial inactivation by particles of different sizes raise intriguing questions about potential pathways for nanotechnology interventions. In addition, nanotechnology can address the challenge of optimizing ozone sterilization (Epelle *et al* 2022). For instance, encapsulation of ozone molecules in nanocarriers or generation of ozone molecules at the nanoscale allows for more controlled and efficient release mechanisms. This controlled release ensures consistent ozone concentration during disinfection, especially when combined

with UVGI, resulting in a synergistic effect of pathogen neutralization. This approach can especially address the observed differences in the deactivation of smaller versus larger bioaerosols, resulting in more consistent disinfection across all particle sizes.

In essence, although nanotechnology is not directly applied to the experimental setup, we propose its integration as a strategy to enhance the overall disinfection effect of the UVGI and ozone approach, especially when dealing with the challenges associated with different bioaerosol sizes. This approach aims to provide innovative solutions for more robust and efficient disinfection of airborne pathogens.

4. Conclusions

We investigated the size-dependent viability of *E. coli* bioaerosols as a function of UVGI and ozone disinfection in a controlled laboratory setting. Our findings demonstrated time- and dose-dependent viability of *E. coli* in aerosols post UVGI and ozone exposure. Furthermore, *E. coli* bioaerosol size was found to be a critical factor in viability, with lower viability observed in the particle size range of 0.25–0.5 μm compared to larger particle sizes ranging from 0.5 to 2.5 μm. The concept of size-dependent viability under UVGI disinfection can be applied for controlling the transmission of pathogenic microorganisms in high-risk indoor environments.

Combining UVGI with ozone disinfection can enhance disinfection efficiency and reduce bacterial growth. The viability of airborne *E. coli* bioaerosols ozone-producing UVGI is higher than the ozone-free UVGI exposure, particularly for smaller particle sizes between 0.25 and 1 μm. Our findings have important implications for improving airborne infection control and preventing the indoor transmission of infectious diseases. It is worth noting that UVGI and ozone have a strong effect on the viability of *E. coli*-containing bioaerosols, but their effectiveness may vary depending on specific types of microorganisms; therefore, disinfection methods should be organism-specific. To protect human health and the living environment, the equipment used need to be properly operated and appropriate safety measures are in place to minimize the risk of accidental UVGI and ozone exposure. In particular, using real-time ozone monitoring equipment and ventilation to monitor or dilute ozone levels is critical to keeping ozone levels within safe limits and creating a health-centered environment. In the end, UVGI and ozone should not be considered as stand-alone solutions to reduce the spread of respiratory infections. They should be used in conjunction with others to ensure more effective protection against airborne pathogens.

Data availability statement

The data cannot be made publicly available upon publication because they are not available in a format that is sufficiently accessible or reusable by other researchers. The data that support the findings of this study are available upon reasonable request from the authors.

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