

# **Control of Aerosol Contaminants in Indoor Air: Combining the Particle Concentration Reduction with Microbial Inactivation**

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Submitted to  
*Environmental Science and Technology*  
June 8, 2006

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An indoor air purification technique, which combines unipolar ion emission and photocatalytic oxidation (promoted by a specially-designed RCI<sup>®</sup> cell), was investigated in two test chambers, 2.75 m<sup>3</sup> and 24.3 m<sup>3</sup>, using non-biological and biological challenge aerosols. The reduction in particle concentration was measured size selectively in real-time, and the Air Cleaning Factor (ACF) and the Clean Air Delivery Rate (CADR) were determined. While testing with virions and bacteria, bioaerosol samples were collected and analyzed, and the microorganism survival rate was determined as a function of exposure time (i.e., duration of the air purifier operation). We observed that the aerosol concentration decreased ~10 to ~100 times more rapidly when the purifier operated as compared to the natural decay. The data suggest that the tested portable unit operating in ~25 m<sup>3</sup> non-ventilated room is capable to provide CADR-values more than twice greater than the conventional close-loop HVAC system with a Rating 8 filter. Approximately 90% of initially viable MS2 viruses were inactivated resulting from 10-60 min exposure to the air purifier. For *B. subtilis*, approximately 75% of viable spores were inactivated in 10 min, and about 90% or greater after 30 min. The particle removal occurred due to unipolar ion emission, while the inactivation of viable airborne microorganisms took place due to photocatalytic oxidation. The biological and chemical mechanisms that led to the inactivation of stress-resistant airborne viruses and bacterial spores were reviewed.

## Introduction

Exposure to respirable airborne particles and microbial agents may cause various health problems. Numerous techniques have been developed to reduce the exposure to indoor particles. Aerosol control in confined, poorly ventilated spaces, when the air exchange with filtration cannot be successfully applied, represents a particular challenge. Another challenge is to decrease the indoor concentration of specific airborne contaminants, e.g., viable biological particles. While some indoor air purification techniques aim solely at the aerosol concentration reduction, others are designed to inactivate viable bioaerosols (e.g., viruses, bacteria, and fungi).

As some commercial air cleaners generate excessive ozone (either as a primary biocidal agent or as a bi-product), these devices have raised considerable public health concerns (1). Among various guidelines for ozone exposures, the following thresholds have been specified for occupational environments: 0.2 ppm for 2 h (2), 0.05–0.10 for 8 h (2), 0.1 ppm for 8 h (3), and 0.05 ppm for instantaneous (no time limit specified) exposure (4). For comparison, the outdoor air standard is 0.08 ppm for 8 h (5). Ozone generators can inactivate viable microorganisms; however, the inactivation occurs at concentrations significantly exceeding health standards (6, 7).

The gas phase detoxification of organic contaminants by photooxidation involving UV radiation and TiO<sub>2</sub> as a photocatalyst has been studied (8, 9). This mechanism has also been applied for inactivating microorganisms in water (10–12). Some effort has been made to explore its application for air cleaning inside a close-loop system (13, 14). The investigators reported significant photocatalytic inactivation of stress-resistant *Serratia marcescens* that occurred when aerosolized bacteria circulated in a

close-loop duct equipped with TiO<sub>2</sub> filter for relatively long period of time. Pal et al. (15) found similar effect for *Escherichia coli*, *Microbacterium* sp. and *Bacillus subtilis*; Keller et al. (16) reported considerable inactivation of airborne *E. coli* passing through a photoreactor coated with TiO<sub>2</sub> film. The biocidal effect of the photocatalytic oxidation can be attributed to photo-generated valence-band holes, hydroxyl radicals, hydrogen peroxide, and other reactive oxygen species. Lin and Li (17) tested the viability change in airborne bacteria and fungi exposed to photooxidation inside a small photoreactor for a very short time, in order of a second. No significant decrease in the CFU count was observed during such a short time.

To our knowledge, no data are available on the effectiveness of portable UV/TiO<sub>2</sub>-based air purifiers to inactivate viable airborne microorganisms in indoor air environments. These data are needed to assess the feasibility of photocatalytic oxidation for air purification in residential and occupational settings. Furthermore, for hybrid air purifiers, which involve several air cleaning mechanisms, no sufficient information is available to differentiate their particle removal efficiency and the biocidal capabilities, which both aim at reducing the bioaerosol exposure in indoor air.

In this study, we investigated a novel air purification technique that combines different aerosol/bioaerosol control mechanisms: the unipolar ion emission and the photocatalytic oxidation promoted by the “Radiant Catalytic Ionization (RCI)” technique. Unipolar ion emission has been shown earlier to reduce the particle concentration in indoor air (18–20), but no scientific data appears to be available on the efficiency of the hybrid-type technique.

## Experimental Section

The indoor air purification process was investigated in the experimental facility schematically shown in Figure 1. The particle removal was determined by measuring the concentration of challenge aerosols, size-selectively in real-time. When testing with viable bioaerosols, the microorganism survival rate was also determined. The experimental protocols validated in our previous studies (18, 19, 21) were adopted. The experiments were conducted when a freestanding hybrid air purifier was operating inside the chamber and when it was turned off. The challenge aerosol was generated from a liquid suspension using a Collison nebulizer (BGI Inc., Waltham, MA) and charge-equilibrated by passing through a 10-mCi Kr<sup>85</sup> charge equilibrator (3M Company, St. Paul, MN). After being mixed with a clean, HEPA-filtered air at a specific temperature (T=24-26<sup>0</sup>C) and relative humidity (RH=21-30%), the aerosol entered the test chamber. Following a 10-15-minute adjustment period established to achieve a uniform aerosol concentration pattern, the experiment began ( $t=0$ ).

In most of the tests, the aerosol concentration,  $C$ , and particle size distribution,  $\Delta C/\Delta \log(d)$ , were measured with an Electrical Low Pressure Impactor (ELPI, TSI Inc./Dekati Ltd, St. Paul, MN). This instrument utilizes the cascade impaction principle and also has a direct-reading capability to determine the concentration of particles of different aerodynamic sizes in 12 channels (each channel = impaction stage), from 0.041 to 8.4  $\mu\text{m}$ . These sizes represent the midpoint diameters of the respective impaction stages (the midpoint = the geometric mean of the stage's boundaries). When the experiments were conducted with viral aerosol that included particles smaller than the lower limit of the ELPI, we used a Wide-Range Particle Spectrometer (WPS; MSP Inc.,

Shoreview, MN). The WPS is a high-resolution real-time instrument combining the principles of differential mobility analysis, condensation particle counting, and laser light scattering to measure the diameter and number concentration of aerosol particles ranging from 10 nm to 10  $\mu\text{m}$ .

For every measured particle size,  $d$ , the aerosol concentration at  $t=0$  was set to exceed the background level (obtained before the challenge aerosol was generated) by about 100-fold. First, the natural concentration decay was characterized by recording  $C_{natural}(d, t)$  every 10 seconds with the ELPI and every 2.5 min with the WPS. Subsequently, the test aerosol was generated and mixed in the chamber again to reach the same initial concentration level. At  $t = 0$ , the air purifier was turned on and the concentration  $C_{AP}(d, t)$  was monitored during up to 120 min (or until the particle count decreased below the limit of detection). To quantify the efficiency of the particle removal exclusively due to the air purifier operation, the air cleaning factor ( $ACF$ ) was determined size-selectively as a function of time:

$$ACF(d, t) = \frac{C_{natural}(d, t)}{C_{AP}(d, t)} \quad (1)$$

In addition, the overall particle removal rate was calculated as

$$\lambda(d, t) = \frac{1}{t} \ln \left[ \frac{C(d, t=0)}{C(d, t)} \right], \quad (2)$$

and the particle removal rate exclusively due to air purifier was calculated as

$$PRR(d, t) = \frac{1}{t} \ln \left[ \frac{C_{AP}(d, t=0)}{C_{AP}(d, t)} \right] - \frac{1}{t} \ln \left[ \frac{C_{natural}(d, t=0)}{C_{natural}(d, t)} \right] \quad (3)$$

In case  $C_{AP}(d, t=0) = C_{natural}(d, t=0)$ ,

$$PRR(d, t) = \frac{1}{t} \ln [ACF(d, t)] \quad (4)$$

This was needed to determine the Clean Air Delivery Rate (CADR), which, according to the ANSI/AHAM (American National Standards Institute/Association of Home Appliance Manufacturers) standard, is defined as

$$CADR(d, t) = V \times PRR(d, t) \quad (5)$$

The CADR concept allows for comparison of air cleaning efficiencies of a freestanding air purifier and a close loop ventilation/air-filtration system in an air volume V (note that PRR is a function of V).

Two non-biological challenge aerosols, NaCl and smoke, were used to study the particle removal by the air purifier. The generated particles were primarily in the size range of 0.02 – 2.0  $\mu\text{m}$ , which includes ultrafine and fine fractions and represents most of the known viruses and bacteria. MS2 virus and *Bacillus subtilis* bacterial spores were the main challenge aerosols. Selected experiments were performed with *Pseudomonas fluorescence* bacteria.

MS2 bacteriophage, a 27 nm tailless non-enveloped icosahedral RNA-coliphage, relatively stable against environmental stress, has been used in the past as a simulant of most mammalian viruses and is known as an indicator for enteric viruses (22–26). Stock suspension of MS2 virus was prepared by adding 9 mL Luria-Bertani broth to freeze dried phage vial (ATCC 15597-B1). This suspension was filtered (using a membrane filter of 0.2  $\mu\text{m}$  porosity) and serially diluted so that the nebulizer suspension had  $10^8$ – $10^9$  PFU/mL (PFU = plaque forming unit). MS2 phage titre was determined by following a modified plaque assay protocol of Adams (27); *Escherichia coli* (ATCC 15597, strain C3000) was used as the host organism.

*B. subtilis* is a gram-positive spore-forming bacterium with rod-shaped spores of approximately 0.7-0.8  $\mu\text{m}$  in width and 1.5-1.8  $\mu\text{m}$  in length (28). *B. subtilis* spores have previously been used in laboratory studies as a surrogate of environmentally resistant, pathogenic bacteria (29–31). Freeze dried bacterial spores of *B. subtilis* (obtained from the US Army Edgewood Laboratories, Aberdeen Proving Ground, Maryland) were activated at 55-60°C for 25 minutes and then washed two times with sterile deionized water by vortexing followed by centrifugation at 7,000 rpm for 7 minutes at room temperature. The total bacterial concentration in suspension was adjusted to  $10^8$ – $10^9$  per mL using a hemacytometer. The viable bacteria were enumerated by cultivating on Trypticase Soy agar (TSA) media at 30°C for 18 hours; the viable (culturable) concentration in the nebulizer suspension was of the same order of magnitude as the total concentration, i.e.,  $10^8$ – $10^9$  CFU/mL (CFU = colony-forming unit). *P. fluorescens* bacteria (used in selected tests) are relatively sensitive to environmental stresses. Prior to aerosolization, vegetative cells of *P. fluorescens* were cultured in Trypticase Soy Broth at 28°C for 18 hours and washed similarly as *B. subtilis* spores.

When testing with biological particles, air samples were collected using Button Samplers (SKC Inc., Eighty Four, PA) equipped with gelatin filters (SKC Inc.) and operated at a flow rate of 4 L/min for 5 min. Eight Button Samplers were utilized in each test generating one blank, one background sample, three samples taken at  $t = 0$ , and the other three taken at a specific time interval,  $t$  ( $t = 10, 15, 30, \text{ and } 60 \text{ min}$ ). Additional selected experiments were performed by using the BioSampler (SKC Inc. Eighty Four, PA) to collect *P. fluorescens* and *B. subtilis*. The BioSampler is efficient to sample particles as large as bacteria (29) while not imposing desiccation stress on viable

microorganisms (as they are collected into a liquid medium). At the same time, since its cut-off size is too high to efficiently sample small MS2 virions, the BioSampler was not used as an alternative to gelatin filters for collecting MS2 virus.

The samples were analyzed for viable airborne microorganisms to quantify the percentage of those survived over time  $t$  as

$$\left[ \frac{PFU / cm^3 (t)}{PFU / cm^3 (t = 0)} \right] \times 100\% \quad (6)$$

for virus and

$$\left[ \frac{CFU / cm^3 (t)}{CFU / cm^3 (t = 0)} \right] \times 100\% \quad (7)$$

for bacteria. The above ratios were obtained with and without operating the air purifier. The above definitions assume that the total bioaerosol concentration (viable + non-viable) remains the same over the testing time. However, our preliminary tests showed that the air purifier's operation considerably reduces the total bioaerosol concentration in the chamber due to ion emission. Therefore, the ion emitter was temporarily disabled in the hybrid unit when testing virus and bacteria inactivation.

An aliquot of 200  $\mu$ L of dissolved gelatin filter extract was used for plaque assay and the number of PFU per milliliter of the extract was determined and converted to the number of airborne active (viable) virions (PFU/cm<sup>3</sup>). Similarly, extract was cultivated on TSA plates and incubated to obtain the airborne concentration of viable bacteria (CFU/cm<sup>3</sup>).

Additional testing was initiated to examine whether the biocidal effect of the air purifier took place indeed in the aerosol phase (and not after microorganisms were collected on filters). For this purpose, aerosolized microorganisms were collected on

eight gelatin filters during 5 min in the chamber without air purifier. Four filters were analyzed for viable microorganisms immediately after this test, while the other four were exposed to the air purifier in the chamber for 10, 15, 30, and 60 min and then analyzed. The comparison of two sets allowed examining if the microorganism inactivation occurred on filters during the collection process.

In addition to measuring the aerosol concentration and the microorganism viability, the ozone level and the air ion concentration were monitored in real-time in the chamber using an ozone monitor (PCI Ozone & Control Systems, Inc., West Caldwell, NJ) and an air ion counter (AlphaLab Inc., Salt Lake City, UT), respectively. The air temperature in the test chamber was  $24\pm 2^{\circ}\text{C}$  and the relative humidity ranged from  $22\pm 2\%$  to  $28\pm 2\%$  as monitored with a thermo/hygrometer pen (Fischer Scientific Co., Pittsburgh, PA).

The purifier prototype (Ecoquest International Inc., Greeneville, TN) used in the study utilized the ion emitter and a specially designed RCI cell. The former produces negative ions and releases them to indoor air, where they are acquired by aerosol particles (it is important to note that this method is different from air cleaning by charging particles at the entrance of the purifier and subsequently collecting them on metal electrodes by electrostatic precipitation). The RCI cell features a flow optimized target structure comprising matrices of elongated tubular elements made of polycarbonate and arranged in a parallel orientation on opposite sides or alternatively on four sides of a broad-spectrum UV light source. The UV lamp utilizes argon gas with mercury and carbide filaments with a flux between 100 and 367 nm. Besides, a coating was applied to the target structure comprising hydrophilic properties and containing the following grouping of materials: titanium dioxide, rhodium, silver, and copper.

The tests were conducted in two indoor test chambers, including a large walk-in test chamber (24.3 m<sup>3</sup>) that simulated a residential room and a smaller chamber (2.75 m<sup>3</sup>) that simulated a confined space (e.g., a bathroom, a small office area, or an automobile cabin). The particle removal was investigated in both chambers, while the bioaerosol viability tests were performed in the smaller chamber that was made of stainless steel and allowed bio-decontamination. The air purifier was tested in non-ventilated chambers (no air exchange) as it has been shown earlier that portable air cleaners are primarily beneficial in poorly ventilated spaces (20, 21). Air exchange was introduced only when testing the close-loop ventilation/air-filtration system equipped with an HVAC filter to compare its performance to that of the portable air purifier in terms of CADR. The ventilation/air-filtration system was also deployed to clean the test chamber between experiments. In most of the tests, the air purifier operated in the corner of the chamber, facing the center. A separate experiment was carried out to examine whether its location and orientation affected the ACF.

## **Results and Discussion**

Figure 2 shows the evolution of the concentration and particle size distribution of NaCl aerosol when the air purifier operated in the large test chamber. As seen from this example, the aerosol concentration of 0.1 µm particles decreased by a factor 28 in one hour and by a factor of about 250 in two hours; the corresponding decreases for 1 µm particles were approximately 10- and 50-fold. When testing with smoke particles, the aerosol concentration decreased even more rapidly. The above levels of the aerosol concentration reduction are considerably greater than those predicted by either tranquil or

stirred natural decay models (32). This result was obtained when both the air ion emitter and the RCI cell operated in the unit. Interestingly, statistically the same particle reduction effect ( $p > 0.05$ ) was observed when the RCI cell was turned off but the ion emitter still operated. The latter finding provides the evidence that the particle removal was achieved as a result of unipolar ion emission but not due to the photocatalytic reaction.

Our estimates suggest that, if the unipolar ion output is sufficient to create at least  $>10^4$  e/cm<sup>3</sup> in a confined space of  $\sim 2\text{--}3$  m<sup>3</sup> or  $>10^5$  e/cm<sup>3</sup> in a room-size volume of  $\sim 20\text{--}30$  m<sup>3</sup>, the concentration of respirable particles decays much faster than according to natural decay. The air purification is particularly efficient at higher initial aerosol concentrations ( $>10^4$  particles/cm<sup>3</sup>) that assure adequate interaction between the air ions and aerosol particles. As mentioned above, the effect is expected to be much more pronounced in non-ventilated environments than in ventilated ones.

The aerosol reduction was especially high for the particles of  $d \leq 0.3$   $\mu\text{m}$ . E.g., when the air purifier with an ion output of  $\sim 10^{12}$  e/sec continuously operated in a corner of the 24.3-m<sup>3</sup> chamber facing the center for two hours, ACF reached  $\sim 30\text{--}70$  for  $d = 0.08\text{--}0.3$   $\mu\text{m}$  and  $\sim 13\text{--}16$  for  $d = 0.8\text{--}2$   $\mu\text{m}$  (in the tests conducted with NaCl and smoke as challenge aerosols). The same ACF levels may be achieved more rapidly in indoor environments of smaller volumes and slower in larger spaces. The experimental trends agree with the ion-induced aerosol removal model (20).

The ACF was found to depend not only on the operation time and the particle size but also on the location/orientation of the purifier in the chamber. For example, a corner location facing the center of the room was found preferable as opposite to the orientation

facing the wall. The difference in ACF obtained for the center and corner locations was significant and increased with the operation time. The shaded area in Figure 3 presents the ion-induced air cleaning factor when the particle size-selective data were integrated over all the measured sizes of NaCl particle up to 2.5  $\mu\text{m}$  (the EPA PM<sub>2.5</sub> standard) and averaged over the three selected locations/orientations in the 24.3-m<sup>3</sup> chamber: in the corner facing the center, in the center, and at 80 cm from the wall facing it.

Figure 4 presents the CADR values achieved by operating the tested air purifier for five selected sizes of NaCl and smoke particles acting as aerosol contaminants in the non-ventilated 24.3 m<sup>3</sup> chamber. The CADR ranges approximately from 42.1 $\pm$ 0.1 to 62.1 $\pm$ 1.8 for NaCl particles of  $d=0.04\text{--}1.99$   $\mu\text{m}$ , and from 72.4 $\pm$ 0.9 to 115.5 $\pm$ 10.8 for smoke particles of the same size range. The difference may be attributed to different ability of NaCl and smoke particles to acquire electric charges from air ions, which results in their different mobilities and subsequently different migration velocities. The above explanation seems valid given that unipolar ion emission was shown to be the major mechanism causing the aerosol particle concentration reduction.

In addition, Figure 4 presents the CADR values achieved by the close-loop air exchange system equipped with a Standard ASHRAE Rating 8 HVAC filter at two air exchange rates, 2.5 and 7.7 ACH. The data suggest that the tested portable air purifier operating in about 25 m<sup>3</sup> non-ventilated room is capable to provide the CADR more than twice greater than the conventional central HVAC system with the Rating 8 filter. Obviously, more efficient particulate filters provide more rapid reduction of aerosol contaminants and may perform better than the tested air purifier. For example, HEPA filter installed in the close-loop air exchange system of the 24.3 m<sup>3</sup> chamber provided

CADRs approximately 4- and 3-fold greater than the portable unit at 2.5 and 7.7 ACH, respectively, when challenged with NaCl particles, and 2.2- and 1.4-fold greater when challenged with smoke particles. However, HEPA filters are rarely used in residential central HVAC systems because of the high pressure drop and the loading effect on their performance.

The particle removal from indoor air by the hybrid air purification technique was also investigated in the smaller (2.75 m<sup>3</sup>) chamber, which otherwise was utilized primarily for assessing the viable microorganism inactivation. The CADR values obtained with MS2 virions from the WPS measurements were  $73 \pm 5$ , i.e., approximately the same as those observed for NaCl and smoke particles in the large chamber. This finding implies that – at least, for the particle size range representing MS2 virions – PRR due to ion emission in indoor air environment is inversely proportional to the air volume [see Eq. (5)]. Furthermore, the removal rates of biological and non-biological particles of the same diameter were within 20% from each other, suggesting feasibility of using non-biological particles to determine the ion-induced aerosol reduction of bio-particles of the same size range.

In both test chambers (non-ventilated), the ozone concentration gradually increased as the purifier was continuously operating. In the 24.3-m<sup>3</sup> chamber, it increased from 0.006 to 0.05 ppm in about 35 minutes, while in a smaller (2.75-m<sup>3</sup>) chamber the same increase occurred in approximately 5 min. However, once an air exchange was introduced (as low as 1 ACH), the ozone concentration in the 24.3-m<sup>3</sup> chamber did not significantly increase as compared to the initial level ( $p > 0.05$ ). Our monitoring data obtained with the tested unit operating in a non-ventilated room of ~100

m<sup>3</sup> suggest that the ozone level can be kept below 0.05 ppm while the unit continuously operates for many hours.

Some air purifiers utilizing ion emission and – to a greater extent – the photocatalytic oxidation may cause greater increase of indoor ozone concentration than the tested one. The use of those devices in confined occupied air spaces may not be appropriate as their continuous operation will likely lead to excessive ozone levels. We believe that the solution can be found by implementing an intermittent regime (as an alternative to continuous one), which allows the air purifier operating until the ozone reaches certain level, after which the ozone-generating element is automatically turned off to allow the ozone concentration to drop; then the cycle can be repeated.

The air ion concentration in the 24.3-m<sup>3</sup> chamber and 2.75-m<sup>3</sup> chamber were approximately  $2.1 \times 10^5 - 6.8 \times 10^5$  e<sup>-</sup>/cm<sup>3</sup> as measured at 1 m upstream of the purifier's front panel. It is comparable to the levels measured with portable ionic air purifiers (18, 19).

Table 1 summarizes the microbial inactivation results. Only approximately 10% of initially viable MS2 virions survived 10-60 min exposure to the purifier in the chamber and about 90% were inactivated. When the natural concentration decay of aerosolized MS2 was monitored in the chamber (with no purifier operating), we found that the concentration of active viruses was relatively stable: the decrease did not exceed  $20.3 \pm 0.9$  % during one hour. The data suggest that the viral inactivation occurs rather quickly since the percent of survived virions did not show dependence on the exposure time for  $t = 10-60$  min. Thus, a relatively short time may be sufficient to reduce the percent of viable viruses in an air volume by a factor of 10 while those survived showed remarkable

resistance to the continuing stress. When aerosolized virions are exposed to photocatalytic oxidation, the hydroxyl radicals can affect the protein capsid and binding sites, thus disabling the virus subsequent interaction with the host and formation of PFUs (33). Additionally, the TiO<sub>2</sub> photocatalytic cell may produce an oxidative damage to the virus capsid (34) and the radicals may cause alteration in the virus' genetic material (35, 36). Our findings suggest that the hybrid air purifier may be used continuously for short time intervals or in intermittent regime to achieve considerable virus inactivation rate. On the other hand, a prolonged operation of the air purifier is believed to be advantageous in environments with a continuous supply of “fresh” active virions.

Approximately 75% of airborne *B. subtilis* spores exposed to the air purifier were inactivated during the first 10 min, 85% during the first 15 min, and about 90% or greater after 30 min (Table 1). Between 30 min and 60 min of exposure, we did not observe significant decrease in the number of survived spores (similar to the trend found for virions), which suggests a non-linearity of the effect. The natural decay in the culturable count was not significant ( $p>0.05$ ) during one hour, as measured using the Button Samplers equipped with gelatin filters. However, the overall standard deviation of the data obtained in these control tests was as high as 58% and the CFU counts from filters were close to the detection limit. To address this issue, we measured the natural decay of viable *B. subtilis* spores with the BioSampler at  $t = 0$  and at  $t = 2h$ . It was confirmed that the viability was constant within about  $\pm 20\%$  in the absence of the air purifier.

In bacteria, the inactivation process by reactive hydroxyl radicals can proceed in five reaction pathways:

- oxidation of Coenzyme A causing inhibition of cell respiration and cell death (37);
- destruction of the outer membrane of bacterial cells (12);
- oxidation of unsaturated phospholipid in bacterial cell membrane (38);
- leakage of intracellular K<sup>+</sup> ions (11); and
- detrimental effects on DNA and RNA (35, 36).

One reason that the inactivation of *B. subtilis* endospores was time-dependent is their thick membrane layer containing peptidoglycans. This is consistent with the study of Matsunaga et al. (39), who found that photooxidation of Coenzyme A by the TiO<sub>2</sub> photocatalyst was not entirely effective against the algae *Chlorella vulgaris* in water because of its thicker cell wall. Some other self-defense mechanisms of bacteria against the oxidation stress, including synthesis of superoxide dismutase enzymes, can also slow down the inactivation process (40).

Although the time was a factor in the bacterial spore inactivation, the viability loss occurred relatively quickly for both the MS2 virus and *B. subtilis*. This can be attributed to rapid interaction of valence-band holes (h<sup>+</sup>) (TiO<sub>2</sub> + hν → h<sup>+</sup> + e<sup>-</sup>.) with the organic substances, which are present in the viral and bacterial outer walls or membranes. The above-mentioned interaction likely occurs before considerable number of hydroxyl radicals (·OH) is generated in the air volume. Although previous studies (11, 12) emphasized the role of hydroxyl radicals (H<sub>2</sub>O + h<sup>+</sup> → ·OH + H<sup>+</sup>), these radicals may not be the primary factor in microbial inactivation, particularly in the air. Furthermore, since our experiments were conducted in relatively dry air (RH<30%), water molecules were not predominant species in contact with the catalyst, and thus the contribution of

hydroxyl radicals was likely much lower than in liquids. Shang et al. (9) have concluded that in the gas phase, organic compounds, such as heptane, can readily interact with photogenerated holes while the interaction with water vapor molecules is not as prominent. Alberici and Jardim (8) have reported that the valence-band holes generated from TiO<sub>2</sub> photooxidation are capable of oxidizing any organic compound. The process also produces hydrogen peroxide ( $O_2 + e^- \rightarrow O_2^-$ ;  $O_2^- + H^+ \rightarrow HO_2\cdot$ ;  $2HO_2\cdot \rightarrow O_2 + H_2O_2$ ), which can freely penetrate into cell membranes and walls and cause microbial inactivation (41). Further biochemical studies on the role of gas phase TiO<sub>2</sub> oxidation on the airborne microorganisms as well as studies on the reaction kinetics at the aerosol phase seem worthwhile to further examine the above interpretations.

Experiments with *P. fluorescens* revealed CFU counts below the detection limit both in the test and control samples. In contrast to *B. subtilis* endospores, even a very short exposure to ambient air (RH<30%) considerably decreased the viability of aerosolized *P. fluorescens* vegetative cells, which are known to be stress-sensitive. Perhaps, microorganisms sensitive to desiccation stress are more usable for this kind of tests if performed at higher relative humidity levels.

Additional control experiments were performed to investigate if the viability decrease found for MS2 virus and *B. subtilis* spores occurred in the aerosol phase or on the sampling filter. For MS2, we found that 1,835±270 PFU/mL and 1,855±325 PFU/mL developed when filter extracts were cultivated from unexposed and 10-min exposed gelatin filters, respectively. For *B. subtilis*, we observed 1,770±275 CFU/mL and 1,125±410 CFU/mL in extracts taken from unexposed and 60-min exposed filters, respectively. No significant changes in either viral or bacterial viability occurred as a

result of a non-aerosol exposure ( $p > 0.05$ ). Thus, these findings confirm that the viral and bacterial inactivation observed in our tests indeed occurred in the aerosol phase and was not associated with the inactivation on filters.

It was concluded that the particle removal took place solely due to unipolar ion emission, while the inactivation of viable airborne MS2 virions and *B. subtilis* spores occurred due to the photocatalytic reaction promoted by the RCI cell (as mentioned above, the ion emitter was disabled during the microorganism inactivation testing). Both mechanisms working simultaneously in a hybrid type air purifier may result in considerable decrease of the exposure to viable aerosol biocontaminants in indoor environment. The following estimate was made based on the experimental data obtained in this study. Assuming that the ion-induced air cleaning removes about 80% of viable airborne pathogens from a room air in 30 min and the RCI-induced photooxidation leaves only 10% of the remaining airborne microorganisms viable, the overall aerosol exposure to the viable pathogen in this room after 30 min is reduced by a factor of about 50.

### **Acknowledgements**

The investigation was partially supported by the EcoQuest International. The participation of Dr. K. Y. Kim was partially funded by the Korea Research Foundation Post-doctoral Fellowship Program. This support is greatly appreciated. The authors also thank Dr. Taekhee Lee for the technical assistance.

## Disclaimer

Reference to any companies or specific commercial products does not constitute or imply their endorsement, recommendation or favoring by the authors or the University of Cincinnati.

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Table 1. Percentage of airborne microorganisms survived over time  $t$  in the 2.75 m<sup>3</sup> chamber with the air purifier operating in it, as measured via PFU count (for MS2 virus) or CFU count (for *Bacillus subtilis* endospores). Bioaerosol sampling was conducted with the Button Sampler equipped with gelatin filters.

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exposure time, t (min)	percentage (mean $\pm$ SD) of airborne microorganisms survived in the chamber with air purifier operating during time $t$	
	MS2 virus, [PFU/cm <sup>3</sup> ] <sub>t</sub> / [PFU/cm <sup>3</sup> ] <sub>t=0</sub>	<i>Bacillus subtilis</i> endospores, [CFU/cm <sup>3</sup> ] <sub>t</sub> / [CFU/cm <sup>3</sup> ] <sub>t=0</sub>
10	9.3 $\pm$ 2.0 (n = 5)	24.1 $\pm$ 3.7 (n = 2)
15	9.2 $\pm$ 4.3 (n = 12)	15.7 $\pm$ 1.7 (n = 3)
30	8.3 $\pm$ 1.1 (n = 8)	7.9 $\pm$ 1.1 (n = 3)
60	10.3 $\pm$ 1.7 (n = 5)	10.1 $\pm$ 1.3 (n = 3)

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n = number of replicates

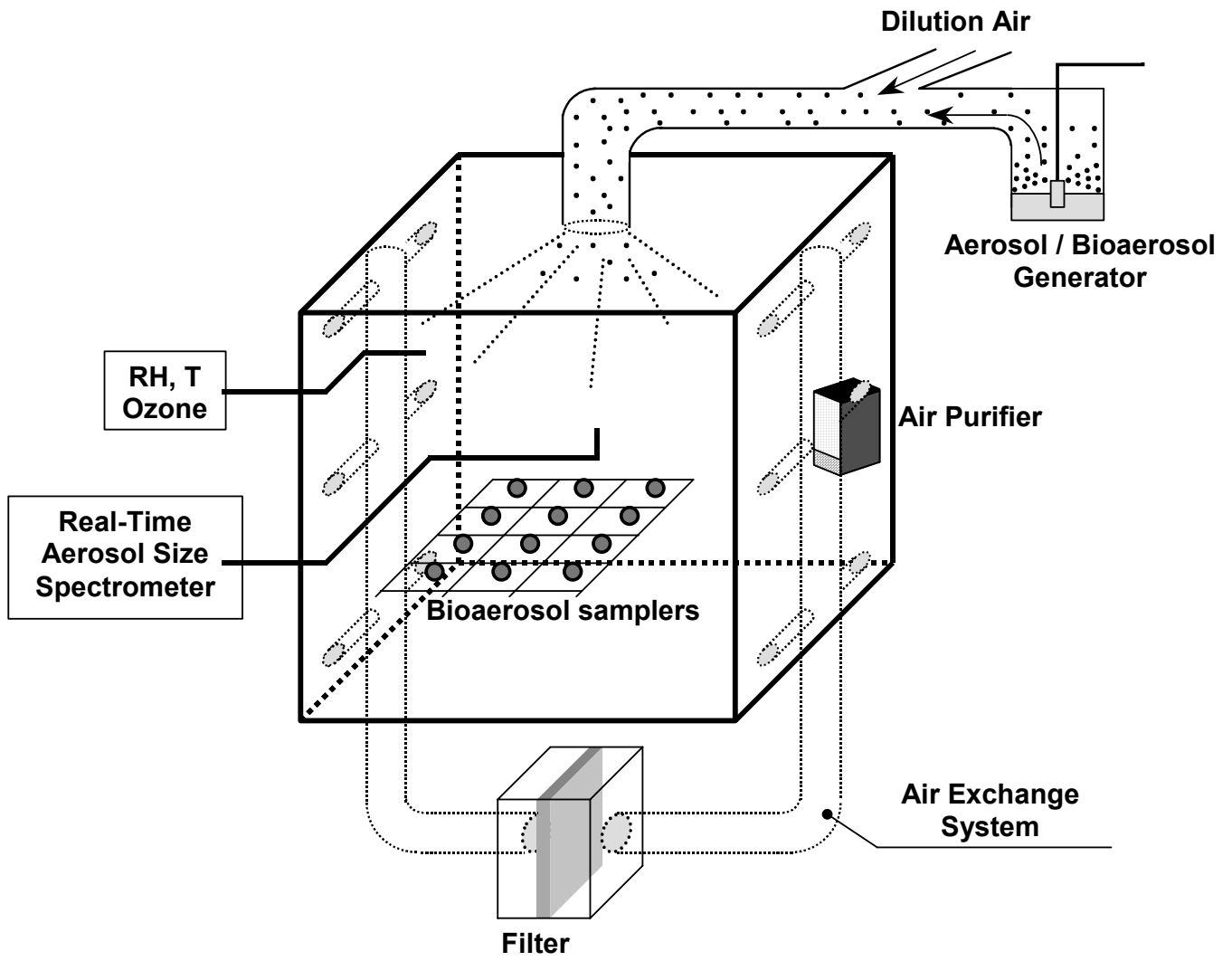
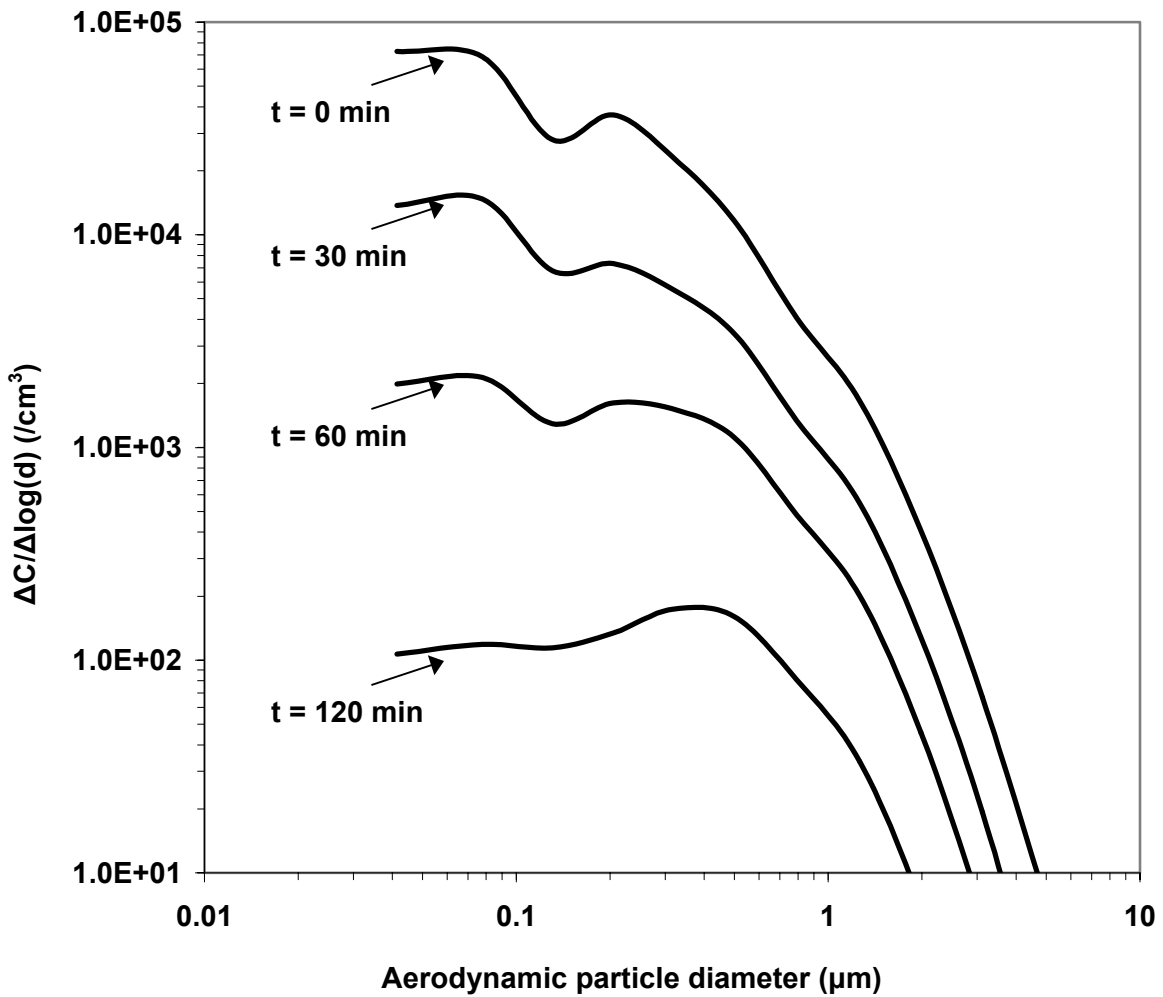
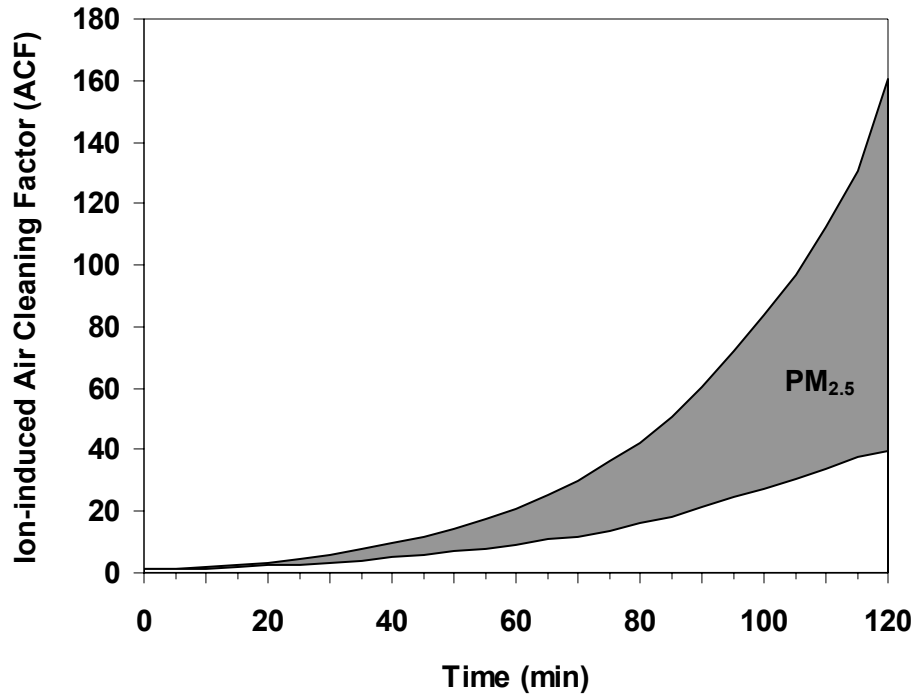


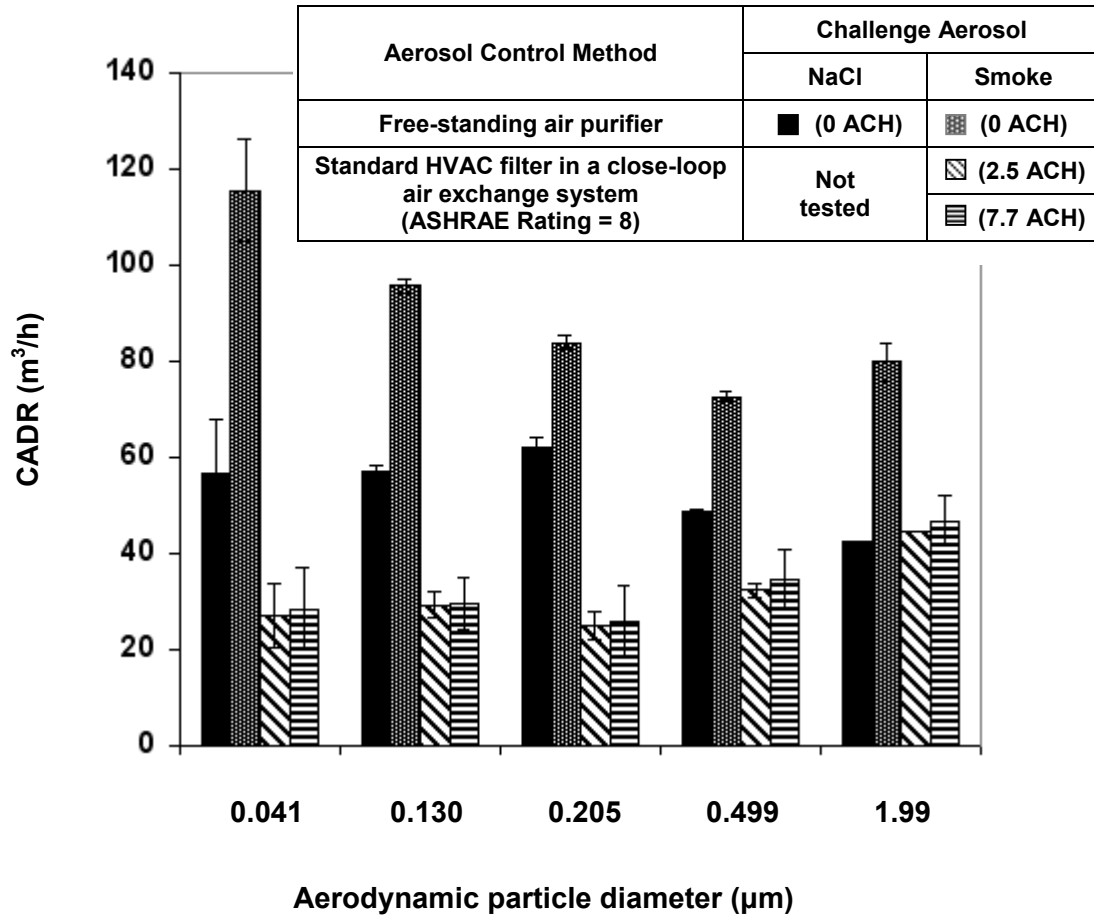
FIGURE 1. Experimental setup.



**FIGURE 2. Particle concentration and size distribution of NaCl aerosol as measured with the ELPI in the  $24.3 \text{ m}^3$  chamber with the air purifier operating facing the chamber's center at 1.7 m from the measurement point. No ventilation in the chamber. The initial total aerosol concentration =  $1.50 \times 10^5 / \text{cm}^3$ .**



**FIGURE 3.** The ion-induced Air Cleaning Factor (ACF) for  $PM_{2.5}$  NaCl as measured with the ELPI and integrated for different locations and orientations of the air purifier in the  $24.3 \text{ m}^3$  chamber. No ventilation in the chamber. The initial  $PM_{2.5}$  aerosol concentration =  $(0.356 - 1.50) \times 10^5 / \text{cm}^3$ .



**FIGURE 4.** Clean Air Delivery Rate (CADR) determined for the NaCl and smoke aerosols as measured with the ELPI in the non-ventilated 24.3 m<sup>3</sup> chamber. The performance of the air purifier is compared to that of a standard HVAC filter (ASHRAE Rating = 8) installed in the close-loop air exchange system of the chamber.