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Biological reductions on Surfaces

Efficacy of EcoQuest Radiant Catalytic Ionization Cell and Breeze AT Ozone Generators at Reducing Microbial Populations on Stainless Steel Surfaces

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Summary and Implications

This study was conducted to determine the potential use of EcoQuest Radiant Catalytic Ionization Cell for the inactivation of *Escherichia coli*, *Listeria monocytogenes*, *Streptococcus* spp., *Pseudomonas aeruginosa*, *Bacillus* spp., *Staphylococcus aureus*, *Candida albicans*, and *S. chartarum*, on stainless-steel surfaces at diverse contact times in a controlled airflow cabinet. In addition, the EcoQuest Breeze AT Ozone generator was evaluated under the same conditions for the inactivation of *Candida albicans* and *S. chartarum*. Better disinfection technologies for food contact surfaces are needed to control food borne pathogens in processing environments. Ozone technologies have only recently been approved for use on food contact surfaces. This study evaluated the application of gaseous ozone and other oxidative gases on stainless-steel surfaces against the microorganisms listed above. Both technologies reduced populations of all microorganisms tested on stainless-steel surfaces by at least 90% after 24 h exposure. The Radiant Catalytic Ionization Cell was more effective at reducing microbial counts for shorter exposure times than was the Breeze AT Ozone Generator.

INTRODUCTION

The food and beverage industries face a number of issues when it comes to producing a safe, wholesome product. Foodborne pathogens such as *E. coli* 0157:H7, *Listeria moncytogenes,* and *Salmonella* spp. have been a growing concern throughout the years. Processors are also concerned about spoilage microorganisms that shorten shelf life and cost companies millions every year in spoiled product. Industries impacted include the meat, seafood, poultry, produce, baking, canned foods, dairy, and almost all other segments of the market.

The U.S. Department of Agriculture estimates the costs associated with food borne illness to be about $5.5 to $22 billion a year. This doesn't include the billions lost every year due to spoiled product, which must be disposed of or sold as a lesser valued product. Better disinfection and microbiological control measures are needed in almost every area of the food industry.

As a disinfectant, ozone has a tremendous ability to oxidize substances. It's thousands of times faster than chlorine and disinfects water three to four times more effectively. As it oxidizes a substance ozone will literally destroy the substance's molecule. It can oxidize organic substances such as bacteria and mildew, sterilize the air, and destroy odors and toxic fumes. Ozone has been used by industry for many years in numerous applications such as odor control, water purification, and as a disinfectant (Mork, 1993). Recent government approval of ozone for use with foods and food contact surfaces has opened the door to many more exciting possibilities for this technology.

In June 2001, the U.S. Food and Drug Administration approved the use of ozone as a sanitizer for food contact surfaces, as well as for direct application on food products. Prior to that time, chlorine was the most widely used sanitizer in the food industry. Ozone may be a better choice for disinfection of surfaces than chlorine. Chlorine is a halogen-based chemical that is corrosive to stainless steel and other metals used to make food-processing equipment. Chlorine can also be a significant health hazard to workers; when mixed with ammonia or acid cleaners, even in small amounts, a toxic gas can form.

Chlorine is a common disinfect used in meat processing and is effective and safe when used

at proper concentrations. However, chlorine is far less effective than ozone and can result in the production of chloroform, carbon tetrachloride, chloromethane, and tri-halomethanes. In contrast, ozone leaves no residual product upon its oxidative reaction.

An important advantage of using ozone in food processing is that the product can be called organic. An organic sanitizer must be registered as a food contact surface sanitizer with the U.S. Environmental Protection Agency (EPA). Ozone has such an EPA registration, and is approved by FDA as a sanitizer for food contact surfaces and for direct application on food products.

Ozone has become more accepted for use in food processing in recent years and is being used in more than just surface applications. A recent U.S. FDA recommendation (2004) stated that "ozone is a substance that can reduce levels of harmful microorganisms, including pathogenic *E. coli* strains and *Cryptosporidium*, in juice. Ozone is approved as a food additive that may be safely used as an antimicrobial agent in the treatment, storage, and processing of certain foods under the conditions of use prescribed in 21 CFR 173.368."

MATERIALS AND METHODS

Preparation of Cultures:

The following bacteria and fungi cultures were used for the study: *Bacillus globigii* (ATCC # 31028, 49822, 49760), *Staphylococcus aureus* (ATCC # 10832D, 25178, 11987), *Candida albicans* (ATCC # 96108, 96114, 96351), *Stachybotrys chartarum* (ATCC # 18843, 26303, 9182), *Pseudomonas aeruginosa* (ATCC# 12121, 23315, 260), *Escherichia coli* (ATCC# 27214, 19110, 67053), *Streptococcus pneumoniae* (ATCC# 27945, 29514, 10782), and *Staphylococcus aureus* - methicillin resistant (ATCC# 33591). Cultures were revived using ATCC recommended instructions.

Bacteria, yeast, and mold strains were individually grown in tripticase soy broth (TSB; Difco Laboratories, Sparks, MD) and YM broth (Difco Laboratories), respectively, to mid-exponential phase followed by a wash and re-suspension in 0.1% peptone water. The cultures were combined by specie type to ca. 10^8 CFU/ml.

Preparation of Samples and Ozone Treatment:

The microbial species used to validate the ozone generators were tested as microbial cocktails inoculated onto 6.3 x 1.8 cm on #8 finish stainless-steel coupons (17.64 cm^2 double sided area). Four stainless steel coupons were dipped per microbial inoculum and vortexed 15 sec to optimize microbial dispersion. Using sterile binder clips, stainless steel coupons were suspended on a cooling rack contained inside a laminar flow cabinet for 1 h to dry. The initial microbial populations attached to the stainless steel coupons ranged from 5 to 6 log CFU/cm^2. The inoculated stainless steel coupons were transferred to a controlled airflow test cabinet (Mini- Environmental Enclosure, Terra Universal, Anaheim, CA) at 26°C and 46% relative humidity (ambient conditions), and treated using the EcoQuest Radiant Catalytic Ionization Cell for 0, 2, 6, and 24 h. The EcoQuest Breeze AT Ozone generator was evaluated separately for treatment periods of 0, 2, 6 and 24 h. Ozone levels were monitored throughout the study (Model 500, Aeroqual, New Zealand).

Sampling:

At the end of the ozone contact time the coupons were vortexed for 30 sec in 30 ml of 0.1% peptone water. Samples inoculated with bacterial cultures were serially diluted, plated on tripticase soy agar (TSA; Difco Laboratories), and incubated for 24 h at 35°C. After preparing serial dilutions, samples inoculated with yeast were plated on potato dextrose agar (PDA; Difco Laboratories) and those inoculated with mold cultures were plated on cornmeal plates. Both PDA and cornmeal plates were incubated 30°C for 5 days. Following incubation, data for each microorganism were reported as colony-forming units per square centimeter (CFU/cm^2).

RESULTS AND DISCUSSION

Reductions in microbial populations on #8 finish stainless steel coupons following 0, 2, 6, and 24 h exposure to the EcoQuest Radiant Catalytic Ionization Cell are presented in Figure 1. Exposure to ozone levels of 0.02 ppm for 2 h reduced all microbial populations tested by at least 0.7 log CFU/cm^2. Longer exposure times resulted in greater reductions, with the greatest reductions found after 24 h exposure. After 24 h exposure, mean microbial reductions for each organism were as follows: *S. aureus* (1.85 log CFU/cm^2), *E. coli* (1.81 log CFU/cm^2), *Bacillus* spp. (2.38 log CFU/cm^2), *S. aureus* met^r (2.98 log CFU/cm^2), *Streptococcus* spp. (1.64 log CFU/cm^2), *P. aeruginosa* (2.0 log CFU/cm^2), *L. monocytogenes* (2.75 log CFU/cm^2), *C. albicans* (3.22 log CFU/cm^2), and *S. chartarum* (3.32 log CFU/cm^2).

Reductions in microbial populations following treatment of stainless steel coupons with the EcoQuest Breeze AT Ozone generator are shown in Figure 2. Reductions of at least 0.2 and 0.4 log CFU/cm^2 were observed after 2 and 6 h of ozone exposure, respectively. After 24 h exposure, mean reductions for *C. albicans* and *S. chartarum* were 1.48 and 1.32 log CFU/cm^2, respectively.

The EcoQuest Radiant Catalytic Ionization Cell and EcoQuest Breeze AT Ozone generators reduced microbial populations on stainless steel surfaces within 2 h under ambient conditions, with greater reductions associated with longer exposure times. The Radiant Catalytic Ionization Cell was more effective than the Breeze AT Ozone Generator at reducing microbiological populations at shorter exposure times of 2 and 6 hours. This study demonstrated that ozone gas has the potential to be an effective surface disinfectant for use in food processing applications. Testing is currently ongoing to evaluate non-treated controls. Phase II of the project, scheduled to be completed by the end of this year, will evaluate the effectiveness of the system for eliminating airborne contamination using the same microorganisms and oxidative technologies.

REFERENCES

Mork, D.D. 1993. *Removing sulfide with ozone.* Water Contamination & Purification. 34-37.

U.S. Food and Drug Administration [FDA] 2004. *Recommendations to processors of apple juice or cider on the use of ozone for pathogen reduction purposes.* Accessed 27 July 2005 at http://www.cfsan.fda.gov/~dms/juicgu13.html.

Fig. 1 Decontamination of highly polished stainless steel surfaces using the *EcoQuest Radiant Catalytic Ionization (ActivePure) Cell*

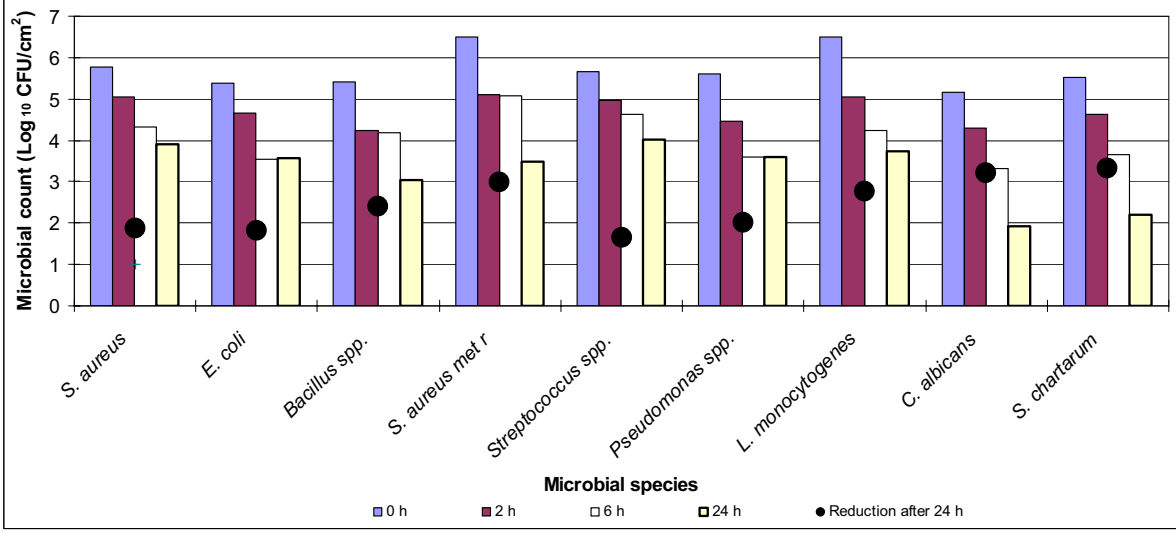
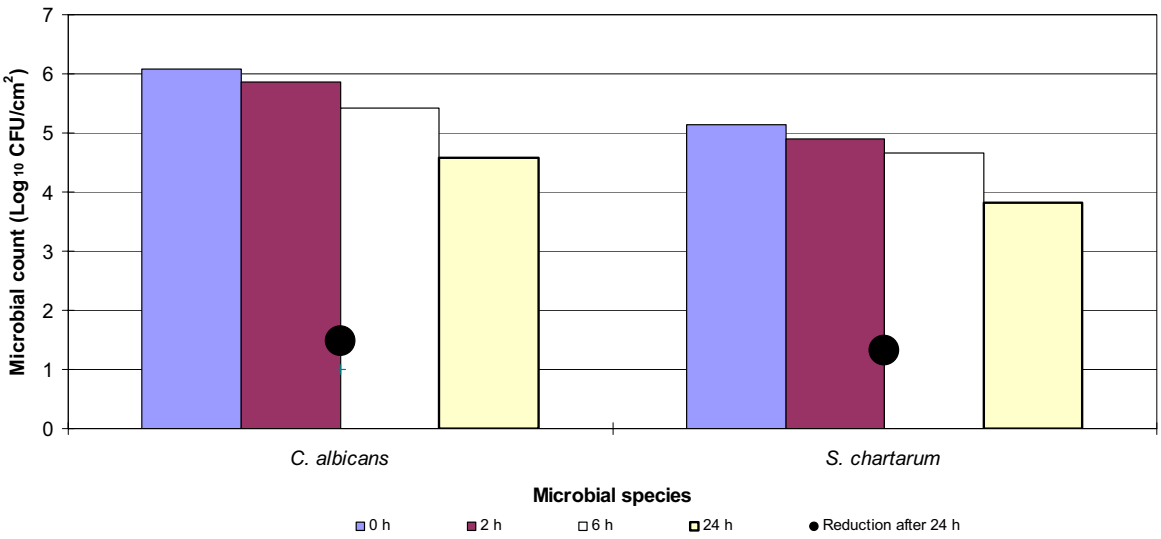


Fig 2. Ozone decontamination on highly polished stainless steel surfaces using the *EcoQuest Breeze AT Ozone* generator



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Control of Contaminants in Indoor Air

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

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An indoor air purification technique, which combines unipolar ion emission and photocatalytic oxidation (promoted by a specially designed RCI cell), was investigated in two test chambers, 2.75 m^3 and 24.3 m^3, using nonbiological and biological challenge aerosols. The reduction in particle concentration was measured size selectively in real-time, and the Air Cleaning Factor 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. 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^3 non-ventilated room is capable to provide CADR-values more than twice as great than the conventional closed-loop HVAC system with a rating 8 filter. The particle removal occurred due to unipolar ion emission, while the inactivation of viable airborne microorganisms was associated with photocatalytic oxidation. Approximately 90% of initially viable MS2 viruses were inactivated resulting from 10 to 60 min exposure to the photocatalytic oxidation. Approximately 75% of viable *B. subtilis* spores were inactivated in 10 min, and about 90% or greater after 30 min. 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).

Some commercial air cleaners generate excessive ozone (either as a primary biocidal agent or as a bi-product); these devices have raised 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*).

Photooxidation involving UV radiation and TiO_2 as a photocatalyst has been applied for gas-phase detoxification of organic contaminants (*8*, *9*) and for inactivating microorganisms in water (*10*−*12*). Some effort has been made to explore its application for air cleaning inside a closed-loop system (*13*, *14*). The investigators reported significant photocatalytic inactivation of stress-resistant *Serratia marcesens* that occurred when aerosolized bacteria circulated in a closed-loop duct equipped with a TiO_2 filter for a 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_2 film. The biocidal effect of the photocatalytic oxidation can be attributed to photogenerated 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, on the order of a second. No significant decrease in the colony forming unit (CFU) count was observed during such a short time.

To our knowledge, no data are available on the effectiveness of portable UV/TiO_2-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: unipolar ion emission and 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 are available on the efficiency of the hybrid-type technique.

Experimental Section

The indoor air purification process was investigated in the experimental facility 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

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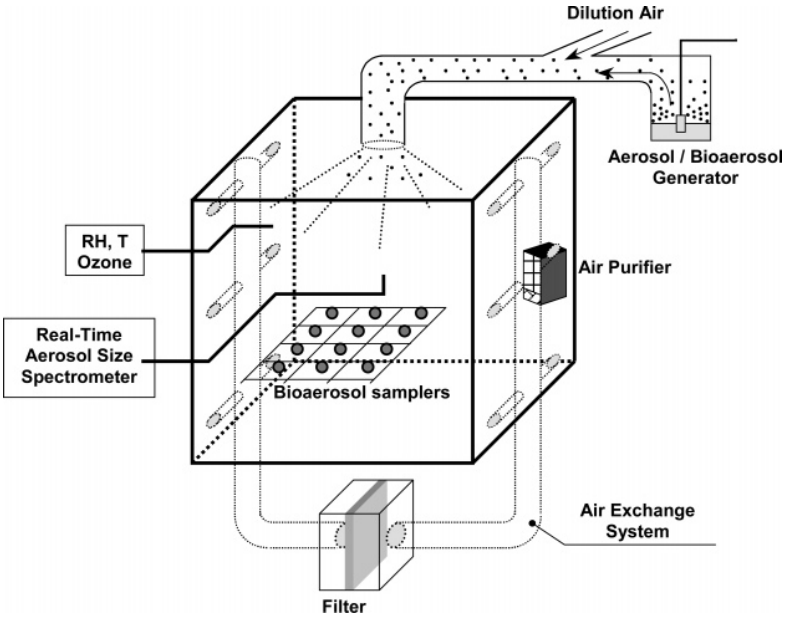

FIGURE 1. Experimental setup.

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^{85} charge equilibrator (3M Company, St. Paul, MN). After being mixed with clean, HEPA-filtered air at a specific temperature (T = 24−26 °C) and relative humidity (RH = 21−30%), the aerosol entered the 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), which 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 μm (midpoint). 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 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 μm.

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 $C_{\text{natural}}(d, t)$ every 10 s 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 $C_{\text{AP}}(d, t)$ was monitored during and 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:

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

In addition, the overall particle removal rate was calculated as

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

and the particle removal rate (exclusively due to air purifier) was defined following the first-order kinetics as

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

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

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

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

$$\text{CADR}(d, t) = V \times \text{PRR}(d, t)\ [\text{m}^3/\text{h}] \tag{5}$$

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

Two nonbiological 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 μm, 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 biological challenge aerosols. Selected experiments were performed with *Pseudomonas fluorescens* 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 it is known as an indicator for enteric viruses (*22*−*26*). Stock suspension of MS2 virus was prepared by adding 9 mL of Luria−Bertani broth to freeze-dried phage vial (ATCC 15597-B1). This suspension was

filtered using a membrane filter of 0.2 μm porosity and serially diluted so that the nebulizer suspension had 10^8-10^9 PFU/mL (PFU = plaque forming unit). MS2 phage titer 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 μm in width and 1.5−1.8 μm 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 U.S. Army Edgewood Laboratories, Aberdeen Proving Ground, Maryland) were activated at 55−60 °C for 25 min and then washed two times with sterile deionized water by vortexing followed by centrifugation at 7000 rpm for 7 min 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 trypicase soy agar (TSA) media at 30 °C for 18 h; 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* (ATCC 13525) were cultured in trypticase soy broth at 28 °C for 18 h and washed similarly as *B. subitilis* 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; four time intervals were tested: t = 10, 15, 30, and 60 min. Additional selected experiments were performed by using a BioSampler (SKC Inc. Eighty Four, PA) to collect *P. fluorescens* and *B. subtilis*. The BioSampler efficiently collects viable bacteria (*29*) while the liquid medium minimizes the desiccation stress. As its cutoff 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 virions (PFU) and bacteria (CFU) to quantify the percentages of those survived over time t. These were obtained with and without operating the air purifier. 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 to ensure sufficient number of microorganisms for determining the viable count at the end of the test.

An aliquot of 200 μL of dissolved gelatin filter extract was used for plaque assay to determine the number of airborne active (viable) virions (PFU/cm^3). Similarly, extract was cultivated on TSA plates to obtain the airborne concentration of viable bacteria (CFU/cm^3).

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.

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 ± 2^0C 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 an ion emitter and a specially designed RCI cell. The former produces negative ions into 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 spectral output between 100 and 367 nm. Besides, a coating was applied to the target structure of the cell comprising hydrophilic properties and containing the following grouping of materials: titanium dioxide, rhodium, silver, and copper. As a result, a photocatalytic oxidation forms reactive species, such as hydroxyl radicals, valence-band holes, superoxide ions, and hydrogen peroxides.

The tests were conducted in two indoor test chambers, including a large walk-in chamber (24.3 m^3) that simulated a residential room and a smaller chamber (2.75 m^3) that simulated a confined space (e.g., bathroom, small office area, or automobile cabin). The particle removal was investigated in both chambers, whereas 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 is known that portable air cleaners are primarily beneficial in poorly ventilated spaces (*20*, *21*). Air exchange was introduced only when testing the closed-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

Particle Removal from Air. 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 of 28 in 1 h and by a factor of about 250 in 2 h; 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 and only the ion emitter operated. The latter finding provides the evidence that the particle removal was achieved as a result of unipolar ion emission but not due to photocatalytic reactions.

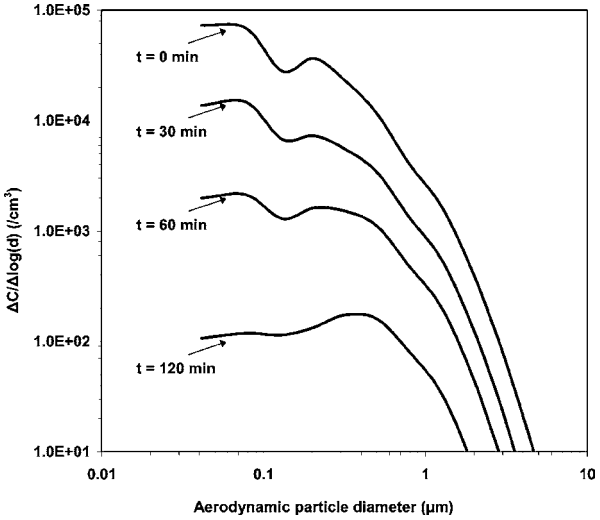

FIGURE 2. Particle concentration and size distribution of NaCl aerosol as measured with the ELPI in the 24.3 m³ chamber with the air purifier operating facing the chamber's center at 1.7 m from the measurement point. No ventilation in the chamber. The initial total aerosol concentration = 1.50 × 10⁵ /cm³.

This finding agrees with previously published data on the effect of unipolar air ionization on the airborne concentration (*18*−*21*). The air purification is particularly efficient at higher initial aerosol concentrations (>10^4 particles/cm^3) that ensure 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 $d \leq 0.3$ μm. E.g., when the air purifier with an ion output of ~10^{12} e/sec continuously operated in a corner of the 24.3-m^3 chamber facing the center for 2 h, ACF reached ~30−70 for $d = 0.08-0.3$ μm and ~13−16 for $d = 0.8-2$ μm (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 the measured sizes of NaCl particle up to 2.5 μm and averaged over the three selected locations/orientations in the 24.3-m^3 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^3 chamber. The CADR ranges approximately from 42.1 ± 0.1 to 62.1 ± 1.8 m^3/h for NaCl particles of $d = 0.04-1.99$ μm, and from 72.4 ± 0.9 to 115.5 ± 10.8 m^3/h 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.

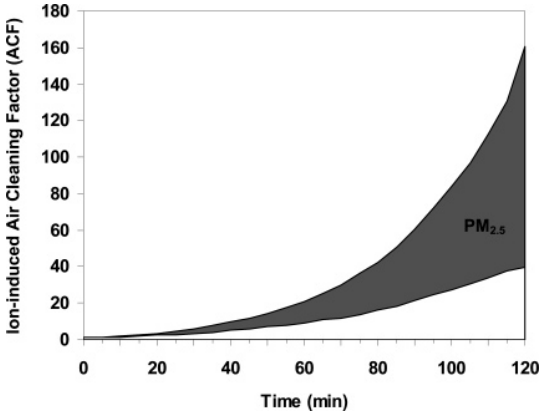

FIGURE 3. The ion-induced Air Cleaning Factor (ACF) for $PM_{2.5}$ NaCl as measured with the ELPI and integrated for different locations and orientations of the air purifier in the 24.3 m³ chamber. No ventilation in the chamber. The initial $PM_{2.5}$ aerosol concentration = (0.356−1.50) × 10⁵/cm³.

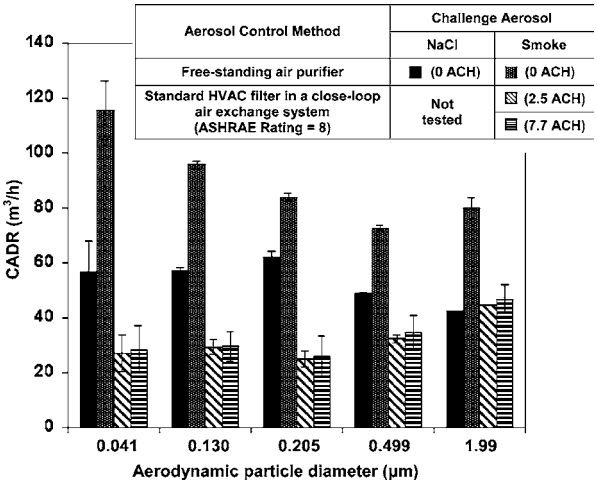

FIGURE 4. Clean Air Delivery Rate (CADR) determined for the NaCl and smoke aerosols as measured with the ELPI in the non-ventilated 24.3 m³ chamber. The performance of the air purifier is compared to that of a standard HVAC filter (ASHRAE rating = 8) installed in the closed-loop air exchange system of the chamber.

In addition, Figure 4 presents the CADR values achieved by the closed-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^3 non-ventilated room is capable to provide a 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, compared to the portable unit, HEPA filter installed in the closed-loop air exchange system of the 24.3 m^3 chamber provided approximately 4- and 3-fold greater CADRs 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^3) 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 ± 5 m^3/h, which is in the CADR-

TABLE 1. Percentage of Airborne Microorganisms Survived over Time *t* in the 2.75 m³ Chamber with the RCI-cell Operating in it, as Measured via PFU Count (for MS2 Virus) or CFU Count (for *Bacillus subtilis* Endospores)[a]

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

[a] Bioaerosol sampling was conducted with the Button Sampler equipped with gelatin filters. n = number of replicates.

range obtained for NaCl and smoke particles in the large chamber for the viral sizes. This suggests the feasibility of using nonbiological particles to determine the ion-induced aerosol reduction of bio-particles of the same size range. Furthermore, 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].

Ozone. In both test chambers (non-ventilated), the ozone concentration gradually increased as the purifier was continuously operating. In the 24.3-m^3 chamber, it increased from 0.006 to 0.05 ppm in about 35 min, while in a smaller (2.75-m^3) 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^3 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^3 (not presented here) 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 such devices in confined occupied air spaces may not be appropriate as their continuous operation may eventually lead to excessive ozone levels and, in the presence of certain chemical compounds, produce nanoparticles (*33*). Although the unipolar ion emission has a potential to suppress this effect, it seems important to keep the ozone level below existing thresholds. 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 a 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.

Microbial Inactivation. 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 ± 0.9% during 1 h. 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 that 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's subsequent interaction with the host and formation of PFUs (*34*). Additionally, the TiO_2 photocatalytic cell may produce oxidative damage to the virus capsid (*35*) and the radicals may cause alteration in the virus's genetic material (*36*, *37*). 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 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 nonlinearity of the effect. The natural decay in the culturable count was not significant ($p > 0.05$) during 1 h, 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 $t = 2$ h. It was confirmed that the viability was constant within about ±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 (*38*);
- destruction of the outer membrane of bacterial cells (*12*);
- oxidation of unsaturated phospholipid in bacterial cell membrane (*39*);
- leakage of intracellular K^+ ions (*11*); and
- detrimental effects on DNA and RNA (*36*, *37*).

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. (*40*), who found that photooxidation of coenzyme A by the TiO_2 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 (*41*).

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^+) ($TiO_2 + h\nu \rightarrow h^+ + e^-$.) 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 ($H_2O + h^+ \rightarrow {}^{\cdot}OH + H^+$), 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_2 photooxidation are capable of oxidizing any organic compound. The process also produces hydrogen peroxide ($O_2 + e^- \rightarrow O_2^{\bullet -}$; $O_2^{\bullet -} + H^+ \rightarrow HO_2^{\bullet}$; $2HO_2^{\bullet} \rightarrow O_2 + H_2O_2$), which can freely penetrate into cell membranes and walls and cause microbial inactivation

(*42*). Further biochemical studies on the role of gas-phase TiO_2 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 test if the test is 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 1835 ± 270 PFU/mL and 1855 ± 325 PFU/mL developed when filter extracts were cultivated from unexposed and 10-min exposed gelatin filters, respectively. For *B. subtilis*, we observed 1770 ± 275 CFU/mL and 1125 ± 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.

Combined Effect (Sample Calculation). 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. Both mechanisms working simultaneously in a hybrid type air purifier may result in considerable decrease of the exposure to pre-existing viable aerosol biocontaminants in indoor environment. Ozone produced by the RCI cell is not believed to cause significant microbial inactivation because its level was not sufficient. Tseng and Li (*43*) referred to 3.43 ppm as an appropriate level for airborne MS2 virus, and Li and Wang (*44*) did not observe any inactivation of airborne *B. subtilis* spores at O_3 as high as 20 ppm.

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 photoxidation 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.

The observed rapid inactivation of microorganisms makes unnecessary to run the RCI cell continuously. The data suggest that it can be used "part-time" for 10−30 min and "rest" for about 1−2 h until the background ozone level is reached (proposed above as an intermittent regime), while the ion emission can take place continuously to keep the aerosol concentration decreasing.

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Literature Cited

(1) EPA (U.S. Environmental Protection Agency). *Ozone generators that are sold as air cleaners: an assessment of effectiveness and health consequences*; http://www.epa.gov/iaq/pubs/ozonegen.html; Accessed on May 22, 2006.

(2) ACGIH (American Conference of Governmental Industrial Hygienists). *Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Indices*; ACGIH: Cincinnati, OH, 2004; p. 44.

(3) OSHA (Occupational Safety & Health Administration). *Regulations (Standards 29 CFR): Air Contaminants, 1915.1000*; http://www.osha.gov/pls/oshaweb; Accessed on May 31, 2006.

(4) FDA (U.S. Food and Drug Administration). *Labeling Regulatory Requirements for Medical Devices*; HHS publication FDA 89-4203; U.S. Governement Printing Office: Washington, DC, 1989.

(5) EPA (U.S. Environmental Protection Agency). *National Ambient Air Quality Standards (1997)*; http://www.epa.gov/air/criteria.html; Accessed on May 24, 2006.

(6) Foarde, K. K.; VanOsdell, D. W.; Steiber, R. S. Investigation of gas-phase ozone as a potential biocide. *Appl. Occup. Environ. Hyg.* **1997,** *12*, 535−542.

(7) Li, C. -S.; Wang, Y. -C. Surface germicidal effects of ozone for microorganisms. *AIHA J.* **2003,** *64*, 533−537.

(8) Alberici, R. M.; Canela, M. C.; Eberlin, M. N.; Jardim, W. F. Catalyst deactivation in the gas phase destruction of nitrogen-containing organic compounds using TiO_2/UV-VIS. *Appl. Catal., B* **2000**, *793*, 1−9.

(9) Shang, J.; Du, Y.; Xu, Z. Photocatalytic oxidation of heptane in the gas-phase over TiO_2. *Chemosphere* **2002**, *46*, 93−99.

(10) Matsunaga, T.; Tomoda, R.; Nakajima, T.; Nakamura, N.; Komine, T. Continuous sterilization system that uses photosemiconductor powders. *Appl. Environ. Microbiol.* **1988**, *54*, 1330−1333.

(11) Saito, T.; Iwase, T.; Horie, J.; Morioka, T. Mode of photocatalytic bactericidal action of powdered semiconductor TiO_2 on Streptococci mutants. *J. Photochem. Photobiol.* **1992**, *14*, 369−379.

(12) Sunada, K.; Kikuchi, Y.; Hashimoto, K.; Fujishima, A. Bactericidal and detoxification effects of TiO_2 thin film photocatalysts. *Environ. Sci. Technol.* **1998**, *32*, 726−728.

(13) Goswami, D. Y.; Trivedi, D. M.; Block, S. S. Photocatalytic disinfection of indoor air. *Trans. ASME* **1997**, *119*, 92−96.

(14) Goswami, T. K.; Hingorani, S.; Greist, H.; Goswami, D. Y.; Block, S. S. Photocatalytic system to destroy bioaerosols in air. *J Adv. Oxidation Tech.* **1999**, *4*, 185−188.

(15) Pal, A.; Min, X.; Yu, L. E.; Pehkonen, S. O.; Ray, M. B. Photocatalytic inactivation of bioaerosols by TiO_2 coated membrane. *Int. J. Chem. Reactor Eng.* **2005,** *3*, A45.

(16) Keller, V.; Keller, N.; Ledoux, M. J.; Lett, M.-C. Biological agent inactivation in a flowing air stream by photocatalysis. *Chem. Commun.* **2005**, *23*, 2918−2920.

(17) Lin, C. -Y.; Li, C. -S. Effectiveness of titanium dioxide photocatalyst filters for controlling bioaerosols. *Aerosol Sci. Technol.* **2003**, *37*, 162−170.

(18) Grinshpun, S. A.; Mainelis, G.; Trunov, M.; Adhikari, A.; Reponen, T.; Willeke, K. Evaluation of ionic air purifiers for reducing aerosol exposure in confined indoor spaces. *Indoor Air* **2005**, *15*, 235−245.

(19) Lee, B. U.; Yermakov, M.; Grinshpun, S. A. Unipolar ion emission enhances respiratory protection against fine and ultrafine particles, *J. Aerosol Sci.* **2004**, *35*, 1359−1368.

(20) Mayya, Y. S.; Sapra, B. K.; Khan, A.; Sunny, F. Aerosol removal by unipolar ionization in indoor environments. *J. Aerosol Sci.* **2004**, *35*, 923−941.

(21) Grinshpun, S. A.; Adhikari, A.; Lee, B. U.; Trunov, M.; Mainelis, G.; Yermakov, M.; Reponen, T. Indoor air pollution control through ionization. In *Air Pollution XII;* Brebbia, C. A., Ed.; WIT Press: Southampton, U.K., 2004; pp 689−704.

(22) Kekez, M. M.; Sattar, S. A. A new ozone-based method for virus inactivation: preliminary study. *Phys. Med. Biol.* **1997**, *42*, 2027−2039.

(23) O'Connel, K. P.; Bucher, J. R.; Anderson, P. E.; Cao, C. J.; Khan, A. S.; Gostomski, M. V.; Valdes, J. J. Real-time fluorogenic reverse transcription-PCR assays for detection of bacteriophages MS2. *Appl. Environ. Microbiol.* **2006,** *72*, 478−483.

(24) Jones, M. V.; Bellamy, K.; Alcock, R.; Hudson, R. The use of bacteriophage MS2 as a model system to evaluate virucidal hand disinfectants. *J Hosp. Infect.* **1991**, *17*, 279−285.

(25) Havelaar, A. H.; van Olphen, M.; Drost, Y. C. F-specific RNA bacteriophages are adequate model organisms for enteric viruses in fresh water. *Appl. Environ. Microbiol.* **1993**, *59*, 2956−2962.

(26) Allwood, P. B.; Malik, Y. S.; Hedberg, C. W.; Goyal, S. M. Survival of F-specific RNA coliphage, feline Calicivirus, and *Escherichia coli* in water: a comparative study. *Appl. Environ. Microbiol.* **2003**, *69*, 5707−5710.

(27) Adams, M. H. *Bacteriophages*; Interscience Publishers, Inc: New York, 1959.

(28) Johnson, B.; Martin, D. D.; Resnick, I. G. Efficacy of selected respiratory protective equipment challenged with *Bacillus subtilis* subsp. *niger*. *Appl. Environ. Microbiol.* **1994**, *60*, 2184−2186.
(29) Lin, X; Reponen, T. A.; Willeke, K.; Grinshpun, S. A.; Foarde, K.; Ensor, D. Long-term sampling of airborne bacteria and fungi into a non-evaporating liquid. *Atmos. Environ.* **1999**, *33*, 4291−4298.
(30) Lin, X.; Reponen, T.; Willeke, K.; Wang, Z.; Grinshpun, S. A.; Trunov, M. Survival of airborne microorganisms during swirling aerosol collection. *Aerosol Sci. Technol.* **2000**, *32*, 184−196.
(31) Mainelis, G.; Grinshpun, S.; Willeke, K.; Reponen, T.; Ulevicius, V.; Hintz, P. Collection of airborne microorganisms by electrostatic precipitation. *Aerosol Sci. Technol.* **1999**, *30*, 127−144.
(32) Hinds, W. C. *Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles,* 2nd ed.; John Wiley & Sons, Inc.: New York, 1999.
(33) Grinshpun, S. A.; Toivola, M.; Lee, S.-A.; Reponen, T. Formation of Nanoparticles in indoor air at an increased ozone level. In *Abstracts of the 24th Annual Meeting of the American Association for Aerosol Research*, Austin, Texas, U.S.A., October 17−21, 2005; AAAR: Mt. Laurel, NJ, 2005; p 327.
(34) Von Sonntag, C. Disinfection by free radicals and UV-radiation. *Water Supply* **1986**, *4*, 11−18.
(35) Turchi, C. S.; Ollis, D. F. Photocatalytic degradation of organic water contaminants: mechanisms involving hydroxyl radical attach. *J. Catal.* **1990**, *122*, 178−192.
(36) Dunford, R.; Salinaro, A.; Cai, L.; Serpone, N.; Horikoshi, S.; Hidaka, H.; Knowland, J. Chemical oxidation and DNA damage catalyzed by inorganic sunscreen ingredients. *FEBS Lett.* **1997**, *418*, 87−90.
(37) Hidaka, H.; Horikoshi, S.; Serpone, N.; Knowland, J. *In vitro* photochemical damage to DNA, RNA, and their bases by an inorganic sunscreen agent on exposure to UVA and UVB radiation. *J. Photochem. Photobiol., A* **1997**, *111*, 205−213.
(38) Vohra, A.; Goswami, D. Y.; Deshpande, D. A.; Block, S. S. Enhanced photocatalytic inactivation of bacterial spores on surfaces in air. *J. Ind. Microbiol. Biotechnol.* **2005**, *32*, 364−370.
(39) Maness, P. C.; Smolinski, S.; Blake, D. M.; Huang, Z.; Wolfrum, E. J.; Jacoby, W. A. Bactericidal activity of photocatalytic TiO_2 reaction: toward an understanding of its killing mechanism. *Appl. Environ. Microbiol.* **1999**, *65*, 4094−4098.
(40) Matsunaga, T.; Tamoda, R.; Nakajima, T.; Wake, H. Photoelectrochemical sterilization of microbial cells by semiconductor powders. *FEMS Microbiol. Lett.* **1985**, *29*, 211−214.
(41) Rincón, A. G.; Pulgarin, C. Photocatalytical inactivation of *E. coli*: effect of (continuous-intermittent) light intensity and of (suspended-fixed) TiO_2 concentration. *Appl. Catal., B* **2003,** *44*, 263−284.
(42) Marquis, R. E.; Sim, J.; Shin, S. Y. Molecular mechanism of resistance to heat and oxidative damage. *J. Appl. Bacteriol. Symp. Suppl.* **1994,** *76*, 40S−48S.
(43) Tseng, C.-C.; Li, C.-S. Ozone for inactivation of aerosolized bacteriophages. *Aerosol. Sci. Technol.* **2006**, *40*, 683−689.
(44) Li, C.-S.; Wang, Y. C. Inactivation effects of airborne ozone for bioaerosols. *J. Environ. Health* **2006**, submitted.

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Efficacy of radiant catalytic ionization to reduce bacterial populations in air and on different surfaces

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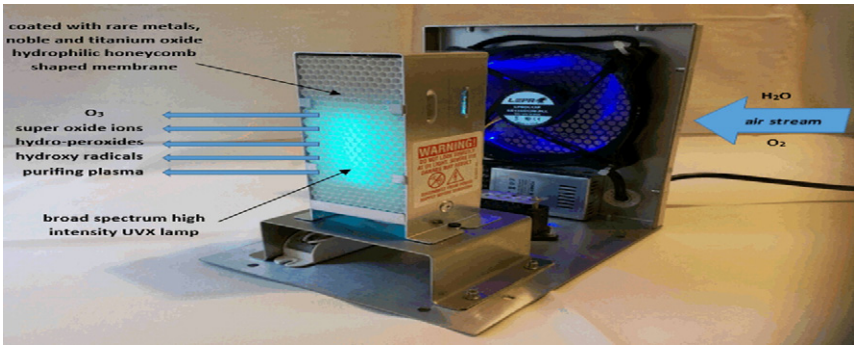
HIGHLIGHTS

- The radial catalytic ionization biocidal efficiency (RCI) has been evaluated.
- The coefficient of microbial elimination from the air was >95% after RCI usage.
- RCI usage causes visible elimination of microorganisms from tested surface.
- RCI biocidal efficiency depends on strain and type of surface.
- Spores of *Clostridium* spp. were more resistant than vegetative form of bacteria and fungi.

GRAPHICAL ABSTRACT



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ABSTRACT

Air contamination by biological agents is often observed in medical or veterinary facilities and industrial plants. Bioaerosols may sediment and pose the surface contamination. Microorganisms present on them may become a source of infections among humans and food contamination. This study determined the use of oxidative gases, including ozone and peroxide, generated by the Radiant Catalytic Ionization (RCI) cell for the inactivation of *Acinetobacter baumannii*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Salmonella* Enteritidis, *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus epidermidis*, *Bacillus subtilis*, *Clostridium sporogenes*, *Candida albicans*, *Aspergillus niger* and *Penicillium chrysogenumon* in air and on different surfaces. Results showed that oxidative gases produced by the RCI cell reduced all tested microorganisms. The full elimination of studied microorganisms from the air was obtained for *E. coli* and *C. albicans*. RCI also proved to be an effective method of eliminating microbes from the examined surfaces. Regarding of the species, strains origin and the type of surface, the reduction rate ranged from 19.0% for *C. albicans* to over 99% for *A. baumanii*. For both, air and surface, the most resistant to RCI was *C. sporogenes* spores, for which the percentage reduction rate ranged from –2.6% to 71.2% on the surfaces and was equal 71.7% in the air.

1. Introduction

One of the major problems of air quality is the presence of microorganisms in it, which include bacteria, molds, and viruses.

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Microorganisms present in the air compose bioaerosol. In this form microorganism may spread over considerable distances, sediment on different surfaces and causing their contamination. Ventilation ducts can be the source of airborne microbial communities (Hayleeyesus and Manaye, 2014; Hospodsky et al., 2012). Air contamination by biological agents is often observed in medical or veterinary facilities, industrial plants (e.g. food processing plants, waste segregation and recycling plants, steel and ironworks) and in agriculture (Brewczyńska et al., 2015; Douwes et al., 2003). Workers dealing with industrial waste recycling or production of highly purified biological substances are exposed to high concentrations of bioaerosols (Rim and Lim, 2014). Most particles of biological aerosols range from nanometric (e.g. bacterial endotoxins), to submicrone (e.g. fragments of bacterial or fungal cells), to particles whose diameter may exceed 100 μm (e.g. plant pollens) (Douwes et al., 2003; Dutkiewicz et al., 2011; Górny, 2010). Bioaerosols with a diameter of 1.0–5.0 μm usually remain in the air, whereas the fraction of large molecules descend on surfaces (Gąska-Jędruch and Dudzińska, 2009). It was proven that in houses the level of bacterial aerosol amounts on average to 10^3 CFU × m^{-3} (Colony Forming Units), and in workplaces 10^2 CFU × m^{-3} (Pastuszka et al., 2000).

Many surfaces, such as stainless steel, plastic, rubber org glass, are used in hospitals or food industry. Microorganisms present on them may become a source of infections in people and food contamination (Bagge-Ravn et al., 2003). The degree of surface contamination depends on their properties, such as the material of which they are made, porosity, hydrophobicity/hydrophilicity, etc. (Ismaïl et al., 2013). Schlegelová et al. (2010) indicated that surfaces that come into contact with food may be contaminated with such bacteria as *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli*, *Bacillus* spp., *Staphylococcus* spp., *Enterococcus* spp.

Ozone or UV-C radiation are commonly used for disinfection of rooms and air. (Kim et al., 1999; Kujundzic et al., 2006). It is necessary to search for and develop new methods for sterilization of air and surfaces which come into contact with patients or food. The technology of radiant catalytic ionization (RCI) is still not well known, but its popularity is gradually increasing. This technology uses the appropriate wavelength and the phenomenon of photooxidation in the presence of UV radiation and appropriate photo-catalysts, such as TiO_2, which compose the hydrophilic coating of surface of matrixes in the RCI module (Grinshpun et al., 2007). This leads to production of superoxide ions and hydroxides, and to generating plasma based on hydrogen peroxide (Cho et al., 2005). In contrast to passive methods of air purification which filter mechanically the air that flows through them, the RCI technology purifies the air outside. The advantage of this solution is the ability to perform constant disinfection of ventilated air, e.g. in food processing plants. In comparison with the effect of UV-C lamps, this technique reduces microbiological contamination and removes odors, and the resulting chemical compounds may sediment on the surface and have microbiocidal effect. It is advantage of these method, but biproducts, which are likely generated when using RCI, are of health concern. United States Environmental Protection Agency (EPA) indicate that ozone generators are not always safe and effective in removing pollutants. Harmful effects can occur following short-term exposure to low levels of ozone. Ozone generators should never be used around the ill, infirm, young or elderly people (US EPA, 1996a, 1996b). However, manufacturers of this type of air purification decelerate their safety for consumers. RCI generates very low level ozone and in the catalytic process breaks ozone down forming other oxidation products. Some authors suggest that low concentration of ozone have no effect on biological contamination (Dyas et al., 1983; Foarde et al., 1997). In this technology ozone is reduced odor, smoke and a wide spectrum of impurities in the air. Biological contamination is reduced by photocatalytic reaction with several other oxidizers. Photo catalytic oxidation must not produce any bi-products of the oxidation reaction. The EPA report confirmed the absence of bi-products using several methods, including gas chromatography, compound-specific detector tubes, and individual gas sensors (US EPA, 2000).

There are few studies in the literature assessing the efficacy of new methods for air and surface purification, such as RCI, therefore it is reasonable to conduct research on their efficacy. The study aimed to assess the efficacy of RCI towards selected microbial species present in the air and on selected solid surfaces.

2. Material and methods

The study involved the assessment of efficacy of microbial inactivation in the air and on selected types of solid surfaces as affected by radiant catalytic ionization. Ionization process was conducted using the device Induct 750 made by ActivTek Sp. z o.o, ensuring an air flow rate of 6 m × s^{-1}. The experiment was carried out in three replications for each studied strain.

2.1. Efficacy of air purification using radiant catalytic ionization

Material used for the study consisted of reference strains of bacteria (*Staphylococcus aureus* ATCC 25213, *Staphylococcus epidermidis* ATCC 35984, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 8159, vegetative forms and spores of *Clostridium sporogenes* ATCC 19404) and fungi (*Candida albicans* ATCC 90028, *Aspergillus niger* ATCC 9142 and *Penicillium chrysogenum* ATCC 10106).

For the study, standardized microbial suspensions in saline with an optical density of 0.5 in McFarland standard were prepared. Next, 4 ml of each suspension was placed in a sterile nebulization chamber of the Medbryt MONSUN MP1 pneumatic inhaler. Nebulization was conducted until the complete removal the suspension from the nebulization chamber of the inhaler (about 15 min).

Nebulization chamber was placed in the testing room which was a hermetically sealed chamber with a capacity of 1.4 m^3 made of steel plates. Prior to each nebulization, the chamber walls were chemically disinfected with a preparation used for disinfection of solid surfaces, and the air contained in it was subjected to the action of the UV-C Philips TUV 36 W/G36 T8 lamp for 20 min. After that time the chamber was opened for about 20 min in order to remove the accumulated ozone. Prior to nebulization, the follow-up assessment of air microbiological purity was performed, to check the initial level of microbiological contamination. The detailed arrangement of the experiment is presented in Fig. 1, and the appearance of the research set in Fig. 2.

Air samples were collected with the compaction method using the device MAS-100 Eco (EMD Chemicals). To assess air microbiological purity after the use of UV-C Philips TUV 36 W/G36 T8 lamp and radiant catalytic ionization, 0.2 and 0.5 m^3 were collected. To assess the air microbiological contamination level in the chamber after nebulization of the microbial suspension, 0.014 and 0.052 m^3 were collected. The list of media used in the study for individual microorganisms and incubation conditions were presented in Table 1.

Colonies grown on media were counted and expressed in CFU × m^{-3} of air. Next the median was calculated for all the media studied for the given microorganism and collected air volumes. The effectiveness was expressed by giving the number of CFU of bacteria and fungi before and after the use of radiant catalytic ionization, as well as calculation of the percentage reduction rate (R[%]) according to the formula:

$$R[\%] = \frac{A-B}{A} \times 100$$

where: A – the output number of microorganisms after nebulization or drying of suspension on the solid surface [CFU × m^{-3}]B – the number of microorganisms after the use of device [CFU × m^{-3}]

Positive control in the experiment was a measurement of the number of microorganisms in the air made at 20 min after nebulization

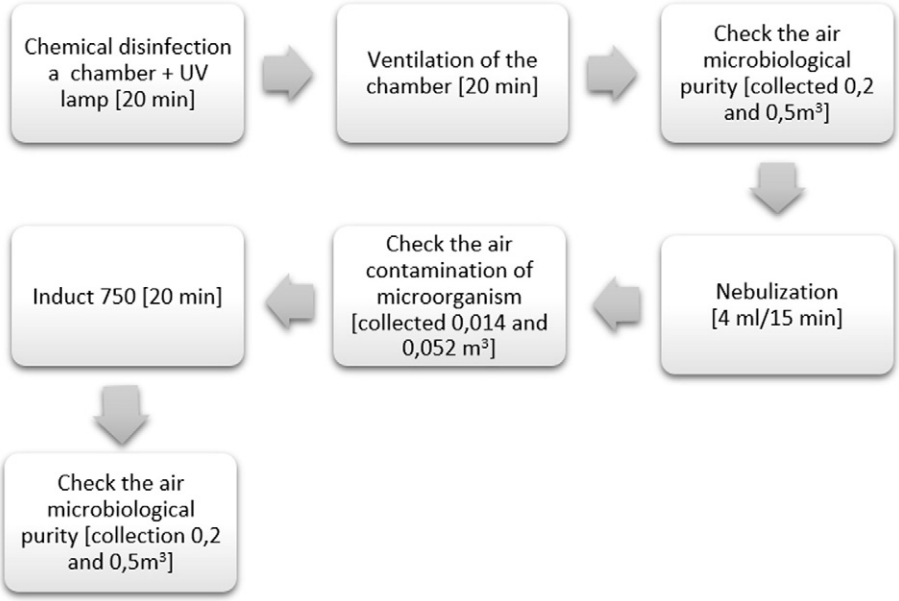

Fig. 1. Detailed arrangement of the experiment concerning the air disinfection effectiveness.

without the operation of the Induct 750. The negative control was air samples taken after preparation of the chamber prior to nebulization.

Statistical analysis of the obtained results was carried out with STATISTICA 12 PL. The calculated values of the percentage reduction rate were analyzed. Shapiro-Wilk test was carried out for the normality of distribution evaluation. The variance analysis was carried out using the one-way ANOVA and the statistical significance of the difference between the percentage reduction coefficients, based on the Tukey post-hoc parametric test at significance level $\alpha = 0.05$, were checked. As an independent variable, the microbial species was considered.

2.2. Assessment of surface disinfection effectiveness with the use of radiant catalytic ionization

Material for the study included reference strains of bacteria (*Acinetobacter baumannii* ATCC 19606, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella* Enteritidis ATCC 13076, *Listeria monocytogenes* ATCC 19111, *Staphylococcus aureus* ATCC 25213, spores of *Clostridium sporogenes* ATCC 19404) and fungi (*Candida albicans* ATCC 90028, *Aspergillus niger* ATCC 9142 and *Penicillium chrysogenum* ATCC 10106) with different biological properties. Moreover, also one clinical strain and one environmental strain were used.

In order to conduct the study, standardized microbial suspensions in 0.5% sterile solution of bovine albumin with an optical density of 0.5 in McFarland standard were prepared. Then 50 μl of each suspension were placed individually on the each studied surface and leave in the lamination chamber until complete drying.

The following solid surfaces were used in the study: steel AISI 304, polypropylene, glazed milled rock tiles, lacquered veneer, rubber and polyamide fitted carpet. Each surface had a size 1 cm × 2 cm. All surfaces before the use in the experiment were sterile.

After drying of the microbial suspension, the surfaces were inducted into the test room (photo 1) and placed at a distance of 1 m from the Induct 750 device, which was turned on for 20 min. After this time,

Fig. 2. The set for assessment of air disinfection effectiveness.

Table 1
Microbiological media used in the study and culture incubation conditions.

Microorganism	Culture media	Incubation conditions
Acinetobacter baumannii	Columbia Agar with addition of 5% sheep blood (Becton Dickinson) MacConkey Agar (Becton Dickinson)	Aerobic 37 °C/24 h
Escherichia coli	Columbia Agar with addition of 5% sheep blood (Becton Dickinson) MacConkey Agar (Becton Dickinson)	
Enterococcus faecalis	Columbia Agar with addition of 5% sheep blood (Becton Dickinson) Enterococcosel Agar (Becton Dickinson)	
Pseudomonas aeruginosa	Columbia Agar with addition of 5% sheep blood (Becton Dickinson) PYA Agar with cetrymide (Becton Dickinson)	
Salmonella Enteritidis	Columbia Agar with addition of 5% sheep blood (Becton Dickinson) XLD Agar (Becton Dickinson)	
Listeria monocytogenes	Columbia Agar with addition of 5% sheep blood (Becton Dickinson) ALOA Agar (Merck)	
Staphylococcus aureus *Streptococcus epidermidis*	Columbia Agar with addition of 5% sheep blood (Becton Dickinson) Chapman Agar (Becton Dickinson)	
Bacillus subtilis	Columbia Agar with addition of 5% sheep blood (Becton Dickinson)	
Clostridium sporogenes (vegetative forms)	Columbia Agar with addition of 5% sheep blood (Becton Dickinson)	Anaerobic 37 °C/48 h
Clostridium sporogenes (spores)		
Candida albicans	Columbia Agar with addition of 5% sheep blood (Becton Dickinson) Sabouraud Agar (Becton Dickinson)	Aerobic 25 °C/72 h
Aspergillus niger *Penicillium chrysogenum*	Columbia Agar with addition of 5% sheep blood (Becton Dickinson) Sabouraud Agar (Becton Dickinson)	Aerobic 25 °C/72 h

fragments of the surfaces were placed in a sterile solution of PBS (20 ml) and subjected to sonication in the Ultrasonic DU-4 (Nickel-Electro) sonicator, and then they were shaken for 5 min with a speed of 400 rpm (rotations per minute). The resulting suspension were 10-fold diluted using a dilution series to 10^{-4}. From each dilution, 100 μl was superficially inoculated on appropriate solid media and incubated in proper conditions (Table. 1).

Grown colonies were counted and their number was determined based on the formula:

$$L = \frac{C}{(N_1 + 0,1N_2)} * d * a$$

where: C - the total number of colonies on all plates chosen for counting,N_1 – the number of plates from the first counted dilution,N_2 – the number of plates from the second counted dilution,d – the dilution factor corresponding to the first (lowest) calculated dilution,a – the ratio of the inoculated material volume,and then was expressed in CFU per 1 cm^2 of the studied surface.

Efficacy was expressed by giving the number of CFU of bacteria and fungi before (positive control) and after the use of radiant catalytic ionization, as well as calculating the percentage reduction rate (R[%]) according to the formula given in Section 2.1.

For the obtained values of the percentage reduction rate the significance of differences were checked in the STATISTICA 12 PL software based on the post-hoc Tukey's test at the significance level $\alpha = 0,05$.

The negative control was the studied surfaces with applied sterile solution of bovine albumin.

Positive control in the experiment was the studied surfaces contaminated with tested microorganisms strains and stored without the operation of the Induct 750. The negative control was the studied surfaces with applied sterile solution of bovine albumin.

Statistical analysis of the obtained results was carried out with STATISTICA 12 PL. The calculated values of the percentage reduction rate were analyzed. Shapiro-Wilk test was carried out for the normality of distribution evaluation. The variance analysis was carried out using the multi-way ANOVA and the statistical significance of the difference between the percentage reduction coefficients, based on the Tukey post-hoc parametric test at significance level $\alpha = 0.05$, were checked. As an independent variables, the microbial strain and type of surface were considered.

Table 2
Number of microorganisms recovered from the air and percentage reduction rate R[%].

Microorganisms	Mean number of microorganisms after nebulization [CFU × m^{-3}](SE)*	Mean number of microorganisms after use of radiant catalytic ionization [CFU × m^{-3}](SE)*	Percentage reduction rate of number of microorganisms [%]
S. aureus ATCC 25213	$3,09 \times 10^5$ ($\pm 4.11 \times 10^5$)	$2,70 \times 10^1$ ($\pm 1.40 \times 10^1$)	99,99[a]
S. epidermidis ATCC 35984	$2,83 \times 10^5$ ($\pm 1.02 \times 10^5$)	$1,50 \times 10^1$ ($\pm 2.25 \times 10^1$)	99,99[a]
E. faecalis ATCC 29212	$4,11 \times 10^5$ ($\pm 2.17 \times 10^5$)	$3,50 \times 10^0$ ($\pm 5.18 \times 10^0$)	99,99[a]
E. coli ATCC 25922	$2,86 \times 10^2$ ($\pm 3.09 \times 10^2$)	n.d.	100,00[a]
B. subtilis ATCC 8159	$2,71 \times 10^5$ ($\pm 2.86 \times 10^3$)	$2,93 \times 10^3$ ($\pm 1.30 \times 10^3$)	98,92[a]
C. sporogenes ATCC 19404	$5,35 \times 10^4$ ($\pm 3.65 \times 10^4$)	$1,80 \times 10^3$ ($\pm 1.50 \times 10^3$)	96,64[a]
C. sporogenes ATCC 19404 (spores)	$4,57 \times 10^4$ ($\pm 8.00 \times 10^3$)	$1,29 \times 10^4$ ($\pm 6.60 \times 10^3$)	71,73[b]
C. albicans ATCC 90028	$4,60 \times 10^3$ ($\pm 1.81 \times 10^3$)	n.d.	100,00[a]
P. chrysogenum ATCC 10106	$9,61 \times 10^4$ ($\pm 5.37 \times 10^4$)	$3,12 \times 10^3$ ($\pm 4.72 \times 10^3$)	96,75[a]
A. niger ATCC 9142	$1,50 \times 10^5$ ($\pm 3.90 \times 10^5$)	$8,39 \times 10^2$ ($\pm 6.45 \times 10^2$)	99,44[a]

* - SE, standard error.
n.d. - not detected.
a,b – values marked with different letters are statistically significantly different ($p \leq 0.05$).

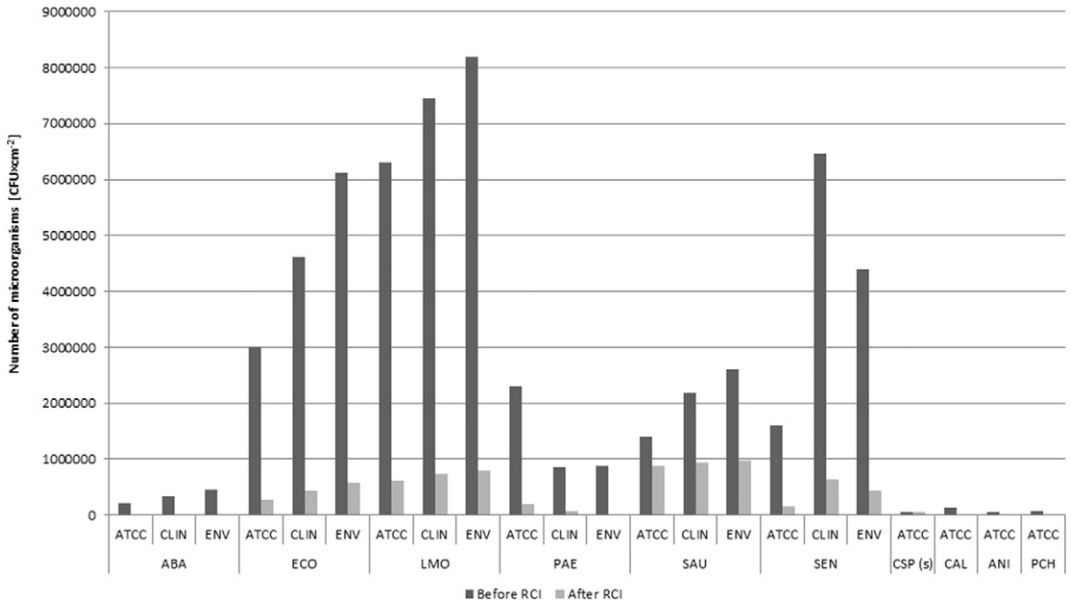

Fig. 3. Number of microorganisms recovered from steel AISI 304 before and after RCI (ABA – *Acinetobacter baumanii*, ECO – *Escherichia coli*, LMO – *Listeria monocytogenes*, PAE – *Pseudomonas aeruginosa*, SAU – *Staphylococcus aureus*, SEN – *Salmonella* Enteritidis, CSP(S) – *Clostridium sporogenes* spores, CAL – *Candida albicans*, ANI – *Aspergillus niger*, PCH – *Penicillium chrysogenum*, ATCC – refrence strain, CLIN – clinical isolate, ENV – environmental isolate).

3. Results

3.1. Assessment of air disinfection efficacy using radiant catalytic ionization

The obtained results made it possible to determine certain fluctuation in the number of CFU of bacteria and fungi present in the air after nebulization of suspensions (Table. 2). For this reason, it was decided to introduce the absolute reduction measure in the form of R[%].

Percentage reduction rates observed for positive control ranged between 1 and 5% and therefore were not showed in the table. In samples that were negative control, the presence of microorganisms was not detected.

The using of radiant catalytic ionization caused a noticeable decrease in the number of all the studied microorganisms (Table 2).

The full elimination of studied microorganisms from the air was obtained for *E. coli* and *C. albicans*. For *S. aureus*, *S. epidermidis* and *E. faecalis* a decrease in number amounted to 4–5 logarithmic units, and the percentage reduction rate was 99.9%. Slightly lower sanitizing effectiveness of radiant catalytic ionization was noted for vegetative forms of spore-forming bacteria (R[%] – from 96.6% to 98.9%) and molds (R[%] – from 96.8% to 99.4%). The lowest effectiveness of radiant catalytic ionization was shown in relations to spores of *C. sporogenes* present in the air. The percentage reduction rate in this case amounted to 71.7% (Table 2). The demonstrated difference in the percentage reduction

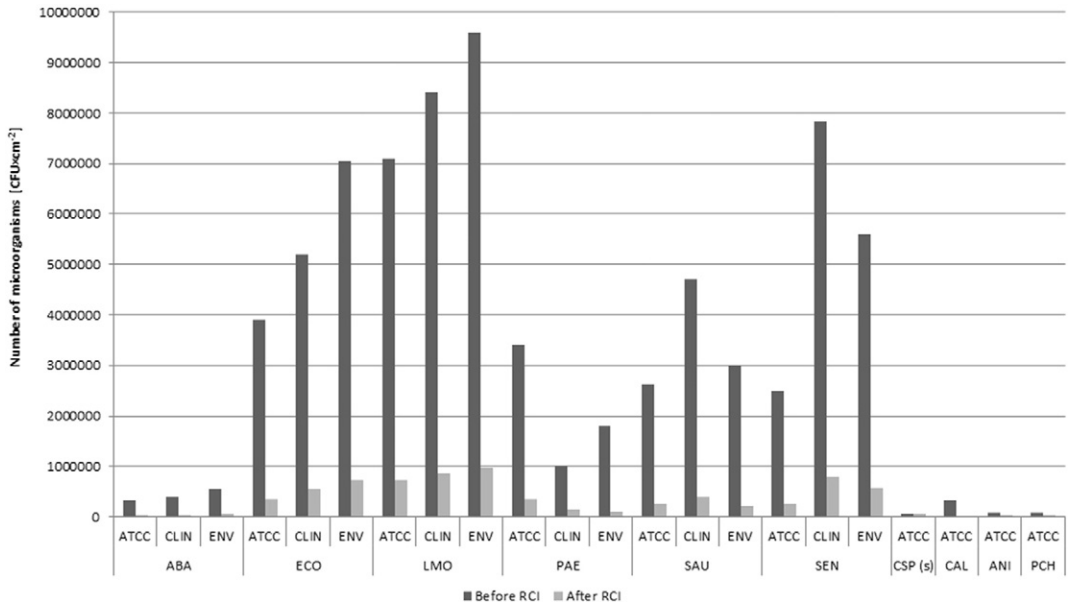

Fig. 4. Number of microorganisms recovered from polypropylene before and after RCI (ABA – *Acinetobacter baumanii*, ECO – *Escherichia coli*, LMO – *Listeria monocytogenes*, PAE – *Pseudomonas aeruginosa*, SAU – *Staphylococcus aureus*, SEN – *Salmonella* Enteritidis, CSP(S) – *Clostridium sporogenes* spores, CAL – *Candida albicans*, ANI – *Aspergillus niger*, PCH – *Penicillium chrysogenum*, ATCC – refrence strain, CLIN – clinical isolate, ENV – environmental isolate).

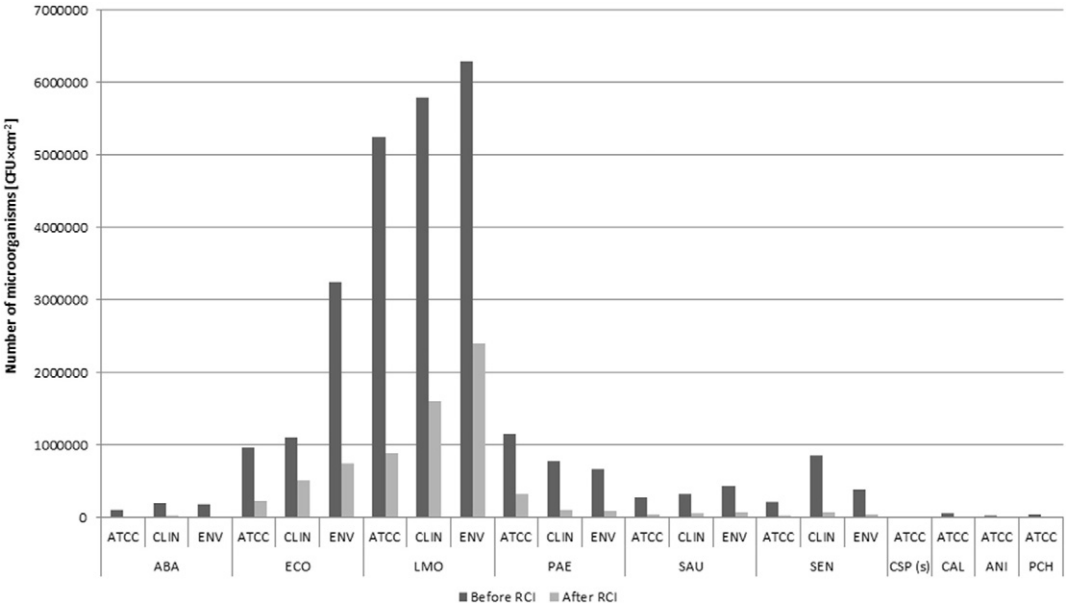

Fig. 5. Number of microorganisms recovered from glazed milled rock tiles before and after RCI (ABA – *Acinetobacter baumanii*, ECO – *Escherichia coli*, LMO – *Listeria monocytogenes*, PAE – *Pseudomonas aeruginosa*, SAU – *Staphylococcus aureus*, SEN – *Salmonella* Enteritidis, CSP(S) – *Clostridium sporogenes* spores, CAL – *Candida albicans*, ANI – *Aspergillus niger*, PCH – *Penicillium chrysogenum*, ATCC – refrence strain, CLIN – clinical isolate, ENV – environmental isolate).

rate of the number of spores able to germinate and the number of vegetative forms of the studied microorganisms was statistically significant (Table 2).

3.2. Assessment of effectiveness of surface disinfection using radiant catalytic ionization

In positive control, any reduction of microorganisms number was not observed. In samples that were negative control, the presence of microorganisms was not detected.

The study allowed to prove a reduction in the number of bacteria as affected by radiant catalytic ionization (Figs. 3–8). Irrespective of the type of studied surface, the most resistant to the action of the used disinfection technique appeared to be *C. sporogenes* spores, for which the percentage reduction rate ranged from –2.6% on glazed milled rock tiles to 71.2% on lacquered veneer (Figs. 3–8).

The application of radiant catalytic ionization appeared to be most effective towards microorganisms present on lacquered veneer (R[%] – 71.2–99.4%) and on steel AISI 304 (R[%] – 6.6–98.9%). The lowest sanitizing effect was obtained on the polyamide fitted carpet (R[%] – 4.3–97.4%) and glazed milled rock tiles (R[%] – –2.6–90.9%) (Table 3).

Differences in the number of re-isolated bacteria resulting from different origin of the strain were observed in the case of *L. monocytogenes* applied on the surface of glazed milled rock tiles and fitted carpet. The reference strain of this species showed a statistically significantly higher reduction rate as compared with the clinical and environmental strains (R[%]: 66.93% vs. 32.86% and 31.17%, respectively), when it was applied on the surface of fitted carpet and as compared with the environmental

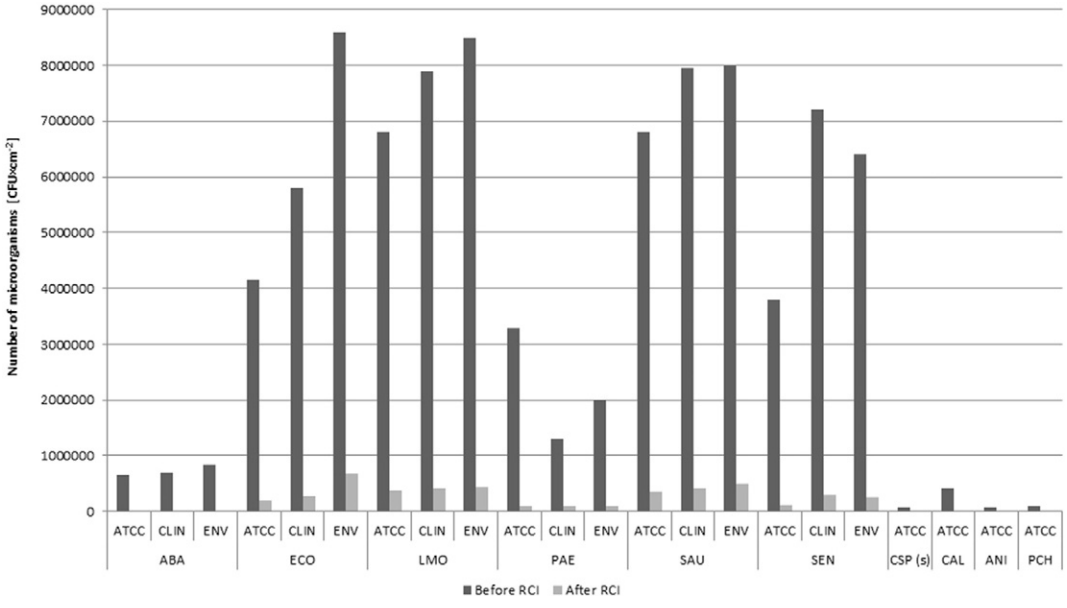

Fig. 6. Number of microorganisms recovered from lacquered veneer before and after RCI (ABA – *Acinetobacter baumanii*, ECO – *Escherichia coli*, LMO – *Listeria monocytogenes*, PAE – *Pseudomonas aeruginosa*, SAU – *Staphylococcus aureus*, SEN – *Salmonella* Enteritidis, CSP(S) – *Clostridium sporogenes* spores, CAL – *Candida albicans*, ANI – *Aspergillus niger*, PCH – *Penicillium chrysogenum*, ATCC – refrence strain, CLIN – clinical isolate, ENV – environmental isolate).

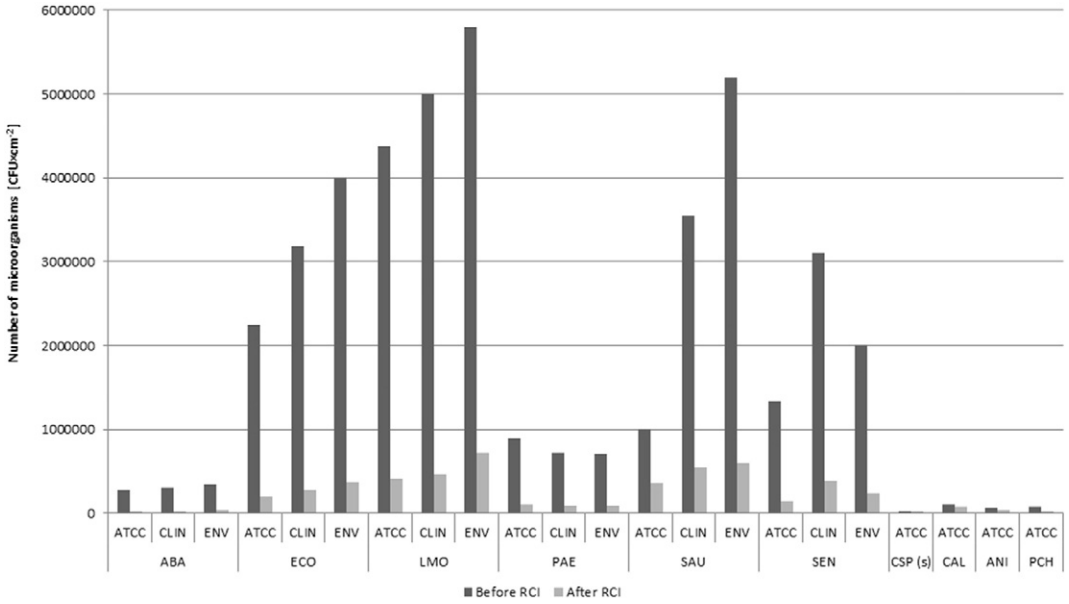

Fig. 7. Number of microorganisms recovered from rubber before and after RCI (ABA – *Acinetobacter baumanii*, ECO – *Escherichia coli*, LMO – *Listeria monocytogenes*, PAE – *Pseudomonas aeruginosa*, SAU – *Staphylococcus aureus*, SEN – *Salmonella* Enteritidis, CSP(S) – *Clostridium sporogenes* spores, CAL – *Candida albicans*, ANI – *Aspergillus niger*, PCH – *Penicillium chrysogenum*, ATCC – refrence strain, CLIN – clinical isolate, ENV – environmental isolate).

strain (R[%] - 83.05% vs. 61.90%), when it was applied on glazed milled rock tiles. The opposite relationship was found in the case of the reference strain of *S. aureus*, which showed a statistically significantly lower reduction rate on rubber, as compared with the clinical and environmental strains (R[%] - 64.00% vs. 84.79% and 88.46%) and on steel, as compared with the environmental strain (R[%] - 37.59% vs. 62.69%) (Table 3).

Of vegetative forms of bacteria, the highest reduction rates, in the case of most studied surfaces, were obtained for *A. baumannii*. The percentage reduction rate determined for these strains stayed within the range 89.22%–99.44% (Table 3). Of the vegetative forms of bacteria, strains of *P. aeruginosa* applied on the surface of fitted carpet appeared to be the most resistant to the action of radiant catalytic ionization (R[%] - 28.00%–66.35%) (Table 3).

The studied yeast-like fungi and molds applied to surfaces of steel AISI 304 and lacquered veneer were characterized by the highest reduction rates under the influence of radiant catalytic ionization from among all the studied surfaces (R[%] 86.91%–97.3%). In most cases, the demonstrated differences were statistically significant (Table 3). Fungi *C. albicans* and *A. niger* showed the highest resistance to the action of radiant catalytic ionization on the rubber surface (R[%] = 19.00% and R[%] = 36.67%), whereas *P. chrysogenum* on the fitted carpet surface (R[%] = 42.19%) (Table 3).

4. Discussion

Disinfection of air and surfaces is necessary to reduce the risk of infections with pathogenic microorganisms in hospitals, food processing

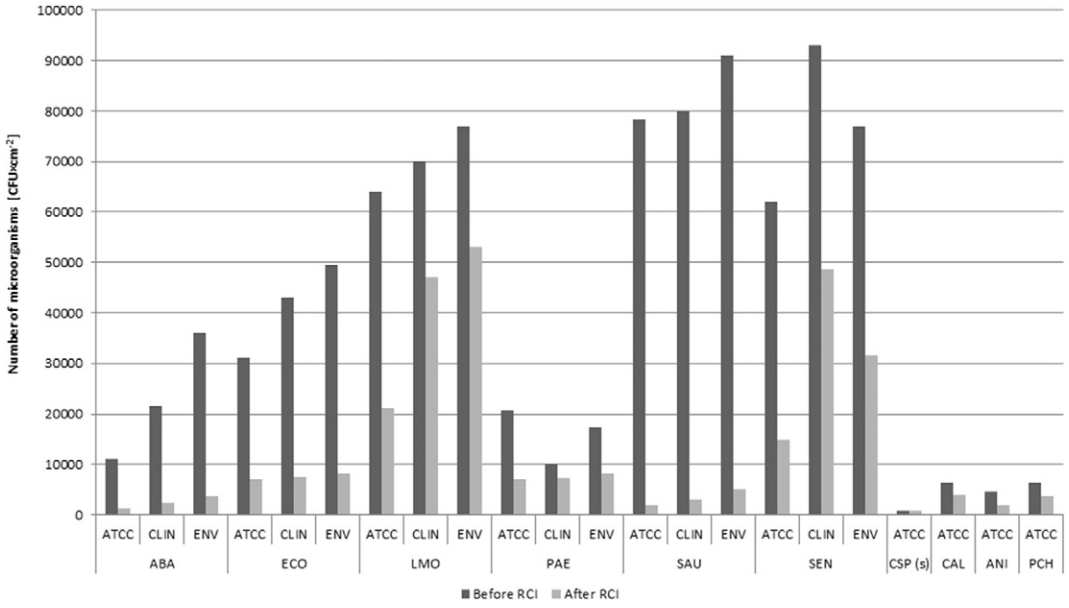

Fig. 8. Number of microorganisms recovered from fitted carpet before and after RCI (ABA – *Acinetobacter baumanii*, ECO – *Escherichia coli*, LMO – *Listeria monocytogenes*, PAE – *Pseudomonas aeruginosa*, SAU – *Staphylococcus aureus*, SEN – *Salmonella* Enteritidis, CSP(S) – *Clostridium sporogenes* spores, CAL – *Candida albicans*, ANI – *Aspergillus niger*, PCH – *Penicillium chrysogenum*, ATCC – refrence strain, CLIN – clinical isolate, ENV – environmental isolate).

plants, public places (schools, offices, etc.) and in houses. Ultraviolet radiation (UV) is a physical process conducted without chemicals. The source of UV-C radiation are low-pressure lamps emitting radiation with a wavelength of 253.7 nm, which inactivate bacteria, viruses, molds and spores causing DNA damage (Bintsis et al., 2000). Released atoms bind to other oxygen molecules producing ozone. It shows excellent oxidizing properties (Li et al., 2003). Artificially produced ozone is as effective as that naturally occurring, provided that its concentration is controlled. In the RCI technology ozone is not a bactericidal agent, since during the operation of the device its level does not exceed 0.05 ppm (Grinshpun et al., 2007; Ortega et al., 2007). Producer of used in this study Induct 750 declare, that this device produce ambient levels of ozone - below 0.04 ppm (ActivTek, n.d.-b, instruction of use Induct 750). This is an important advantage of the RCI technology, due to the toxicity of ozone and undesirable side effects of exposure to high ozone levels (Güzel-Seydima et al., 2004). However, EPA note that in close, non-ventilated room, level of ozone may be decrease above health concern (US EPA, 1996a, 1996b). Grinshpun et al. (2007) indicated that the generated ions (unipolar ion emission), and not photocatalytic reactions make it possible to remove contaminations such as cigarette smoke from the air. Inanimate particles are removed from the air by electrostatic precipitation, which is excited by the ionization process. Generated ions combine on the basis of electrostatic interactions with airborne contaminants, creating large and fast sediment conglomerates (Małecka and Borowski, 2011). In turn, reactive oxygen forms eliminate microbial contamination, such as bacteria, viruses or molds. Emitted chemical particles generate oxidative damage of the viral genetic material, and also impairs the functionality of the capsid protein. In bacterial cells, the coenzyme A molecules are oxidized, which results in the inhibition of cellular respiration, the oxidation of unsaturated phospholipids and the destruction of the outer membrane, and the accumulation of harmful DNA or RNA mutations (Grinshpun et al., 2007).

Sanitizing surfaces with UV-C light requires a higher dose of radiation emitting for a longer time as compared with methods utilizing the phenomenon of photo-oxidation (Ha et al., 2009; Saini et al., 2014).

The present study assessed the effectiveness of RCI towards microorganisms in the air and on different surfaces. It was proved to be the effective elimination system for many microbial species. The full elimination of microorganisms from the air was obtained for *E. coli* and *C. albicans*. The percentage reduction rate for *S. aureus*,

Table 3
Percentage reduction rate of the number of microorganisms R[%].

Strain	Percentage reduction rate [%]					
	Steel AISI 304	Polypropylene	Glazed milled rock tiles	Lacquered veneer	Rubber	Fitted carpet
ABA ATCC	98,79^{a}	90,63^{a}	89,22^{a}	99,44^{a}	90,39^{a}	89,36^{a}
	(15,12)*	(9,37)	(14.03)	(15,06)	(7,79)	(12,26)
ABA CLIN	98,82^{a}	90,75^{a}	89,35^{a}	99,30^{a}	90,53^{a}	89,40^{a}
	(15,86)	(12,27)	(10,17)	(13,17)	(10,18)	(11,48)
ABA ENV	98,86^{a}	90,34^{a}	90,86^{a}	99,39^{a}	89,91^{a}	89,44^{a}
	(13,12)	(7,39)	(11,71)	(15,22)	(12,29)	(4,16)
ECO ATCC	90,67^{a}	91,28^{a}	76,29^{a,c}	95,18^{a}	91,16^{a}	77,56^{c,e}
	(10,09)	(9,38)	(2,15)	(10,34)	(5,61)	(5,00)
ECO CLIN	90,33^{a}	89,23^{a}	53,00^{c}	95,38^{a}	91,22^{a}	82,33^{a,e}
	(12,21)	(6,45)	(1,06)	(11,61)	(8,20)	(8,03)
ECO ENV	90,52^{a}	89,65^{a}	77,16^{a,c}	92,21^{a}	90,75^{a}	83,60^{a,e}
	(11.03)	(7,18)	(5,19)	(13,07)	(11,00)	(9,14)
LMO ATCC	90,16^{a}	89,72^{a}	83,05^{a,e}	94,56^{a}	90,62^{a}	66,93^{c,e}
	(9,57)	(9,09)	(6,52)	(8,46)	(9,31)	(0,72)
LMO CLIN	90,20^{a}	89,76^{a}	72,41^{c,e}	94,75^{a}	90,80^{a}	32,86^{b}
	(12,03)	(11,56)	(2,31)	(8,26)	(7,17)	(3,11)
LMO ENV	90,37^{a}	89,84^{a}	61,90^{c}	94,94^{a}	87,67^{a}	31,17^{b}
	(9,22)	(11,63)	(1,62)	(9,28)	(13,30)	(9,67)
PAE ATCC	91,30^{a}	89,71^{a}	72,26^{c}	96,95^{a}	88,89^{a}	66,35^{c,e}
	(10,31)	(8,29)	(3,37)	(13,30)	(1,01)	(11,19)
PAE CLIN	90,75^{a}	84,00^{a}	87,01^{a}	93,08^{a}	87,64^{a}	28,00^{b}
	(10,14)	(3,15)	(3,38)	(10,14)	(7,38)	(8,07)
PAE ENV	90,23^{a}	94,44^{a}	86,02^{a}	95,00^{a}	87,07^{a}	52,02^{b,c}
	(11.19)	(9,30)	(6,21)	(10,44)	(4,17)	(7,77)
SAU ATCC	37,59^{b}	89,69^{a}	82,75^{a,e}	94,65^{a}	64,00^{c}	97,44^{a}
	(10,29)	(12,13)	(10,11)	(11,19)	(12,52)	(12,38)
SAU CLIN	56,88^{b,c}	91,70^{a}	80,94^{a,e}	94,76^{a}	84,79^{a}	96,08^{a}
	(11,07)	(10,55)	(8,18)	(12,33)	(8,22)	(8,15)
SAU ENV	62,69^{c,e}	92,67^{a}	81,61^{a,e}	93,75^{a}	88,46^{a}	94,40^{a}
	(9,47)	(8,12)	(3,46)	(10,37)	(10,52)	(6,06)
SEN ATCC	90,38^{a}	89,52^{a}	86,84^{a}	97,16^{a}	88,95^{a}	75,97^{c,e}
	(10,17)	(14,03)	(8,26)	(10,52)	(8,13)	(2,62)
SEN CLIN	90,28^{a}	89,90^{a}	90,75^{a}	95,83^{a}	87,74^{a}	47,74^{b,c}
	(10,36)	(9,21)	(6,63)	(15,02)	(13,08)	(4,84)
SEN ENV	90,23^{a}	89,59^{a}	88,30^{a}	95,95^{a}	88,00^{a}	58,83^{c}
	(10,01)	(8,28)	(8,07)	(13,20)	(10,75)	(3,06)
CSP (s)	6,61^{d}	3,23^{d}	-2,56^{d}	71,23^{c}	1,14^{d}	4,30^{d}
	(1,02)	(0,51)	(0,40)	(11,30)	(0,30)	(0,53)
CAL ATCC	92,93^{a}	97,02^{a}	88,00^{a}	96,84^{a}	19,00^{b,d}	37,50^{b}
	(13,25)	(8,03)	(7,12)	(11,59)	(13,21)	(3,17)
ANI ATCC	86,91^{a}	60,00^{c}	68,85^{c,e}	95,91^{a}	36,67^{b}	56,52^{b,c}
	(14,12)	(2,16)	(8,50)	(12,13)	(2,15)	(0,97)
PCH ATCC	90,13^{a}	53,85^{c}	75,06^{c,e}	97,30^{a}	66,71^{c}	42,19^{b,c}
	(15,52)	(1,09)	(5,88)	(11,08)	(1111)	(1,27)

ABA – *Acinetobacter baumanii*, ECO – *Escherichia coli*, LMO – *Listeria monocytogenes*, PAE – *Pseudomonas aeruginosa*, SAU – *Staphylococcus aureus*, SEN – *Salmonella* Enteritidis, CSP(S) – *Clostridium sporogenes* spores, CAL – *Candida albicans*, ANI – *Aspergillus niger*, PCH – *Penicillium chrysogenum*, ATCC – refrence strain, CLIN – clinical isolate, ENV – environmental isolate.
a,b,c, … - values marked with different letters are significantly different ($p \leq 0.05$).
* - standard deviation.

S. epidermidis and *E. faecalis* was 99.9%. Lower effectiveness of RCI was recorded in the case of vegetative forms of spore-forming bacteria (R[%] – from 96.6% to 98.9%) and molds (R[%] – from 96.8% to 99.4%). The lowest effectiveness of RCI was proved in relations to *C. sporogenes* spores present in the air (R[%] – 71.7%). Also Grinshpun et al. (2007) indicated a reduction of effectiveness of the RCI technology towards spores of *B. subtilis* in the 2.75 m^3 chamber. 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. In present study a hermetically sealed chamber had a capacity of 1.4 m^3. The observed high rates of reduction in the number of germination able spores may be due to the fact that both experiments were carried out in relatively small cubic chambers. These results are promising for real indoor environments, where air pollution is much lower. Coverage study air purification amounts to 70 m^3 (ActivTek, n.d.-b, instruction of use Induct 750). Experiments carried in University of Agriculture in Krakow indicated that RCI was effective in rooms of cubature 20 and 45 m^3. The percentage reduction rate for total number of bacteria, *Staphylococcus* spp. and fungi ranged from 73,1% to 82,0% The results of the research carried out in University of Agriculture in Krakow about efficacy of RCI technology (Barabasz et al., 2014). This study indicated that RCI technology can be used in real rooms with different cubature. This technology was tested in museums, hospitals and hotels (ActivTek, n.d.-a, documents about use of product, http://activtek.pl/dokumenty/, accessed 10.06.2017).

In the present study it was found that the effectiveness of RCI on the surfaces was the lowest in the case of *C. sporogenes* spores, for which the percentage reduction rate ranged from –2.6% (glazed milled rock tiles) to 71.2% (lacquered veneer). One reason that spores were more resistant to RCI may be their thick membrane layer containing peptidoglycans (Berberidou et al., 2012). To the best of our knowledge the mechanism of act the RCI on spores is not fully understood. It must also be stressed that time of exposure is very important for effective inactivation of spores. In present study action time of RCI was only 20 min, which may be insufficient to kill bacteria spores. Scanning electron microscopy of *B. stearothermophilus* endospores indicated that photocatalytic oxidation affected negatively on the shape and structure of the spore. The sporicidal effect of oxidation is increasing over time (Berberidou et al., 2012).

The use of radiant catalytic ionization appeared the most effective in the case of microorganisms present on lacquered veneer (R[%] – 71.2–99.4%) and on steel AISI 304 (R[%] – 6.6–98.9%). The lowest effectiveness of RCI was shown on the polyamide fitted carpet (R[%] – 4.3–97.4%) and glazed milled rock tiles (R[%] – –2.6–90.9%).

On the surface of steel AISI 304, the most resistant to RCI were strains of *S. aureus* (R[%] - 37.6%–62.7%. The most susceptible were strains of *A. baumannii*, for which R[%] was almost 99%. Ortega et al., 2007 indicated >90% efficacy of RCI towards *S. aureus*, *Bacillus* spp., *E. coli*, *L. monocytogenes*, *C. albicans* on the surface of steel. In the present study, on different surfaces, such as rubber and fitted carpet, a considerably lower effectiveness of RCI towards *C. albicans* was shown (R[%] - 19%–37.5%). This may be related to the structure of the studied surfaces and their different properties. Moreover, contaminated surfaces were exposed to RCI products for only 20 min. In further experiments, the effect of exposure time on change in the number of microorganisms on the studied surfaces should be determined. Other authors indicated a decrease in the number of microorganisms along with growing time of exposure (Ortega et al., 2007). The efficacy of photooxidation towards *L. monocytogenes* on the surface of steel was also proved by Saini et al. (2014) (4.37 log CFU/coupon on stainless steel after 15-min treatment).

The application of UV-C light to reduce superficial microbial contamination of food is commonly known. The effectiveness of this method has been demonstrated to reduce contamination of fruits and vegetables and meat (Adhikari et al., 2015; Chun et al., 2010; Martínez-Hernández et al., 2015). Saini et al. (2014), also proved the effectiveness of photo-oxidation in food disinfection (ready-to-eat cheese and turkey). Microorganism reduction amounted to >2 log CFU/sample after 5 min of exposure. This is an important advantage of devices utilizing the phenomenon of photooxidation, UV light and ozone.

In the available literature there is no information concerning the disinfection of different surfaces with the RCI technique. In the present study such a comparison was made, since the RCI technology may be used in various environments, both in industry, where elements of steel and rubber predominate, and in everyday life, in houses, offices, etc., where there are more fitted carpets or surfaces made of lacquered veneer.

The present study proves that the RCI technology is an effective method for air and surface disinfection, although its effectiveness is varied depending on the microbial species. The possibility of widespread use of devices utilizing RCI, even when there are some people in the room, is their undoubted advantage, which may contribute to improving working conditions both in places where the air is contaminated and also where there is a risk of surface contamination.

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References

ActivTek. documents about use of product. http://activtek.pl/dokumenty/ (accessed 10.06.17).
ActivTek. instruction of use Induct 750 product. http://activtek.pl/wp-content/uploads/2014/12/INDUCT-750-Spec-Sheet.pdf (accessed 10.06.17).
Adhikari, A., Syamaladevi, R.M., Killinger, K., Sablani, S.S., 2015. Ultraviolet-C light inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on organic fruit surfaces. Int. J. Food Microbiol. 210:136–142. http://dx.doi.org/10.1016/j.ijfoodmicro.2015.06.018.
Bagge-Ravn, D., Ng, Y., Hjelm, M., Christiansen, J.N., Johansen, C., Gram, L., 2003. The microbial ecology of processing equipment in different fish industries-analysis of the microflora during processing and following cleaning and disinfection. Int. J. Food Microbiol. 87 (3), 239–250.
Barabasz, W., et al., 2014. The Results of the Research Carried Out in University of Agriculture in Krakow about Efficacy of RCI Technology. http://activtek.pl/wp-content/uploads/2014/07/Uniwersytet-Rolniczy.pdf (accessed 10.06.17).
Berberidou, C., Paspaltsis, I., Pavlidou, E., Sklaviadis, T., Poulios, I., 2012. Heterogenous photocatalytic inactivation of *B. stearothermophilus* endospores in aqueous suspensions under artificial and solar irradiation. Appl. Catal. Environ. 125:375–382. http://dx.doi.org/10.1016/j.apcatb.2012.06.005.
Bintsis, T., Litopoulou-Tzanetaki, E., Robinson, R.K., 2000. Existing and potential applications of ultraviolet light in the foodindustry—a critical review. J. Sci. Food Agric. 80 (6):637–645. http://dx.doi.org/10.1002/(SICI)1097-0010(20000501)80:6<637::AID-JSFA603>3.0.CO;2-1.
Brewczyńska, A., Depczyńska, D., Borecka, A., Winnicka, I., Kubiak, L., Skopińska-Różewska, E., Niemcewicz, M., Kocik, J., 2015. The influence of the workplace-related biological agents on the immune systems of emergency medical personnel. Cent. Eur. J. Immunol. 40 (2):243–248. http://dx.doi.org/10.5114/ceji.2015.52838.
Cho, M., Chung, H., Choi, W., Yoon, J., 2005. Different inactivation behaviors of MS-2 phage and *Escherichia coli* inTiO_2 photocatalytic disinfection. Appl. Environ. Microbiol. 71 (1):270–275. http://dx.doi.org/10.1128/AEM.71.1.270-275.2005.
Chun, H.H., Kim, J.Y., Lee, B.D., Yu, D.J., Song, K.B., 2010. Effect of UV-C irradiation on the inactivation of inoculated pathogens and quality of chicken breasts during storage. Food Control 21:276–280. http://dx.doi.org/10.1016/j.foodcont.2009.06.006.
Douwes, J., Thorne, P., Pearce, N., Heederik, D., 2003. Bioaerosol health effects and exposure assessment:progress and prospects. Ann. Occup. Hyg. 47 (3), 187–200.
Dutkiewicz, J., Cisak, E., Sroka, J., Wójcik-Fatl, A.A., Zając, V., 2011. Biological agents as occupational hazards– selected issues. Ann. Agric. Environ. Med. 18, 286–293.
Dyas, A., Boughton, B.J., Das, B.C., 1983. Ozone killing action against bacterial and fungal species; microbiological testing of a domestic ozone generator. J. Clin. Pathol. 36, 1102–1104.
Foarde, K., van Osdell, D., Steiber, R., 1997. Investigation of gas-phase ozone as a potential biocide. J. Occup. Environ. Hyg. 12 (8), 535–542.
Gąska-Jędruch, U., Dudzińska, M.R., 2009. Zanieczyszczenia mikrobiologiczne w powietrzu wewnętrznym, w Polska Inżynieria Środowiska pięć lat po wstąpieniu do Unii Europejskiej. In: Ozonka, Janusza, Pawłowskiego, Artura (Eds.), Monografie Komitetu Inżynierii Środowiska, tom 2 59, pp. 31–40 (Article in Polish).
Górny, R.L., 2010. Biological aerosols – a role of hygienic standards in the protection of environment and health. Environmental Medicine 13, 41–51.
Grinshpun, S.A., Adhikari, A., Honda, T., Kim, K.Y., Toivola, M., Rao, K.S., Reponen, T., 2007. Control of aerosol contaminants in indoor air: combining the particle concentration reduction with microbial inactivation. Environ. Sci. Technol. 41 (2):606–612. http://dx.doi.org/10.1021/es061373o.
Güzel-Seydima, Z., Bever, P.I., Greeneb, A.K., 2004. Efficacy of ozone to reduce bacterial populations in the presence of food components. Food Microbiol. 21 (4), 475–479.

Ha, J., Lee, Y., Na, B., Bae, D., Ha, S., 2009. Effects of ultraviolet radiation to reduce the numbers of foodborne pathogenic microorganisms on stainless steel chips. J. Korean Soc. Appl. Biol. Chem. 52 (3):301–304. http://dx.doi.org/10.3839/jksabc.2009.053.

Hayleeyesus, S.F., Manaye, A.M., 2014. Microbiological quality of indoor air in university libraries. Asian Pac. J. Trop. Biomed. 4 (Suppl. 1):S312–S317. http://dx.doi.org/10.12980/APJTB.4.2014C807.

Hospodsky, D., Qian, J., Nazaroff, W.W., Yamamoto, N., Bibby, K., Rismani-Yazdi, H., Peccia, J., 2012. Human occupancy as a source ofindoor airborne bacteria. PLoS One 7 (4). http://dx.doi.org/10.1371/journal.pone.0034867.

Ismaïl, R., Aviat, F., Michel, V., Le Bayon, I., Gay-Perret, P., Kutnik, M., Fédérighi, M., 2013. Methods for recovering microorganisms from solid surfaces used in the food industry: a review of the literature. Int. J. Environ. Res. Public Health 10 (11):6169–6183. http://dx.doi.org/10.3390/ijerph10116169.

Kim, J.-G., Yousef, A.E., Chism, G., 1999. W. Use of ozone to inactivate microorganisms on lettuce. J. Food Saf. 19 (1):17–34. http://dx.doi.org/10.1111/j.1745-4565.1999.tb00231.x.

Kujundzic, E., Matalkah, F., Howard, C.J., Hernandez, M., Miller, S.L., 2006. UV air cleaners and upper-room air ultraviolet germicidal irradiation for controlling airborne bacteria and fungal spores. J. Occup. Environ. Hyg. 3 (10):536–546. http://dx.doi.org/10.1080/15459620600909799.

Li, C.S., Wang, Y., C., 2003. Surface germicidal effects of ozone for microorganisms. AIHA J. 64 (4):533–537. http://dx.doi.org/10.1202/559.1.

Małecka, I., Borowski, G., 2011. Disinfecting air with UV and radial catalytic ionization in ventilation systems. Zeszyty Naukowe – Inżynieria Lądowa i Wodna w Kształtowaniu Środowiska. 3, pp. 25–30 (Article in Polish).

Martínez-Hernández, G.B., Huertas, J.P., Navarro-Rico, J., Gómez, P.A., Artés, F., Palop, A., Artés-Hernández, F., 2015. Inactivation kinetics of foodborne pathogens by UV-C radiation and its subsequent growth in fresh-cut kailan-hybrid broccoli. Food Microbiol. 46:263–271. http://dx.doi.org/10.1016/j.fm.2014.08.008.

Ortega, M.T., Franken, L.J., Hatesohl, P.R., Marsden, J.L., 2007. Efficacy of ecoquest radiant catalytic ionization cell and breeze at ozone generator at reducing microbial populations on stainless steel surfaces. J. Rapid Methods Autom. Microbiol. 15 (4):359–368. http://dx.doi.org/10.1111/j.1745-4581.2007.00107.x.

Pastuszka, J.S., Paw, U.K.T., Lis, D.O., Wlazło, A., Ulfig, K., 2000. Bacterial and fungal in indoor environment in Upper Silesia, Poland. Atmos. Environ. 34, 3833–3842.

Rim, K.T., Lim, C.H., 2014. Biologically hazardous agents at work and efforts to protect workers' health: a review of recent reports. Saf Health Work. 5 (2):43–52. http://dx.doi.org/10.1016/j.shaw.2014.03.006.

Saini, J.K., Marsden, J.L., Getty, K.J., Fung, D.Y., 2014. Advanced oxidation technology with photohydroionization as a surface treatment for controlling *Listeria monocytogenes* on stainless steel surfaces and ready-to-eat cheese and turkey. Foodborne Pathog. Dis. 11 (4):295–300. http://dx.doi.org/10.1089/fpd.2013.1512.

Schlegelová, J., Babák, V., Holasová, M., Konstantinová, L., Necidová, L., Šišák, F., Vlková, H., Roubal, P., Jaglic, Z., 2010. Microbial contamination after sanitation of food contact surfaces in dairy and meat processing plants. Czech J. Food Sci. 28 (5), 450–461.

U.S. Environmental Protection Agency (US EPA), 1996a. Air Quality Criteria for Ozone and Related Photochemical Oxidants. report nos. EPA/600/P-93/004aF-cF, 3v. NTIS, Springfield, VA; PB-185582, PB96-185590 and PB96-185608National Center for Environmental Assessment-RTP Office, Research Triangle Park, NC.

U.S. Environmental Protection Agency (US EPA), 1996b. Review of National Ambient Air Quality Standards for Ozone: Assessment of Scientific and Technical Information. OAQPS Staff PaperOffice of Air Quality Planning and Standards, Research Triangle Park. NC EPA-452/R-96-007.

U.S. Environmental Protection Agency (US EPA), 2000. Final Report: Photocatalytic AIR Cleaner for Indoor Air Pollution Control. https://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.highlight/abstract/1357/report/F (accessed 10.06.17).