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Rapid Inactivation of Biological Species in the Air using Atmospheric Pressure Nonthermal Plasma

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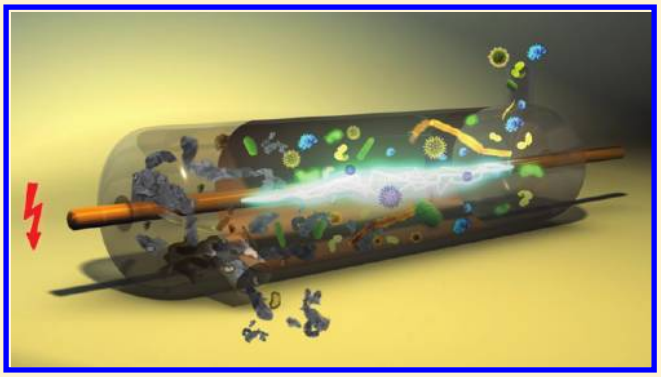
Supporting Information

ABSTRACT: Here, nonthermal plasma generated by a dielectric barrier discharge (DBD) system was applied to inactivating aerosolized *Bacillus subtilis* cells and *Pseudomonas fluorescens* as well as indoor and outdoor bioaerosols. The culturability, viability, and diversity losses of the microorganisms in air samples treated by the plasma for 0.06−0.12 s were studied using culturing, DNA stain as well as polymerase chain reaction-denaturing gradient gel electrophoresis (PCR−DGGE) methods. In addition, the viable fraction of bacterial aerosols with and without the plasma treatment was also quantified using qPCR coupled with ethidium monoazide (EMA). It was shown that less than 2% of *B. subtilis* aerosols survived the plasma treatment of 0.12 s, while none of the *P. fluorescens* aerosols survived. Viability tests, EMA-qPCR results, and Scanning Electron Microscopy (SEM) images demonstrated that both bacterial species suffered significant viability loss, membrane, and DNA damages. Exposure of environmental bacterial and fungal aerosols to the plasma for 0.06 s also resulted in their significant inactivations, more than 95% for bacteria and 85−98% for fungal species. PCR-DGGE analysis showed that plasma exposure of 0.06 s resulted in culturable bacterial aerosol diversity loss for both environments, especially pronounced for indoor environment. The results here demonstrate that nonthermal plasma exposure could offer a highly efficient air decontamination technology.



INTRODUCTION

Airborne biological particles are ubiquitous in the environments, including a variety of microorganisms (bacteria, fungi, and viruses), allergens, plant debris, endotoxin, glucans, and skin scales. Exposure to those pathogenic microbes or derivatives was shown to cause numerous adverse health effects.[1] In addition, the contamination of the environments as a result of either intentionally or accidentally released biowarfare agents can induce great harm and fear among the public as manifested by the anthrax events in 2001 in the United States. Biological aerosol exposure has become one of the major concerns for the residential, healthcare, and government sectors. The outbreaks of SARS in 2003 and influenza H1N1 viral infections in 2009 across the globe prompted worldwide attention for effective biological monitoring and control measures.

In general, particulate filters inside heating, ventilating, and air-conditioning (HVAC) systems are widely utilized as a method to control airborne microorganisms. However, the collected biological agents are not inactivated but are possibly accumulated on the filter surface and even proliferate during long periods of high relative humidity (>80%).[2] To inactivate bioaerosols, different types of technologies have been investigated over the past years. They include carbon nanotube filter,[3,4] ion emissions,[5−7] ultraviolet irradiation,[8] electrostatic field,[9] and a combination of unipolar ion emission and photocatalytic oxidation.[10] Recently, thermal treatment[11,12] and microwave irradiation[13−15] were also studied in inactivating bioaerosols.

In addition to these microbial decontamination technologies, nonthermal (cold) plasma (known as the fourth state of matter, a collection of free charged particles moving at random direction) has also been extensively investigated but primarily focused on liquids, foods, or surfaces.[16−21] Recently, cold plasma was increasingly being investigated for air sterilization.[22−24] It was shown that a 1.5 and 5.5 log reductions of the airborne *E. coli* were achieved, respectively, after single plasma exposure of 10-s and 2 min.[22] In another study, 89% bioaerosol removal efficiency was obtained using the plasma with an energy dose of 38 J/L.[24] It was shown that the plasma inactivation of microbes was attributed to both cell wall rupture and DNA damages.[25] Both

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the leakage of the cytoplasm contents and even a complete rupture of the membrane were observed for *B. subtilis* spores residing at filter surface after the plasma treatment of 5 min.[26] In most of these studies, single bacterial species were tested. However, environmental bioaerosols are shown to consist of a diverse set of species, including at least 1800 bacterial types,[27] and some of them even belong to bacterial families with pathogenic members including environmental relatives of select bioterrorism agents.[27] Apart from a limited number of studies on airborne exposure, the effects of nonthermal plasma exposure on viability, diversity, and DNA damages of environmental bioaerosols are still lacking. Equally important, the effects of plasma exposure on fungal aerosols, believed to have caused many adverse health effects, are not available in the literature.

Here, in addition to aerosolized hardy and sensitive bacterial species, environmental bacterial and fungal aerosols both indoors and outdoors as well as chemical pollutants were subjected to nonthermal plasma treatment. The culturability, viability, DNA damages, and diversity of atmospheric nonthermal plasma treated bacterial and/or fungal aerosols were studied using culturing, EMA-qPCR, DNA stain as well as PCR-DGGE methods. The results obtained here could lead to further development of cold plasma based decontamination technology for environmental pollutants.

MATERIALS AND METHODS

Nonthermal Plasma Generation and Experimental Setup. In this study, a dielectric barrier discharge (DBD) system shown in Figure 1 was used to generate plasma at a voltage of 14 kV with a frequency of 10 kHz supplied by a High Voltage High Frequency Power Supply (CTP-2000K, Suman, Nanjing, China) under an ambient temperature of 20 °C. Figure 1 (A) shows the device, and Figure 1 (B) shows the dimensions and components of the DBD system as well as the procedure in which air samples were exposed to the plasma. The contents of the plasma were also analyzed by an optical emission spectroscopy (OES) using a Multi Channel Fiber Optic Spectrometer (AvaSpec-2048-8-USB2, Avantes, Eerbeek, The Netherlands). Here, an energy output of 24 W was used, and the inside temperature was measured around 60 °C when the device was being operated.

EXPERIMENTAL PROCEDURE

Bioaerosol Inactivation by Nonthermal Plasma. To investigate bioaerosol inactivation by nonthermal plasma, *Pseudomonas fluorescens* (a sensitive bacterial species) and *Bacillus subtilis* var. niger cells (a hardy bacterial species) were used. They were first grown on Petri dishes with trypticase soy agar (Becton, Dickson and Company, Sparks, MD) under 26 and 30 °C, respectively, for 18 h. Before the experiments, freshly purified water (Milli-Q, Millipore, Billerica and MA) was added to the agar plate, and colonies of *B. subtilis* var. niger were removed from the agar surfaces using a pipet tip. The obtained bacterial suspension was poured into a tube and centrifuged at 7000 rpm (Eppendorf Centrifuge 5804 R, Eppendorf, Hamburg, Germany) for 7 min. The subsequent pellet was resuspended in freshly purified water and centrifuged again. The final pellet of bacteria from the second centrifugation was suspended in the freshly purified water for subsequent aerosolization and exposure experiments.

In this study, the aerosolized bacterial species were exposed to the nonthermal plasma. A Collison nebulizer (BGI, Inc.) operated at a flow rate of 4.2 L/min was used to aerosolize *B. subtilis* cells and *P. fluorescens*. The resulting bioaerosols were further dried and diluted by an additional pure N_2 airflow about 10 L/min. The bioaerosol flow was further drawn into the exposure chamber and exposed to the nonthermal plasma produced using the system setup shown in Figure 1. The control and exposed air samples were collected using a BioSampler (SKC Inc., Eighty four, PA) continuously for 15 min at a sampling flow rate of 12.5 L/min. The air samples with and without the plasma treatment were diluted 10 times and cultured for

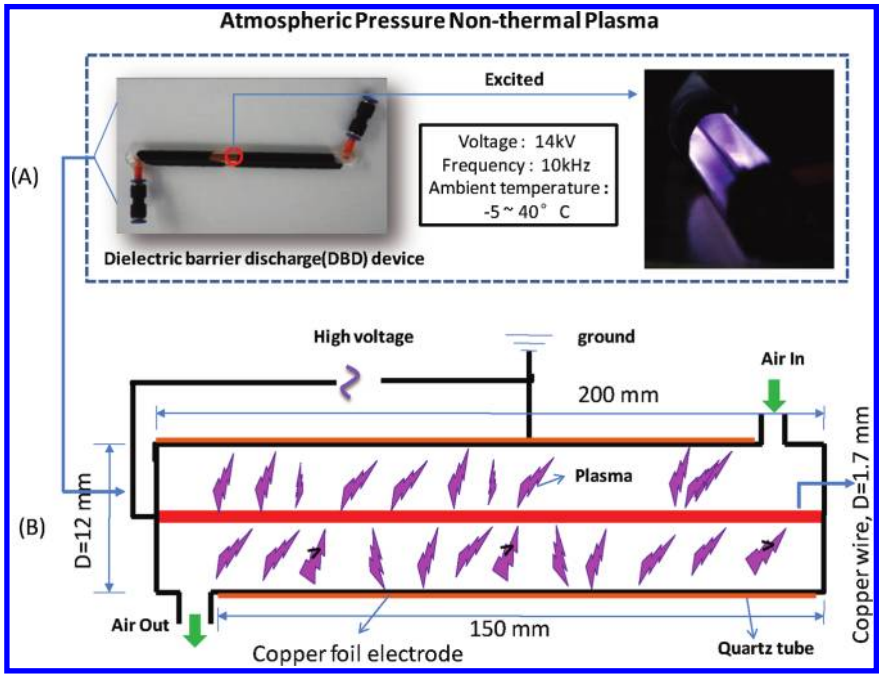

Figure 1. A) Experimental setup for airborne pollutants (biological and chemical species) treatments using atmospheric pressure nonthermal plasma. Plasma was produced at a voltage of 14 kV with a frequency of 10 kHz under ambient temperature of 5−40 °C; B) Dimensions of the DBD device and illustration of the experimental procedure.

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18 h on Trypticase Soy Agar (TSA) (Becton, Dickson and Company, Sparks, MD) plates at 26 °C for *P. fluorescens* and 37 °C for *B. subtilis*. According to the dimension of the DBD device and the air sampling flow rate, the plasma exposure time was about 0.12 s . At least six independent repeats were performed for each of the bacterial species tested. The relative humidity (RH) level was measured around 35% for all environments. Due to DBD system stability issue, higher RH levels were not investigated here. To investigate the inactivation mechanisms, liquid samples of *B. subtilis* and *P. fluorescens* were treated by another specifically designed DBD device, which consists an outer copper foil, serving as a single electrode, and a Teflon tube with outer and inner diameters of 10 and 7 mm, respectively, and their microbial images before and after the treatment were taken using a Scanning Electron Microscope (Hitachi, S-3000N). During the experiment, the plasma torch was placed 5 mm above the bacterial suspensions and the temperature ranged from 25 to 31 °C. Besides, chemical species such as HC and NO were also treated by the plasma to investigate its oxidizing capacity.

In addition, bacterial and fungal aerosols (composed of both sensitive and hardy cells) from both indoor (lab office) and outdoor (outside of a two-story building) were also exposed to the nonthermal plasma. The air samples from both environments with and without the plasma exposure were alternately impacted directly on agar plates using a BioStage impactor (SKC Inc., Eighty four, PA) connected to a SKC pump for 15−25 min at a flow rate of 28.3 L/min. This in turn resulted in a plasma exposure time of 0.06 s. Use of the BioStage impactor would not only test shorter exposure time but also eliminate the postsample processing steps such as centrifugation and filtration as a result of diluted environmental bioaerosol concentrations. Currently, the BioSampler and BioStage impactor are two widely used bioaerosol samplers, but both of them have a standard air sampling flow rate (12.5 L/min and 28.3 L/min, respectively). Accordingly, use of these samplers here resulted in different exposure times. However, use of filtration method could extend the exposure time to various values.

The bacterial aerosols collected were grown on Trypticase Soy Agar (TSA) (Becton, Dickson and Company, Sparks, MD) plates for 2−3 days, and fungal aerosols were grown on Malt Extract Agar (Becton, Dickson and Company, Sparks, MD) for 3−5 days. The sampling head for the BioStage sampler has 400 holes, and accordingly bioaerosol particles (two or more) could be collected onto the same spot on the agar plate but eventually growing into one CFU. Therefore, a statistical probability is applied using the formula developed by Feller (1968) to adjust such a coincidence.[28] The experiments were independently conducted three times in both indoor and outdoor environments on three different dates. For each time, three independent air samples were taken for control and exposure experiments.

Both for the aerosolized bacterial species and environmental bioaerosols, colony forming units (CFUs) were manually counted and bioaerosol concentrations were then calculated as CFU/m^3, and the survival rate was calculated using the equation below

$$S = CFU_{exposed}/CFU_{control} \times 100\% \quad ([1])$$

where S is the survival rate, $CFU_{exposed}$ is the culturable bioaerosol concentration after the plasma treatment, and $CFU_{control}$ is the culturable bioaerosol concentration without the plasma treatment.

EMA-qPCR and DNA Stain of Aerosolized Bacterial Species. For aerosolized bacterial species exposure, ethidium monoazide (EMA) coupled with real-time quantitative PCR (qPCR) was also applied to quantifying the viable fraction of control and exposed bioaerosol samples. EMA is a double-stranded DNA intercalating dye which can penetrate nonviable cells, or cells with compromised membranes, form covalent bonds with DNA, and cleave the DNA into pieces upon photoactivation,[29−31] thus preventing the DNA of nonviable cells from being amplified in subsequent polymerase chain reactions. While for those viable cells, or cells with intact membranes, the EMA is kept outside and inactivated after several minutes of halogen light exposure.[29] Therefore, the EMA-qPCR method, or qPCR analyses of bioaerosol samples with or without EMA pretreatment, yields both the viable and total quantities of bacterial cells.

In this study, for both control and exposed samples, 1 mL of the air sample collected by the BioSampler was transferred to 1.5 mL sterile centrifuge tube) and then subjected to centrifugation of 8200 rpm (8000*g*, 2 min, 4 °C), bringing the concentrated samples to a final volume of 150 μL after supernatant removal. The 150 μL concentrated samples are gently vortexed to obtain well-mixed cell suspensions. Then EMA was added to the samples at a final concentration of 100 μg/mL. Afterward, these 1.5 mL tubes containing concentrated bacterial samples with and without EMA treatment were kept in the dark for 5 min, then placed in an ice bath, and exposed to a 500 W halogen light 20 cm above the ice for 2 min according to a previous study.[32] Then, the suspensions in the 1.5 mL tubes were centrifuged under 13000 rpm twice (20000*g*, 5 min × 2, 4 °C) according to a previous study,[32] to a final volume of 100 μL for direct DNA extraction (TIANamp Bacterial Genomic DNA Extraction Kit, Cat#RP302-02). In this study, bacterial aerosol samples collected from three independent repeats under the same conditions were pooled together after the DNA extraction, and 3 μL of the original DNA from the mixture was used as the template in the qPCR analysis. The 50 μL qPCR reaction mixture includes 1 μL ultrapure dNTPs, 1 μL probe, 1 μL forward primer, 1 μL reverse primer, 5 μL 10 × Taq Reaction Buffer, 0.2 μL Taq DNA polymerase, 4 μL DNA template, and 37.8 μL ddH2O. The cycle conditions were set as the following: 50 °C 2 min (Stage 1, 1 cycle), 95 °C 10 min (Stage 2, 1 cycle), 95 °C 15 s and 60 °C 1 min (Stage 3, 40 cycles). For each mixture of DNA samples, two qPCR and two EMA-PCR tests were performed.

Viability and PCR-DGGE Analysis. In addition, the viabilities of both *B. subtilis* and *P. fluorescens* in the air samples (control and exposed) were also studied using a Live/Dead Bacterial Viability Kit (Molecular Probes) according to a procedure outlined in the Supporting Information. The PCR-DGGE analysis was also applied to analyzing the culturable bacterial aerosol communities in the air samples (control and plasma treated) including pure *B. subtilis* cells and *P. fluorescens* collected according to a procedure outlined in the Supporting Information.

Statistical Analysis. Due to the non-normal distribution of the control and plasma-treated bioaerosol concentrations, nonparametric analysis method Wilcoxon Signed Ranks Test (2-tailed) was used. A p-value of less than 0.05 indicated a statistically significant difference at a confidence level of 95%.

RESULTS AND DISCUSSION

Figure 2 shows the chemical analysis of the nonthermal plasma produced using a spectroscopic method. As observed in Figure 2, the spectra was dominated by N_2 (C→B) emissions as a result

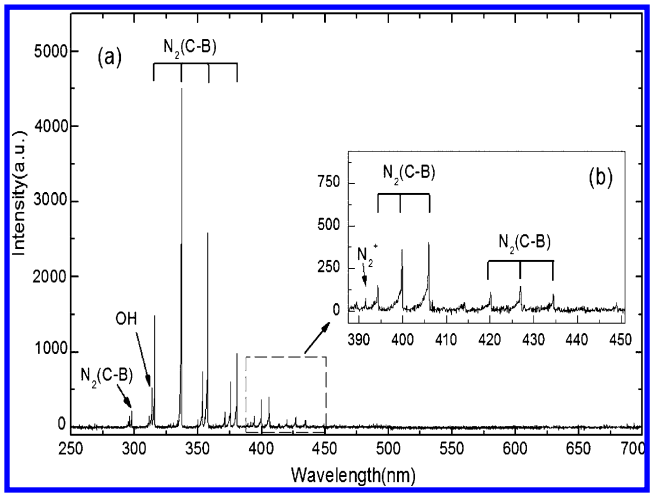

Figure 2. The emission spectra of the ambient air discharge plasma produced at a voltage of 14 kV with a frequency of 10 kHz under ambient temperature of 20 °C: (a) 250−700 nm; the inset (b) shows the magnified emission spectra for indicated wavelength range; N_2(C−B) indicates the second positive system.

of many excitation processes such as electron impact excitation from the molecular ground state N2 (X1∑g+) and first metastable state N2 (A3∑u+), pooling reaction, and transfer of energy between collisional partners.[33,34] The OH emission line was also visible, which is due to the direct electron impact dissociation of H_2O molecules.[35]

In our study, we have observed significant inactivations both for *B. subtilis* and *P. fluorescens* aerosols. Only 1.6% of *B. subtilis* survived the plasma treatment of 0.12 s, while none of the *P. luorescens* survived such treatment with about a 7-log inactivation. Statistical analysis showed that there was a statistically significant difference between the control and plasma-exposed groups for both species (p-value = 0.043 and 0.002, respectively). The results were averages from six independent aerosolization and exposure experiments. *B. subtilis*, believed to be very resistant to the environmental stress, are often used as a surrogate for anthrax causing species *B. anthracis* given their similar characteristics. In a recent study, an inactivation of 65% for *B. subtilis* was achieved using microwave irradiation at a power level of 700 W for about 1.5 min.[13] Here, such a high level of inactivation of *B. subtilis* within subseconds, superior to microwave irradiation, implies that nonthermal plasma could be very useful in inactivating stress resistant species such as anthrax spores which are agents possibly used during a bioterrorism event.

For aerosolized bacterial species (*B. subtilis* and *P. fluorescens*), EMA-qPCR was used to quantify the viable fraction in the air samples with and without the plasma treatment. From Figure 3, it was observed that about 10% of *B. subtilis* in the control aerosol samples were viable by EMA-qPCR estimate. After the plasma treatment, about 94% of viable *B. subtilis* cells (10% viable in initial sample) were found dead. It was also observed that after the plasma treatment the total number of *B. subtilis* cells decreased about 75% as estimated by the qPCR. This would be mainly due to the damages of *B. subtilis* DNA in addition to the culturability and viability loss. For *P. fluorescens* exposure, it was found that about 1% of them in the control samples were viable, and after the plasma treatment about 65% of viable *P. fluorescens* (1% viable in initial sample) were found dead. Such killing differences observed for *B. subtilis* and *P. fluorescens* could be attributed to the initial viability and PCR amplification limitations. A study indicated that qPCR is capable of detecting bacterial concentration differences of at least 1.3 to 3.2 times in air samples.[36] Similar to *B. subtilis*, after the plasma treatment the total number of *P. fluorescens* cells significantly decreased by more than 90% as estimated by qPCR, which was more severe compared to that of *B. sutiliz*. *B. subtilis* is a Gram-positive cell with a thicker cell wall, thus providing it more protection than that of *P. fluorescens*, a Gram-negative cell.

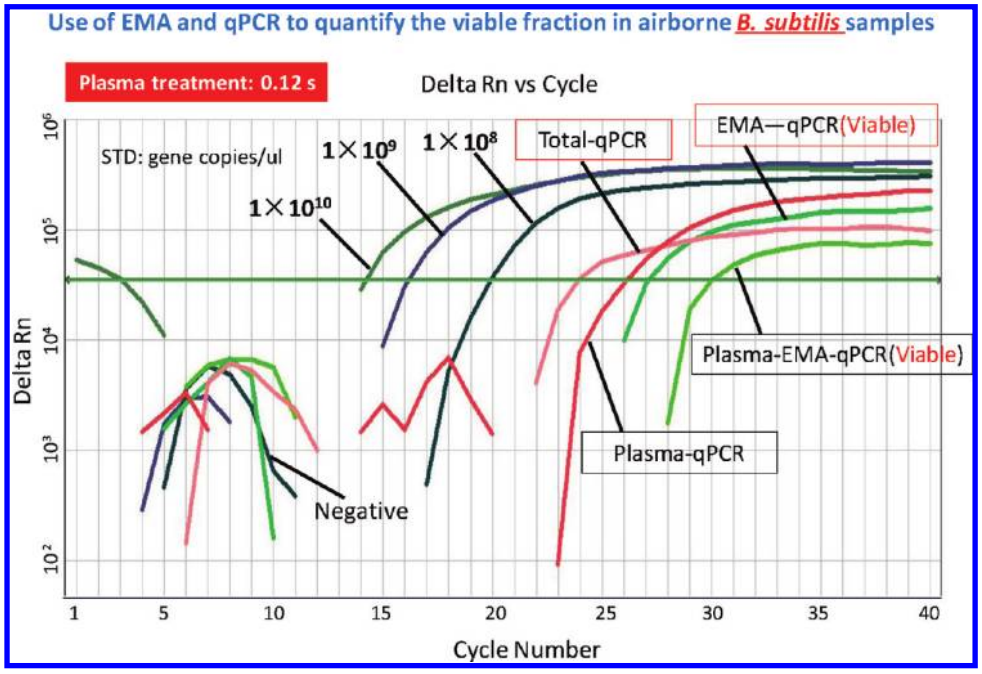

Figure 3. Total and viable fractions of airborne *B. subtilis* aerosol samples collected using the BioSampler with and without plasma treatment; Total-qPCR indicates the total *B. subtilis* concentration; EMA-qPCR (viable) indicates the total viable *B. subtilis* concentration without plasma treatment; Plasma-qPCR indicates the total *B. subtilis* concentration after the plasma treatment; Plasma-EMA-qPCR indicates the total viable *B. subtilis* concentration after the plasma treatment.

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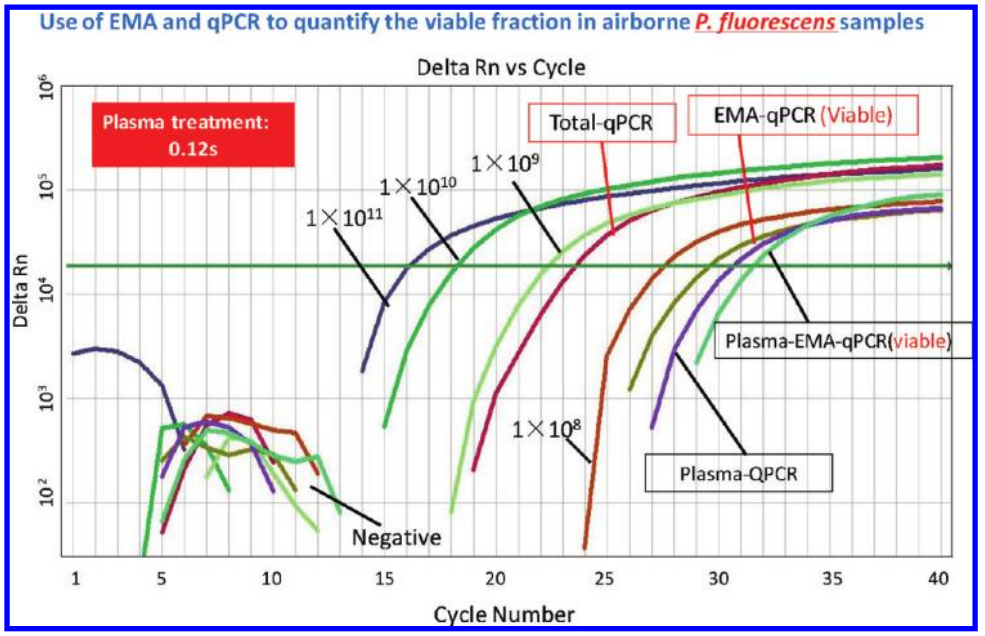

Figure 4. Total and viable fractions of airborne *P. fluorescens* aerosol samples collected using the BioSampler with and without plasma treatment; Total-qPCR indicates the total *P. fluorescens* concentration; EMA-qPCR (viable) indicates the total viable *P. fluorescens* concentration without plasma treatment; Plasma-qPCR indicates the total *P. fluorescens* concentration after the plasma treatment; Plasma-EMA-qPCR indicates the total viable *P. fluorescens* concentration after the plasma treatment.

Accordingly, more membrane ruptures were observed for *P. fluorescens* cells. Compared to the *P. fluorescens* culturability loss (7-log), its viability loss was smaller, suggesting that nonculturable cells might be still viable. These results indicated that the plasma exposure not only reduced the viability but also damaged the integrity of bacterial DNA. In a previous study, DNA damage by the plasma exposure was also observed, but no DNA fragmenting was found.[25] Similar to the DNA stain method, use of EMA-qPCR can provide a quantitative estimate for the viable fraction of the bacterial aerosol sample as demonstrated in Figure 3 and Figure 4.

Figure S1 (Supporting Information) shows the viability tests for *B. subtilis* and *P. fluorescens* aerosols with and without the plasma treatment of 0.12 s. For those control samples, both viable (green) and nonviable (yellow) cells were observed for both species; however, for the plasma treated samples no whole cells were observed using the Live/Dead Viability Kit. Low number of bacterial cells observed was due to the decrease in total undamaged cells after the plasma treatment. The findings from viability tests shown in Figure S1 seemed to agree with the inactivation tests (culturability) and EMA-qPCR results shown in Figures 3and 4. In a previous study, both the leakage of the cytoplasm contents and even a complete rupture of the spore membrane were observed for *B. subtilis* spores residing at filter surface after the plasma treatment of 5 min.[26] In our study, SEM images shown in Figure 5 and Figure S2 and Figure S3 (Supporting Information) also demonstrated that after the plasma treatment both *B. subtilis* and *P. fluorescens* suffered severe membrane ruptures. Here, in addition to the biological aerosol inactivation we have also observed rapid reductions of HC and NO after the plasma treatment as shown in Figure S4 (Supporting Information). This suggests that the produced plasma has high oxidizing capacity.

Sharma et al. (2005) also observed a 5-log reduction of *E. coli* and one log reduction of *B. atrophaeus* (*B. subtilis*) residing on agar surface after the plasma exposure of 1 s, while for *B. atrophaeus* spores 3-log reduction was observed after 10 min exposure.[25] The inactivation was attributed to both cell wall rupture and DNA damages.[25] In another study, a 1.5-log reduction was achieved for airborne *E. coli* when exposed to the plasma for 1 ms.[22] They indicated that hydroxyl itself has a significant effect, responsible for ~13.5% of those killed; however, ozone and UVC had a negligible effect at very short exposure times (~1 ms) inside plasma.[22] In another study, it was suggested that ozone alone might not be the major player for the inactivation.[23] Similar to *E. coli, P. fluorescens* is a very sensitive bacterium, and here a 7-log reduction was achieved after 0.12 s plasma exposure. Results from this study suggest that plasma exposure of milliseconds results in not only culturability loss but also viability loss and membrane ruptures of airborne *B. subtilis* and *P. fluorescens*. In contrast to bioaerosol thermal treatments,[11,12] inactivation of bioaerosols by nonthermal plasma occurred under lower temperature (below 60 °C).

Different from other studies, the inactivation of environmental bacterial and fungal aerosols by the plasma was also investigated. Similar to aerosolized exposure experiments, low survival rates of total bacterial aerosols, close to 1%, were observed both for indoor and outdoor environments after 0.06 s plasma exposure. *B. subtilis* and *P. fluorescens* could generally represent those hardy and sensitive species present in the natural environments. Thus, it is expected that the inactivation rate for environmental bioaerosols would fall between those of *B. subtilis* and *P. fluorescens*. However, among culturable environmental bioaerosols sensitive cells, e.g., *P. fluorescens* type ones, account for a very small fraction. Accordingly, the inactivation rates observed for environmental bioaerosols were those largely for hardy species, e.g., *B. subtilis* type ones. As shown in our study, the survival rates for pure *B. subtilis* aerosols and environmental bioaerosols were comparable (1.6% and 1%, respectively). For fungal aerosols, the survival rate, about 15%, was found higher in outdoor environment compared to ~2% for indoor environment. For outdoor fungal aerosol exposure, higher survival rate was due to the survival of the hardy fungal species within a very short time plasma exposure (0.06 s). The survival rates for environmental bioaerosols were averages from

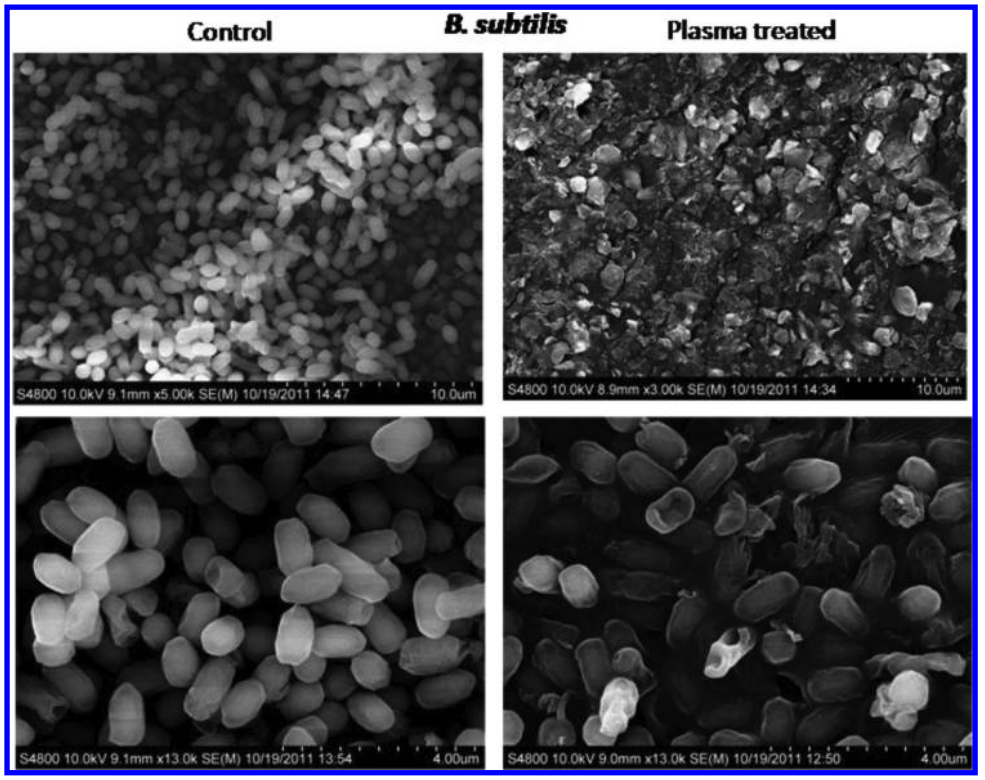

Figure 5. Scanning electron microscopy (SEM) images (top: low resolution, bottom: high resolution) of control and plasma-treated *B. subtilis* samples.

total 9 repeats obtained on three different dates. Wilcoxon tests showed that the differences in control and treated bacterial and fungal aerosols in two environments were all statistically significant (p-value < 0.028). In a recent study, it was also shown that nonthermal plasma could achieve effective inactivation of antifungal resistants *Candida* albicans, *Candida* krusei, and *Candida* plated on agar plates by a 10 min treatment, and it was suggested that reactive oxygen species were responsible for the inactivation.[37] Lesser degree of fungal aerosol inactivation obtained here for outdoor environment was mainly due to the short plasma exposure time and thicker fungal cell wall structure. Different from peptidoglycan of bacterial membrane, the main component of the fungal cell wall is chitin, a more rigid material leading to more resistance to the stress. In addition to the rapid culturability loss of the environmental bioaerosols, we have also observed a decrease in culturable bacterial aerosol diversity (fewer gel bands) both for the indoor and outdoor environments as shown in Figure 6. The diversity loss (decrease in the number of gel bands) was observed more pronounced for indoor environment compared to those of outdoor environment. The difference resulted from different environmental conditions and species composition in different environments. These results implied that for certain species there were complete destruction after the plasma treatment. For *P. fluorescens*, after the plasma treatment no visible bands were observed as shown in Figure 6. This suggests that in addition to the culturability/viability loss there were also DNA damages for this sensitive bacterial species. To investigate the potential bacterial cell losses on the plasma tube wall due to the charging effects, we monitored the size distribution of particles exiting the plasma device operated with the BioStage impactor using an Optical Particle Counter. As observed from Figure S5 (Supporting Information), when the plasma was applied the particle number concentration increased regardless of particle sizes. Higher increases (20−40%) were observed for particles of 0.65−3 μm, which are the size ranges of most microbial species. Peak increase was found around 2 μm. The particle number increase was due to cell fragmentation as a result of the plasma exposure. From the results shown in Figure S5, we can conclude that use of plasma might not result in particle loss in the plasma tube wall. However, we could not exclude the possibility that the potential loss of bacteria could be offset by the particle number increase due to the cell fragmentation. In this case, the inactivation rate under the exposure time tested could be slightly overestimated only if the bacteria cells deposited on the plasma wall are still viable (which is less likely).

Plasma consists of photons, electrons, positive and negative ions, free radicals, and excited or nonexcited molecules and atoms. Nonthermal plasma has been extensively used in inactivating biological species. Some studies propose that UV radiation, free radicals, as well as the bombardment of the cell wall by charged particles or synergistic effects of these factors could contribute to the overall inactivation of the microorganisms as discussed by Fernándeza and Thompson.[38] In our study, spectroscopic analysis showed that there was also a high level of OH species present in the plasma produced under the experimental conditions used. It is certain that OH species played a role in the inactivation observed in this study. Another study indicated that the reactive oxygen species (ROS) in plasma play a dominant role in the inactivation of Gram-negative *E. coli* and Gram-positive *S. aureus*.[39] In another study, it was suggested that ozone alone might not be the only dominant factor, but the synergetic action of short-living plasma agents (direct plasma treatment) and ozone, in inactivating airborne *E. coli*.[23] Here, we have also observed severe membrane ruptures of the bacterial species tested. However, the exact mechanisms of

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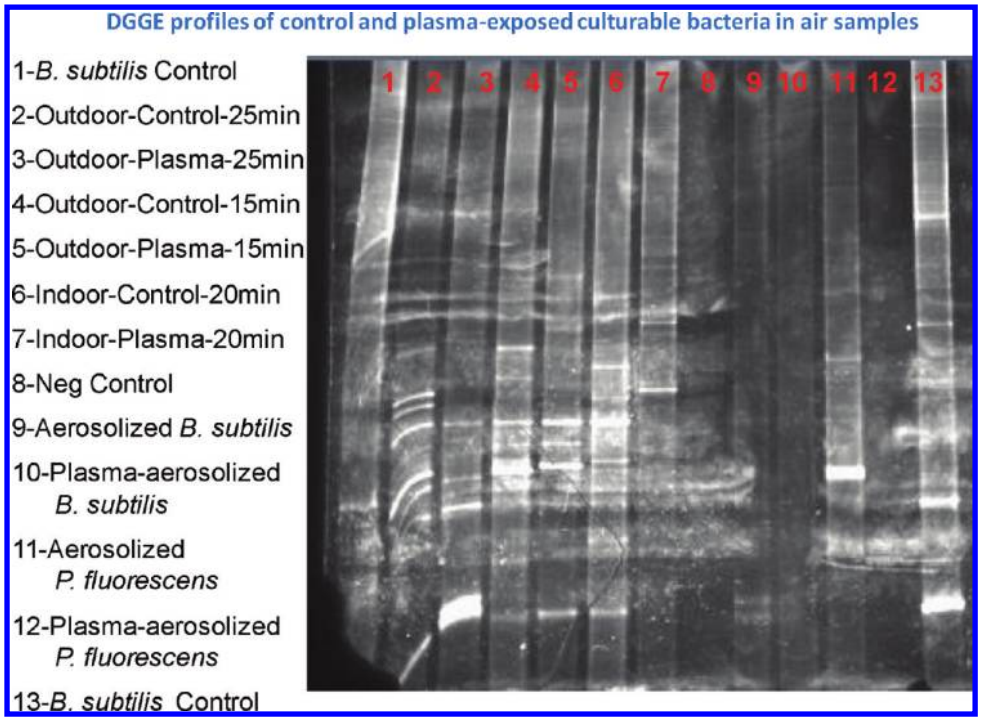

Figure 6. DGGE profiles of environmental bacterial aerosols (control and treated by plasma) and total aerosolized *B. subtilis* and *P. fluorescens* (culturable and nonculturable); bacterial colony forming units used were pooled from at least three repeats for each of the environments tested.

the microbial inactivation by the plasma exposure are are still under debate.[40] The debate arises from the conditions including the gas mixture at which the plasma is produced, the species tested, and environmental conditions such as relative humidity (RH) level. For laboratory generated bioaerosols, the charges imparted by the nebulization process could not only affect their viability[41,42] but also could influence the inactivation efficiency by DBD system according to recent discussion about the interactions between plasma charged species and cells.[43]

In most of the previous studies, surface and liquid-borne states of pure species inactivation were conducted. Here, we assessed the inactivation efficacies of nonthermal plasma when treating environmental bioaerosols in both indoor and outdoor environments. In addition, we applied molecular tools such as EMA-qPCR and PCR-DGGE methods to study the viability and diversity loss of treated microbial species and air samples. Increasing threats from bioaerosol exposure necessitate the development of efficient air decontamination technology. Experimental data here indicated that plasma exposure resulted in significant inactivations of environmental bioaerosols (bacteria and fungi) both indoors and outdoors within milliseconds. Results here were from single-pass plasma device, and in the future multiple passes or different configurations with longer exposure time should be explored for enhanced inactivations. In addition, different energy doses should also be investigated for inactivating biological aerosols, especially for fungal species. Besides, the inactivation efficiencies should be also studied under different RH levels controlled by a desiccant together with a hygrometer. The nonthermal plasma method holds great promise in inactivating bioaerosols, particularly useful in environments that are water-damaged or contaminated by accidentally or intentionally released biowarfare agents. Such applications could also extend to air sterilization in hospitals, high value buildings, military bases, flight cabins, and other public health facilities. However, its applications could be limited by its secondary pollutants such as ozone and NO_2 which are not only harmful to the people but also to the environments. Combination of catalysts with plasma to some extend could minimize such problems.

■ ASSOCIATED CONTENT

Supporting Information

Figures S1−S5 and text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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