# Rapid Inactivation of Airborne Bacteria Using Atmospheric Pressure Dielectric Barrier Grating Discharge

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***Abstract*—Dielectric barrier discharge plasma has been known to inactivate many different microorganisms on surfaces when treatment times are on the order of seconds or minutes in duration. In this paper, a unique plasma air cleaning facility was created which combines a dielectric barrier grating discharge (DBGD) with a filterless laboratory-scale ventilation system and is used to treat concentrated bacterial bioaerosol in a moving air stream at air flow rates of 25 L/s. Results indicate that plasma treatment times on the order of milliseconds corresponding to one pass through the DBGD device can achieve 1.5-log reduction in culturable *E. coli* immediately after contact with plasma and 5-log reduction totally following in the minutes after the plasma treatment. A numerical characterization study was performed to help predict and understand the mechanism of bacteria inactivation in the DBD plasma from a variety of plasma factors.**

***Index Terms*—Airborne microorganism, air sterilization, dielectric barrier discharge, E. coli, nonthermal plasma.**

## I. Introduction

NONTHERMAL plasma-based technologies have demonstrated success in inactivating many different types of microorganisms such as viruses, Gram-negative, and Gram-positive bacteria on the surfaces and in aqueous solutions [1]–[6]. Several inactivation mechanisms by atmospheric pressure plasma have been proposed and are still under continuous debate in current review papers [4]. The most common plasma inactivation mechanisms that cause lethal effects to microorganisms are summarized in the following.

1) UVC and VUV irradiation in the wavelength range ($< 300$ nm) leads to the inactivation of microorganisms as a result of dissociation and/or crosslinking of DNA strands.
2) The diffusion of oxygen species (O, $O_3$, and $O_2^*$) or oxygen-containing radicals (e.g., OH and NO) through the bacteria cell wall causes the local damage possibly by oxidation of cytoplasmic membrane, proteins, and DNA strands.
3) Bombardment on the cell wall by charged particles (electrons and ions) could induce breaking of chemical bonds, erosion, and localized occurrence of openings in the membrane with further penetration of plasma toxic compounds inside the cells.
4) Localized, periodical, and short-term heating of bacteria by their contact with plasma channels could enhance all of the previously listed lethal effects.
5) All of the various plasma active components can synergistically interact, resulting in a combination of the previously listed mechanisms that when combined will significantly enhance or even mainly determine the overall process of inactivation.

In comparison with plasma-based surface and water sterilization, only a few plasma researchers have focused on air decontamination using nonthermal plasma. Most of them have been successful only when coupling plasma technology with high-efficiency particulate-air (HEPA) filters to both trap and kill microorganisms [6]–[8]. The downside of relying on HEPA filters is that they have a limited efficiency in trapping submicrometer-sized airborne microorganisms [9] and they also cause significant pressure losses in heating, ventilation, and air conditioning systems, giving rise to higher energy and maintenance costs.

In this paper, a unique experimental facility, named the Pathogen Detection and Remediation Facility (PDRF), was designed to perform air-decontamination experiments using a dielectric-barrier-grating-discharge (DBGD) plasma device combined with a laboratory-scale ventilation system with bioaerosol sampling capabilities. Dielectric barrier discharge plasma was chosen as the primary discharge for air-decontamination experiments because it has been proven as microbial disinfectant in many surface sterilization studies [10]–[12], and it is a low-power consuming nonthermal

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discharge that is relatively easy to construct, requiring simple power supplies.

The results of this paper show that direct contact of bioaerosol with the DBGD device with very short duration can cause a 1.5-log reduction (97%) in culturable *Escherichia coli* and an ∼5-log reduction (99.999%) finally measured in the 2-min following exposure. Fast treatment times within plasma are due to a high air flow rate and high velocity of the bioaerosol particles in flight and a small discharge length of the DBGD device, which results in a residence time of treatment of approximately 1 ms. These findings are somewhat remarkable because, in most DBD surface sterilization studies, treatment times are always at least 1000 times longer on the order of seconds and, in some cases, even minutes in duration [1]–[5]. Standard colony-forming-unit (CFU) culture techniques and flow cytometry were used to detect culturability and presence of bacteria in the air flow before and after passing through the DBGD. Flow cytometry, in conjunction with different fluorescent dyes, represents a sensitive and culture-independent method to rapidly detect bacteria in environmental samples [13], [14]. This method, in addition to the CFU culturing technique, allows the quantification of the whole bacterial population, including both the culturable and inactivated fractions with high accuracy even for low microbial concentrations. It is important to verify the presence of inactivated bacteria because it eliminates the possibility that the DBGD device acts as an electrostatic precipitator, which could charge the bioaerosol droplets and remove them from the air flow inside the PDRF system. The results of this paper show that our DBGD plasma device is acting not as an electrostatic precipitator but as a device that is capable of deactivating high concentrations of bacterial bioaerosol in flight at high flow rates in a ventilation system.

To understand the mechanism of rapid deactivation, a numerical characterization study has been performed to identify which plasma factors (active chemical species and charged and excited species) are most effective for sterilization and help predict the success of DBD plasma inactivation on other types of airborne microorganisms. Since each type of individual microorganism has a different cellular composition and each responds differently to external factors, it is our ultimate goal to construct a model that can describe disinfection kinetics for all types of microorganisms in the DBD plasma. However, in this paper, we begin with simple kinetic estimations to identify trends and reactions based on disinfection empirical data from other researchers, as well as our own, and establish a methodology to quantify the sterilization effect of different plasma factors on *E. coli* bacteria.

## II. Design of the PDRF

The PDRF system is a bioaerosol treatment facility that was designed to provide a recirculating air flow environment for the DBD air-decontamination experiments. One obvious feature of a recirculating system is that the bioaerosol can be treated with repeated passes through the same plasma discharge. Additionally, a sealed recirculating system allows for complete control over relative humidity (RH) inside the system, which is important because even small fluctuations in RH have been shown to significantly decrease the survivability of airborne bacteria [15]. The PDRF system was designed as a plug flow reactor, i.e., air flow inside the system is turbulent so that radial variation of the bacterial concentration in the airflow is minimized. Fig. 1 shows a general scheme of the PDRF. The PDRF system has a total volume of 250 L and is designed to operate at high air flow rates (∼25 L/s or greater), which are typical for indoor ventilation systems. The system has an inlet with attached Collison nebulizer for bioaerosol generation and two air sampling ports connected to a vacuum air sampling system. The system also has a large volume barrel that contains a series of aluminum baffle plates and a variable speed centrifugal blower motor that drives the air through the DBGD treatment chamber. The system recirculation time, i.e., the time for one bioaerosol particle to make one complete revolution through the system, is approximately 10 s.

### A. Air Sampling System

To separate the decontamination effect of direct exposure to the DBD plasma from the remote exposure of ozone and other long-lived chemical species that can interact with bioaerosols downstream of the discharge, a sampling method was devised so that air samples are taken just before and after the bioaerosol passes through the discharge area. As there are only two sample ports (located on either side of the DBGD device), each set of two air samples measures the change in viability of bacteria on a "per pass" basis through the discharge. For each of the subsequent sets of air samples, the sample taken "before plasma" can give a measurement of the change in viability due to the effect of residual ozone from the previous "after plasma" sample. Liquid impingement was the chosen air sampling method because it minimizes desiccation stress on the bacteria by directly depositing them into a buffered saline solution. Liquid impingers operate by drawing a sample of air through an inlet tube submerged in a solution, thereby causing the air stream to strike the liquid bed trapping aerosols in the solution through forces of inertia [16]. The AGI-30 is the most commonly used liquid impinger which contains a critical orifice that limits the maximum air sampling rate to 12.5 L/s. The vacuum air sampling system was designed to take as large volume air sample as possible (∼1 L) in the shortest period of time (∼1 s) so as not to significantly disturb the flow inside the system. To accommodate this high air sampling rate, the AGI-30 impinger was modified by replacing the standard critical orifice with a hollow tip with several jet ports. The efficiency of these modified liquid impingers was measured in several control experiments in the PDRF system and was found to be reproducible. The efficiency of the samplers was calculated by comparing the theoretical amount of nebulized bacteria into the system for each experiment with the CFUs recovered in the modified impingers. A total of eight modified impingers were constructed, and variations in sampling efficiency between them were found to be negligible for the same bacteria; however, the overall efficiency of this type of sampler was in the range of 6% ± 3%. To some, this may seem to be a low efficiency rating; however, when sampling

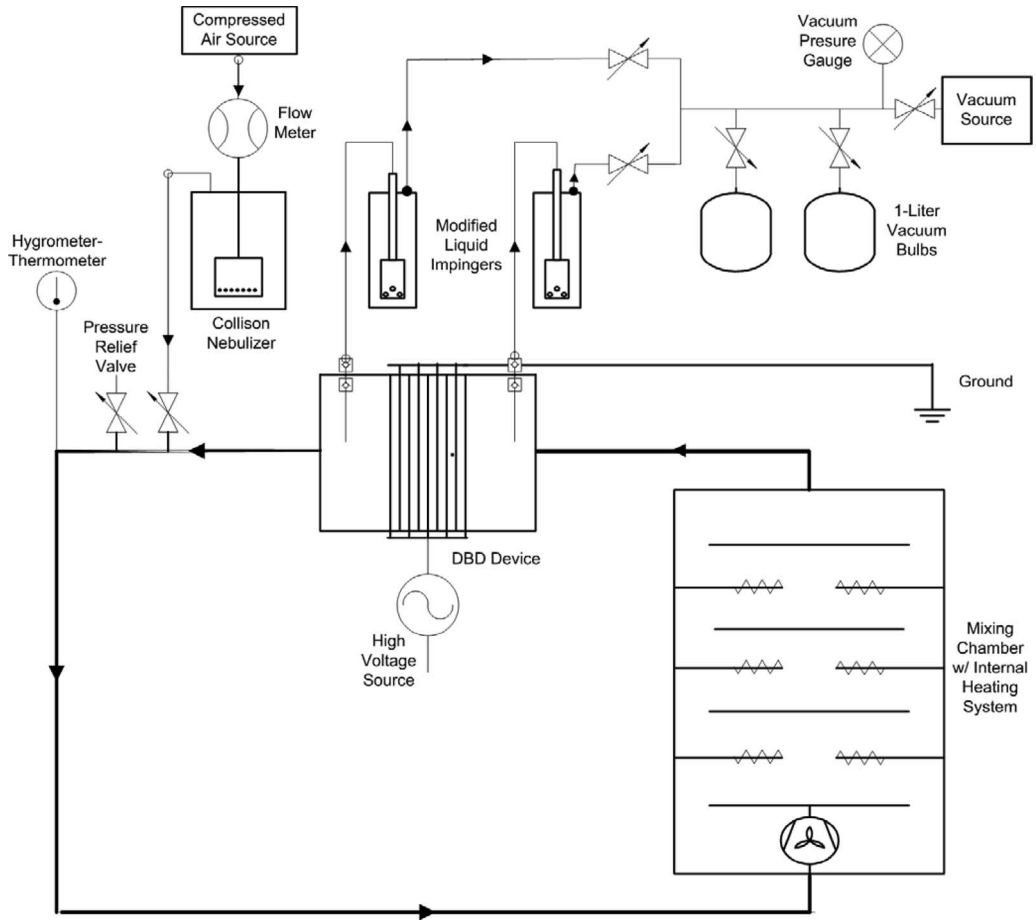


Fig. 1. Schematic of the PDRF.

bioaerosols, reproducibility is often considered more important than the efficiency rating because the final conclusions are derived from the internal comparisons between various data collected using the same samplers [17]. Additionally, the efficiency of the modified impingers was also dependent on the growth phase (exponential, stationary, etc.) and the strain type for microorganisms used in experiments (*E. coli* type K-12 was used in all trials reported here).

### *B. Dielectric Barrier Grating Discharge (DBGD)*

The DBGD consists of a thin plane of wires with equally spaced air gaps of 1.5 mm. The high-voltage electrodes are 1-mm diameter copper wire shielded with a quartz capillary dielectric that has an approximate wall thickness of 0.5 mm. The total area of the DBGD discharge including electrodes is 214.5 $cm^2$ and without electrodes is 91.5 $cm^2$. Fig. 2 shows an image of the DBGD device. The DBGD device has two air sample ports located at a distance of 10 cm from each side of the discharge area so that the bioaerosol can be sampled right before and after it enters the plasma discharge. When the PDRF system is operated at a flow rate of 25 L/s, the air velocity between the electrodes of DBGD is 2.74 m/s, and the residence time of treatment, i.e., the duration of one bioaerosol particle (containing one *E. coli* bacterium) passing through the DBGD device, is approximately 0.73 ms, assuming a plasma thickness of 2 mm which is equal to the quartz capillary diameter.

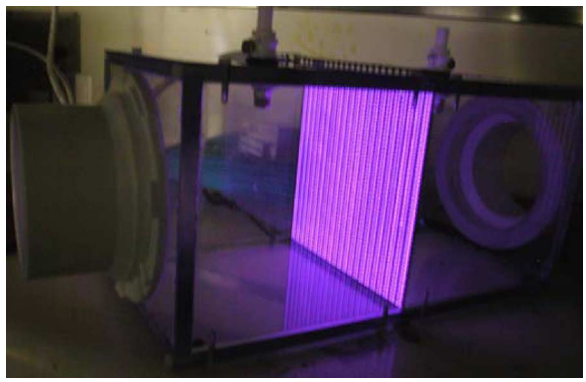

Fig. 2. DBGD air sterilization chamber.

The DBGD device is operated using a quasi-pulsed power supply that delivers large voltage pulses with the following damped oscillations. An image of the current and voltage waveforms is shown in Fig. 3. Oscilloscope measurements indicate that the duration of one pulse period is approximately 600 $\mu$s, the maximum peak-to-peak voltage is 28 kV, and the pulsed current is nearly 50 A (peak-to-peak value). The average power of the discharge over one pulse period is approximately 330 W, and considering the discharge area of 91 $cm^2$, the power density is 3.6 W/$cm^2$. Since the majority of power is discharged within the pulse duration (within the duty cycle of the pulse period), it is useful to describe the power in terms of the pulse

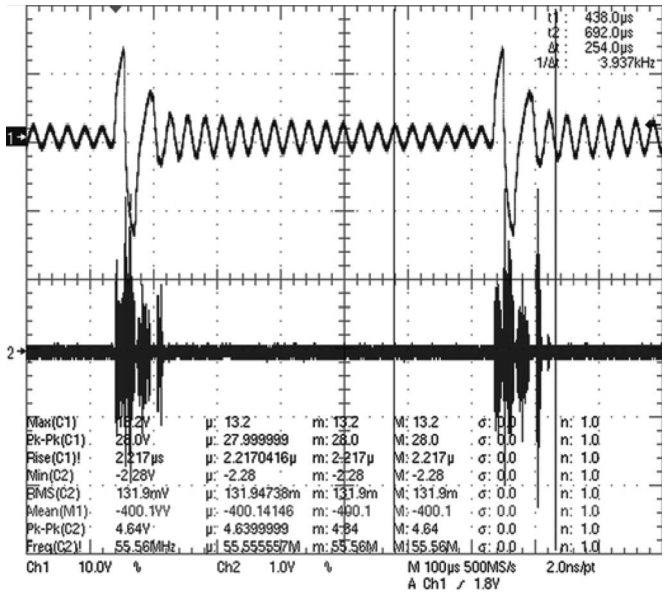


Fig. 3. Voltage and current waveforms of the DBD device, as shown on channels 1 and 2, respectively.

duration itself since there is essentially no discharge between pulses. Measurements indicate that the pulse duration is 77 $\mu$s, nearly an order of magnitude less than the complete pulse period, which gives a duty cycle of 0.1283. The average power in the pulse duration is then 2571 W. Given that the residence time of a bioaerosol particle passing through the discharge area is 730 $\mu$s and the pulse period is 600 $\mu$s, this means that each bioaerosol particle that passes through the DBGD area experiences about one pulse of DBD discharge power. The typical concentration of bioaerosol in an experiment is approximately $5 \times 10^5$ bacteria per liter of air, which translates to approximately $9 \times 10^3$ bacteria within the cross section of discharge area at any given time (in each 2-mm wide cross section of flow passing through the DBGD, assuming plug flow conditions in the DBGD chamber).

A steady-state concentration of ozone of 28 ppm was measured in the PDRF system by the calibrated optical ozone meter MedOzon-254/5 ("MedOzone," Russia) after 10 s of DBGD operation (time required for one volume treatment). UV radiation intensity from the discharge in the UVC spectral region was measured with a Radiometer IL1700 equipped with sun-blind SED220 photodiode (International Light, USA). The measured UVC intensity was approximately 30 $\mu$W/cm$^2$.

## III. Materials and Methods

### A. Nebulization and Air Sampling Procedures

The PDRF system was initially presterilized using an internal heating system and prehumidified to 70% RH. The bacterial culture was placed into a BGI 24-jet Collison nebulizer, and the nebulizer was operated at 40 psi for a period of 45 s (nebulizing rate: 1.1 ml/min). According to the manufacturer's specifications, the Collison nebulizer generates bioaerosol droplets with a median diameter of 2 $\mu$m at the operating conditions that are used in experiments (40-psi static pressure of air that drives the nebulizer). The DBGD device was then switched ON for a period of 10 s so that the entire volume of bioaerosol in the system is treated with one pass through the discharge. Subsequent volume treatments are made within a 2-min interval to allow for time to remove used air samplers and replace them with sterile samplers. Air samples are taken in pairs and in sequential order: before and after passing through plasma. Therefore, the decontamination effectiveness of the DBGD device is measured on a per pass basis with each set of air samples, and subsequent volume treatments can show an additive effect of multiple passes through the discharge. Each of the presterilized air samplers was initially filled with 30 ml of sterile phosphate-buffered-saline (PBS) solution, and after sampling, each sample solution was serially diluted in accordance with the standard practices outlined in Section III-B.

### B. Culture Preparation and Assay

*Escherichia coli* (K-12 strain) was used in all trials. Following each experiment, liquid samples from each impinger were serially diluted in the PBS, plated onto Luria–Miller agar plates, and incubated at 37 °C overnight. Visible colonies were counted and recorded within the following 24-h period. All preparation activities were followed in accordance with the standard microbiological procedures outlined in [18].

### C. Flow-Cytometry Procedures

Flow-cytometric measurements were made using FACS calibur (Becton Dickinson, USA) flow cytometer with a 488-nm excitation from an argon ion laser at 15 mW. Fluorochromes with a high affinity for nucleic acid SYBR Green I and propidium iodide (PI) (molecular probes) were used for flow cytometry. The SYBR Green I, which is a green fluorescent nucleic acid stain, has been shown to stain the living and dead Gram-positive and Gram-negative bacteria [19]. PI is a red fluorescent dye that intercalates with dsDNA and only enters permeabilized disintegrated cytoplasmic membranes [20].

Liquid samples taken from the nebulizer and from each impinger were divided into five subsamples. Two of the subsamples were stained by the SYBR Green I, one with the PI, one with the SYBR Green I and PI, and the last one was nonstained and used as a control. For total bacteria counts, 5 $\mu$l of SYBR Green I and (or) 5 $\mu$l of PI were added to 500 $\mu$l of the sample and incubated for 15 min in the dark at room temperature. Concentrated culture prepared for nebulization and stained by SYBR Green I was used as a positive control, and PBS with SYBR Green I or PI was used as a negative control to provide the total bacteria count. The positive control sample for the PI staining of only dead bacteria was prepared by adding 50 $\mu$l of disinfectant (97% ethanol) in the bacteria solution and waiting for a period of 5 min to ensure 100% dead *E. coli*. The sensitivity of flow-cytometric measurements to *E. coli* was determined by measuring the additional five samples obtained by serial dilution of the concentrated original culture used for nebulization ($10^2$, $10^3$, $10^4$, $10^5$, and $10^6$ times diluted in the PBS). The flow-cytometry method in these particular

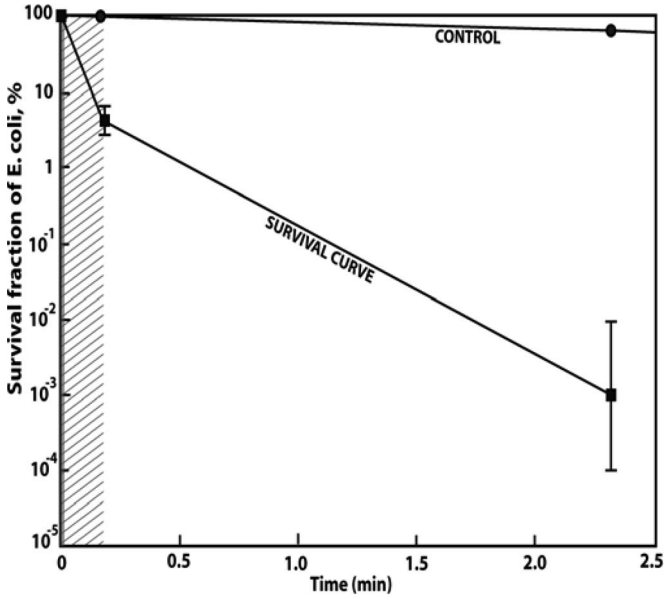


Fig. 4. Survival curve showing results of the DBD-treated *E. coli* bioaerosol in PDRF system. The gray shaded area of the curve depicts one plasma treatment cycle in which the entire system volume was treated with one pass through the discharge. The plasma treatment time is 10 s, but during this treatment, each bioaerosol particle passes once through the discharge with a duration of approximately 1 ms (due to the large volume and high air flow rate inside the system). Samples 4 to 6 were omitted from this figure because no culturable bacteria were detected. Control experiments show a negligible change in viable *E. coli* concentration.

conditions was shown to be able to detect $\leq 10^2$ bacteria (*E. coli*) per milliliter of PBS solution.

## IV. Results and Discussion

### A. Experimental Results

The culture test results from seven replicate trials of the DBGD-treated *E. coli* bioaerosol in the PDRF system are shown in Fig. 4. Control experiments indicate a small but negligible change in the surviving fraction of *E. coli* over the total experimental period. In the DBGD-treated trials, an approximate 1.5-log reduction (97%) in the surviving fraction of *E. coli* was measured between samples 1 and 2, which corresponds to an approximate 1-ms treatment time (one pass through the DBGD plasma). It is important to restate here that the plasma was ignited for 10 s for each set of two samples (as indicated by the gray shaded area on Fig. 4), and this is the time required to treat the entire volume of bioaerosol in the system once. It is our assumption that, with each 10-s plasma treatment cycle, each airborne *E. coli* makes only one pass through the DBGD, and due to the high velocity in the system, their residence time within the discharge is estimated to be on the order of 1 ms. An interesting second decrease in the surviving fraction of *E. coli* is shown between samples 2 and 3, which occurred in the time between the plasma treatments. In this second decline, the number of culturable bacteria decreased by an additional 99.95% (3.5 logs) in the time when the plasma discharge was switched off. Sample 4 was taken after the second plasma treatment (not shown), and samples 5 and 6 were taken before and after the third plasma treatment. Samples

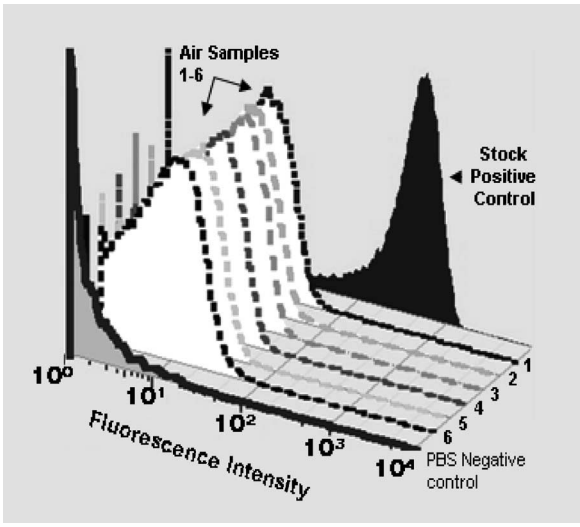


Fig. 5. Flow-cytometric histograms for the total number of *E. coli* (alive + dead) stained by SYBR Green I. Stock solution used for nebulization was considered as a positive control, while PBS solution with the same amount of SYBR Green I was considered as a negative control.

4 to 6 did not reveal any culturable bacteria; therefore, those experimental points were omitted from Fig. 4.

Flow cytometry was also employed to detect the presence of *E. coli* in each of the six air samples taken during experiments. While colony counting techniques are limited to detect only the culturable (i.e., visibly growing) bacteria, flow cytometry is capable of detecting the physical presence of bacteria in a sample regardless of culturability. The flow cytometry utilizes two florescent dyes: SYBR Green I to detect the presence of all bacteria (dead and alive) and PI to detect the bacteria with disintegrated cytoplasmic membranes. Fig. 5 shows the flow-cytometry results for the DBGD-treated air samples using only the SYBR Green I florescent dye. The florescent intensity peak for air samples one through six is identical, which means that there are the same numbers of total bacteria present for each air sample taken during experiments. The stock positive control sample is a pure untreated sample of *E. coli* whose intensity (horizontal axis) peak was two orders of value greater than the intensity of the air samples. Additionally, the intensity of PI red fluorescence (not shown) was found to be negligible in comparison with the expected PI positive control, and therefore, the outer membranes of treated *E. coli* were not disintegrated after an interaction with the DBGD.

Flow-cytometry analysis of air samples taken during these trials indicates that the total number of bacteria (both active and inactive) remains almost constant; therefore, the DBGD device is not acting as an electrostatic precipitator, and the concentration of bioaerosol particles remains undisturbed for the duration of each experiment. The flow-cytometry results also showed that bacterial outer membranes of *E. coli* were not damaged with up to three passes of direct exposure in the DBGD plasma device. However, culture test results demonstrated a 97% reduction in culturable *E. coli* with a millisecond exposure time in the DBGD plasma (one pass through discharge) and a subsequent 3.5-log reduction in the 2-min following treatment. The direct plasma exposure time of 0.73 ms (per pass) allows enough time

for bacteria to be attacked by all chemically active components of plasma: charged particles, UV radiation, OH radicals, atomic oxygen, and ozone, which is one explanation for the initial 97% reduction in culturability. Subsequent remote exposure to the remaining ozone in the 2-min following direct plasma treatment may account for the additional 3.5-log reduction.

### B. Plasma-Based Sterilization: A Numerical Characterization

The plasma-based sterilization community has performed a great deal of effort to study the sterilizing effects of various types of plasmas on microorganisms of all types. However, there is still a lack of understanding to describe the mechanism causing the sterilization effect observed in experiments. There are many theories as to which plasma active species are responsible for inactivation, but there is no real numerical model to quantify the role of each specie and/or a synergistic coupling of species which can accurately describe the experimentally observed phenomena. In this section of this paper, a numerical characterization of the sterilizing effect of plasma factors responsible for sterilization will be established as a basis for a larger more comprehensive model to be developed and refined sometime in the near future. This numerical characterization study utilizes a chemical kinetics approach that uses rate equations to describe the change in concentration of a viable microorganism by considering the effect of each plasma factor individually. The following differential equation describes the rate of change in concentration of a viable microorganism $[M]$ with time

$$\frac{d[M]}{dt} = -\sum_{i=1}^{n} k_i[M][A_i] \tag{1}$$

where $n$ is the number of plasma species interacting with microorganisms, $k_i$ is the reaction rate constant given as volume per unit time, $[M]$ is the concentration per unit volume of viable microorganisms, $A_i$ is the concentration per unit volume of $i^{\text{th}}$ species. Here, concentration $[M]$ is specific to a given experiment, the number $n$ and concentration $[A]$ of interacting species are dependent on the type of plasma discharge, and $k_i$ is specific to the type of species $A_i$.

The foundation of this approach is the reaction rate constant $(k)$ that is invoked to describe the sole interaction of each plasma factor with the microorganism of interest. The reaction rate constants can be derived from basic experimental data based on the following formula:

$$k_{i,m} = \frac{\ln\left(\frac{1}{S}\right)}{[A_i] \cdot t} \tag{2}$$

where $S$ is the surviving fraction of microorganism population, and $t$ is the time of exposure. The product $[A_1] * t$ is also known as the CT value or the contact time. It is important to state here that the reaction rate constants are each specific to one plasma factor and one microorganism. For example, Table I shows the reaction rate constants for the sterilizing effect of three plasma factors O3, OH, and UV on the *E. coli* bacteria.

TABLE I
RATE CONSTANTS CALCULATED FROM EMPIRICAL DATA FOR THE INTERACTION OF *E. coli* BACTERIA WITH O3, OH, AND UV

| $k_{\text{factor, microorganism}}$ | Derived Reaction Rate Constant | Empirical data reference |
|---|---|---|
| $k_{\text{O3, E.coli}}$ | $1.5\text{x}10^{-16}$ ($\text{cm}^3$/s) | [21] |
| $k_{\text{OH, E.coli}}$ | $3.6\text{x}10^{-13}$ ($\text{cm}^3$/s) | [21] |
| $k_{\text{UV, E.coli}}$ | $3.8\text{x}10^{-3}$ ($\text{cm}^2$/μJ) | [22] |

Because the reaction rate constants are derived from empirical data, they are not always available for all plasma factors and all microorganisms. There is, however, a great deal of empirical data (available in the food and water decontamination literature) in which the well-known plasma factors of O3, UV, and OH were used to destroy a variety of bacteria, viruses, and spores in various liquids and surfaces [23]–[25]. In the next section, the reaction rate constants from Table I and species concentration measurements from our DBGD discharge are combined in (1) to yield a numerical estimation of the sterilizing effect from each plasma factor on the survivability of *E. coli* in direct contact with the DBGD.

### C. Numerical Characterization of the PDRF

In this section, the numerical characterization principles previously described are applied to the PDRF system, and a comparison is made to our experimental results, taking into account the concentrations of plasma factors in our system. The plasma factors considered here will be those of O3, OH, and UV with the *E. coli* bacteria. The effect of other very important plasma factors—charged species (electrons and ions) and other chemically reactive species like radicals (atomic oxygen and nitrogen oxide) and excited molecules was not taken into account at this time simply for a lack of empirical data to formulate a rate constant. It is our goal to continue to search for empirical data involving those factors so that they may be incorporated in this characterization study in the near future.

The concentrations of each plasma factor in the DBGD are quantified, and the reaction rate constants for these plasma factors and *E. coli* bacteria are established in the following.

1) Ozone $(\text{O}_3)$. In a recent publication [21], the CT value for the exposure of *E. coli* to the ozone is $4 \cdot 10^{-2}$ mg · min/l with a two-log reduction in the viable microorganism concentration. Hence, the reaction rate constant for the interaction of ozone with *E. coli* was calculated as $1.56 \cdot 10^{-16}$ $\text{cm}^3/\text{s}$. This is further confirmed from other experimental data in [26]. Based on our experimental measurements, the steady-state ozone concentration in the PDRF system after 10 s of DBGD operation is about 28 ppm.
2) Hydroxyl (OH) radical. According to [21], the CT value for the exposure of *E. coli* to OH is $0.8 \cdot 10^{-5}$ mg · min/l for a two-log reduction in the viable microorganism concentration. Based on (2), the reaction rate constant is $3.6 \cdot 10^{-13}$ $\text{cm}^3/\text{s}$. Hence, it is suggested that the rate of inactivation of *E. coli* by OH is about $10^3$–$10^4$ times faster than $\text{O}_3$. This is an expected result due to the highly

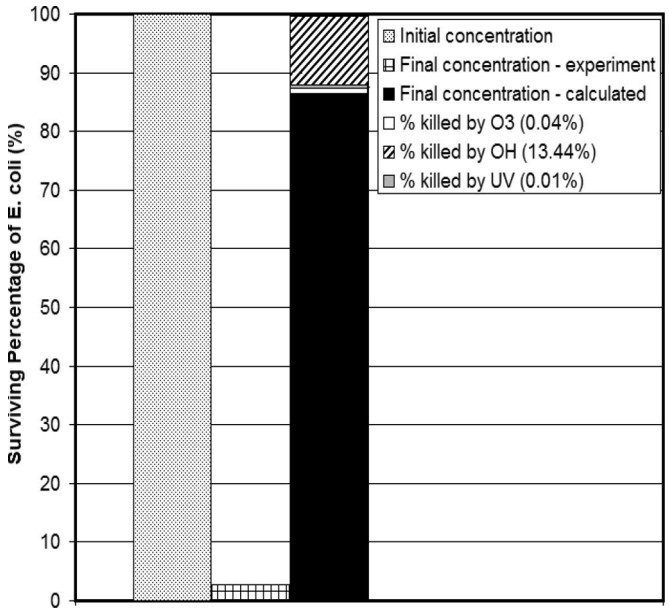


Fig. 6. Comparison of experimental results from the PDRF system for the DBGD treatment of *E. coli* and results of the numerical characterization study showing the sterilizing effect from only three selected species in DBD plasma: ozone, hydroxyl, and UV radiation. In all cases, *E. coli* is treated with one pass through the discharge with a particle residence time of $\sim$1 ms. Hydroxyl is the largest contributor of the species investigated; ozone and UV are almost negligible with these very short residence times.

reactive nature of hydroxyl radicals. The concentration of OH radicals in the PDRF system was not measured; however, Laroussi [4] estimated the OH concentration in DBD based on relative spectroscopic measurements and reported a linear increase of OH with power, and therefore, we scaled these estimations to our corresponding power level arrived at a value of $10^{14}$ cm$^{-3}$. Others report typical concentrations of OH in atmospheric pressure discharges in the range of $10^{12}$–$10^{15}$ cm$^{-3}$ [28].

3) Ultraviolet (UV) radiation. Studies of inactivation of microorganisms using UVC radiation ($\leq$ 300 nm) have long been conducted by researchers. There is an abundant amount of empirical data available for inactivation of various microorganisms from the UVC radiation [22]. For UV interaction with *E. coli*, we have a reaction rate constant of $3.76 \cdot 10^{-3}$ cm$^2/\mu$J [22]. Measurements of UV in the PDRF system showed an intensity of approximately 30 $\mu$W/cm$^2$.

A 1-ms timescale was used for *E. coli*'*s* direct contact with the DBGD plasma as this corresponds to the approximate time of exposure estimated in the experimental system. The effect of *E. coli* in contact with postplasma products (namely, ozone) during recirculation through the system is not estimated at this time. Control losses, or physical losses of bioaerosol particles on the walls of the PDRF system, were incorporated in these estimations; however, these losses are almost negligible both in comparison to the effect of the chemical species and due to the very short timescale used here. The rate constant of control loss is approximately $4.5 \cdot 10^{-3}$ per CFU per second.

The simulation results shown in Fig. 6 predict a 13.49% decrease in viable *E. coli* with a 1-ms exposure time in DBGD plasma with a consideration of ozone, hydroxyl, and UV only. Hydroxyl itself is shown to cause a 13.44% reduction in viable *E. coli*, while ozone and UV have an almost negligible impact with reductions of 0.04 and 0.01%, respectively. It is apparent that these results cannot completely explain the experimental result achieved with the PDRF experimental system (97% decrease during the same times and modes of treatment). However, the simulation results both coincide with and quantify the conclusions of many plasma sterilization researchers, which is that ozone, hydroxyl, and UV radiation have a limited role in the bacterial sterilization process in direct exposure with the DBD plasmas. Charged species, such as ions and electrons, atomic oxygen, and electronically excited species have been shown to have a significant role in sterilization and may act in tandem with other radicals and chemical species in a synergistic way. Choi *et al.* [30] have shown the importance of physical damage to the structure of bacteria from ions present in DBD discharges. One explanation of this mechanism is the initial negative charging of a bioaerosol droplet (containing microorganisms) within plasma due to the attachment of fast-moving electrons, followed by ion bombardment onto the microorganism's surface at relatively high energies. This is analogous to etching ("strong etching," as described by Park [31] and Moisan [32]). Such etching can provide openings for active species to interact faster with the microorganism, leading to a synergistic sterilization effect.

Additionally, Hermann *et al.* [33] discuss the possibility of inactivation by excited species from the afterglow of an atmospheric pressure plasma jet. Usually, operated in helium to avoid instability, the discharge is doped with a few percent of molecular gases such as $O_2$. These oxygen-containing active species may play a role via deexcitation and subsequent energy transfer onto the microorganism's surface. This may further provide assistance to overcome energy barriers, if any, during synergistic chemical interactions between the active species and the microorganisms. Similarly, Pointu *et al.* [34] showed that the excited species play a more important role in comparison to UV in their atmospheric plasma system.

While the numerical characterization scheme presented here is somewhat oversimplified, in that it only takes into account the effect of ozone, hydroxyl, and UV radiation, it remains a useful tool that researchers can exploit to identify which species and combinations of species are responsible to inactivation. Further investigation and development is needed, with an emphasis on empirical data of the sterilizing effect of individual plasma factors on microorganisms, in order to advance these estimations into a full working model.

## V. Conclusion

The PDRF is a bioaerosol decontamination installation that combines DBGD with a filterless ventilation system for the purpose of destroying high concentrations of bacterial bioaerosols from indoor air.

The PDRF system is unique and has the capability of experimenting with virtually any type of airborne microorganism. The results presented here show that the PDRF system can achieve an $\sim$5-log reduction (99.999%) in viable *E. coli* with

a millisecond direct exposure time in the DBD ventilation grating (DBGD) device without the use of a filter to trap and treat airborne particles. These results are unique because, in most DBD surface sterilization studies, treatment times are always at least 1000 times longer on the order of seconds and, in some cases, even minutes in duration. This concept of a filterless system has shown that a very short exposure time of bioaerosol to DBGD plasma can cause rapid inactivation of microorganisms.

A simple numerical characterization study was performed in an attempt to quantify the sterilizing effect of three well-known plasma factors (O3, OH, and UV) on the *E. coli* bacteria when treated inside our DBGD plasma device. The results show that hydroxyl itself has a significant effect (responsible for $\sim$13.5% of those killed); however, ozone and UVC have a negligible effect at very short exposure times ($\sim$1 ms) inside plasma. These numerical estimations must be expanded to include other chemically active species: radicals (e.g., atomic oxygen), charged particles (ions and electrons), and electronically excited molecules in order to completely characterize and quantify the sterilization effect from the DBD plasma. In order to achieve this, using our methods, experimental data of the individual effect of additional species on microorganisms must be provided. A refined and expanded kinetic model that considers all biologically active plasma components and can accurately predict sterilization could have a strong and lasting impact in many areas of biomedical applications of nonthermal plasma.

Additionally, the experimental conditions of this paper closely mimic the conditions that might exist during a bioterrorist attack, namely, the release of a high concentration of bioaerosol moving at high flow rate inside of a ventilation duct. If proven robust and safe enough for use indoors, nonthermal plasma air cleaning technology could be employed in commercial and military buildings for the purpose of mitigating the detrimental effects of a pathogenic bioaerosol release by terrorists. Currently, there is no such bioterrorism prevention technology used in the majority of buildings in the U.S.

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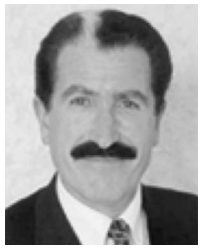

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