Bacterial Inactivation Using an RF-Powered Atmospheric Pressure Plasma

Ashish Sharma, Amy Pruden, Ovidiu Stan, Member, IEEE, and George J. Collins, Fellow, IEEE

Abstract—Cells of Escherichia coli were exposed to a downstream plasma afterglow plume emitted from a slotted plasma device operating in open air at atmospheric pressure. Various feed-gas mixtures were capacitive-ally excited, as they flowed into the open air past radio frequency-powered electrodes. To estimate the underlying inactivation pathways, various experimental conditions were tested by incorporating ultraviolet filters, varying parameters such as electrical power and frequency, feed-gas composition, position and flow rates, and the distance of the samples from the electrode. Experimental results demonstrated a colony-forming unit reduction of well over five logs with less than 2 s of exposure per unit area. These results offer a promising means of wide-area inactivation of harmful microbes in a practical environment, where the sample is neither a part of the electrical circuit nor placed in an enclosure. The device is electrically grounded and could be held like a wand applicator.

Index Terms—Atmospheric pressure plasmas, radio-frequency (RF), sterilization.

I. INTRODUCTION

The elimination of disease-causing agents from surfaces of equipment, which can sometimes be challenging to fulfill without using toxic materials or high temperatures, is an absolutely necessary requirement in many fields. Historically, many different approaches have been used to inactivate pathogens. Two widely used inactivation methods, especially in the medical field, are autoclaving and exposure to gases such as ethylene oxide (EtO). Although effective, both methods suffer from drawbacks such as exposure to extremely high temperatures (> 100 °C) in the case of autoclaves and toxic exposure in the case of EtO. Another concern with these methods is the long treatment times, which can vary from about half an hour to almost 30 h [1].

Nonequilibrium atmospheric pressure plasmas have a gas temperature of hundreds of degrees Kelvin, an electron temperature of about 1 eV, and electron number densities in the order of $10^{11−14}/cm^3$ with metastable densities that are 1–2 orders of magnitude greater than ion densities. A straightforward way of generating nonequilibrium atmospheric pressure plasmas is to operate a glow discharge at a current density below the threshold for glow-to-arc transitions. This was first demonstrated in the 1950s [2].

Several research groups have been investigating the development of atmospheric plasma technology for inactivation of bacteria [3]–[8]. Many different electrode configurations, feed-gas mixtures, and operating conditions have been tested with varying results [3]. Some groups achieve operations at very high close to room temperature, but the treatment methodology is not suitable for wide-area treatment as the plume area is relatively small [4]. Other researchers have created chambers that can be used for treating the contaminated specimen [5], [6]. However, a drawback with this approach is that device portability and convenience of use are compromised. A research group has addressed this issue by creating a portable backpack decontamination wand that involves remote exposure of samples [7], [5]. Still, other research groups have developed needle plasmas [8], [9] that work at relatively low voltages but are not suitable for wide-area treatment.

Our research focuses on radio-frequency (RF)-driven open-air slot microplasmas that are tens of centimeters in length. The 62 active electrodes excite a rare gas mixture, and the effluents flow into open air as an afterglow plasma plume at near room temperature. The powered wedge electrode is recessed within a volume behind the grounded slot (Fig. 1), as is much of the associated interelectrode plasma, so that (external) work pieces require no electrical connection or sealed chamber to operate. The two electrodes of extended length are spaced fractions of millimeters from each other. This close-coupled plasma-electrode design facilitates surface electrode phenomena, as cold emission of electrons from surfaces crucial to a sustainable plasma operation via photo- and ion-induced secondary emission.

The slot plasma has unique geometry and operating conditions compared to prior works [10]–[13]. First, operation in 76 open air with a line source plasma afterglow of a high (> 900) aspect ratio that is spatially homogeneous even at hundreds of watts of delivered RF power. Second, the work piece is neither placed on an electrode nor a part of the electrical circuits but is placed rather in the afterglow plume. Third, the work pieces can be about 1 cm away from the active plasma with a clear optical path to the surfaces to be treated, allowing for high fluxes of plasma species and high-energy photons from the active plasma. It is judged that this electrode geometry is scalable to meter-long lengths, allowing for wide-area treatment. The features previously listed make the device highly attractive to be used as a portable handheld decontaminating wand.
power applied. (Color version available online at http://ieeexplore.ieee.org.)

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II. PROCEDURES AND EXPERIMENTAL SETUP

A. RF Hollow-Slot Plasma Device

Recent publications present the detailed technical aspects96
of the hollow-slot plasma reactor [10]–[14]. The hollow-slot
98 electrode configuration used for this paper consisted of two
99 electrodes, as shown in Fig. 1, which are coming out and into
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101 plume emerging from the slot into open air is shown in the inset
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103 gas flow, and applied RF voltage locations are also indicated
104 in Fig. 1. An external open slot electrically grounded hollow
105 electrode opposes an internal RF-powered wedge-shaped elec-
106 trode. It is judged that this electrode design allows a corona-
107 initiated ignition and subsequent glow confinement of the linear
108 plasma to the electrode area. This allows for two distinct mi-
109 crodischarge regions to be formed, namely: 1) a luminous glow
110 of active discharge between the electrodes and 2) a downstream
111 afterglow plume. In between the two regions is a gas expansion
112 region. The open slot width w is fixed at 200 µm for current
113 studies but is variable from 50 to 800 µm. Similarly, for the
114 results presented in this paper, the interelectrode spacing d was
115 fixed at 500 µm, but other values are possible. The length of the
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Fig. 1. Schematic diagram of the device, showing critical dimensions w (slot width) and d (interelectrode spacing). The major regions are active discharge and downstream afterglow plume. The work piece surface, where bacterial inactivation occurs, is 2.5–10 mm away from the active plasma in the afterglow plum. A photo of the plume emerging from the linear slot is presented in the bottom right inset. The length of the linear slot can be extended to 30 cm, depending on the electrode design, gas flow, rare gas employed, and total RF power applied. (Color version available online at http://ieeexplore.ieee.org.)
Fig. 2. Schematic of the inactivation methodology.

Dishes containing 9 mL of LB agar. The Petri dishes with the filter membranes placed on the LB agar were exposed to the afterglow plume emitted from the grounded hollow-slot electrode for the required time. After the plasma treatment, the petri dishes were incubated at 37°C for one day, prior to determining the resulting number of colony-forming units (CFU). The experimental process is depicted in Fig. 2.

C. Varied Conditions

To better understand the underlying mechanism of inactivation and to investigate the roles played by different plasma constituents, a variety of experiments were performed, testing various configurations.

1) Gas ON, Plasma OFF: To ensure that the bacteria were being inactivated by the actual plasma (radical and photon species) and not merely being blown off the surface by the gas flow, a control experiment was performed, in which the bacteria-laden filter membranes were exposed only to gas flowing through the electrodes with the power turned off.

2) Distance From the Electrode: As the plume exits from the electrodes into open air, the conditions downstream are expected to vary with the distance from the electrode. To study the impact of the sample distance from the electrode on inactivation, an experiment was performed, in which the distance of the samples was varied from 0.25 to 1.00 cm. The temperatures to which the samples were exposed were measured using temperature strips (±1°C, Omega, Stamford, CT).

3) Frequency: The plasma characteristics can change dramatically with change in the frequency of the RF power supplied to the powered electrode. To determine the exact nature of this change on the inactivation levels, a comparison of inactivation was done by powering the device at two different frequencies: 13.56 and 60 MHz. For this range of frequencies, rare gas flow was required, but at 160-MHz excitation, it must be noted that the required rare gas flow is much reduced, but these results will be reported elsewhere.

4) Oxygen: For the baseline experiments, the main gas fed to the device was argon, supplemented with a fixed and minute amount of oxygen. To determine the effect of oxygen on inactivation, an experiment was conducted, where varying amounts of oxygen were mixed with the main argon feed-gas. This was done in order to explore the possibility of enhancing the formation of oxygen radicals, which are powerful species with the potential to destroy bacterial cells.

5) UV Effect: A significant amount of UV radiation is known to be produced by the plasma reactor. UV is well known for its ability to inactivate microbes and is thus used widely in the drinking water industry as a final disinfectant. Two different approaches were used to estimate the role of UV in bacterial inactivation by the plasma plume. First, a magnesium fluoride (MgF₂) window was placed between the device and the samples. Magnesium fluoride allows UV light above 100 nm while blocking particles such as radicals and ions. This provided a means to isolate the effect of UV. A variation of this experiment was also conducted by covering the sample with the polystyrene lid of the petri dish, which blocked both particles and UV below 300 nm.

Second, spectroscopic analysis of the plasma plume was performed in order to qualitatively determine the nature of the...
Fig. 3. Survival curve of *E. coli* when exposed at a distance of 0.5 cm from the electrode.

UV flux emitted. The optical spectra $I(\lambda)$ were obtained using a 0.2-m McPherson VM 502 scanning monochromator, with a grating of 1200 g/mm blazed at 120 nm. Light was detected using an Acton 781 photomultiplier tube. The optical emission was measured by placing the linear slot plasma in close proximity, and parallel, to the entrance slit of the spectrometer. The spectral resolution of the detection system (full-width at half-maximum (FWHM) of slit function) was about 0.7 nm. An optical window (MgF$_2$) and a mask were employed to define the emitting area. The output spectra correspond to light emitted from a 4-mm-long slot (defined by the mask).

6) **Gas Composition and Flow:** Discharge characteristics are known to vary with the type of feed-gas used. To determine the role of gas composition and flow in the inactivation, argon was compared with helium as the primary feed-gas at different flow rates.

7) **Power:** Finally, different levels of power were transmitted through the electrodes to estimate the amount of energy needed to achieve the desired level of inactivation.

III. RESULTS AND DISCUSSION

The exposure of *E. coli* to the downstream plasma plume generated by the RF-powered hollow-slot device was observed to cause a total inactivation of well over five logs in less than 2 s (per unit area). A survival curve for the baseline condition of the argon gas at a flow rate of 20 L/min powered by a 60-MHz device is shown in Fig. 3. This demonstrated that the 60-MHz plasma was capable of microbial inactivation, as was observed previously for the 13.56 MHz [14], and defined the baseline for subsequent experiments. In the following sections, the effect of changing various operational parameters is presented, and insights with respect to the inactivation pathways are discussed.

A. **Gas ON, Plasma OFF**

The gas flow with the instrument switched off was not observed to have an effect on bacterial inactivation (Fig. 4). This confirmed that the plasma plume, which contains radicals and photons, was the driving force in the bacterial inactivation.

B. **Distance From the Electrode**

As shown in Fig. 5, as the distance of the sample was varied from 0.25 to 1 cm away from the electrode, there was no significant change in the relative inactivation of *E. coli*. This was in spite of the gradient in temperature, which was observed between 40 °C and 70 °C. This indicates that heat does not play a significant role in inactivation, considering that the temperature was less than 40 °C at a distance of 1 cm away from the electrode.

C. **Frequency**

A comparison of the inactivation while powering the device with two different power supplies, one operating at 13.56 MHz and the other at 60 MHz, is shown in Fig. 6. It can be deduced that the higher fluxes produced by the 60-MHz one resulted in a higher level of inactivation. It is judged that this is due to the different radical and photon distributions measured for the two different RFs.
Fig. 6. Comparison of the inactivation of \textit{E. coli} by 60- and 13.56-MHz-powered devices.

Fig. 7. Plot comparing the effect of oxygen flow on inactivation.

D. Oxygen

Oxygen can play an important role in the inactivation process because of its decomposition into atomic oxygen, which is a powerful oxidant. In addition, radicals such as \textit{OH} may form from atmospheric water vapor dissociation, which may physically attack the cells. To test this, minute quantities of oxygen were added to the main feed-gas of argon. However, because the device operates in open air, there is always some atmospheric oxygen present. As shown in Fig. 7, the inactivation rate was not significantly affected by oxygen addition. In fact, the average inactivation rate was slightly lower when the highest amount of oxygen was added to the feed. A reason for this might be that the addition of oxygen made the discharge slightly unstable and hence had a net negative influence on the flux of active species.

E. UV Effect

The germicidal effects of UV light are well known. UV light inactivates organisms by absorption of the photons, which causes a photochemical reaction that alters molecular components that are essential to cell function. As UV rays penetrate the cell wall of the microorganism, the energy reacts with nucleic acids and other vital cell components, resulting in the injury or death of the exposed cells [15]. As shown in Fig. 8, the inactivation in the sample covered with MgF$_2$ was much higher than in the sample covered with the polystyrene lid. This suggests that UV played a major role in the inactivation. In addition, as polystyrene is known to filter out light below 300 nm, the results further emphasize the role of UV in the range of 100–300 nm.

UV ranging from about 180 to about 280 nm can be very destructive to microbes for a variety of reasons. There is a sizeable amount of production of lethal ozone at about 185 nm. Ozone, like UV, is commonly used as a disinfectant in drinking water treatment. In addition, the range of 240–280 nm is known as the “germicidal range,” where the microbial deoxyribonucleic acid (DNA) absorbs the most energy, with the region around 265 nm being the “peak germicidal range” (depends on the guanine–cytosine (GC) content of the DNA). The spectroscopic analysis of the device is shown in Fig. 9 and indicates that the transitions observed are within the ozone production range as well as the germicidal range.
IV. Conclusion

Based on the results, the principal conclusions that can be drawn are given as follows: UV plays a primary role in the inactivation process, whereas plasma radicals play a secondary role. However, a synergistic effect was observed in the combination of UV and radicals, which caused a higher rate of inactivation than possible using the two treatments in isolation. Gas temperature within the range observed in this paper had a negligible effect on the inactivation.

Further research needs to be carried out to understand the impact of the plasma constituents on entities at the cellular and molecular levels. The use of air in place of rare gases is also possible by operating at even higher frequencies but needs to be probed further.

This technology holds significant promise to overcome the current obstacles of existing sterilization approaches.

ACKNOWLEDGMENT

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REFERENCES

Ashish Sharma received the B.E. degree in electronics and power engineering from Nagpur University, Nagpur, India, and the M.S. degree in electrical engineering from Colorado State University, Fort Collins, in 2000 and 2004, respectively. He is currently working toward the Ph.D. degree in electrical engineering at Colorado State University. His research interests include biological and environmental applications of nonthermal atmospheric pressure plasmas.

Amy Pruden received the B.S. degree in biology and the Ph.D. degree in environmental science from the University of Cincinnati, Cincinnati, OH, in 1997 and 2002, respectively. She is currently an Assistant Professor with the Department of Civil and Environmental Engineering, Colorado State University, Fort Collins. Her research interests include environmental microbiology, and waste treatment. Dr. Pruden is a member of the American Society for Microbiology, the American Water Works Association, and the American Chemical Society. She has also received the National Science Foundation CAREER Award.

Ovidiu Stan (M’06) received the M.S. degree in electronics engineering from the Polytechnic Institute, Bucharest, Romania, in 1990. He is currently working toward the Ph.D. degree in electrical engineering at Colorado State University, Fort Collins. In 1990, he joined the Research Institute for Electronic Components, where he was engaged in analog design. Since 2000, he has been a Test Engineer with Advanced Energy, Inc., Fort Collins, CO. His research interests include high-power radio-frequency measurement techniques.

George J. Collins (S’62–M’72–SM’75–F’87) received the B.S. degree from Manhattan College, Riverdale, NY, and the M.S. and Ph.D. degrees from Yale University, New Haven, CT, in 1964, 1965, and 1970, respectively, all in electrical engineering. He is currently a Professor with the Department of Electrical and Computer Engineering, Colorado State University, Fort Collins. His research interests include lasers, quantum electronics, and semiconductor processing. Dr. Collins is a Fellow of the American Physical Society and the Optical Society of America, and a Sloan Fellow (physics).
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I. INTRODUCTION

THE ELIMINATION of disease-causing agents from surfaces of equipment, which can sometimes be challenging to fulfill without using toxic materials or high temperatures, is an absolutely necessary requirement in many fields. Historically, many different approaches have been used to inactivate pathogens. Two widely used inactivation methods, especially in the medical field, are autoclaving and exposure to gases such as ethylene oxide (EtO). Although effective, both methods suffer from drawbacks such as exposure to extremely high temperatures (> 100 °C) in the case of autoclaves and toxic exposure in the case of EtO. Another concern with these methods is the long treatment times, which can vary from about half an hour to almost 30 h [1].

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Several research groups have been investigating the development of atmospheric plasma technology for inactivation of bacteria [3]–[8]. Many different electrode configurations, feed-gas mixtures, and operating conditions have been tested with varying results [3]. Some groups achieve operations at very close to room temperature, but the treatment methodology is not suitable for wide-area treatment as the plume area is relatively small [4]. Other researchers have created chambers that can be used for treating the contaminated specimen [5], [6]. However, a drawback with this approach is that device portability and convenience of use are compromised. A research group has addressed this issue by creating a portable backpack decontamination wand that involves remote exposure of samples [7], [5]. Still, other research groups have developed needle plasmas [8], [9] that work at relatively low voltages but are not suitable for wide-area treatment.

Our research focuses on radio-frequency (RF)-driven open-air slot microplasmas that are tens of centimeters in length. The 62 active electrodes excite a rare gas mixture, and the effluents flow into open air as an afterglow plasma plume at near room temperature. The powered wedge electrode is recessed within a volume behind the grounded slot (Fig. 1), as is much of the associated interelectrode plasma, so that (external) work pieces require no electrical connection or sealed chamber to operate. The two electrodes of extended length are spaced fractions of millimeters from each other. This close-coupled plasma electrode design facilitates surface electrode phenomena, such as cold emission of electrons from surfaces crucial to a stable plasma operation via photo- and ion-induced secondary emission.

The slot plasma has unique geometry and operating conditions compared to prior works [10]–[13]. First, operation in 76 open air with a line source plasma afterglow of a high (> 900) aspect ratio that is spatially homogeneous even at hundreds of watts of delivered RF power. Second, the work piece is neither placed on an electrode nor a part of the electrical circuits but is placed rather in the afterglow plume. Third, the work pieces can be about 1 cm away from the active plasma with a clear optical path to the surfaces to be treated, allowing for high fluxes of plasma species and high-energy photons from the active plasma. It is judged that this electrode geometry is scalable to meter-long lengths, allowing for wide-area treatment. The features previously listed make the device highly attractive to be used as a portable handheld decontaminating wand.
A. RF Hollow-Slot Plasma Device

Recent publications present the detailed technical aspects of the hollow-slot reactor [10]–[14]. The hollow-slot electrode configuration used for this paper consisted of two electrodes, as shown in Fig. 1, which are coming out and into the plane of this paper. A photograph of the actual plasma emerging from the slot into open air is shown in the inset of the figure. Electrode shapes, critical electrode spacing, feed gas flow, and applied RF voltage locations are also indicated in Fig. 1. An external open slot electrically grounded hollow electrode opposes an internal RF-powered wedge-shaped electrode. It is judged that this electrode design allows a corona-initiated ignition and subsequent glow confinement of the linear plasma to the electrode area. This allows for two distinct micro-discharge regions to be formed, namely: 1) a luminous glow of active discharge between the electrodes and 2) a downstream afterglow plume. In between the two regions is a gas expansion region. The open slot width is fixed at 200 µm for current studies but is variable from 50 to 800 µm. Similarly, for the results presented in this paper, the interelectrode spacing \( d \) was fixed at 500 µm, but other values are possible. The length of the electrode in this paper is approximately 75 mm, but it has also been extended to 300 mm for other applications of the device. It must be emphasized that the surfaces are processed in open air and are neither part of the electrical circuit nor placed on 119 electrodes in enclosures, in which all considerable practical advantages well beyond the present focus on bacterial treatment. In the interelectrode region, the representative operating conditions are given as follows: PD \( \sim 10 \) torr \( \cdot \) cm, average root-meansquare (rms) \( E \sim 20 \) kV/cm, average rms \( E/N \sim 70 \) Td, \( d \) current density \( \sim 0.7 \) A/cm\(^2\), associated power density \( (EJ \cos \theta) \sim 14 \) kW/cm\(^2\), and energy per volume delivered to the flowing gas \( \sim 100–200 \) J/L. A plasma afterglow plume with a linear shape of 1–30 cm long exits the grounded slot and extends 1–8 mm from the slot. Moreover, the active plasma is 129 millimeters away and delivers a strong flux of both ultraviolet (UV) and vacuum UV (VUV) photons.

The experiments were conducted in open air and without 132 windows at atmospheric pressure. An external gas flow was applied through the electrode regions using a mixture of rare gas (argon or helium) and oxygen flowing at rates of 5–20 L/min and 6–20 sccm, respectively. The reactor was powered by a 136 60-MHz RF power supply (Advanced Energy, Fort Collins, CO), and a matching network was connected between the 138 power supply and the plasma reactor to optimize power transfer. The power delivered to the reactor was varied from 140 50 to 150 W.

The samples to be exposed were kept at a fixed position, varying from 2.5 mm to 1 cm below the open reactor slot. A 143 motor drive was used to achieve the translational motion of the 144 afterglow plasma plume in order to uniformly sweep the entire 145 surface area of the target sample with the afterglow plasma, 146 creating a “push-broom” source of photons, radicals, and ions, 147 all of which may play an active role in bacterial inactivation. 148 Varying exposure times were achieved by passing the plasma 149 over the sample at various rates and varying the number of 150 passes using the motor to move the plasma slot. For instance, a 151 single pass lasting 2.5 min resulted in an effective exposure of 152 about 0.6 s/unit area.

B. Escherichia Coli (E. coli) Strain Information and Sample Preparation

\( E. coli \) (ATCC 9637, Biosafety level 1, Invitrogen, Carlsbad, CA) was used to test the sterilizing capabilities of the plasma. \( E. coli \) is a gram-negative bacterium and a common standard of reference in the development of new sterilizing technologies [3]. \( E. coli \) cultures were grown overnight in a petri dish containing Luria–Bertani (LB) agar (Difco, Sparks, MD) at 37 °C, and then, a single colony was transferred into 250 mL of 163 Bacto tryptic soy broth (Becton, Dickinson and Company, Sparks, MD), which was maintained for 8 h at 37 °C. This 165 allowed the cells to reach exponential log phase. Exponential-166 log-phase cells (10 mL) were harvested and transferred from the 167 broth under sterile conditions to the phosphate buffer solution 168 (90 mL and pH 7.0). The solution was serially diluted further to the required concentration range. Five milliliters of the 170 diluted solutions were filtered in triplicate for each 171 lution onto presterilized filter membranes [0.45-µm mixed cellulose esters, 47-mm diameter (Millipore)]. Following the 173 filtration, the membranes were placed on sterile 10-mL Petri 174
Fig. 2. Schematic of the inactivation methodology.

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177 the afterglow plume emitted from the grounded hollow-slot
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5) UV Effect: A significant amount of UV radiation is
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7) Power: Finally, different levels of power were transmitted through the electrodes to estimate the amount of energy needed to achieve the desired level of inactivation.

III. RESULTS AND DISCUSSION

The exposure of E. coli to the downstream plasma plume generated by the RF-powered hollow-slot device was observed to cause a total inactivation of well over five logs in less than 2 s (per unit area). A survival curve for the baseline condition of the argon gas at a flow rate of 20 L/min powered by a 60-MHz device is shown in Fig. 3. This demonstrated that the 60-MHz plasma was capable of microbial inactivation, as was observed previously for the 13.56 MHz [14], and defined the baseline for subsequent experiments. In the following sections, the effect of changing various operational parameters is presented, and insights with respect to the inactivation pathways are discussed.

A. Gas ON, Plasma OFF

The gas flow with the instrument switched off was not observed to have an effect on bacterial inactivation (Fig. 4). This confirmed that the plasma plume, which contains radicals and photons, was the driving force in the bacterial inactivation.

B. Distance From the Electrode

As shown in Fig. 5, as the distance of the sample was varied from 0.25 to 1 cm away from the electrode, there was no significant change in the relative inactivation of E. coli. This was in spite of the gradient in temperature, which was observed between 40 °C and 70 °C. This indicates that heat does not play a significant role in inactivation, considering that the temperature was less than 40 °C at a distance of 1 cm away from the electrode.

C. Frequency

A comparison of the inactivation while powering the device with two different power supplies, one operating at 13.56 MHz and the other at 60 MHz, is shown in Fig. 6. It can be deduced that the higher fluxes produced by the 60-MHz one resulted in a higher level of inactivation. It is judged that this is due to the different radical and photon distributions measured for the two different RFs.
D. Oxygen

Oxygen can play an important role in the inactivation process because of its decomposition into atomic oxygen, which is a powerful oxidant. In addition, radicals such as \( \text{OH} \) may form from atmospheric water vapor dissociation, which may physically attack the cells. To test this, minute quantities of oxygen were added to the main feed-gas of argon. However, because the device operates in open air, there is always some atmospheric oxygen present. As shown in Fig. 7, the inactivation rate was not significantly affected by oxygen addition. In fact, the average inactivation rate was slightly lower when the highest amount of oxygen was added to the feed. A reason for this might be that the addition of oxygen made the discharge slightly unstable and hence had a net negative influence on the flux of active species.

E. UV Effect

The germicidal effects of UV light are well known. UV light inactivates organisms by absorption of the photons, which causes a photochemical reaction that alters molecular components that are essential to cell function. As UV rays penetrate the cell wall of the microorganism, the energy reacts with nucleic acids and other vital cell components, resulting in the injury or death of the exposed cells [15]. As shown in Fig. 8, the inactivation in the sample covered with \( \text{MgF}_2 \) was much higher than in the sample covered with the polystyrene lid. This suggests that UV played a major role in the inactivation. In addition, as polystyrene is known to filter out light below 300 nm, the results further emphasize the role of UV in the range of 100–300 nm.

UV ranging from about 180 to about 280 nm can be very destructive to microbes for a variety of reasons. There is a sizeable amount of production of lethal ozone at about 185 nm. Ozone, like UV, is commonly used as a disinfectant in drinking water treatment. In addition, the range of 240–280 nm is known as the “germicidal range,” where the microbial deoxyribonucleic acid (DNA) absorbs the most energy, with the region around 265 nm being the “peak germicidal range” (depends on the guanine–cytosine (GC) content of the DNA). The spectroscopic analysis of the device is shown in Fig. 9 and indicates that the transitions observed are within the ozone production range as well as the germicidal range.
F. Gas Composition and Flow

The results of the experiments comparing argon and helium at different flow rates are presented in Fig. 10. The results indicate that helium was not as effective as argon in inactivating the bacteria. A possible reason could be because of the lack of UV generation in the germicidal range. With respect to flow rates, it was observed that in the case of argon, the inactivation rate was higher for lower flow rates. This could be attributed to the fact that the plasma constituents remain in the vicinity at lower flow rates. On the contrary, average inactivation levels actually increased, however only slightly, with increased He flow. This could be due to the minor role that temperature plays in the inactivation, as observed in Fig. 5.

G. Power Variation

As evident in Fig. 11, increasing the power yielded increased inactivation. This can be attributed to more dissociation of radicals and higher photon fluxes at higher power levels, as gas temperature measurements confirmed that there was only a minor effect on gas heating (data not shown).

IV. Conclusion

Based on the results, the principal conclusions that can be drawn are given as follows: UV plays a primary role in the inactivation process, whereas plasma radicals play a secondary role. However, a synergistic effect was observed in the combination of UV and radicals, which caused a higher rate of inactivation than possible using the two treatments in isolation. Gas temperature within the range observed in this paper had a negligible effect on the inactivation.

Further research needs to be carried out to understand the impact of the plasma constituents on entities at the cellular and molecular levels. The use of air in place of rare gases is also possible by operating at even higher frequencies but needs to be probed further.

This technology holds significant promise to overcome the current obstacles of existing sterilization approaches.
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