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Bacterial Inactivation in Open Air by the Afterglow Plume Emitted from a Grounded Hollow Slot Electrode

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Escherichia coli, *Bacillus atrophaeus,* and *Bacillus atrophaeus* spores were exposed to a downstream plasma afterglow plume emitted from a slotted plasma device operating in open air at atmospheric pressure. The reactor electrodes were RF powered at 13.56 MHz to excite a mixture of argon and oxygen gases by a capacitive discharge as it flowed past the electrodes into open air. Bacterial inactivation experiments on surfaces exposed to the plasma afterglow were conducted with varying plasma exposure times. Experimental results demonstrated a colony forming unit (CFU) reduction of almost 5 $\log_{10}$ of *E. coli* with only 1 s of exposure per unit area. One log CFU reduction was observed in *B. atrophaeus* with the same treatment time of 1 s per unit area. *B. atrophaeus* spores showed a reduction of 3 $\log_{10}$ with exposure time of 10 min. Comparison on various growth media suggests that cells are killed rather than sublethally injured, while the mechanistic action of the plasma appears to affect both nucleic acids as well as the cell wall structure. These results present a promising means of inactivation of harmful microbes in a practical environment with an electrically grounded device that is handheld, much like a wand applicator. Results are applicable to the development of plasma sterilization tools for various environmental purposes.

Introduction

Eradication of harmful microorganisms in the environment has been an important goal since they were first discovered and understood. Now with increasing concern over problems such as bioterrorism and the recent rise of antibiotic resistant pathogens, new technologies for sterilization are especially in demand. Different approaches have been employed over the years to inactivate pathogenic microorganisms. Many of the more reliable conventional techniques used until now for sterilization involve exposure to toxic compounds such as ethylene oxide or chlorine gas or exposing the specimen to high temperatures and pressures in an autoclave (*1*). Besides being time-consuming, these techniques can damage sensitive materials and also lead to the inadvertent creation and release of poisonous compounds. Subsequently, such techniques can pose a hazard to the operating personnel and also become a significant problem for waste disposal (*2*). These drawbacks especially render such approaches counterproductive to the environmental field, which seeks to reduce hazardous compound use and production and eliminate risk to humans. These problems are equally undesirable in the food and pharmacology packaging industries and in the sterilization of medical instrumentation.

Atmospheric pressure glow discharges provide a means of microbial inactivation which eliminates harmful gas emissions and is capable of operation at high rates with reduced temperature (*3*). Moreover, operation at atmospheric pressure makes the process feasible for use in open air, which significantly reduces the cost of application. Several research groups have developed atmospheric plasmas driven by DC, AC, pulsed, RF, and microwave (*4*−*7*), and these recent advances in plasma research present the possibility of promising new tools for the environmental field. One research group has developed a one atmosphere uniform glow discharge plasma (OAUGDP) reactor (*1*) in which the electrodes are housed in a container of dimensions 40 × 35 × 35 cm and inactivation rates of 6 $\log_{10}$ were achieved with 5 min of exposure using *Escherichia coli* embedded in agar medium. Another commercial system that employs a plasma combined with hydrogen peroxide as the active mechanism of inactivation has recently become available, but its requirement to operate under vacuum is not ideal. [Johnson and Johnson Advanced Sterilization Products (ASP) sells the STERRAD 50 sterilizer, which operates in a vacuum.]

The approach presented in this study is focused on open-air operation with wedge-open slot electrodes that are driven by RF at frequencies from 4 to 60 MHz with a flow of rare gas through the electrodes forming an afterglow plume. The powered electrode is enclosed within a grounded but slotted electrode so the device may be handheld. The work piece to be sterilized is placed in the plasma plume, a few millimeters away from the slotted electrode. A remarkable feature of this new wandlike device was that only 1 s was required to achieve close to 5 $\log_{10}$ reduction in *E. coli*.

Experimental Procedures

Open-Air Hollow Slot Plasma Reactor with Afterglow Plume. Recent publications of our work present the technical detail of the conceptual design and development of the hollow slot plasma reactor (*8*, *9*). The hollow slot electrode plasma reactor used for this study consisted of two electrodes as shown in Figure 1 which are coming out and into the plane of the paper. Included in this figure inset is a photograph of the actual plasma plume emerging from the slot. Electrode shapes, critical electrode spacing, feed gas flow, and applied RF voltage locations are also indicated in Figure 1. An open-slot electrically grounded hollow electrode opposes an RF powered wedge-shaped electrode. This electrode design creates a corona-initiated ignition and glow confinement to the electrode area, which is a main feature of this device. This allows for two distinct microdischarge regions to be formed: a luminous glow of active discharge between electrodes and a downstream afterglow plume. The open-slot width (w) is fixed at 200 μm for current studies but is variable from 50 to 800 μm. For the results presented in this report, the interelectrode spacing (d) has been varied from 400 μm to 600 μm in steps of 100 μm, but other values are possible. The length of the electrode in this study is approximately 75 mm (3 in.), but it has also been extended to 300 mm for other practical applications of the device. The open slot through which plasma species flow is electrically grounded and the powered electrode is recessed safely inside the slot structure, thereby allowing the placement of sensitive work pieces near the open slot. In the interelectrode region,

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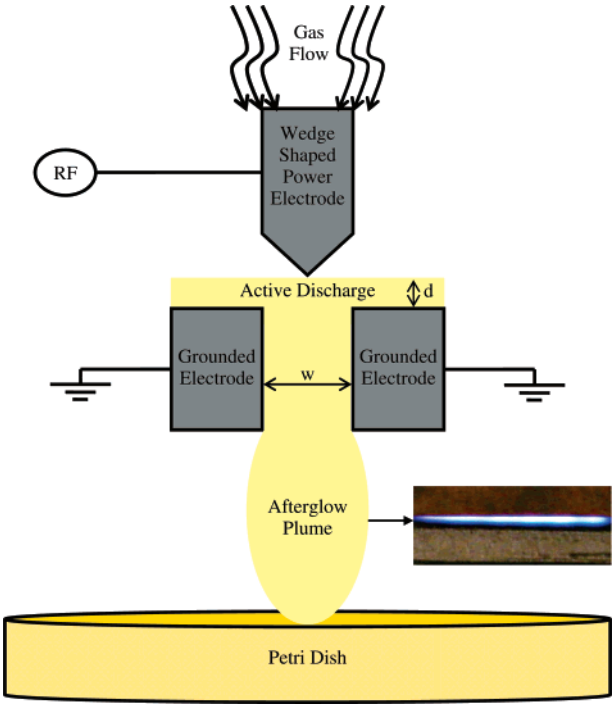

FIGURE 1. Schematic diagram of the reactor employed in this work showing critical dimensions *w* (slot width) and *d* (wedge to grounded slot interelectrode spacing) as well as the active discharge, downstream plume regions, and the location of the work piece surface where bacterial inactivation occurs. A photo of the actual plume emerging from the slot is presented in the lower right inset. The length of the slot is 2−8-mm long, depending on gas flow, the rare gas employed, and total RF power applied. The Petri dish is 4−6 mm away from the grounded electrodes.

representative operating conditions are pressure-distance product (PD) ~ 10 Torr-cm, average root mean square (RMS) $E \sim 20$ kV/cm, average RMS $E/N \sim 70$ Townsend (Td), current density ~ 0.7 A/cm^2, associated power density (E J cos θ) ~ 14 kW/cm^3, and energy per volume delivered to the flowing gas ~ 50−150 J/L. A plasma afterglow plume of linear shape exits the grounded slot and extends 1−8 mm from the slot.

The experiments were conducted in open air and without windows at atmospheric pressure. External gas flow was applied through the electrode regions using a mixture of argon and oxygen flowing at rates of 32 L/min and 6 standard cm^3/min, respectively. The reactor was fed by an RF power supply and a matching network connected between the power supply and the plasma reactor to optimize power transmission. Power delivered to the reactor was 177 W and current (RMS) was 1.5 A. The RF voltage at which the plasma formed was 203 V.

The samples to be exposed were kept at a fixed position between 1 and 8 mm under the open reactor slot. A motor drive was used to achieve translational motion of the afterglow plasma plume to uniformly sweep the entire surface area of the target sample with the afterglow plasma, creating a "push-broom" source of photons, radicals, and ions, all of which may play an active role in bacterial inactivation. Varying amounts of exposure time were achieved by passing the plasma slot over the sample at various fixed rates and varying the number of passes using a motor to move the plasma slot.

Microbial Strains and Preparation. *Escherichia coli. E. coli* (ATCC 9637, Biosafety level 1) served as the primary strain used to test the sterilizing capabilities of the plasma under various conditions. *E. coli* is a Gram-negative bacterium and is a common standard of reference in the development of new sterilizing technologies (*3*). The strain was obtained from Invitrogen (Carlsbad, CA) with the following genotype: F− *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lac*ZΔM15 Δ*lac*X74 *deo*R *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (*Str*R) *end*A1 *nup*G. The strain also carried a low-copy-number F plasmid containing resistance genes both to ampicillin and kanamycin antibiotics.

E. coli cultures were grown on LB agar (Difco, Sparks, MD) for 2 days at 37 °C prior to experimentation. Cells were subsequently transferred from the plate under sterile conditions to 250 mL of phosphate buffer solution (PBS, pH 7.0). The solution was serially diluted to the required concentration range. Five milliliters of the solution was filtered in triplicate for each dilution onto presterilized filter membranes (0.45-μm mixed cellulose esters, 47-mm diameter (Millipore, Pittsburgh, PA)). Following filtration, the membranes were placed on sterile 10-mL Petri dishes containing 9 mL of LB agar. The Petri dishes with the filter membranes in place 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 1 day prior to determining the resulting number of colony forming units (CFU).

Bacillus atrophaeus. Bacillus atrophaeus is a gram-positive bacterium and thus contains thicker cell walls than *E. coli*. *B. atrophaeus* is also a commonly used reference strain in sterilization experiments. The thicker layers of peptidoglycan in the cell walls of *B. atrophaeus* are considered to render the strain more resistant to sterilization than *E. coli*. *B. atrophaeus* was obtained from the American Type Culture Collection (ATCC, Manassas, VA) and grown under recommended conditions at 30 °C. For our experiments, we maintained the cultures on LB agar plates. *B. atrophaeus* cells were prepared for experimentation as described above for *E. coli*.

Bacillus atrophaeus Spores. Bacillus atrophaeus spores (ATCC No. 9372) were in the form of strips (3.81 cm × 0.64 cm) commonly used for verifying autoclave efficiency. The strips were obtained from Raven Biological Laboratories, INC, NE. These strips were certified to have an average initial CFU count of 1.2×10^7. The experiment on the *B. atrophaeus* spores was conducted by exposing the test strips in triplicate lengthwise under the plasma slot for 1, 5, or 10 min on each side. The control strip was unexposed to the plasma. After exposure, the strips were immersed in 5 mL of sterile tryptic soy broth (TSB, Difco) in 10-mL glass culture tubes and maintained on a rotary shaker for 21 h at ambient temperature. Thereafter, the resulting broth was serially diluted and the turbidity was determined using an HACH (Loveland, CO) 2100 N turbidimeter. The diluted solutions were also plated onto LB agar plates and grown for 24 h at 30 °C to determine the resulting CFU.

Experimental Conditions. *Industrial Grade Argon versus Purified Argon.* One of the goals in the development of this sterilization device is a reduction of cost. Therefore, the relative effect of operating the plasma with either purified grade or industrial grade argon was determined using *E. coli* as the target strain. The instrument was originally configured for operation with purified argon; however, if industrial grade argon is comparable, this would represent a significant reduction in cost. All gases used in this study were obtained from General Air, Inc., Fort Collins, CO.

Comparison of Growth Media. To distinguish lethal from sublethal effects of the plasma, the effect of different growth media was determined with *E. coli*. First LB agar, a highly nutrient-rich medium, was compared with R2A agar (Difco), a nutrient poor medium commonly used to enumerate cells in drinking water. Because high nutrient concentrations can actually inhibit the growth of injured cells, using R2A is a common method of preventing this inhibition and enumerating cells which have been sublethally injured. Second, the effect of adding ampicillin to the LB medium was determined.

Ampicillin is a β-Lactam antibiotic which blocks the final cross-linking stage of the pathway which occurs in bacterial cell walls. It acts by inhibiting the synthesis of peptidoglycan, an important component of bacterial cell walls (*10*). The purpose of this experiment was to determine whether antibiotic present in the medium would further inhibit growth of sublethally injured cells which may have been capable of growth on LB medium. For these experiments, filter-sterilized ampicillin (Fisher, Fairlawn, NJ) was added to the LB agar at a concentration of 0.1 mg/L after autoclaving. *E. coli* containing the ampicillin/kanamycin resistance plasmid were grown on LB/ampicillin plates prior to experimentation. Finally, the effect of adding kanamycin to the LB medium was determined. In contrast to ampicillin, kanamycin is an aminoglycoside−aminocyclitol type of antibiotic, which disrupts 30S ribosomal subunit of the bacteria (*10*). Kanamycin was added to LB agar at a concentration of 0.03 mg/L prior to experimentation.

Effect of Plasma on Bacterial DNA. Because the plasma is known to produce UV light, it was considered that UV damage to nucleic acids present in the cells may present a functional aspect of the plasma action. The DNA tested was a 1500 bp polymerase chain reaction (PCR) amplification product of microbial community DNA (from soil). The 1500 bp product was chosen, rather than genomic DNA, so that a segment of microbial DNA of defined length could be tested. This allowed the possibility to identify DNA fragmenting by the plasma. To test the effect of the plasma on DNA, two different experiments were conducted: In the first experiment, the same pool of 1500 bp DNA was loaded onto an agarose gel at the same concentration and volume into eight separate wells. The gel was run until sufficient separation allowed 1500 bp DNA gel slices to be excised. The gel slices were then exposed to (i) low power (57 W) for 5 min, (ii) medium power (114 W) for 1, 3, or 5 min, or (iii) high power (177 W) for 1, 3, or 5 min. The DNA was then extracted from the treated gel slices using a GeneClean Spin Kit (QBiogene, Carlsbad, CA). The extracted DNA was then run on a second agarose gel, stained with ethidium bromide (Bio-Rad, Hercules, CA), and visualized under UV light to determine the effect. In the second experiment, the same DNA was transferred in liquid form to nitrocellulose filter membranes slices. The membranes were then exposed to the plasma at high power in triplicate for 1, 3, or 5 min. The DNA present on the membranes was then extracted into purified water and an agarose gel was run to determine the effect.

Electron Microscopy. Scanning electron microscopy (SEM) was used to determine whether the plasma had a physical effect on the structure of the cells. *E. coli* cells were prepared as described above except that Nuclepore (Whatman, Clifton, NJ) membranes were used, which are specialized for microscopy. Both plasma-exposed (2 s per unit area) and unexposed cells were investigated using SEM. SEM was carried out with the assistance of the Colorado State University Microscopy Center using JEOL JSM6500F thermal field emission scanning electron microscope.

Results and Discussion

Inactivation of Various Microbes. The plasma plume clearly had a lethal effect on all microbes tested with a treatment time of about 1 s for nonsporulated forms. The effect of the plasma plume was observed to differ with the kind of microbe tested. In Figure 2, a comparison of control and plasma-treated *E. coli* is shown. In this experiment, a reduction of almost 5 $\log_{10}$ was observed. The *B. atrophaeus* experiment is shown in Figure 3. Here, it was observed that *B. atrophaeus* was more challenging to inactivate, as expected considering the thicker cell walls. However, one log removal was observed in a little over 1 s per unit area. The survival curve of the experiment on *B. atrophaeus* spores is shown in Figure 4.

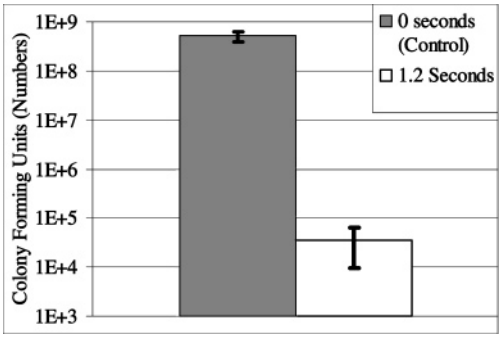

FIGURE 2. Log survivors of *E. coli* on LB agar after exposure to open-air plasma plume.

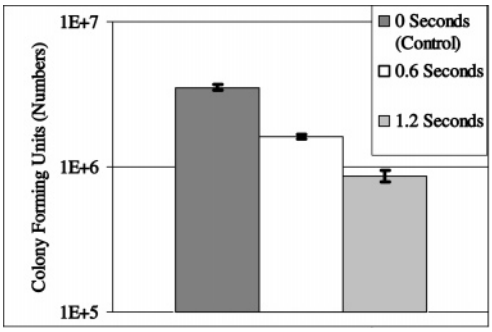

FIGURE 3. Log survivors of *B. atrophaeus* on LB agar after exposure to open-air plasma plume.

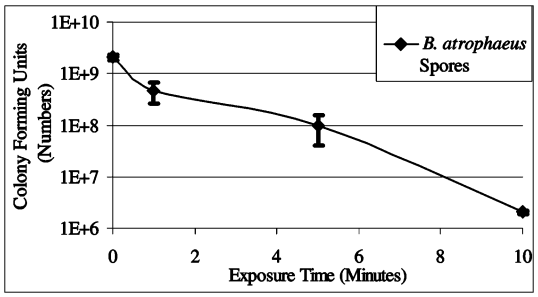

FIGURE 4. Survival curve of *B. atrophaeus* spores on LB agar after exposure to open-air plasma plume and 24 h of regrowth.

Bacterial spores are considered to be one of the most difficult forms of bacteria to kill (*11*) and are commonly used to test the efficiency of sterilization devices, such as autoclaves. An impressive 3 $\log_{10}$ removal of spores was observed with 10 min of exposure on each side of the spore strip. The differences observed with the three kinds of microbes investigated suggest that cell wall structure plays an important role in the inactivation efficiency of the plasma. This is of interest considering that an alternative application of this particular plasma configuration is surface etching, and thus one function of the plasma may be to "etch" the cell walls of the microbes.

Effect of Industrial versus Purified Grade Argon. The plasma showed no noticeable changes in performance when operated with industrial grade rather than the prepurified grade argon. The difference in its inactivation rate was not significant, but on average inactivation of *E. coli* actually improved when the industrial grade argon was used (Figure 5). One possible cause of this effect was that the presence of water vapor in the industrial grade argon formed additional radicals such as OH in the plasma which enhance its sterilizing effect. This result was very encouraging considering that the cost of industrial grade argon was 8.5 cents per cubic foot, where as the ultrahigh purity grade argon was 56.7 cents per cubic foot, which represents almost a 7-fold increase in

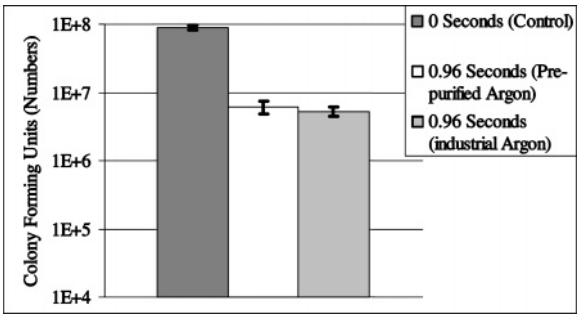

FIGURE 5. Comparison of log survival of *E. coli* using plasmas operated with either industrial grade or prepurified argon.

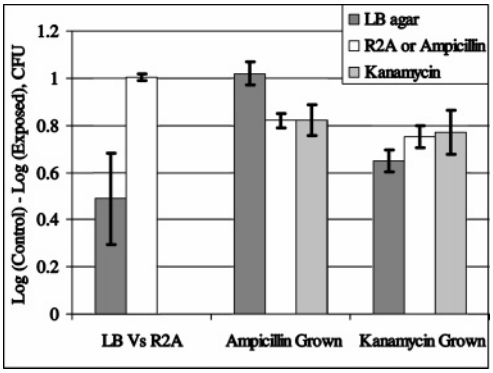

FIGURE 6. Log kill of *E. coli* after 0.48-s exposure to afterglow plasma plume under different growth conditions.

cost. Therefore, the remaining experiments were conducted with industrial purity argon. While the present study has provided proof-of-concept that the plasma configuration is effective for microbial inactivation and uses a rare gas as baseline, it is anticipated that future experiments will explore operation with alternative gases. Air is promising and would be ideal in terms of cost. The use of amendments, such as water vapor and hydrogen peroxide, is also worthy of further investigation.

Effect of Growth Medium. To verify that the CFU counts were representative of both viable and any sublethally injured cells, as well as to investigate the mechanistic action of the plasma, the effect of various growth media was tested. Figure 6 compares the log removal observed on the various growth media for the three sets of experiments. Here, it is demonstrated that there was no difference between the log removal of cells grown on any of the three media with their respective controls, with the exception of the R2A medium versus LB. LB actually showed slightly more growth after treatment, indicating that if there were sublethally injured cells present, it was better at fostering their growth. This was unexpected, since R2A is commonly used to grow sublethally injured cells, but in this case it could be that the higher nutrient concentrations present in LB actually helped the injured cells to grow.

The effect of antibiotic addition can provide some indication of the mechanism of action of the plasma. Because ampicillin acts by suppressing the production of peptidoglycan, an essential component of bacterial cell walls, any difference in results when treated cells are grown on ampicillin may indicate an effect on the cell walls. In this case, greater removal for exposed cells grown on ampicillin would be expected. Kanamycin, on the other, has an entirely different mode of action than ampicillin and acts by directly inhibiting the 30S subunit of the bacterial ribosome. If the effect were directly on the nucleic acids of the cell, then greater log removal for plasma-treated cells grown on the kanamycin would be expected. The experiments both with ampicillin and kanamycin, however, showed no significant difference in the log removal with respect to each other or their respective controls (Figure 6). While this provided further confirmation that LB was sufficient for growing sublethally injured cells, it did not provide significant information regarding the mechanism of the plasma inactivation. The action of the plasma may thus affect either or both cell walls and nucleic acids. Future work will measure both the absolute photon flux from these devices as well as the radical flux levels of oxygen radicals to better understand inactivation mechanisms under the experimental conditions (*12*).

Effect of Plasma on DNA. Both experiments in which DNA was exposed to the plasma clearly demonstrated that it had a direct effect on DNA. In the first experiment in which the exposed DNA was present in agarose gel slices, an obvious difference could be seen in the remaining DNA with respect to power (low, medium, high) and exposure time (1, 3, and 5 min) (Figure 7). The observed effect was that the ethidium bromide stained DNA lost signal intensity with increased power and exposure time. This is an indicator of degradation of the DNA. No DNA fragmenting was observed, however, and any DNA remaining at the end of the experiment was the same molecular weight (1500 bp) as the original DNA. An interesting note is that the gel slices containing DNA were observed to "glow" under the plasma, which provides further confirmation that UV was produced by the plasma.

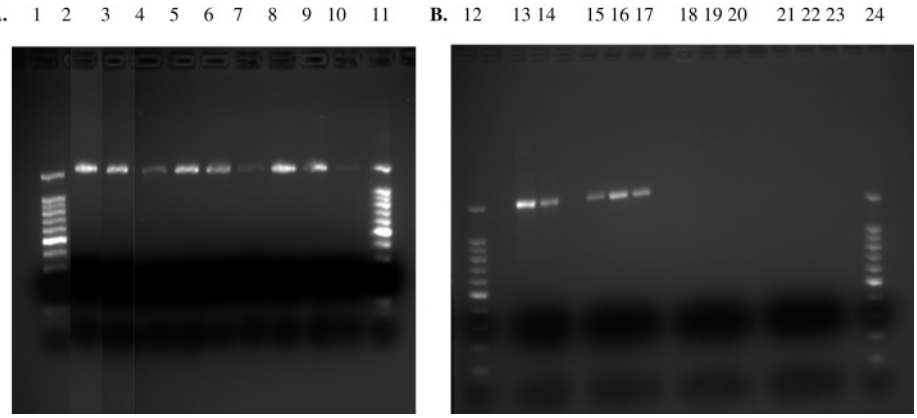

FIGURE 7. Exposure of ~1500 bp PCR product of microbial community DNA run by agarose gel electrophoresis stained with ethidium bromide and visualized under UV light. Lanes 1, 11, 12, 24: 1500 bp ladder, topmost band is 1500 bp, (A) DNA exposed in agarose gel slices: 2, 3: untreated control; 4: 5 min at low power (57 W); 5: 1 min at medium power (114 W); 6: 3 min at medium power; 7: 5 min at medium power; 8: 1 min at high power (177 W); 9: 3 min at high power; 10: 5 min at high power (B) DNA exposed directly on nitrocellulose filter at high power: 13, 14: untreated control; 15–17: 1 min; 18–20: 3 min; 21–23: 5 min.

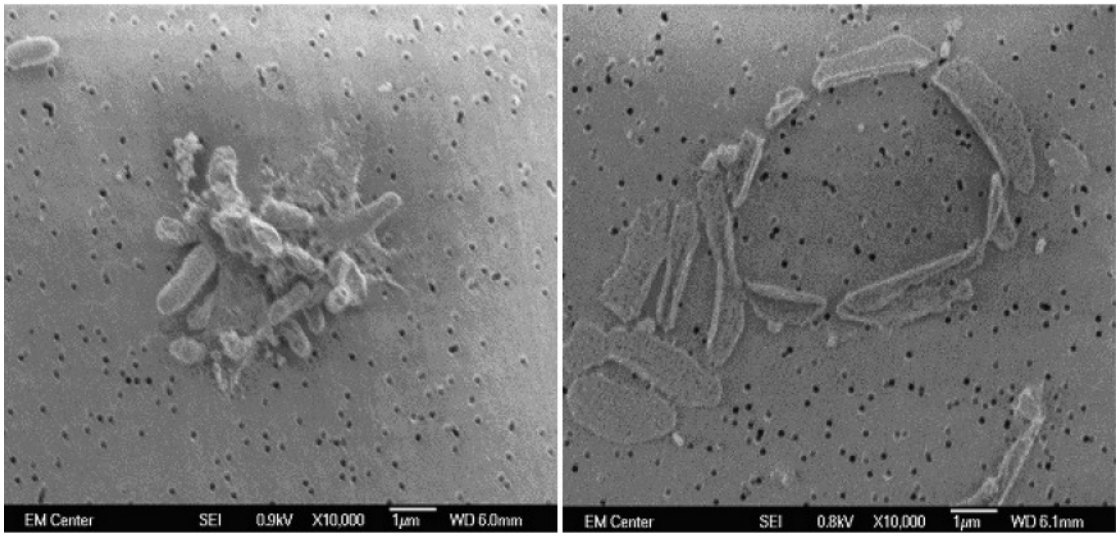

FIGURE 8. Scanning electron micrograph of untreated (left) and treated (right) cells exposed to plasma for 2 s. The instrument used was JEOL JSM6500F thermal field emission scanning electron microscope.

In the second experiment, it was considered that the agarose gel may have sheltered the DNA from the direct effects of the plasma. Therefore, the DNA was bound directly to nitrocellulose membranes prior to exposure to the plasma. Here, the effect was even more dramatic: after 1 min of exposure, the visible DNA was markedly decreased from the control, and after 3 and 5 min of exposure, no DNA could be visualized at all by agarose gel electrophoresis and ethidium bromide staining.

These experiments demonstrate that the plasma does have a direct effect on DNA, though it is unknown whether the bacterial cell wall and membrane provide some protection from this action. The fact that the effect of exposure when the DNA was present within the gel slice was milder than when directly exposed suggests that it is possible that the cell walls could provide some shielding of the UV or other active species produced by the plasma. However, cell walls can be removed by the oxygen radicals converting carbon to volatile gases (*3*), thus providing a means of direct exposure of nucleic acids.

Scanning Electron Microscopy of Treated cells. The SEM of the treated and untreated *E. coli* cells clearly indicated that the plasma treatment caused the cell walls to rupture (Figure 8). This demonstrates that the plasma has a direct physical impact on the cells. This is relevant considering that the results from growing the cells on different media suggested that there is a threshold in which cells are either active or killed, with no accountable production of sublethally injured cells. Judging by the electromicrographs, it appears that the cells have "burst" open. Researchers have suggested different reasons for the bursting of the cells (*4*) but most agree that gasification of cell walls plays an important role (*3*). In this process, oxygen radicals gasify carbon in cell walls into volatile carbon oxides, thereby destroying cell walls.

Advantages and Significance of Plasma Inactivation. Microbial inactivation has become a critical issue considering threats such as bioterrorism, in addition to everyday needs such as treatment of drinking water (*13*). While the results of this study focus on bacterial inactivation on surfaces, we have already begun adapting the technique to water treatment (*14*). Plasma sterilization of water has been suggested as an attractive means of water treatment (*15*) considering that it involves no addition of chemicals and employs highly reactive radicals working synergistically with the UV that is also produced. It is judged that latest advances in plasma technology such as those presented in this paper will lead to a notable increase in its application in environmental technology, not only with respect to microbial inactivation (*13*), but also to contaminant degradation (*14*, *16*, *17*) and surface modification.

The use of open-air grounded hollow slot plasma as described here presents several technical advantages. For example, in this study the afterglow plume portion of the plasma was implemented for microbial inactivation, which is significantly different from using the active discharge (*3*). First, the afterglow plume temperature is less than 50 °C, which is especially valuable for treating heat-sensitive samples. Also, the active species which it is composed of are less likely to harm sensitive equipment than the active discharge. These include very few charged particles, as the afterglow plume is essentially comprised of neutral atoms, radicals, and molecules, some of which are in an excited state. This greatly reduces the chance that the work piece surface would be damaged by positive ions accelerating in the floating sheath (an interfacial region between the plasma and any surface immersed in it where the ion density exceeds that of electrons). Finally, the afterglow can fill larger volumes than the active discharge present in the original slot between the electrodes, providing a larger active surface area and volume.

The wedge-slot design used in this study allows for corona-initiated ignition with a post-breakdown diffuse glow as well as tailored gas flows including a gas expansion region. However, the larger the slot electrode opening to surrounding air, the less the ability to reduce O_2 in the interelectrode region. As a consequence, for any open-air plasma, it is not possible to precisely set the mix of gas species present in the active microplasma or in the plasma plume. It is considered that this is not a shortcoming because the approach is more than justified by the unique ability to simultaneously provide both photon and radical fluxes from the electrically grounded and spatially open slot to sterilize work pieces placed in open air. The advantages of application without employing a sealed enclosure containing only rare gases are readily apparent, which include cost, practicality, and ease of use compared to previous techniques using electrodes housed in a sealed enclosure (*1*). Further, windowless operation avoids well-established window transmission degradation over time, especially for photons in the VUV (vacuum ultraviolet) (*9*). It also allows the unimpeded flux of radicals from the active discharge region through the open slots via the plasma plume to the work piece containing active microbes to be inactivated.

The requirement of pressurized gas bottles (argon and oxygen) in this work is a disadvantage compared to other plasma inactivation techniques previously discussed in the literature (*1*) that use ambient air. We are presently working to operate in ambient air with success at 1-cm lengths of slot plasma running only on ambient air but not beyond those dimensions. We judge that by changing from 13 MHz to higher frequencies (60 and 160 MHz) and by new electrode designs this dimension may be extended beyond 1 cm.

Plasma technology presents several potential advantages over current sterilizing technologies. One notable aspect for the environmental field is that toxic gases or other hazardous compounds are not required. Radicals generated by the device are highly potent but recombine to their original harmless form when the device is switched off. The configuration presented in this study is especially advantageous because it is operated in open air and is grounded, allowing it to be handheld like a wand applicator. However, the results are considered to be widely applicable and may be configured for the desired application, such as water treatment or contaminant degradation.

Acknowledgments

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