Bactericidal Effect of a Dielectric Barrier Discharge Plasma Jet Generated in Laminar and Preturbulent Helium Flows

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ABSTRACT: The bactericidal effect of a helium dielectric barrier discharge plasma jet generated in laminar (at 3 m/s) and preturbulent (at 10 m/s) helium flows is considered in terms of average discharge power and distance of treatment of *Escherichia coli* cells grown on a nutrient agar surface in a Petri dish. Sizes of bacteria inhibition zones are estimated and morphological changes in ultrastructure of bacterial cells are revealed using electron microscopy. This study suggests that the gas flow regime is an important factor in the production of the helium plasma jet used to inhibit bacteria. We reveal the different responses of *E. coli* cells treated by the helium plasma jet generated in laminar and preturbulent gas flows. Numerous focal destructions appear in the cells after 2 min of treatment with the laminar gas flow regime. Along with these focal destructions, expansion of cytoplasm and a superhelix of nucleoids are revealed with the preturbulent regime. We explain differences in behavior of cell morphology under the plasma jet formed at different gas flow regimes by the different gas-dynamic conditions of mixing ambient air into the helium flow.

KEY WORDS: dielectric barrier discharge, helium plasma jet, laminar gas flow, preturbulent gas flow, bactericidal effect, morphological properties, electron microscopy

I. INTRODUCTION

Plasma jet generators based on dielectric barrier discharge (DBD) are classified as a type of low-temperature (strongly nonequilibrium) plasma source that can operate at atmospheric pressure. Such generators can be used in laboratory and clinical investigations that are aimed at examining bactericidal efficacy of low-temperature atmospheric pressure plasma and developing ways to enhance it.[1–4] The generators usually produce plasma jets after a gas flow passes through a cylindrical discharge cell made of dielectric material, inside which the DBD is ignited.[5–9] Inert gases (helium in particular) are often used to create a cold atmospheric plasma jet. Typical parameters of alternating current voltage supply for helium are as follow: pulse repetition frequency in tens of kilohertz and voltage amplitude in kilovolts.[7–9] The helium plasma jet propagates from the nozzle of the generator into the surrounding air. The formation of the plasma jet at the outlet of the generator depends on a number of factors including gas outlet velocity,[10] impurity composition of a total gas flow,[11] and peak-to-peak value of discharge voltage.[12]

Currently, a dominating idea about the agents having the main role in antimicrobial activity of cold atmospheric plasma is related to the action of reactive oxygen and nitrogen species.[9,13] To provide their generation, it is preferable to use air as the plasma-supporting gas. But even small amounts of air or oxygen (~1%–2%) added to the total flow of inert gas cause complications in the production the stable plasma jet propagating in air due to the electronegative nature of O_2 molecules.[11,14,15]

On the other hand, the use of pure helium without any admixtures also produces a bactericidal effect. The concern is that chemically active species may be generated from the ambient air molecules involved into the helium plasma jet when it is propagating in the environment[16]; mixing ambient air into the helium plasma jet may be responsible for the generation of chemically active species. This is determined by the gas outlet velocity (m/s) that is assigned by gas flow rate (L/min) and the inner diameter of the nozzle (mm). While maintaining other conditions of the plasma jet formation (such as the type of gas and the geometrical sizes of the discharge tube and nozzle), the gas outlet velocity provides a corresponding gas flow regime (laminar or turbulent) and, consequently, influences the mixing of ambient air with helium.

Researchers[17,18] noted that the gas flow regime influences the bacteria-killing pattern and distinguished three different shapes of a killing spot (solid circle, ring, and ring with a concentric central spot) that depends on the gas flow conditions for the generation of the helium plasma jet. Killing patterns can be explained by experimental data revealing density distributions of ground-state atomic oxygen near a treated surface, obtained using two-photon absorption laser-induced fluorescence spectroscopy.[19] The data agree with simulation results obtained using a coupled model between neutral gas flow and plasma dynamics.[20]

Thus, gas flow regime is an important factor that influences bactericidal efficacy of the helium plasma jet and should be taken into account when developing certain plasma medicine technologies. This study is devoted to the investigation of the bactericidal effect of the helium plasma jet generated in a laminar (at 3 m/s) and preturbulent (at 10 m/s) regime, in terms of the appearance of inhibition zones and morphological changes in the ultrastructure of bacterial cells.

In addition, the dimensions (diameters) of the inhibition zones are presented reflecting the average power discharge that strongly influences the heating of gas. Because the most promising applications of cold plasma jets are disinfection of living materials and antibacterial treatments for wounds, heating gas in a plasma jet and the thermal effect on the treated object must be carefully controlled. This has been achieved with the use of a high-voltage supply to control the average discharge power and has been applied to study the bactericidal effect of a helium plasma jet.

II. MATERIAL AND METHODS

A. DBD Helium Plasma Jet Generator

To generate a helium plasma jet, DBD was ignited in a helium flow inside a quartz tube. A depiction showing the electrical components of the experimental setup is shown in

Fig. 1. The plasma jet generator consists of a cylindrical discharge cell and pulsed high-voltage power supply.

The power component of the generator was realized according to the scheme of a symmetrical half-bridge. Field-effect transistors were controlled by a driving generator (2), for which the running frequency of the driving generator could be set in the range of 50–200 kHz. A low-frequency pulse generator (1) allowed us to modulate high-voltage pulses and control the duty cycle from 10% to 90%; this runs similarly to a pulse chopper. Thus, we were able to control the average power deposited into the discharge from the secondary winding of the high-voltage transformer (4).

A plasma jet was formed when the helium flow passed through the cylindrical discharge cell with a coaxial electrode system "inner rod–outer ring" (Fig. 2). A quartz tube with an inner diameter of 5.58 mm and wall thickness of 1 mm was used as a dielectric barrier to ignite the DBD. High-voltage pulses were applied to the inner electrode, which was made from copper wire that was 1.5 mm in diameter. The outer electrode, made from a copper foil strip that was 5 mm wide, was grounded. Gas was supplied via a pipe fitting.

B. Electrical Parameters and Gas Flow Regimes for Plasma Treatment of Bacterial Cells

Electrical and gas-dynamics conditions of plasma jet generation were controlled for all of the experiments.
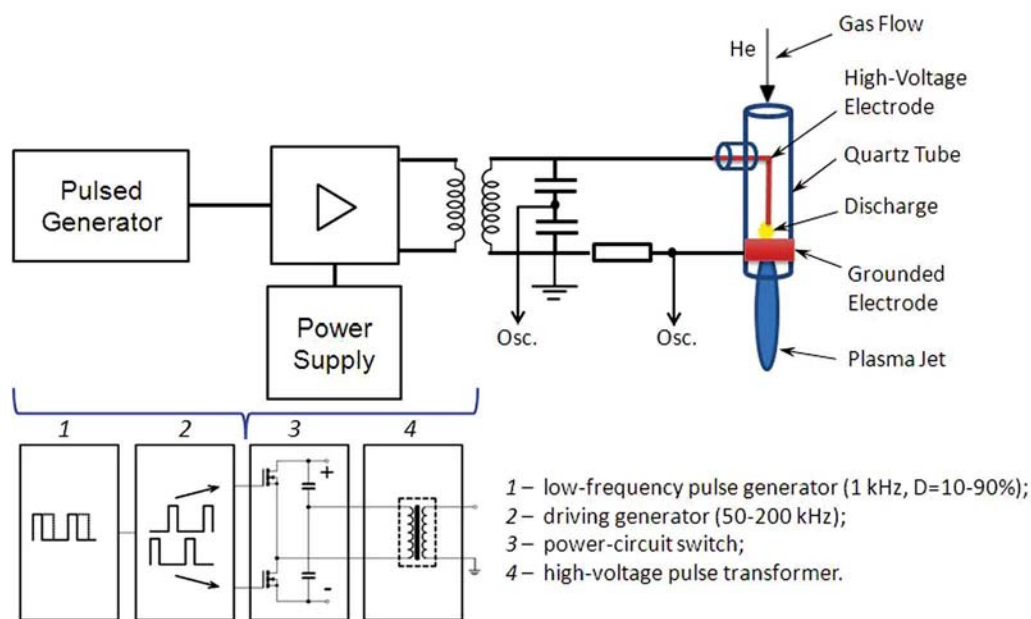

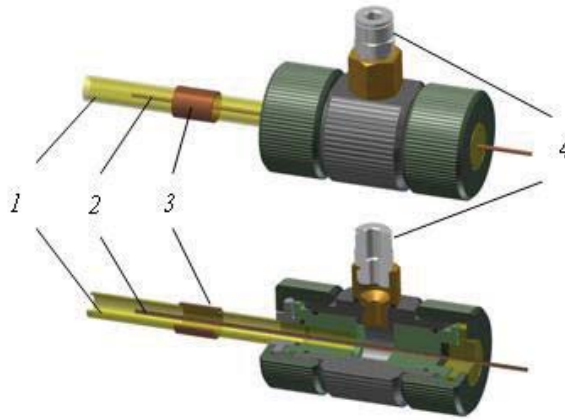**FIG. 1:** Schematic showing electrical components of the experimental setup

FIG. 2: Three-dimensional draft of the discharge cell. (*1*) Quartz tube (inner diameter, 5.58 mm; wall thickness, 1 mm); (*2*) high-voltage electrode (copper wire, 1.5 mm diameter); (*3*) grounded electrode (copper foil strip, 5 mm wide); (*4*) gas flow pipe fitting.

1. Electrical Parameters

The electrical parameters of the discharge were measured according to Fig. 1. Electrical signals were recorded with an oscilloscope, and the obtained discharge cell voltage and current oscillograms are presented in Fig. 3. The voltage signal was modulated, varying with a duty cycle D in the range of 10%–90% to save the parameters of a pulse inside a pulse burst. This varied the average discharge power Pd from 20 to 220 mW. Figure 4 shows the voltage signals at the duty cycles of 10% (Fig. 4, top) and 90% (Fig. 4, bottom). The average discharge powers were ~24 and 220 mW, respectively. Thus, the parameters of the high-voltage supply were set as follows:

- peak-to-peak discharge cell voltage $U \approx 5$ kV
- pulsed frequency $f = 38$ kHz
- amplitude of discharge cell current $I \approx \pm 2.5$ mA
- duty cycle of voltage signal $D = 10\%$–90%
- average discharge power $Pd \approx 24$–220 mW

Varying the duty cycle revealed how the average discharge power affected the behavior of the plasma jet at the treatments of biological samples as well as the bactericidal effect.

2. Gas Flow Regimes

The experimental gas outlet velocity Vg was controlled with a gas flow meter that was set to 3 m/s for a laminar regime (Fig. 5, top) and 10 m/s for a preturbulent regime (Fig. 5, bottom). Reynolds numbers were, correspondingly, 155 and 520.
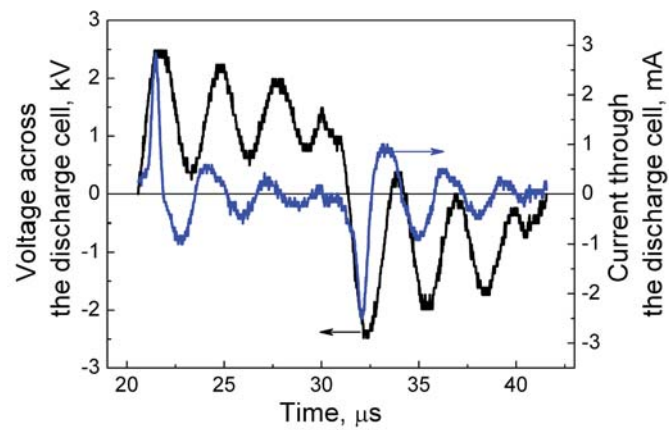
FIG. 3: Oscillograms of the voltage across the discharge cell and current through the discharge cell, recorded according to the process depicted in Fig. 1
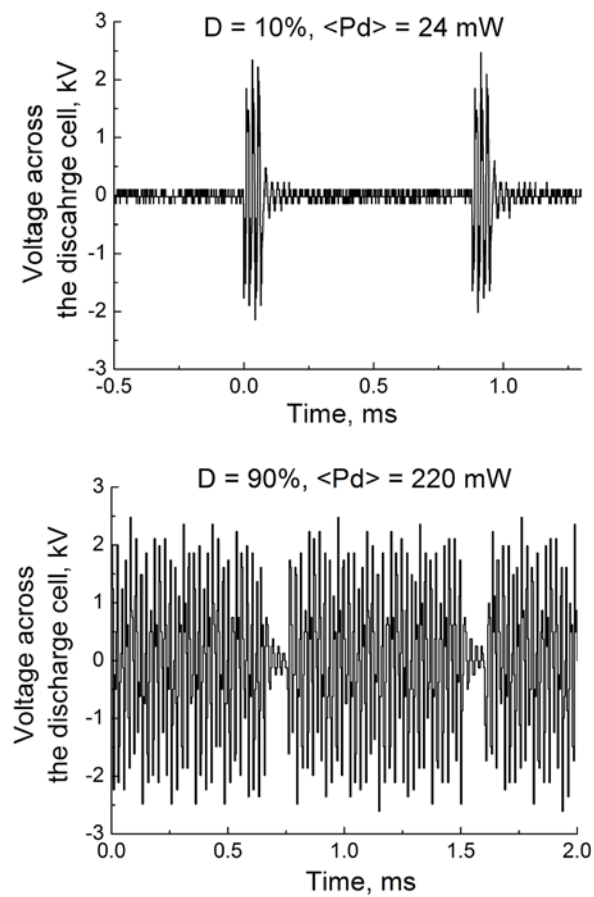

FIG. 4: Modulated voltage signals at a duty cycle of 10% (top) and 90% (bottom)

Gas flow regimes were previously investigated in terms of the length of plasma jet. Analysis of the dependence of plasma jet length on gas outlet velocity revealed the transition of the laminar mode into the turbulence mode (Figs. 5 and 6). This was accompanied by decreasing plasma jet length (e.g., from ~60 mm to ~20 mm for the tube with a diameter of 5.58 mm) and a blurring of the plasma jet shape (Fig. 5). Figure 5 shows how the appearance of the plasma jet changes when the laminar regime changed to the preturbulent regime. The chosen gas-dynamics conditions for forming a plasma jet provided a comparatively similar length of plasma jet, but its shape varied as a result of differing conditions for mixing the helium flow with the surrounding air. Mixing ambient air into a helium flow is thought to influence the production of active species toward bacterial cells and determine the bactericidal effect of the helium plasma jet.

C. Cultivation of Microorganisms

The bactericidal effect of helium plasma jet was examined using *Escherichia coli* as the model biological sample. Planktonic cells of *E. coli* were cultured in Brain-Heart Infusion broth (Sigma-Aldrich; St. Louis, MO). The bacterial culture was incubated for 24 hr at 37°C to a concentration of $~10^9$–10^{10} colony forming units (CFU)/mL. We used the pour plate method to count the number of living cells. During the streak plate procedure, we spread a mixture of cells over the surface of a semisolid, agar-based nutrient medium (*Mueller–Hinton*; Merck, Germany) in a Petri dish, such that fewer and fewer bacterial cells were deposited at widely separated points on the surface of the medium. Following incubation, the cells developed into colonies.[21]

Two types of bacterial biofilms were prepared with differing CFU: The first sample had a concentration of 10^9 CFU/mL and the second had 10^3 CFU/mL (a 1:10^6 mixture of
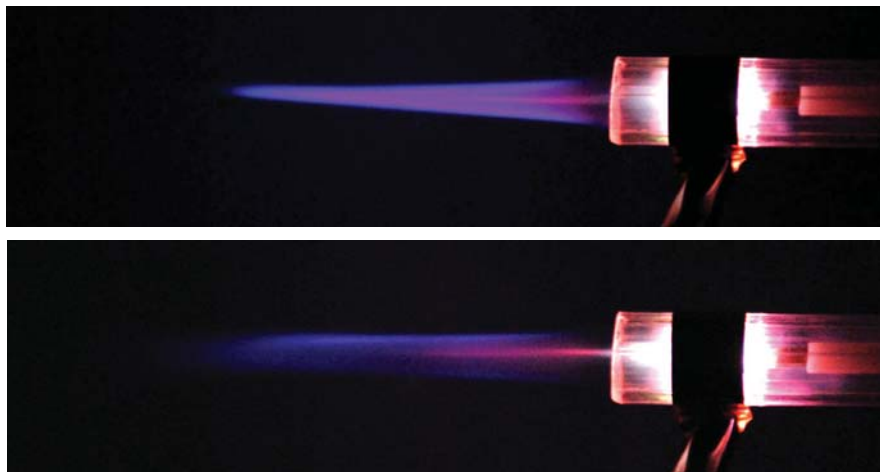

FIG. 5: Images of the helium plasma jet generated in a laminar gas flow (top) and a preturbulent gas flow (bottom)
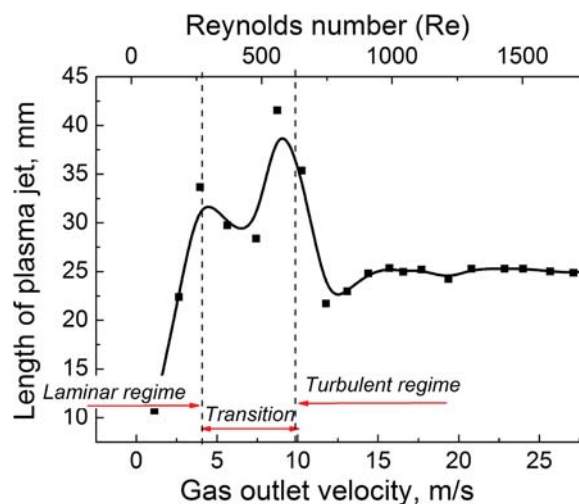
FIG. 6: Length of plasma jet in terms of gas outlet velocities and corresponding Reynolds numbers

bacterial cultures 10^9 CFU/mL and broth growth media). To a form a bacterial biofilm, we dispensed a small volume of bacterial sample (0.1–0.2 mL) aseptically onto the center of the Petri dish using a micropipettor. The spreader was gently moved through the sample across the dish while the turntable was slowly spinning. After the sample was completely absorbed into the agar, we divided the Petri dish into four quadrants (see Fig. 7) that were then exposed to the helium plasma jet. To identify bactericidal plasma jet effects, all samples were incubated for 24 hr at 37°C after plasma treatments. Finally, we detected inhibition zones that formed where the plasma jet was applied. These zones were measured to the nearest millimeter.

D. Treatments of Bacterial Cells by Helium Plasma Jet

To treat inoculated bacterial cells with the helium plasma jet and examine its bactericidal effect, the Petri dish with the agar inoculated by bacterial strains was placed under the plasma generator. The distance between the edge of the discharge cell and agar surface was fixed but varied from 5 to 35 mm (Fig. 8). The duration of plasma treatments was 2 min in all experiments. During treatments, the appearance and shape of the plasma jet was photographed using a Nikon D80 camera equipped with a Sigma 28-200 Macro lens.

E. Preparation of Biological Specimens for Electron Microscopy Investigation

We used transmission electron microscopy (TEM) to investigate the ultrastructure of the cells that were treated with the helium plasma jet. Microbiological specimens for
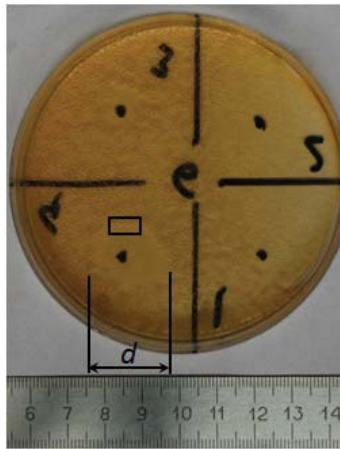
FIG. 7: The Petri dish 24 hr after treatments. (*d*) Diameter (dimension) of the cell inhibition zone; (rectangle) zone selected for TEM
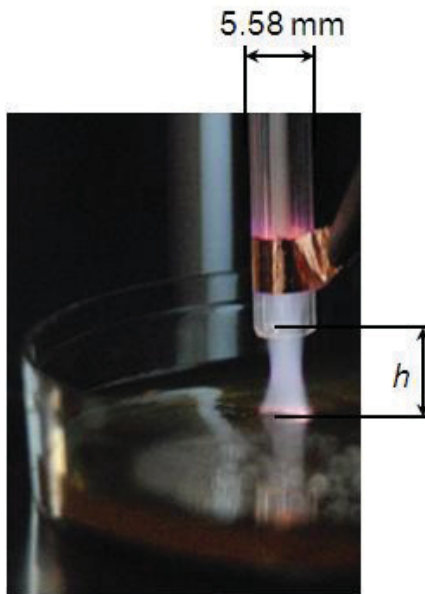

FIG. 8: Image of the helium plasma jet treatment of an agar surface inoculated with *E. coli* strains. The inner diameter of the cylindrical discharge cell is 5.58 mm; distance from the outlet of the plasma generator to the surface of agar *h* is 5–35 mm.

TEM were prepared by means of an ultrathin section method described elsewhere.[22] Specimens were selected from the edges of inhibition zones, including the zones without any visually detectable microorganism growth (Fig. 7). Microbiological specimens prepared with the ultrathin section method were observed using a JEM-100C TEM at an accelerating voltage of 80 kV.

III. RESULTS AND DISCUSSION

A. Examination of Bactericidal Effect with Some Treatments Conditions Variations

To estimate the relative efficacy of the bactericidal effect, we measured the sizes of the inhibition zones 24 hr after the treatments. The dimension or diameter of an inhibition zone was considered to be related to the response of the bacterial biofilm on the bactericidal effect of the helium plasma jet. All trials were conducted with varying average Pd from 24 to 220 mW; that is, at a 10%–90% duty cycle of high-voltage pulses D and a 3–40 mm distance of treatment h.

The influence of the distance between the edge of the plasma generator nozzle and agar surface at differing average discharge powers is shown in Fig. 9. We observed the transition of plasma jet to arc at reduced distances that varied along with changing treatment conditions. For example, Fig. 10 presents how the plasma jet changed with reduced treatment distance. The images showed the preturbulent gas flow regime at $Vg = 10$ m/s and average discharge power $Pd = 98$ mW. The data on inhibition zones obtained at the action of the arc are highlighted in Figs. 11 and 12 with dashed circles; these were not taken into consideration for analysis of the bactericidal effect of helium plasma jet.

With increased average power of the discharge, the efficacy of the bactericidal effect of helium DBD plasma jet increased; the closer the plasma jet generator was to the treated surface, the more likely the transition of the discharge into the arc occurred. The approximate location of the edge of the plasma generator nozzle for safe (without
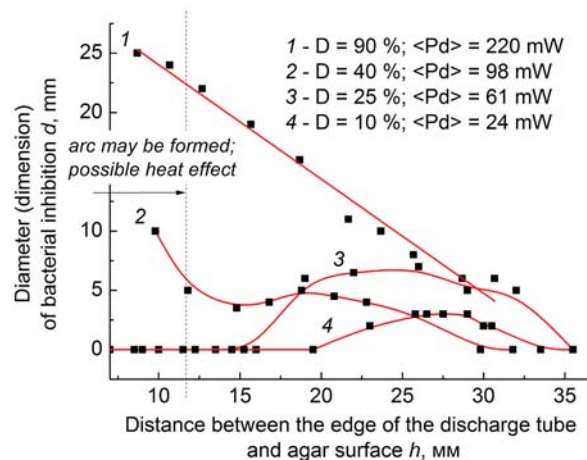

FIG. 9: Diameter (dimension) of the inhibition zone d versus the distance between the edge of the discharge tube and agar surface h at various average discharge powers. The gas flow regime was preturbulent ($Vg = 10$ m/s); treatment duration is 2 min.
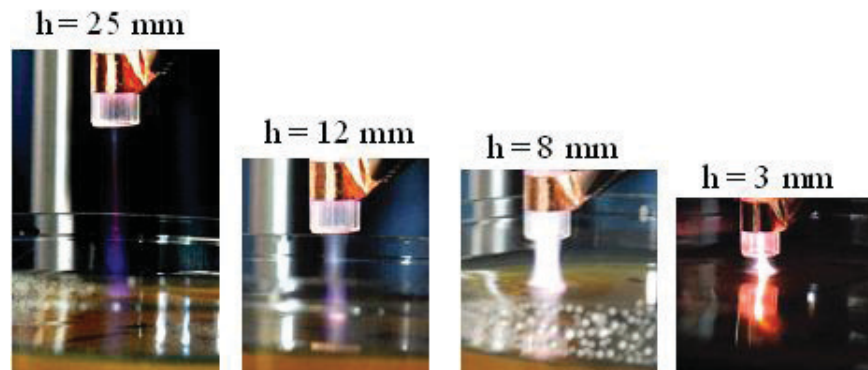
FIG. 10: The helium plasma jet at reduced treatment distances. The gas flow regime was preturbulent ($Vg = 10$ m/s); average discharge power Pd is 98 mW.
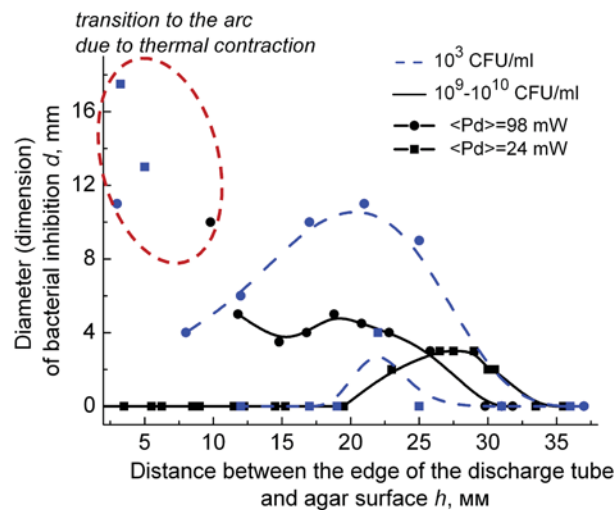

FIG. 11: Diameter (dimension) of the inhibition zone d versus the distance between the edge of the discharge tube and agar surface h at the average discharge powers of 24 and 98 mW for the cell concentrations of 10^3 and 10^9–10^{10} CFU/mL, respectively. The gas flow regime was preturbulent ($Vg = 10$ m/s).

arc formation) treatment is shown by the dashed line in Fig. 9. It was very important to control this distance; for future experiments, maintaining a certain height for every set of treatment conditions is strongly recommended.

An additional feature of the dependence of inhibition zone size on the distance of treatment is the presence of a maximum. This appears at the different densities of cell inoculation (Fig. 11). We observed it for both samples of bacterial cultures (with the concentrations of 10^9–10^{10} and 10^3 CFU/ml).
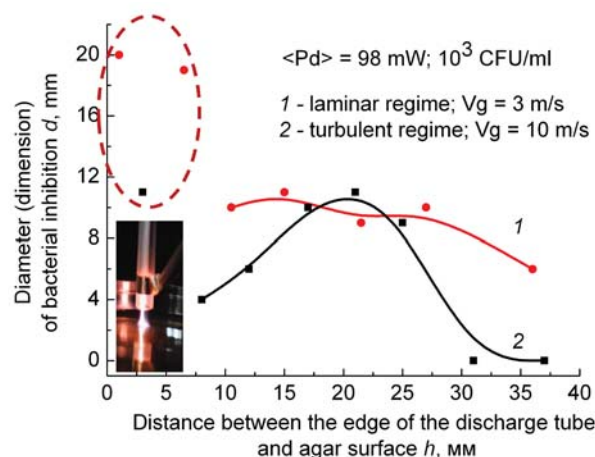
FIG. 12: Diameter (dimension) of the inhibition zone d versus the distance between the edge of the discharge tube and agar surface h at an average discharge power of 98 mW. The cell concentration was 10^3 CFU/mL for laminar and preturbulent gas flow regimes.

It is evident that this maximum shows the thickness of the air layer, which is optimal for the possible generation of reactive oxygen and/or nitrogen species. The species are formed when involving particles of air into the space of plasma jet with strong electrical field. This area, called a head of streamer, has a high strength of electrical field of 80–100 kV/cm[7] that is sufficient to produce energetic electrons. These are responsible for excitation, ionization, and dissociation reactions due to electron impact collisions.[18] Thus, different reactive agents can be created by the helium plasma jet in a thin air layer near the bacteria-inoculated agar surface.

As mentioned above, the gas-dynamics structure of the plasma jet can significantly influence the produced bactericidal effect. In terms of sizes of inhibition zone, a bactericidal effect of helium plasma jet at the laminar gas flow regime is characterized by reduced dependence on distance of treatment; the maximum is less likely to be expressed (Fig. 12). This can be linked to a more uniform mixing of air into the helium flow at laminar regime for the entire investigated range of treatment distances.

B. Morphological Properties of Bacterial Cells Treated by Helium Plasma Jet: Effect of the Gas Flow Regime

We used TEM to reveal morphological changes in the ultrastructure of bacterial cells treated by helium plasma jet at laminar (3 m/s) and preturbulent (10 m/s) gas flow regimes. These changes in the cells selected from the edges of inhibition zones clarify some mechanisms of destroying bacteria under the action of a helium plasma jet.

The revealed effects for the two gas flow regimes significantly differed. With the laminar regime, we observed focal multiple destructions that presented voids in cyto-

plasma around cell perimeters (Fig. 13A). These voids appeared because the cytoplasmatic membrane began to move away from the cell membrane.

The turbulent gas flow regime not only caused formation of focal destructions in the cells, but the cytoplasm and nucleoid also underwent significant expansion with the formation of superhelical DNA. This was possibly related to the action of reactive oxygen species that likely originated more intensively at the preturbulent regime than at the laminar regime due to a more intensive mixing of surrounding air into the plasma jet.

The impressive difference in the action of the helium plasma jet formed by the laminar and preturbulent gas flows indirectly suggests that controlling the gas flow regime may initiate specific changes in the morphological properties of the cells. This phenomenon requires additional research to be described in greater detail. We are not currently ready to state that a turbulent gas flow yields a plasma jet with stronger bactericidal effects than available at a laminar gas flow. But the effect of gas flow regime on plasma jet production should clearly be investigated together with responses of the treated bacterial cells.

IV. CONCLUSION

We considered the efficacy of the bactericidal effect of helium plasma jet in terms of average discharge power and distance to sample. These were described by sizes of in-
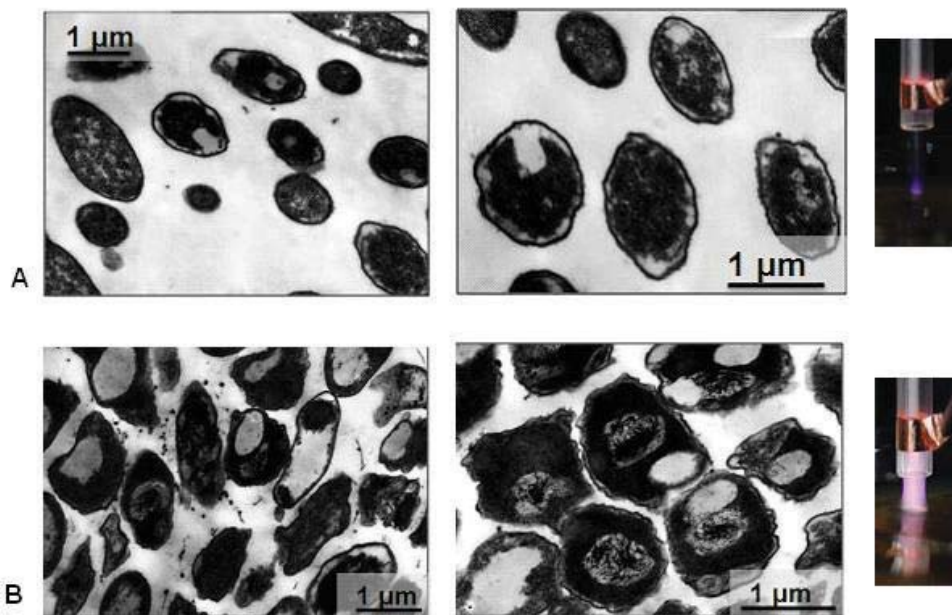

FIG. 13: Electron microscope images of *E. coli* cells treated by helium plasma jet at laminar (A) and preturbulent (B) gas flow regimes. The average discharge power is 98 mW; treatment distances are 10 (A) and 8 (B) mm.

hibition zones and changes in morphological properties of treated cells. In this study, we obtained results of plasma treatment of *E.coli* cells for laminar and preturbulent helium flows.

With increasing of the average discharge power, the efficacy of the bactericidal effect of helium DBD plasma jet increases. Moreover, this depends on the gas-dynamics condition of mixing ambient air into the helium plasma jet. A specific maximum was revealed in the dependence of the inhibition zone sizes on the treatment distance, which may be related to the optimal thickness of the air layer to generate chemical reactive species, such as atomic oxygen, that can determine the bactericidal effect of plasma jet.

The morphology of treated cells greatly depends on the gas-dynamics conditions of the formation of the plasma jet. At a laminar gas flow regime, morphological changes in the ultrastructure of the treated cells are mainly focal destructions, whereas at a turbulent regime, besides focal destructions, changes include rarefied cytoplasm and expanded nucleoids with superhelical DNAs. This may be caused by the possible effect of reactive oxygen species that might be generated as a result of involving air particles into the plasma jet.

ACKNOWLEDGMENT

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