



Cold atmospheric argon plasma jet source and its application for bacterial inactivation

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Abstract

In this work, the efficiency of inactivation of a cold argon plasma jet at atmospheric pressure (APPJ) on the basis of a low-current spark discharge into microorganisms with different characteristics of the cell walls was evaluated. Gram-negative bacteria *Escherichia coli* M17, gram-positive bacteria *Bacillus subtilis* 534 and *Bacillus cereus* IP 5832, and the yeast *Saccharomyces cerevisiae* were seeded on cultured Petri dishes. A plasma jet with an average power of 0.85 W and a flow rate of argon of 6.7 l/min was directed perpendicular to the Petri dishes with agar. The distance to the agar varied from 0.5 to 3 cm, and the treatment time varied from 5 to 300 s. The efficiency of inactivation was assessed by measuring the area of inactivation zones (where there was no growth of microorganisms). It was shown that gram-negative bacteria *E. coli* M17 is most susceptible to exposure to the plasma jet, and gram-positive bacteria *B. cereus* IP 5832 and yeast *S. cerevisiae* are most stable. It is established that an increase in the treatment time of plasma jets of a low-current spark allows effective inactivation of microorganisms over a much larger area.

Keywords Argon plasma · Microorganisms · Low-current spark · Plasma jets · Plasma inactivation · Atmospheric-pressure glow discharge

Introduction

Investigation of properties of gas-discharge processes aimed at determining the possibility of their application for sterilization and disinfection treatment in protecting industrial materials, equipment, and electronics from biological damage and microbiologically induced corrosion has become of vital importance at present [1, 2]. Plasma treatment of living tissues produces the desirable therapeutic effect in sterilization and arrest of bleeding, bleeding control, and cure of some skin diseases [3, 4]. This trend has acquired special importance in recent years due to the increasing need of mankind in new high-productive sterilization and disinfection technologies that do not require high temperatures, are simple in operation, and are characterized by high effectiveness and reliability.

Among plasma technologies, analysis of discharges generating low-temperature (cold) nonequilibrium plasmas under atmospheric pressure is of special importance [5–11]. Various types of gas discharges that can serve as sources of a low-temperature nonequilibrium plasma include the creeping discharge, corona, barrier, and pulsed discharges under atmospheric pressure. In spite of the large number of publications (see, for example, [8, 9, 12, 13]) devoted to analysis of various characteristics of discharges and the well-proven high effectiveness of application of discharges for biomedical purposes on the laboratory scale, the treatment with cold plasma under atmospheric pressure aimed at inactivation of microorganisms has not found wide application. This is primarily due to the fact that cold plasma sources require technically complicated equipment with a low economic efficiency. Second, the atmospheric-pressure discharges for treatment of biological objects require high voltages (10–40 kV), which necessitates a high safety level to be ensured. For this reason, the choice of discharge parameters for which safe and nondestructive action is ensured is one of the main physical problems in plasma medicine. Most plasma jets have a diameter of a few millimeters. On the basis of the low-current spark discharge in argon at

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atmospheric pressure, it is possible to receive homogeneous enough plasma jets of big diameters which are perspective, for example, in medicine for treatment of vast wounds [14].

In the present work, three species representative of different microbial groups were treated in order to evaluate the inactivation efficiency of cold atmospheric-pressure plasma jet. Gram-positive bacteria possess thick cell walls composed by several layers of peptidoglycan. Differently from gram-negative bacteria, they are absent of bacterial outer membrane. Gram-negative bacteria exhibit clearly layered structure with three main sections: the outer membrane, the peptidoglycan cell wall, and the inner membrane. The outer membrane plays important role in protecting the cell against toxic molecules and providing an extra stabilizing layer around the cell [15, 16]. Fungi are eukaryotic cells, and their cell walls are completely different from the prokaryotic ones (bacteria). Fungal cell walls are very thick and composed by rigid polysaccharide layers, such as chitin. It is responsible for providing structural strength to fungi cell walls making them more resistant to harmful extracellular agents [17]. Due to these noticeable structural differences of the selected microorganisms, it is expected that different responses to the plasma treatment would occur. Here, the gram-positive bacterium *Bacillus subtilis* 534 and *Bacillus cereus* IP 5832, the gram-negative bacterium *Escherichia coli* M17, and the fungus *Saccharomyces cerevisiae* were plated on standard Petri dish filled with culture media and then exposed to plasma. The effect of the distance between plasma plume and agar surface was also investigated.

Materials and methods

Experimental setup

We have developed a nonequilibrium plasma source operating under atmospheric pressure for generating low-temperature (cold) plasma based on plasma jets of a low-current spark discharge [10, 14] under the atmospheric pressure (Fig. 1). The cathode (point 1 with radius of curvature $r = 30 \mu\text{m}$) is placed in an insulating casing in the form of a tube of radius $R = 2 \text{ cm}$. The anode is a metal cylinder 1.5 cm in length and 2 cm in diameter. For stabilizing the discharge, the point is loaded with a controllable high ballast resistance $R_b (> 1 \text{ M}\Omega)$. The casing has intake orifices for puffing argon, which are arranged so that cold argon plasma produced by a system of plasma jets from low-current spark propagates in the direction from the electrode gap with an argon flow like a flame. Argon flow rate G is measured by PM-A-0.16 GUZ rotameter up to $5 \times 10^{-5} \text{ kg/s}$.

The averaged current of a discharge was measured with M 906 pointer microammeter. The waveforms and amplitudes

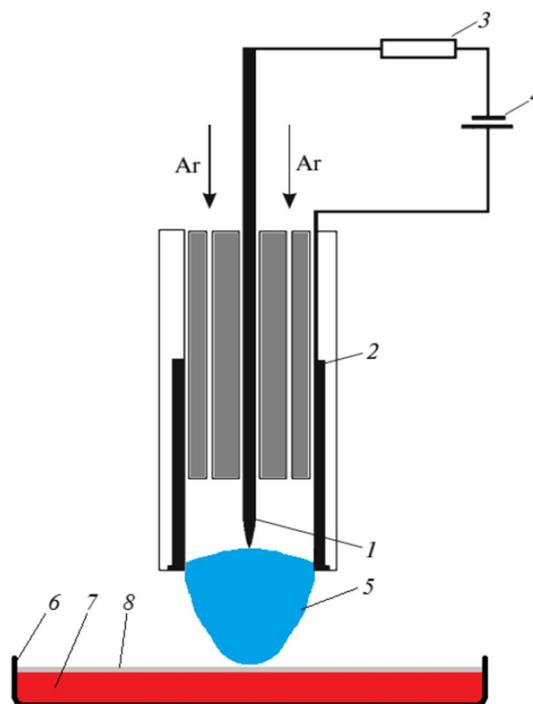


Fig. 1 Schematic diagram of experimental setup: (1) point cathode; (2) cylindrical anode; (3) ballast resistance; (4) power supply (5) plasma jet; (6). Petri dish; (7) culture medium; and (8) microbial suspension

of the pulsed current component were monitored using low-inductive current shunts and S1-65 oscilloscope.

Microorganisms and preparation of inoculum

Bacterial strains

Bacterial strains used in the study: *E. coli* M17, *B. subtilis* 534, *B. cereus* IP 5832, and *S. cerevisiae*, were obtained from the collection of microorganisms from the Biotechnology Department, Institute of Food Engineering and Biotechnology, East-Siberian State University of Technology and Management (Ulan-Ude, Russia). Cultures were stored on nutrient media at 4 °C.

Preparation of inoculum

To prepare the inoculum, the studied bacteria were incubated on nutrient media for 48 h at 28 °C (*S. cerevisiae*) and for 24 h at 37 °C (*E. coli* M17, *B. subtilis* 534, *B. cereus* IP 5832), and then, the daily cell culture was suspended in sterile water. The results of the quantitative determination of microorganisms, carried out according to the Koch method, were expressed in colony forming units (CFU). Initial cell concentrations were obtained: 9.14 log CFU/ml for *E. coli* M17, 7.83 log CFU/ml for *B. subtilis* 534, 8.47 log CFU/ml

for *B. cereus* IP 5832, and 7.14 log CFU/ml for *S. cerevisiae*. All samples were analyzed in triplicate, and the given value is the average value of three determinations.

To evaluate the sensitivity of microorganisms to cold argon plasma generated by low-current spark discharge plasma jets, a method was used based on measuring the diameter of the inactivation zone, which manifests itself as a clean area on a nutrient medium. Therefore, a medium was inoculated with test microorganisms. About 100 μl of working suspension was added into a Petri dish with nutrient medium and carefully rubbed with a spreader. *Bacillus subtilis* was seeded on RPA, and *E. coli*, *B. cereus*, and *S. cerevisiae* were seeded on MPA. The plates with medium inoculated microorganisms were placed in a discharge chamber under plasma jets. Plasma-treated media were incubated in an incubator within 24 h at 37 °C, and then, the diameters of inactivation zone were measured.

Treatment conditions

Plasma jet operated at current of 0.5 mA, voltage of 1.7 kV, and argon flow rate of 6.7 l/min for all treatments. An increase in the gas temperature at such discharge parameters does not exceed 0.01 °C. The plasma jet length was ~ 1.0 cm. Each experiment was performed in triplicate to ensure reproducibility, and the plasma plume was directed perpendicular to the surface of the agar plates. Microorganisms were exposed to the plasma jet for 5, 10, 30, 60, 120, 240, and 360 s. The treatments were carried out at three different distances between the nozzle and the agar (0.5, 2.0 and 3.0 cm). For the control experiments, the samples were exposed to argon flow at the same flow rate without plasma ignition.

Results and discussion

The bactericidal properties of low-current spark discharge plasma jets were studied for their effects on the *E. coli* strains. Figure 2 shows a picture of low-current spark

discharge cold argon plasma jet generator. Low-current spark discharge plasma jetting time ranged from 5 to 60 s. Distance h from the plasma source to the surface of microorganism growth ranged from 0.5 to 3 cm.

The current–voltage characteristic of discharge is dropping, and the nature of current flow in the plasma channel is an established mode of periodical current pulses (Fig. 3). In the formation of discharge current pulse, two specific areas can be marked out: the initial narrow peak with amplitude $I_m \sim 280 \mu\text{A}$ (area 1) and the second longer area ($T \sim 70 \mu\text{s}$) that essentially determines the period T of discharge current pulse. The low-current spark plasma jetting influence on microorganisms is registered as round transparent areas, which are the microorganism's growth inactivation zones.

The data obtained show high sensitivity of microorganisms to cold argon plasma treatment (Fig. 4). Minimal plasma inactivation time of the *E. coli* cells is 5 s at 0.5 cm from the side section of electrode structure. At a distance increased to 3 cm, the number of survived microorganism

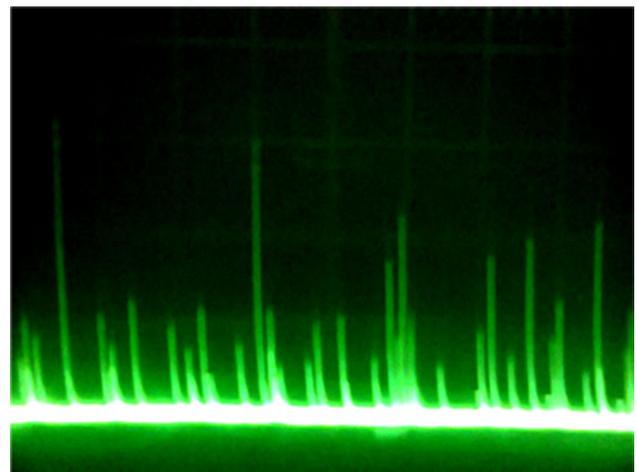
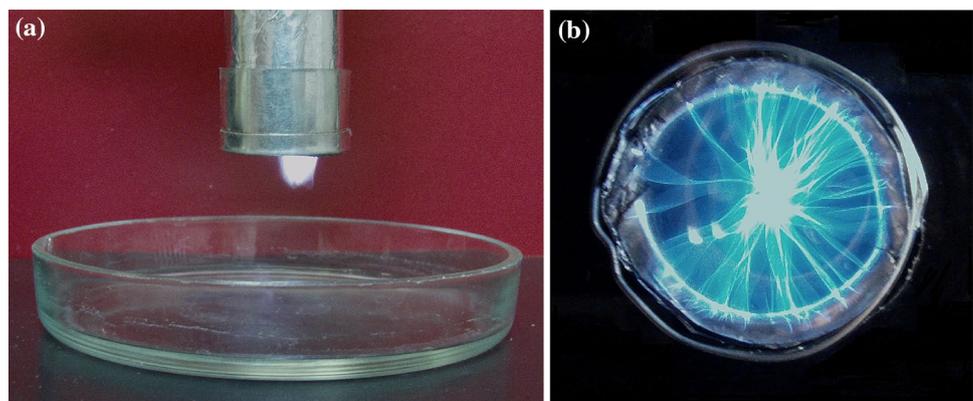


Fig. 3 Low-voltage spark discharge current pulses. Interelectrode spacing $d = 1.25$ cm, amperage $I = 500 \mu\text{A}$, $R_6 = 21 \text{ M}\Omega$, $[I] = 50 \mu\text{A/div}$, $[t] = 0.2 \text{ ms/div}$

Fig. 2 Cold argon plasma generator based on low-current spark plasma jets: **a** microorganism inactivation in a Petri dish and **b** side view of discharge



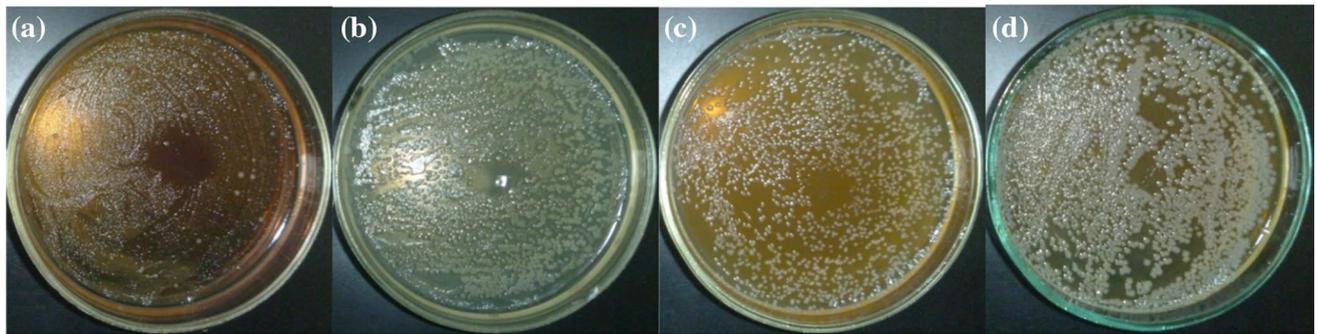


Fig. 4 Bacterial growth inactivation area's treatment time $t=4$ min, distance $h=0.5$ cm: **a** *Escherichia coli*; **b** *Bacillus subtilis*; **c** *Saccharomyces cerevisiae*; **d** *Bacillus cereus*

is considerably greater. Increasing the plasma treatment time up to 40 s at a distance of 3 cm causes a considerable reduction (by 74%) in survived microorganisms (Fig. 5). The determination of argon plasma inactivation ability carried out by counting the colonies shows that after a minute of treatment, only a few grown-up microorganisms survive.

It should be noted that the inactivation area is not restricted by the anode diameter, within which low-current spark plasma jets are formed. As it can be seen in Fig. 6, increasing the treatment time with low-current

spark plasma jets allows efficient inactivation of a much larger area. At that, the diameter of inactivation area in direct contact of plasma jets with the surface of microbial environment increases more than in remote exposure. The results obtained correspond to the results shown in [18]. So, the diameter of inactivation area increases with increasing time of treatment of *Pseudomonas aeruginosa* bacteria on solid agar with atmospheric-pressure cold plasma jets (99.5% helium and 0.5% oxygen).

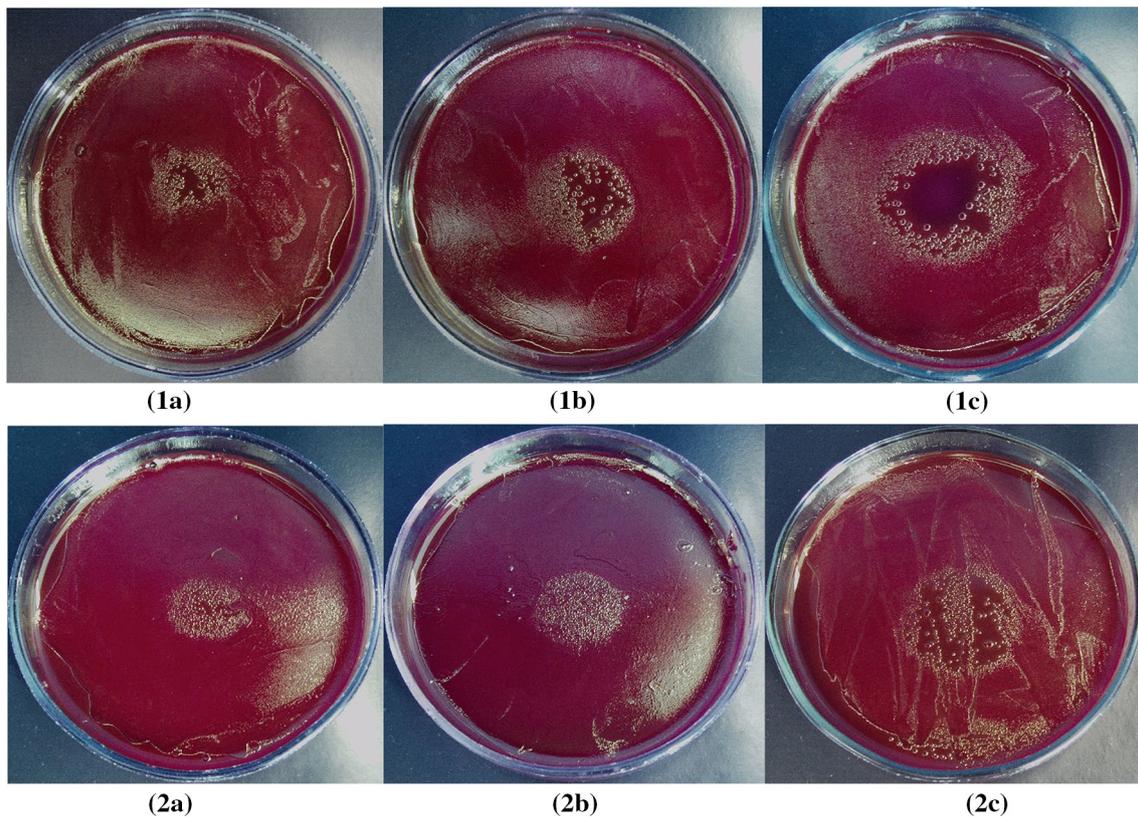


Fig. 5 *E. coli* growth inactivation areas in relation to h : (1) $h=1.0$ cm, (2) $h=2.0$ cm, **a** $t=5$ s; **b** $t=20$ s, **c** $t=60$ s

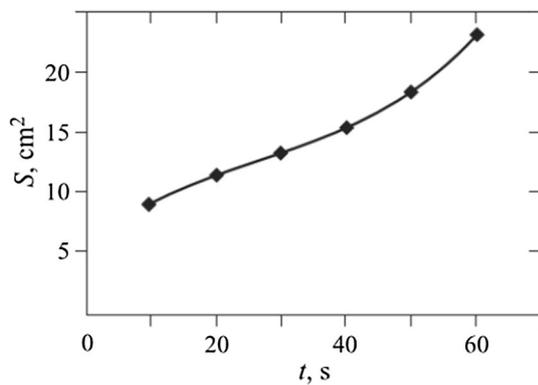


Fig. 6 The relationship of inactivation area of *Escherichia coli* and exposure time, $h=0.5$ cm

Conclusions

The high bactericidal efficiency of low-current high-voltage discharge cold argon plasma is displayed. The study of bacterial survival in the repetitively pulsed negative corona plasma and atmospheric-pressure glow discharge showed that after treating the wafers for 2 min in each mode, the microorganisms are totally inactivated. It has been found that increasing the time of microbial environment treatment with low-current spark plasma jets allows efficient inactivation of a much larger area than the anode electrode cross section.

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