Topic number: Workshop 1

Effect of Gas Composition and Humidity on Bactria Inactivation Efficiency by DC Corona Discharge

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Plasma inactivation of bacteria is one of the oldest applications in the field of biomedical applications of plasmas. Different types of discharges were successfully and effectively applied for sterilization of both gram-positive and gram-negative bacteria, as well as spores on surfaces, in air and liquids, and have been described in literature [1]. Inactivation efficiency of plasmas was investigated various chemical systems, such as air, nitrogen, oxygen, noble gases. The nature of plasma interactions with bacteria is rather complex, and is clearly different for different conditions, for example, arc discharge at atmospheric pressure, and low pressure glow discharge. Non-thermal plasma generated at atmospheric pressure produces a complex mixture of reactive molecules, charges, electric fields, and radiation. The role of all these components has been widely studied, and it was noticed that inactivation efficiency highly depends on presence of charged species for room air conditions [1, 2], while radiation effect is almost negligible. Nevertheless we still did not understand the role of different plasma species and selectivity of plasma effect even in simplest cases. To clarify the role of different plasma species one should separate the role of heating, electrical field, reactive oxygen species - oxidants (ROS), nitrogen oxides, UV radiation. It is very difficult task in the case of DBD when the streamers simultaneously generate all active components. Probably, the best discharge to separate the role of plasma-produced active components is DC corona, where stable, uniform and controllable generation of various neutral active species, charges, and global electric fields is possible.

Experiments were conducted using DC corona discharge at atmospheric pressure. Seventeen needles were uniformly fixed in cylindrical plastic enclosure with inner diameter of 5 cm (Figure 1). Grounded metal plates filled with agar or grounded metal coupons were placed 4 cm below the needles, and were playing role of second electrode. The corona needles were powered with either positive or negative dc voltage through a 10 MOhm resistor to produce ion flow. By variation of applied voltage from 5 to 30 kV, the current of ions was varied from 10 to 250 μA. In order to check sterilization efficiency of neutral active species generated by the discharge, a grounded metal mesh was introduces into the system. The effects of gas composition and presence of water were studied by introducing either dry or wet (gases were bubbled through distilled water, which was boiled prior the experiment for ~20 min and then cooled to room temperature) air, O_2, N_2, Ar, and He into the discharge volume at rate of 10 slpm. In this study we have used an *E. coli* suspension in phosphate buffered saline (PBS) at initial concentration of about $3 \cdot 10^9$ cells/ml. Bacteria were treated on brain heart infusion (BHI) agar surface: 400 μl of bacteria solution were inoculated on BHI agar prepared in aluminum plates (plate diameter 6.5 cm) and spread on agar surface to obtain uniform layer of bacteria. After inoculation plates were allowed to dry in room air for about 30 minutes to allow evaporation of excess water. In such a case, bacteria were never completely dry and were covered by a minute amount of water, a condition which we refer to as “moist”. To quantify number of bacteria per unit area of agar bacteria were properly diluted, inoculated on agar surface as described above, and after incubation at 37^0C for 15 hours resulting colonies were counted.

Initial experiments were done with bacteria of various initial concentrations dried on agar (“moist” condition) utilizing corona discharge in room air (~60% humidity) with constant current of 100 μA. Results for corona of both positive and negative polarities of applied voltage are shown on Figure 2 in terms of colony number density as a function of dose, where exposure time of treatment area at constant current was recalculated to charge density ($\mu C/cm^2$). It is shown, that positive corona discharge treatment of bacteria dried on agar surface at constant

current of 100 μA permits up to about 6-log reduction of viable bacteria in about 90 seconds of treatment. Interestingly, negative corona has in general the same effect on bacteria viability, however the same level of inactivation may be achieved at 1.5 – 3 times higher doses of treatment. Inactivation efficiency in case of "indirect" treatment with mesh was negligible (less than a 1-log reduction in one minute of treatment).

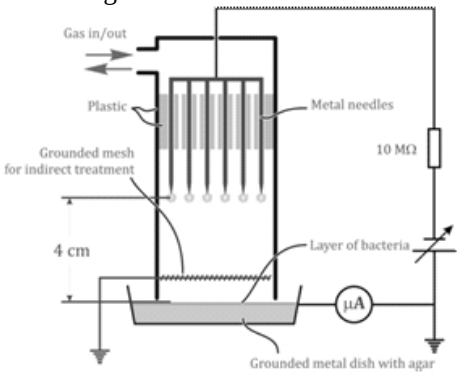

Figure 1. Experimental set up

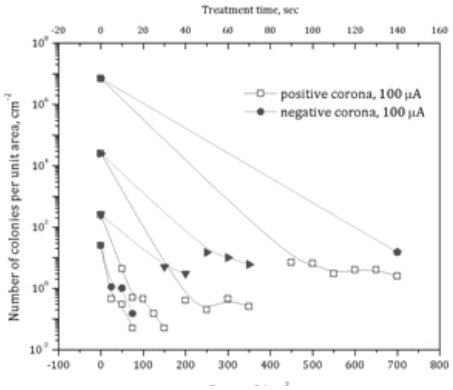

Figure 2. Bacteria inactivation in room air

The results of experiments with different gas composition are shown on Figure 3. Compared to room air (~60% RH), only in O_2/H_2O mixture were achieved the same inactivation efficiency, while no visible inactivation was observed in all other cases. These results show that neither UV radiation, ozone, H_2O_2, nor other neutral active species alone produced by corona in dry oxygen or other gases with water have an effect on bacteria viability. Also, it is clear that charged particles alone or in combination with water do not provide any bacteria inactivation.

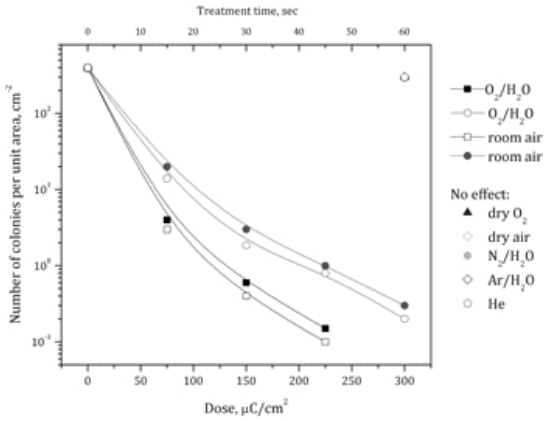

Figure 3. Bacteria inactivation by corona discharge in different gases.

The results clearly show that inactivation of bacteria may not be explained by effect of only neutral active species or charged particles acting independently. Moreover, simultaneous presence of oxygen and water molecules in the discharge is required. This means that the inactivation is not due to positive (O_4^+) or negative (O_2^-) ions, and cannot be explained by OH generation only. Therefore the mechanism of peroxidation of bacterial membrane catalyzed by plasma should take into account that both positive and negative ions have approximately the same effect; effect of charged species is chemical and not related to such physical phenomena as sheer stress, ion bombardment damage, or thermal effects; ions catalyze peroxidation processes of bacterial membrane composed of polysaccharides; simultaneous presence of oxygen and water vapor is necessary for effective bacteria inactivation.

References:

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2. D Dobrynin, G Fridman, G Friedman and A Fridman, "Physical and biological mechanisms of direct plasma interaction with living tissue", 2009 New J. Phys. 11 115020 (26pp)