

Comparison of various types and parameters of corona discharges for decontamination of surfaces and liquids

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Résumé

We studied the decontamination of surfaces and water suspension of microorganisms by low temperature plasma generated in the DC corona discharge of an open-air type in the point-to-plane or point-to-point arrangement. We found, that different types of inhibition zones appeared on surfaces, which indicates the different mechanism of action of the point-to-point and point-to-plane discharges. In liquids, the efficiency of decontamination is more or less equal for various bacteria and much lower for the eukaryotic yeast.

Introduction

The action of the plasma generated by electric discharges is one of the possible methods of inactivation of bacteria and other microbes, mediated by the bactericidal action of UV light and reactive particles. The various experimental arrangements, advantages and status of research in this field were reviewed in details in many works, e.g., [1], [2], [3], [4] and [5]. This contribution is a part of our systematic study of properties and differences of various types and arrangements of DC discharges and their microbial effects.

Apparatus, methods and the microorganisms under study

We studied the inactivation of microbial suspensions in wells of a dot plate or on wet surfaces by low temperature plasma generated in the DC corona discharge. The used simple apparatus of an open-air type enabling the point-to-plane or point-to-point arrangement was previously described in [6]. The point-to-plane corona discharge was generated on the point electrode represented by the tip of a hypodermic needle and situated 4 mm over the sample. The plane anode was either the suspension in the well or the conducting surface of an agar cultivation medium. The bipolar point-to-point corona discharge was generated on a pair of hypodermic needles arranged in an angle of 30° with tips approx. 4-6 mm apart and situated 4 mm over the sample.

For the decontamination of liquids, 0.5 ml of the suspension of microorganisms was pipetted into the sterile wells of a dot plate, grounded and exposed to the discharge. Following the exposition, the content of each well was diluted, spread onto the surface of cultivation medium and after the overnight cultivation at 37 °C, the numbers of survival colonies were counted.

For the surface decontamination, 1 ml of the microbial suspensions were inoculated onto the surface of Sabouraud or Mueller-Hinton agar (Lab M, Ltd.) diluted to obtain the microbial concentration of 10⁶ cfu cm⁻². After the suspension soaked, the samples were exposed to the corona discharge, incubated at 37 °C for 24 hours and the inhibition zones were measured.

All expositions were performed under laminar flow of HEPA-filtered air to prevent the airborne contamination, the ambient conditions were controlled by an air-conditioning of the laboratory.

The microorganisms under study were "wild" strains of the following species isolated at the Institute of Immunology and Microbiology: a yeast *Candida albicans*, Gram-negative bacterium *Escherichia coli* and Gram-positive bacterium *Staphylococcus epidermidis*.

Experiments

In the case of decontamination of liquids, the parameters of discharges were adjusted as follows: for the positive transient spark corona to $U = 10$ kV, 8 kV or 6 kV and $I = 200$ μA; for the negative pulseless glow regime to $U = 9$ kV and $I = 180$ μA; for the negative glow discharge to $U = 9$ kV and $I =$

370 μA . Using the different parameters respects the different character of particular discharges. The samples were exposed up to 8 min.

In the case of decontamination of surfaces, the point-to-plane discharge was adjusted to the current $I = 50 \mu\text{A}$, its voltage was $U = 4.6 \text{ kV}$. The point-to-point corona discharge was adjusted to the current $I = 200 \mu\text{A}$ and voltage $U = 10 \text{ kV}$. The different parameters for particular discharge types are determined by the different character and geometry of discharges. The values represent a compromise between discharge stability (transition into spark) and its energy and enable the qualitative comparison of all discharge types. The samples were exposed for 8 minutes.

Results and discussion

In liquids, the total inactivation of bacteria becomes after 60 seconds of exposition to the positive discharge and after 75 seconds of exposition to the negative one. On the other hand, exposition times of 8 or 10 minutes are necessary for the total inactivation of yeast by positive or negative discharge, respectively. The best efficiency of decontamination was found for the positive transient spark corona discharge at voltage in the interval of $U = 8 - 10 \text{ kV}$ and current of $I = 200 \mu\text{A}$. The negative glow discharge is equally or more effective than the pulseless one. The inactivation of the eukaryotic yeast is less effective and requires 8 - 10 times longer expositions.

Interesting results were obtained after exposition of inoculated agar surfaces. The point-to-plane discharge produced circular and sharply bordered inhibition zones with diameters of 5 - 6 mm, which were completely clear and contained no growing microbes. In contrast to liquids, the greater zones and thus greater sensitivity to the discharge were observed for *Candida* than for bacteria. After exposition to point-to-point discharge, we obtained two types of asymmetric fan-shaped zones of incomplete inhibition. In the case of *Candida albicans* yeast and Gram-positive bacterium *Staphylococcus epidermidis*, the inhibition zones were well bordered and almost clear of any surviving colonies, whereas for the Gram-negative bacterium *Escherichia coli* we obtained larger fan-shaped zones containing the reduced number of surviving colonies and very small, if any, zone of total inhibition only. The areas of total inhibition were approximately of $S = 3 \text{ cm}^2$ for *Candida albicans* and $S = 1 \text{ cm}^2$ for *Staphylococcus epidermidis*, the zone of incomplete *Escherichia coli* inhibition was of $S = 10 \text{ cm}^2$. The different plasma-chemical processes in the discharges and the different reaction mechanisms with the microbial cell wall are probably responsible for these effects.

Conclusions

It can be concluded that the positive transient spark corona displays the best efficiency of liquids decontamination. The efficiencies of used discharges are very similar, so that the precise control of their parameters is not necessary in the possible practice.

The results of decontamination of surfaces imply the different mechanisms of microbial inactivation by the point-to-point and point-to-plane discharges. Whereas point-to-plane discharge produces uniform and nearly circular inhibition zones of total inhibition, the character and appearance of two zone types produced by the point-to-point discharge was different. This fact supports the assumption that the sterilization agents are of different nature in point-to-point and point-to-plane discharges, displaying different efficiency and probably different mechanisms of action on microbial structures.

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