STAPHYLOCOCCUS AUREUS INACTIVATION BY USING ATMOSPHERIC PRESSURE GLOW DISCHARGE WITH PLASMA CATHODE

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Non-equilibrium plasma at atmospheric pressure can present an alternative to traditional methods of sterilization and disinfection /1/. At present for non-equilibrium plasma generation at atmospheric pressure various sources are widely used: surface and volume dielectric barrier discharges, atmospheric pressure plasma jets, etc. Despite the huge number of papers on plasma sterilization, the full understanding of the mechanisms of microorganisms inactivation in a wide range of characteristics of nonequilibrium plasma is still absent, which hinders the development of technology of plasma decontamination and especially plasma medicine. In this paper vegetative bacteria *Staphylococcus aureus* inactivation using atmospheric pressure glow discharges (APGD) with plasma cathode in the three-electrode system is considered /2/. The discharges are stationary that makes easier the identification of linkages between inactivation characteristics and plasma parameters, which is important for elucidating the mechanisms of plasma inactivation.

Schematic diagram of experimental setup is shown in Fig. 1*a*. The discharge chamber has two sections (upper section A and lower section B) separated by 1 mm thick copper plate with 2 mm central hole. Working gas at a flow of ~1 L/min at atmospheric pressure is fed through section A, central hole and section B. The working gas was helium grade B (the total concentration of impurities

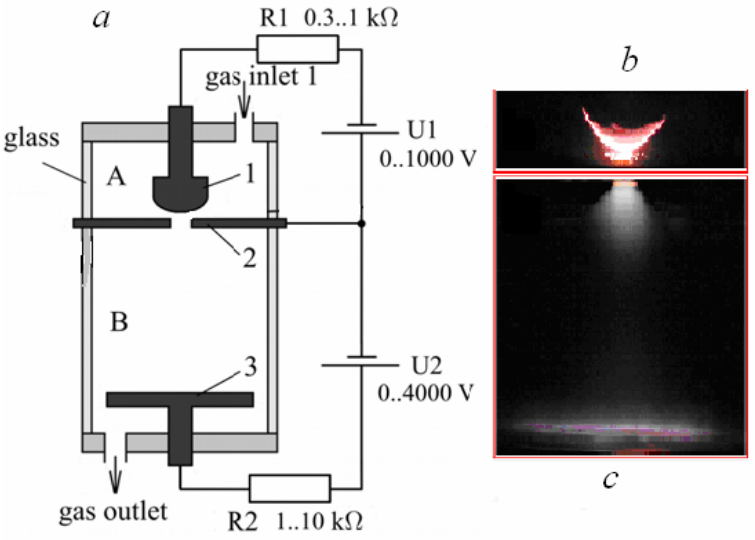

Fig.1. Sketch of discharge system (*a*) and images of self (*b*) and non-self discharge (*c*)

H_2, N_2, H_2O and others does not exceed 0.01% with a maximum H_2O content of 0.005%). In the upper section a self-sustained normal atmospheric pressure glow discharge is initiated between weakly rounded tungsten cathode 1 and copper plate anode 2. For better discharge stability the gap is usually on the order of 1 mm. The Fig. 1*b* shows an image of the discharge in section A at a current of 300 mA. The discharge serves as plasma cathode for the main discharge which is created in section B by applying a positive potential to the copper planar electrode 3. In this paper a helium large-volume diffuse non-self-sustained APGD at a current 1 mA and 5 mA at 3cm interelectrode gap is used for surface decontamination from bacteria. An image of non-self-discharge with plasma cathode at a current of 5 mA is shown in Fig. 1c. Two power supplies and corresponding ballast resistors are used to maintain the discharges.

Bactericidal effectiveness of helium plasma of non-self-sustained discharge was investigated on Gram-positive *Staphylococcus aureus*. They cause many diseases, including superficial and deep abscesses, poisoning, urinary tract infections. A strain of *Staphylococcus aureus ATCC 6538* has a typical biochemical characteristics of the genus, is highly resistant to drying and exposure to other environmental factors. Microorganisms were deposited on stainless steel 2x2 cm samples with average surface density of about 10^6 cm^{-2}.

After preparation a procedure of sample exposure to plasma was performed. The samples for plasma treatment were placed on the anode (bottom electrode) for an exposure time. For quantitative determination of inactivation effect a direct cell counting method was used. Fig. 2 shows the inactivation curves for vegetative bacterial cells *Staphylococcus aureus* ATCC 6538 at 0 mA (only plasma cathode), 1 mA and 5 mA. It can be seen that the plasma treatment in a low-current APGD the number of viable microorganisms decreases exponentially with time. D-times of inactivation decrease with discharge current increase.

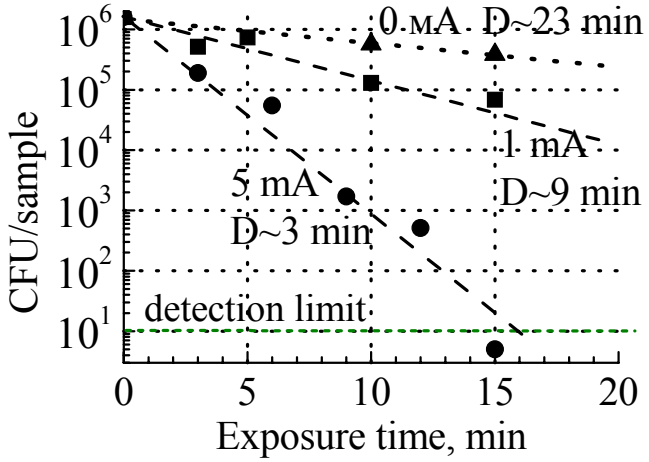

Fig. 2. The inactivation curves for vegetative bacterial cells *Staphylococcus aureus* ATCC 6538 at 0 mA, 1 mA and 5 mA

Today's conventional belief is that in the process of inactivation using plasma mainly four components are important, those are heat, ultraviolet radiation, charged particles and neutral oxygen-and nitrogen-containing chemically reactive species (OH, NO, O_3 and

etc.) /3/. On the basis of this, we define the parameters of the plasma and determine the role of different mechanisms that lead to the inactivation of bacteria.

Gas temperature T_g was determined by the relative intensities of the rotational lines of the vibrational-rotational band of hydroxyl (0,0) OH (A-X). At a current of 5 mA the gas temperature T_g ~ 50 ºC at the vicinity of the sample with microorganisms. Therefore, in our case the thermal action of the plasma can not have a significant effect on the inactivation of microorganisms.

Ultraviolet radiation with wavelengths of 205–315 nm has a high bactericidal capacity causing dimerization of thymine in DNA molecules. The quantitative value that characterizes the UV radiation impact on microorganisms is bactericidal surface irradiance representing the convolution of the spectral efficiency of bactericidal efficiency /4/ to the spectral density of the energy flow surface density. UV flux incident on the sample with microorganisms consists of a plasma radiation flow form non-self-sustained discharge and the flow from the plasma cathode through the opening between the sections. Inactivation D-time changes at transfer from bacteria exposure only to plasma cathode (Fig. 2, 0 mA) to the cumulative effects of the plasma cathode and non-self-sustained discharge (Fig. 2, 1 mA and 5 mA) and the charge is much greater than the ratio of bacterial surface irradiances of the sample caused by these discharges. This suggests that ultraviolet radiation is not one of the main mechanisms that cause the death of bacteria in our experiments.

Two mechanisms of charged particles inactivation effects on microorganisms should be marked: direct chemical effect of electrons and ions and breaking of bacterial cells membranes due to the accumulation of charged particles /5,6/. As noted in /5/, the mechanism of electrostatic damage can occur to Gram-negative organisms, and it is unlikely to Gram-positive bacteria which includes *Staphylococcus aureus*. As in our case the microorganisms are at the anode then the charged particles that can influence bacteria should be attributed to electrons. In practice electron beams with energies of hundreds of keV and higher /3/ are used to disinfect which is not the case.

In the anode region the reduced field strength increases sharply to 15 Td which corresponds to the average electron energy of about 7 eV. This leads to an appearance of thin luminous disk on the anode surface. Fig. 3 shows the emission spectrum of the plasma at a discharge current of 5 mA at the vicinity of the sample surface on which the micro-organisms are deposited. The presence of OH and NO molecules in the plasma near bacteria follows from Figure 3. Since the effectiveness of OH bactericidal impact on microorganisms is greater than two orders of magnitudes than for NO /6/, then Fig. 3 indicates that in this case the primary neutral chemically active species leading to bacterial death are OH molecules. At a current of 5 mA the calculated concentration of OH molecules

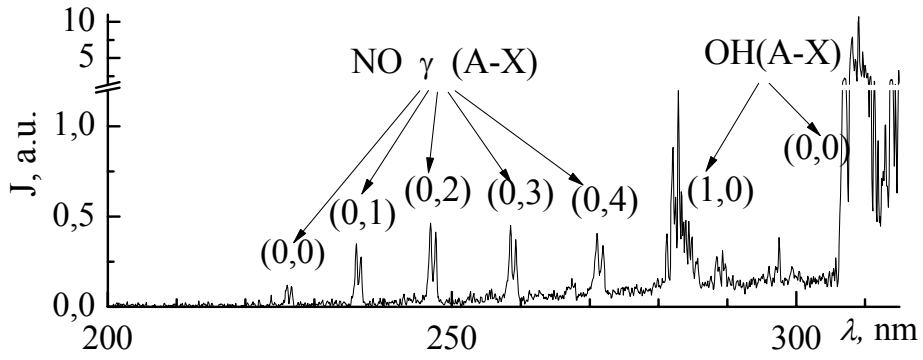

Fig. 3. Spectrum of plasma radiation near the surface of the sample

near the sample with microorganisms and the diffusion flux of OH onto its surface are $4.5 \cdot 10^{11} cm^{-3}$ and $5.0 \cdot 10^{13} cm^{-2} c^{-1}$. It was assumed that OH molecule are formed due to dissociation of water molecules by electron impact with a cross section of the reaction taken from /7/. The electron energy distribution function was calculated with computer program BOLSIG+ /8/. The electron density near the sample surface corresponding to the field strength 15 Td was estimated as 10^{10} cm^{-3} and the concentration of water in helium was assumed as maximum for the used grade B gas. Using the data for the constants of *Escherichia coli* inactivation presented in /6/ and the D-time values obtained in our case gives the surface concentration of OH molecules about $3.5 \cdot 10^{10}$ cm^{-3}. Taking into account that inactivation time of *Escherichia coli is* 1.5 times less than one for *Staphylococcus aureus* we find that our calculated OH concentration is 8 times higher than one obtained using the data from /6/. Perhaps it is due to excessive water concentration used in our calculations.

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