UDK 533.9

PLASMA STERILIZATION IN LOW-PRESSURE RF DISCHARGE

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The present paper clarifies the conditions under which the process of plasma sterilization of medical tools may be efficiently performed in the RF capacitive gas discharge of low pressure in air. Experiments were performed with a number of gram-positive and gram-negative bacteria as well as with fungi. The process of sterilization in the RF discharge is shown to possess a threshold pattern. Probably the bombardment of bacteria with positive ions and hot molecules of the neutral gas is the main sterilizing factor in the low pressure RF discharge, and the UV radiation of plasma plays the auxiliary role.

1. Introduction

STERILIZATION in microbiology and medicine stands for total destruction of micro-organisms and their spores with the help of physical and chemical means. Most frequently one uses such physical means as temperature, ultraviolet rays, high-energy radiation, ultrasound and filtration.

Methods of contemporary sterilization:

- **High-temperature method [1]:**

1) over the flame, 2) through boiling, 3) with dry heat, 4) with flowing vapour, 5) with vapour under pressure.

- **Low-temperature sterilization [2]:**

1) with ionising radiation, 2) with liquid chemical substances, 3) with hydrogen peroxide, 4) with ozone, 5) plasma sterilization.

Plasma methods of sterilization:

- In the plasma of a chemical mixture [2].
- In the low-temperature plasma of hydrogen peroxide [2 – 5].
- In the gas discharge under ambient pressure [6 – 11].
- In the DC glow discharge under low gas pressure [12].
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Plasma sterilization is the "youngest" and the most promising way to disinfect medical tools. The surge of the developments in the plasma sterilizer design in various countries indicates the vital nature of this topic. However, as a rule one applies in plasma sterilizers the hydrogen peroxide vapour and some costly mixtures of gases and vapours thus making the operation costs of such a sterilizer higher. Therefore this paper devotes the main attention to the feasibility of using the RF capacitive low-pressure gas discharge in air for sterilizing medical tools.

The theme of this paper lies at the interface between the gas discharge physics and medicine. The limited number of publications on this theme has made it necessary to perform a number of experiments with the cultures of various bacteria. The experiments have resulted in establishing the conditions under which the RF capacitive low-pressure discharge in air may be applied for sterilizing medical tools. We have also obtained a number of new results, i.e., establishing the almost threshold pattern of the plasma sterilization process as well as the parameters of the RF capacitive discharge in the presence of a gauze spanning inside the discharge volume.

2. Experimental details

Experiments on plasma sterilization were performed in the discharge chamber of 100 mm in diameter and the interelectrode gap of 54 mm. A RF capacitive gas discharge in air was ignited in the chamber within the pressure range $p = 0.1$-1 Torr. The RF voltage with the 13.56 MHz operation frequency was supplied from the generator to one of the electrodes. Another electrode was grounded. Gauze with 0.5-cm mesh size was installed between electrodes occupying all the cross-section of the discharge tube. Samples under study with bacterial cultures were put on this gauze (we used needles for blood tests as samples). The chamber was evacuated via a preliminary vacuum pump down to pressures below 0.1 Torr, then air was puffed up to the operating pressure and the RF discharge was ignited.

The following cultures were put on the needles: gram-positive (S. aureus, S. epidermidis, Str. mitis) and gram-negative (K. pneumoniae, P. mirabilis, E. cloacca, E. coli) bacteria, as well as fungi (Candida). First we sterilized the samples (needles) in the RF discharge during 5 min to remove possible contamination of samples with environmental microbes.

3. Experimental results

Figure 1 shows the air pressure dependence of the number of K. pneumoniae left after sterilization at the RF voltage $U_{rf} = 500$ V and the processing period $t = 1$ min. One sees from this figure that the pressure range $p > 0.4$ Torr is the most advantageous for performing sterilization.

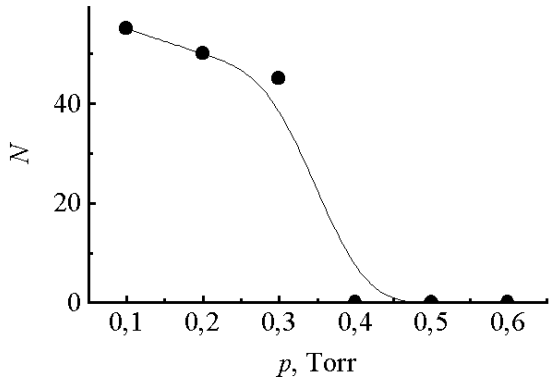

Fig.1. Air pressure dependence of the number of K. pneumoniae left after sterilization at the RF voltage U_{rf} = 500 V and the processing period of t = 1 min

Figure 2 shows the ratio of the number of microbes left after sterilizing the sample to the initial number of K. pneumoniae against the U_{rf} voltage value for the processing period of t = 0.5 min. This figure exhibits the threshold-like pattern of the sterilization process. For the given processing period of t = 0.5 min the RF voltage values required for sterilizing are U_{rf} > 350 V. At lesser RF voltage values these microbes remain alive, their number being approximately equal to the initial number of microbes put on the sample.

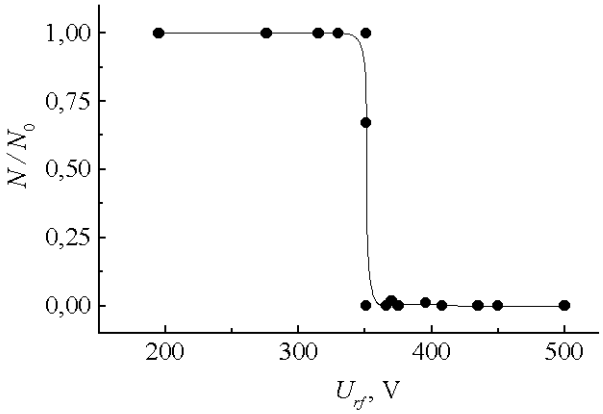

Fig.2. Ratio of the number of microbes left after sterilizing the sample to the initial number of K. pneumoniae against the U_{rf} voltage value for the processing period of t = 0.5 min and the air pressure of p = 0.6 Torr

Figure 3 depicts the ratio of the number of S. aureus microbes N left after sterilization to the initial number N_0 against processing period at three different RF power levels. Figure 3 also indicates the threshold-like pattern of the N/N_0 versus processing period. The more is the RF discharge power level, the less is the time needed for sample sterilizing.

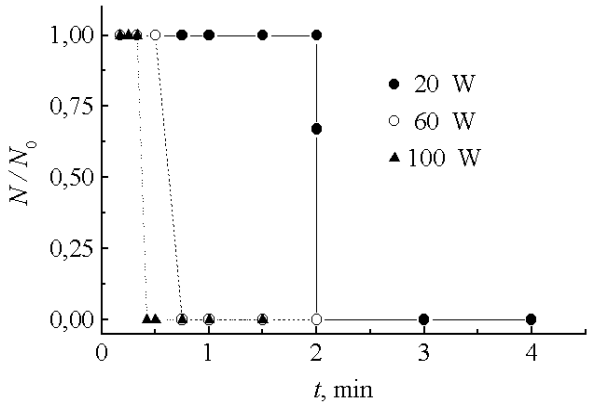

Fig. 3. Ratio of the number of S. aureus microbes N left after sterilization to the initial number N_0 against the processing period at three different RF power levels W = 20 W (U_{rf} = 195 V), W = 60 W (U_{rf} = 350 V) and W = 100 W (U_{rf} = 450 V) and the air pressure p = 0.6 Torr

Figure 4 depicts the dependence of the ratio N/N_0 of K. pneumoniae and E. coli microbes against processing period at the RF power value W = 100 W. We see from the figure that the quantity N/N_0 for these microbes sharply decreases with time at $t \geq 0.5$ min, and the pattern is also threshold-like.

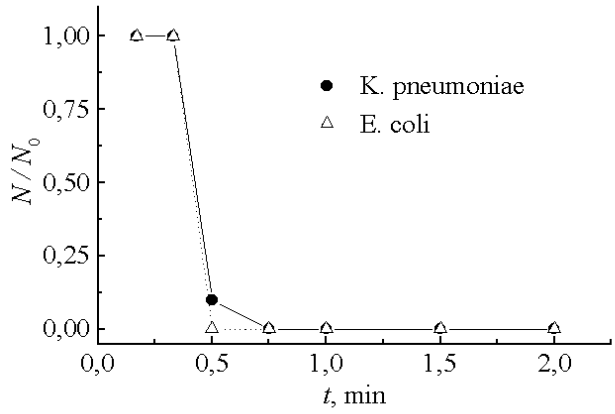

Fig.4. Ratio N/N_0 of K. pneumoniae and E. coli microbes against processing period at the RF power value W = 100 W (U_{rf} = 450 V) and the air pressure p = 0.6 Torr.

Figure 5 shows the dependence of the ratio N/N_0 for the Candida fungus culture against processing period at RF power levels W = 20 W and W = 100 W. It follows from the figure that at the RF power level W = 20 W the sample becomes sterile with the processing period $t \geq 2$ min, whereas with the RF power W = 100 W it is sufficient to burn the discharge for 10 seconds to sterilize the sample.

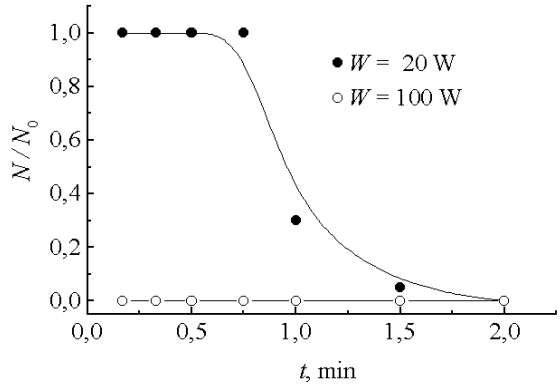

Fig.5. Ratio N/N_0 for the Candida fungus culture against processing period at RF power levels W = 20 W (U_{rf} = 195 V) and W = 100 W (U_{rf} = 450 V) and the air pressure p = 0.6 Torr.

It follows from our results that among the gram-negative bacteria the K. pneumoniae happened to be the most resistant to the action of the RF discharge. It has not only the outer casing (all bacteria have it) but a capsule protecting it from the effect of unfavourable conditions. Other gram-negative bacteria (P. mirabilis, э E. cloacca, E. coli) are less resistant to the action of the RF discharge. Therefore one requires less time for a complete sterilization of a specimen. Among the gram-positive bacteria S. Aureus is the most resistant, whereas other microbes we have studied (S. epidermidis, Str. mitis) have perished quickly in the RF discharge. The fungi (Candida) are killed easily with a burning RF discharge even at low RF power levels.

We also obtained the dependencies of the gas temperature and plasma density against air pressure near the gauze surface (Fig.6). The gas temperature was measured with a thermocouple located at the 2-mm distance from the gauze surface. The plasma density was measured simultaneously with a cylindrical probe.

The temperature values measured with a thermocouple represent not only the gas temperature, because the thermocouple is heated in the discharge by the molecules of the neutral gas hitting its surface as well as by the flows of ions, electrons and radiation (infrared, visible and ultra-violet light always present in the radiation spectrum of the RF capacitive discharge). The thermocouple was at the floating potential; thus its heating by the flows of charged particles was reduced. Actually the temperature values obtained corresponded to the temperature an isolated body can acquire in the discharge. In what follows, we will call it a "gas temperature".

As is seen from Fig. 5, under low pressures and moderate values of the applied RF voltage (U_{rf} = 200-300 V) the gas temperature is comparatively low ($T \approx 50 \div 60$ ^{o}C), but on increasing the RF voltage, the gas temperature grows and approaches the values $T \approx 100 \div 150$ ^{o}C. Increasing the pressure (up to 0.6-0.7 Torr) leads to the monotonous growth of the gas temperature, but at higher pressure values and small RF voltages the gas temperature decreases. The increase of the RF voltage at all pressure values was accompanied by the increase of the gas temperature. Within the pressure range up to 0.4 Torr and with RF voltages up to 450 V the plasma density did not actually depend on air pressure, by the subsequent increase of pressure led to the decrease in plasma density. In Fig. 6 the first three curves (U_{rf} = 210-450 V) relate to the weak-current mode of the RF discharge burning. At U_{rf} = 600 V and $p > 0.7$ Torr the RF discharge assumes the strong-current mode accompanied by a sharp increase of the gas temperature as well as of the plasma density near the gauze surface.

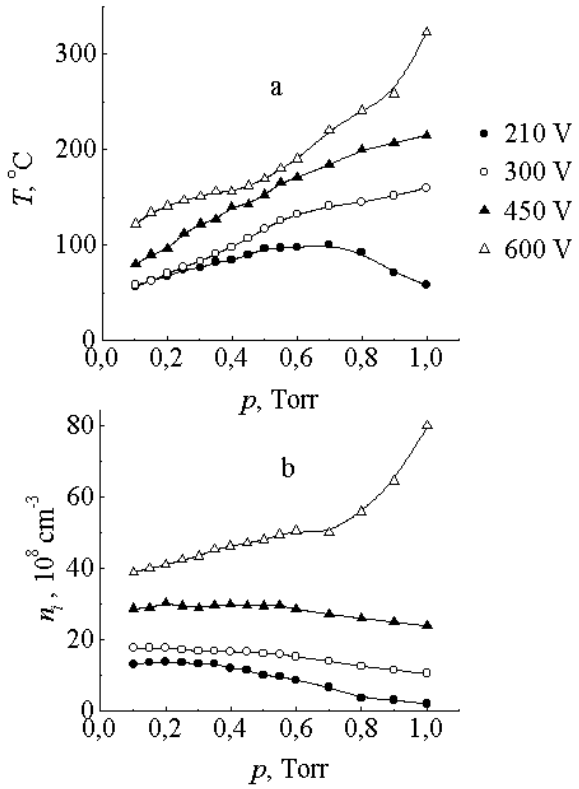

Fig.6. Gas temperature (a) and plasma density (b) near the gauze surface against air pressure at different RF voltages.

The abrupt decrease of the gas temperature and plasma density at $p > 0.7$ Torr and low RF voltage values may be attributed to approaching the RF discharge quenching. Therefore the ohmic power consumed by the RF discharge decreases. Consequently, at the constant RF voltage U_{rf} = 210 V the ohmic discharge current decreases leading to the decrease in the gas heating and plasma density lowering. Further increase of the air pressure at U_{rf} = 210 V leads to quenching the RF discharge.

The electron temperature and plasma density vaues

near the gauze surface shown in Fig. 7 have been determined from the current-voltage characteristics of the cylindrical probe (5 mm in length and 0.18 mm in diameter). One sees from this figure that the plasma density near the gauze surface is approximately proportional to the RF voltage applied in the weak-current regime. After the transition of the RF discharge to the strong-current regime the growth rate of the plasma density increases abruptly. The electron temperature near the gauze surface is practically constant in the weak-current regime (T_e = 3.6 eV). On increasing the RF voltage the electron temperature incrases up to 5 eV before the quenching of the discharge. The transition of the RF discharge to the strong-current regime of burning leads to the decrease of the electron temperature [12-14].

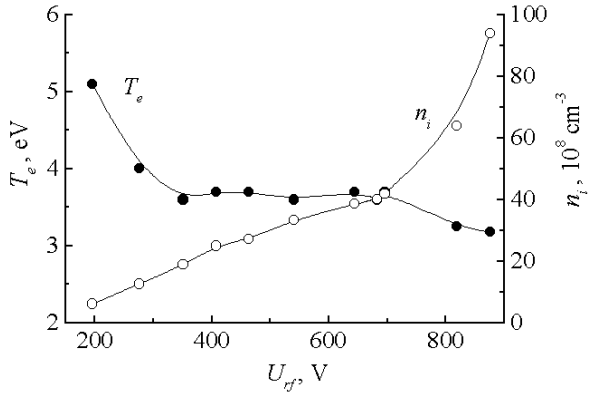

Fig.7. The electron temperature and plasma density near the gauze surface against the RF voltage at the air pressure p = 0.6 Torr.

Figure 8 shows the axial profiles of the plasma density $n_i(z)$ at various values of the RF voltage applied. In this figure the broken line shows the position of the gauze on which the samples we have put. The axial profiles possess clearly pronounced maxima in the vicinity of the boundaries of near-electrode layers even in the weak-current regime of the RF discharge burning. One also observes a not very large maximum near the gauze. On increasing the RF voltage, the plasma density increases within the whole discharge gap. First, at low RF voltage values the discharge exhibits the approximately uniform luminosity in the whole volume of the chamber with a slight increase of luminosity near the boundaries of the near-electrode layers and near the gauze surface. The increase of the RF voltage leads to the picture when the sharp maxima of luminosity are observed near the layers and the gauze whereas in all other regions the discharge luminosity is weak. In the weak-current regime of the discharge burning the characteristics of the luminosity near the gauze are similar to those of the positive column of the DC glow discharge.

The following main factors may affect the microorganisms in the RF discharge in air at low pressures:

- flows of charged particles (electrons, ions);

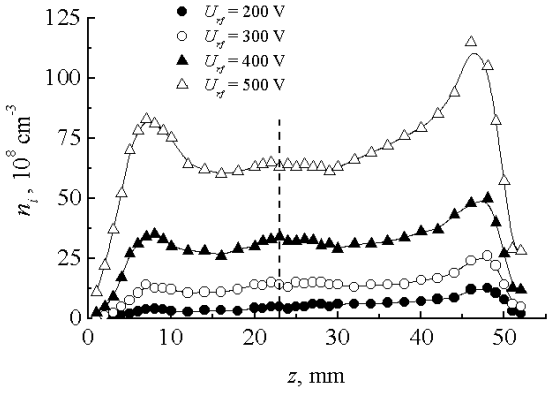

Fig. 8. Axial profiles of the plasma density at various values of the RF voltage applied U_{rf} and the air pressure p = 0.6 Torr.

- heated neutral gas;
- UV radiation;
- low pressure (vacuum).

Our studies show that storing samples in the vacuum chamber with the inner air pressure order of 0.1 – 10 Torr during 12 hours does not decrease the quantity of microbes put on the sample initially. Therefore vacuum has no noticeable effect on bacterial cultures stored in it. Some samples with the cultures on them were processed only with the discharge radiation because we had put them outside the chamber but close to the quartz wall in the region of maximum radiation at W = 400 W during 5 min. Such a processing technique also did not affect the initial number of bacteria on the sample. For comparison it may be stated that under the same conditions the samples inside the chamber were sterilized already after 5 s after switching on the discharge. Therefore the discharge UV radiation itself does not kill bacteria, and some other factors are also needed for the sample sterilization.

This somewhat unexpected result may be explained as follows. In order to sterilize with the UV radiation, one requires the time period order of some ten minutes [12], i.e., this process is comparatively slow. In our case the required period of sterilization was in the range of ten seconds to some minutes. Therefore other faster mechanisms were at work under conditions of our experiments

As the gauze with the samples on it was under the floating potential, the decelerating potential of 10-15 V stopped the bulk of electrons, and only a small portion of the fastest electrons could reach the samples. The gauze with samples was located sufficiently far from the boundaries of near-electrode layers, and sterilizing was performed most frequently in the weak-current mode of the discharge. Therefore the beams of fast electrons generated by the ions hitting the electrodes and gaining energy in the near-electrode layers as well as the electrons gaining energy under stochastic heating near the layer boundaries actually did not reach the sample surface.

Consequently, the contribution of the electron flow to the sterilization process could not be substantial.

A flow of positive ions was also incident on the samples. For the ions this voltage drop 10-15 V was accelerating. The accelerated ions bombarded the surface of bacteria demolishing their protective outer shells. If one measures the flux of positive ions on the probe at the RF voltage values given in Figs 2-5 and multiplies the threshold time t_s, at which one observes the step-like decrease of the number of bacteria on the sample by the ion current value I_i, then the product $D = t_s\ I_i$ remains approximately constant. For example, for the cylindrical probe applied (0.18 mm in diameter, 5 mm in length) we have obtained the value D = 770-800 μA·s. The quantity D is the dose of ion irradiation. Probably for a bacterium to be killed it should obtain a certain dose of ion irradiation, i.e., its shell should experience a certain amount of collisions with accelerated ions.

Hot gas molecules may cause heat damage to the outer shells of bacteria involving their death. At the air pressure p = 0.6 Torr even at the lowest RF voltage of RF discharge burning U_{rf} = 210 V the neutral gas temperature is approximately 100 ^{o}C (Fig.6), and at U_{rf} = 600 V the gas temperature is already 200 ^{o}C. For the instantaneous death of S. aureus bacteria one should heat them to the temperature of 100 ^{o}C. The processing occurs at the lowered gas pressure, then for heating bacteria one requires more time than in air at atmospheric pressure. The more is the temperature of the neutral gas, the less time is required for heating bacteria to death temperatures.

Consequently, bombardment of bacteria with the flows of positive ions and hot molecules of the neutral gas is the main sterilizing factor in the low-pressure RF discharge. Probably the UV radiation of plasma plays the auxiliary role.

4. Conclusions

Thus the present paper clarifies the conditions under which the process of plasma sterilization of medical tools may be efficiently performed in the RF capacitive gas discharge of low pressure in air. Experiments were performed with a number of gram-positive and gram-negative bacteria as well as with fungi. The process of sterilization in the RF discharge is shown to possess a threshold pattern. Probably the bombardment of bacteria with positive ions and hot molecules of the neutral gas is the main sterilizing factor in the low pressure RF discharge, and the UV radiation of plasma plays the auxiliary role.

References

1. V.I. Vashkov Sredstva i metody sterilizatsii, primenyaemye v meditsine. M.: "Meditsina", 1973 (In Russian).
2. The Future of Low-Temperature Sterilization Technology. // Advanced Sterilization Products, 1996.
3. P.T. Jacobs // J. Healthcare Materiel Management, 1989, vol. 7, p. 49.
4. K.B. Frey // Surgical Technology, 1994, p. 8 .
5. P.T. Jacobs STERRAD Sterilization System: A new technology for instrument sterilization. // Advanced Sterilization Products, 1994.
6. Y. Ku, C. Brickman, K. Wintenberg, T.C. Montie, P. Tsai, L. Wadsworth, J.R. Roth // Record-Abstracts of IEEE Intern. Conf. On Plasma Sci., 1996, Boston, USA, p. 2IP15.
7. M. Laroussi // IEEE Trans. Plasma Science, 1996, vol. 23, p. 1188.
8. A.K. Carr, J.R. Roth, C. Brickman, K. Kelly-Wintenberg, T.C. Montie, P. Tsai, L. Wadsworth // Record-Abstracts of IEEE Intern. Conf. On Plasma Sci., 1997, San Diego, USA, p. 4Q21.
9. D.M. Sherman, R.B. Gadri, F. Karakaya, Z.Che, T.C. Montie, K. Kelly-Wintenberg, P.P.Y. Tsai, J.R. Roth // Record-Abstracts of IEEE Intern. Conf. On Plasma Sci., 1999, Monterey, USA, p. 2B08.
10. K. Kelly-Wintenberg, T.C. Montie, J.R. Roth, Z. Chen // Record-Abstracts of IEEE Intern. Conf. On Plasma Sci., 1999, Monterey, USA, p. 4P09.
11. E. Garate, O. Gornostaeva, I. Alexeff // Record-Abstracts of IEEE Intern. Conf. On Plasma Sci., 1999, Monterey, USA, p. 4P10.
12. V.A. Khomich, I.L. Mikhno, I.A. Soloshenko, V.V. Tsiolko // Abstracts of 25th EPS Conf. on Controlled Fusion and Plasma Physics, Praha, 1998, p. 209.
12. Yu.P. Raizer Gas Discharge Physics. Berlin: Springer, 1991.
13. Yu.P. Raizer, M.N. Shneider and N.A. Yatsenko Radio-Frequency Capacitive Discharges New York: CRC Press, 1995.
14. V.A. Lisovskiy // Technical Physics, 1998, vol. 43, p. 526.