Features of Sterilization Using Low Pressure DC Discharge Hydrogen Peroxide Plasma

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Abstract.

This investigation studies the theoretical and practical features of sterilization using glow discharge plasma in hydrogen peroxide vapor. It is determined that, in such a system, most sterilization is performed by active particles formed in the plasma, rather than by ultraviolet (UV) radiation (as is the case in gas discharges like air and oxygen). This study shows that sterilization by discharge plasma in hydrogen peroxide vapor is more efficient than sterilization by plasma of the discharge in gases: sterilization time is 2-3 times shorter in open surfaces and 10 times shorter in packed articles. This study's calculations indicate that this enhanced efficiency might be due to fact that concentrations of the main biologically active particles, such as atomic oxygen and oxygen molecules excited to lower metastable states, reach values of $\sim 10^{14}$-10^{15} cm^{-3}, which is 2-3 order of magnitude higher than that in oxygen plasma at the same parameters of the discharge.

Index words: plasma, discharge, hydrogen peroxide, sterilization.

1. Introduction.

In modern medical practice, a wide variety of heat-sensitive instruments and materials is used that requires cold sterilization techniques. Up to now, sterilization of such articles was performed by means of toxic gases—pure ethylene oxide or its mixture with fluorochlorocarbons. This sterilization technique requires a long aeration process (up to 24 hours) and, importantly, creates a serious threat for both personnel and the environment. For these reasons, development of new cold sterilization techniques is extremely problematic. One of the most serious current alternatives of gaseous sterilization is the use of gas discharge plasma as a sterilizing agent. The plasma technique is advantageous because, as chemically active medium, plasma is formed by excitation, dissociation, and ionization of any gaseous or vaporous substance, including non-toxic substances and even inert gases. Such active particles exist only while the discharge glows, and they disappear almost immediately after turning off the discharge. These two circumstances completely solve the problem of safety and ecology.

For an unbiased estimation of the efficiency and application range of the plasma sterilization technique, it is necessary to first investigate the main sterilizing factors of gas discharge plasma. In recent studies [1, 2], the factors determining sterilizing action of DC glow discharge plasma [3] in oxygen, air, carbon dioxide gas, hydrogen, argon, nitrogen and their mixtures were studied both theoretically and experimentally. These investigations show that the main role in plasma sterilization of open surfaces is performed by the plasma's UV radiation, whereas sterilization of the instruments having complex shape is determined by action of electrically neutral chemically active plasma particles.

Other recent studies [4, 5] have investigated the features of the use of flowing afterglow of microwave gas discharge for efficient inactivation of bacterial spores. These studies show that, in such systems, atomic oxygen and UV radiation from flowing afterglow are both required for effective sterilization of microorganisms and their residuals. However, as has been shown in a third group of studies [6, 7], the discharges in hydrogen peroxide are more promising than that in gases. In accordance to these studies, the present research is devoted to experimental and theoretical investigation of the main sterilizing factors of the plasma of DC glow discharge in hydrogen peroxide vapor. Unlike this third group of studies, our investigations are performed with essentially higher concentrations of hydrogen peroxide and with another kind of discharge.

2. Experimental technique and methods.

This investigation employed a chamber volume of 25 liters, and the following sterilization algorithm was used:

1. The chamber was evacuated by a forevacuum pump down to pressure lower than 0.1 Torr.
2. The pump was turned off, and the hydrogen peroxide aqueous solution vapor (with either 30% or 60% concentration) was supplied to the chamber.
3. The chamber was then evacuated down to an operating pressure of ~0.2 Torr, and the DC discharge was ignited for the sterilization duration. (Density of the power W_d introduced into the discharge was varied from 0.001 to 0.01 W/cc.)

Supply of hydrogen peroxide aqueous solution vapor into the preliminarily evacuated chamber was performed in two ways. In the first case, a H_2O_2 solution was injected into the chamber at the pressure of several atmospheres using a special nozzle. Dispersion of the solution reached the desired concentration, filling the chamber with vapor within 5-10 seconds. Shields mounted on the nozzle prevented direct contact of the H_2O_2 solution droplets with sterilized articles. In the second case, evaporation occurred within a special evaporator. The evaporator consisted of a massive hollow cylinder (70 mm long by 10 mm in diameter) heated up to $70\text{-}80^0$ C. The hydrogen peroxide solution was supplied into one end of the evaporator, entering the chamber as a vapor. The maximum amount of solution to be evaporated was estimated using the amount of vapor required to create a saturated vapor pressure in the chamber. For 30% H_2O_2 aqueous solution, this amount comprised about 0.5 ml with the chamber walls at 20^0 C.

The evacuation time—the time the chamber filled with vapor of H_2O_2 solution to the point of operating pressure at ~0.1-0.3 Torr—never exceeded one minute. During the sterilization process, the working pressure was maintained at this level due to evaporation of hydrogen peroxide and water from the chamber walls with simultaneous decrease of pumping out rate. The sterilization time was counted starting from the moment of discharge ignition. Density of power W_d introduced into the discharge was varied from 0.001 to 0.01 W/cc.

Metallic Petri dishes with internal surface of ~10 cm^2 were used as test-objects. Medical studies have been performed using spores of Bac. subtilis and Bac. stearothermophilus as the most resistant kinds of microorganisms.

3. Experimental results.

Since hydrogen peroxide is itself an efficient sterilizing agent, the first series of our experiments determined the role of hydrogen peroxide molecules in the overall sterilizing features of the plasma. The relative contributions of pure plasma sterilizing factors—UV radiation and biologically active particles formed in the plasma—were also determined. For that purpose, three groups of test objects were used: the first group was exposed only to hydrogen peroxide vapor; the second group was exposed to the combined action of hydrogen peroxide vapor and plasma; and the third group was exposed only to the UV radiation of the plasma. (In the last group, the test objects were vacuum covered by a filter made of KU-1 quartz, so that neither peroxide vapor nor active plasma particles could penetrate to the contaminated surface.) In figure 1, survival curves of the spores (depending on the number of survived spores on the sterilization time) are presented for three these cases. This illustration shows that the use of plasma decreases the sterilization time by about 3 times, as compared to sterilization by only hydrogen peroxide vapor. It should be noted that actual role of hydrogen peroxide molecules in sterilization is yet smaller since, as it will be shown below, they burn away in the discharge rather quickly and are converted to other particles (including biologically active ones in result of electron hits). The figure also shows that, unlike the case of plasma discharges in gases [1,2], ultraviolet radiation in this system does not contribute greatly. It is also important to note that sterilization time of open surfaces by plasma of the discharge in peroxide vapor is 2-3 times less than when using the discharge plasma in oxygen, which is the most efficient gaseous medium.

The second set of experiments was devoted to studying the efficiency of sterilization of articles packed in porous paper (which is used in gaseous sterilization technique), and also to sterilization of articles having complex shapes (particularly probes, catheters, etc.). Figure 2 illustrates survival curves obtained by sterilization of test objects packed in paper TPT-0260 (Type 1073-B Tyvek) with various doses of injected 30% H_2O_2 solution (and various concentrations of peroxide in the chamber, respectively). This figure demonstrates that, with the initial growth of peroxide dose evaporated in the chamber from 0.125 to 0.5 ml, the sterilization time decreases quickly, and with further dose increase up to 1.0 ml, the sterilization time remains practically unchanged. One possible reason for this is the saturation of the packaging paper by water vapor, which decreases the paper's penetrability. The experiments performed with injection of the same hydrogen peroxide amount by means of water solutions having various peroxide concentrations indirectly confirms these results.

Figure 3 illustrates the survival curves obtained at sterilization of the test objects packed in paper TPT-260 (Type 1073-B Tyvek) by oxygen and peroxide (using 30% H_2O_2 solution) plasmas at various powers introduced into the discharge. The figure shows that the sterilization time for packed articles in hydrogen peroxide use is about 10 times less than that in oxygen use. The figure also suggests that sterilization time decreases essentially with the growth of power introduced into the discharge. However, the surface temperature of typical articles made of plastics (catheters, endoscopes, etc.) indicate that specific power increase above the level of 0.01 W/cc is unreasonable, because it leads to heating processed articles over $50\text{-}55^0$ C.

Figure 4 illustrates survival curves obtained at sterilization of test objects packed in paper TPT-0260 (Type 1073-B Tyvek) by hydrogen peroxide with supply of 0.25 ml 60% H_2O_2 solution (7.5 mg/l concentration) for various powers introduced into the discharge. Comparison of survival curves in figures 3 and 4 shows that, in spite the fact that peroxide concentrations in the chamber were practically the same, sterilization efficiency in 60% solution injection was essentially higher. A possible reason for this effect is that the saturation of packaging paper by water vapor, which decreases its penetrability for particles formed in the plasma.

The accomplished experiments have shown that:

1. The main role in sterilization of open surfaces by plasma of the discharge in hydrogen peroxide solution vapor is performed by active particles formed in the plasma, not by UV radiation of the plasma.
2. Sterilization by hydrogen peroxide plasma is more efficient than sterilization by plasma of the discharge in oxygen, especially for packed articles and articles with complex shapes.
3. Optimal specific power introduced into the discharge comprises about 0.01 W/cm^3.
4. Efficiency of sterilization by hydrogen peroxide plasma depends not only on power introduced into the discharge and peroxide dose, but also on concentration of H_2O_2 solution injected into the chamber.

4. Results of computer simulation.

We have calculated the plasma component content for used discharge in order to determine the kind of active particles, which define sterilizing features of hydrogen peroxide plasma. This calculation was performed on a basis of kinetic equations, which were solved together with the Boltzman equation, using scheme described in existing research [2]. Elementary processes, which were taken into consideration while modeling the component content, are presented in table 1. At present time, there are practically no experimental data in the literature regarding cross sections of interaction between electrons and hydrogen peroxide molecules. For this reason cross, sections of non-elastic scattering of electrons on H_2O_2 molecules were calculated by Thompson-Gryzinski formulas [9, 10]. Values of rate constants for molecular processes were taken from several accepted studies [11-13].

Figure 5 shows temporal evolution of neutral components of the mixture for the discharge parameters W_d = 0.01 W/cc, P = 0.1 Torr used in the experiment. The ratio of initial concentrations of hydrogen peroxide and water vapors corresponded to the saturation pressures of these components over the surface of 60% aqueous solution of hydrogen peroxide at 20^0 C. As figure 5 illustrates, decomposition of hydrogen peroxide is completed in about 1 second because of the low dissociation energy of the H_2O_2 molecule (energy needed to break a HO-OH bond is ~2.2 eV, and that needed to break a HO_2-H bond is ~3.8 eV). Final products of H_2O_2 decomposition are oxygen and hydrogen molecules, and the concentrations of these molecules become equal to the initial hydrogen peroxide density. Among biologically active particles, as well as in case of the discharge in oxygen, oxygen atoms and oxygen molecules excited to the initial metastable levels (ε = 0.98 and 1.64 eV) demonstrate the highest concentrations. However, their concentration in hydrogen peroxide plasma is 2-3 order of magnitude higher than those with the discharge in oxygen. This difference is due to two reasons: 1) low bond energies in H_2O_2 molecule; 2) essentially higher concentrations of electrons possessing energies in 1-6 eV range in hydrogen peroxide plasma. The difference between electron energy distribution functions (EEDF) in two those media is due to the following circumstances. As it was shown in [14], EEDF in oxygen discharge plasma is cut off at energies about 1-2 eV due to excitation of the lowest metastable electron levels of O_2 molecule. As to plasma of the discharge in vapor of hydrogen peroxide aqueous solution, EEDF is determined first of all by properties of water molecules, because H_2O concentration in the discharge is essentially higher than concentrations of other particles, including O_2. In this case EEDF cutoff due to excitation of electron levels of water molecules occurs at energies $\geq$ 6 eV, which in turn results in generation rate growth for atomic oxygen and oxygen molecules excited to the lowest metastable states.

Thus on a basis of results of the calculations one can make the following conclusions:

1. Main sterilizing factors in hydrogen peroxide plasma are represented by oxygen atoms and oxygen molecules excited to the lowest metastable states.
2. High sterilization efficiency in hydrogen peroxide plasma, as compared to that in plasma of the discharge in the most efficient gaseous medium – oxygen, is due to fact that concentration of active particles in the first case is two to three orders of magnitude higher than that in the second case.
3. High concentration of active particles in hydrogen peroxide plasma is due to low bond energies in H_2O_2 molecules and appropriate EEDF in hydrogen peroxide plasma.

It should be also noted that the sterilization efficiency in hydrogen peroxide plasma may be enhanced at the expense of fact that active plasma particles stimulate disintegration of hydrogen peroxide molecules adsorbed at surfaces of sterilized articles with formation of very active OH and OH_2 radicals. Such possibility occurs due to existence of a mechanism of undivided chain reaction of hydrogen peroxide disintegration at its interaction with OH and OH_2 radicals. Formation of initial OH radical in this chain may occur, first of all, at coming oxygen atom to the surface of sterilized article by reaction (46) (see Table 1). Thus, the most probable chain of reactions at the surface is looking as follows:

$$O + H_2O_2 \rightarrow OH + HO_2$$

$$H_2O_2 + OH \rightarrow H_2O + HO_2$$

$$H_2O_2 + HO_2 \rightarrow H_2O + O_2 + OH$$

Thus, action on microorganisms in hydrogen peroxide plasma can be provided not only by active particles generated in plasma volume, but as well by those created at the surface of sterilized article.

5. Acknowledgements.

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6. References.

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Table 1.

NN	Reaction	Rate
1	$e + H_2O \rightarrow OH + H^-$	$9.0 \cdot 10^{-15}$ cm^3s^{-1} *
2	$e + H_2O \rightarrow H_2 + O^-$	$9.5 \cdot 10^{-16}$ cm^3s^{-1} *
3	$e + H_2O \rightarrow H + OH^-$	$3.1 \cdot 10^{-16}$ cm^3s^{-1} *
4	$e + H_2O_2 \rightarrow H_2O_2^+ + e + e$	$3.2 \cdot 10^{-11}$ cm^3s^{-1} *
5	$e + H_2O_2 \rightarrow HO_2^+ + H + e + e$	$2.2 \cdot 10^{-11}$ cm^3s^{-1} *
6	$e + H_2O_2 \rightarrow OH^+ + OH + e + e$	$2.2 \cdot 10^{-11}$ cm^3s^{-1} *
7	$e + H_2O \rightarrow H_2O^+ + e + e$	$2.4 \cdot 10^{-10}$ cm^3s^{-1} *
8	$e + H_2O \rightarrow H + OH + e$	$6.8 \cdot 10^{-12}$ cm^3s^{-1} *
9	$e + H_2O \rightarrow H_2 + O + e$	$7.2 \cdot 10^{-12}$ cm^3s^{-1} *
10	$e + H_2O_2 \rightarrow OH + OH + e$	$1.4 \cdot 10^{-9}$ cm^3s^{-1} *
11	$e + H_2O_2 \rightarrow HO_2 + H + e$	$3.1 \cdot 10^{-11}$ cm^3s^{-1} *
12	$O_2 + e \rightarrow O_2^+ + e + e$	$1.44 \cdot 10^{-10}$ cm^3s^{-1} *
13	$O_2(a) + e \rightarrow O_2^+ + e + e$	$3.6 \cdot 10^{-11}$ cm^3s^{-1} *
14	$O_2(b) + e \rightarrow O_2^+ + e + e$	$3.8 \cdot 10^{-11}$ cm^3s^{-1} *
15	$O + e \rightarrow O^+ + e + e$	$1.44 \cdot 10^{-10}$ cm^3s^{-1} *
16	$O(d) + e \rightarrow O^+ + e + e$	$2.9 \cdot 10^{-11}$ cm^3s^{-1} *
17	$O(s) + e \rightarrow O^+ + e + e$	$3.8 \cdot 10^{-11}$ cm^3s^{-1} *
18	$O_2 + e \rightarrow O_2(a) + e$	$8.0 \cdot 10^{-12}$ cm^3s^{-1} *
19	$O_2 + e \rightarrow O_2(b) + e$	$6.7 \cdot 10^{-13}$ cm^3s^{-1} *
20	$O + e \rightarrow O(d) + e$	$2.3 \cdot 10^{-9}$ cm^3s^{-1} *
21	$O + e \rightarrow O(s) + e$	$1.41 \cdot 10^{-11}$ cm^3s^{-1} *
22	$O_2 + e \rightarrow O + O + e$	$2.2 \cdot 10^{-11}$ cm^3s^{-1} *
23	$O_2^+ + e \rightarrow O + O$	$1.39 \cdot 10^{-8}$ cm^3s^{-1} *
24	$O_2 + e \rightarrow O^- + O$	$3.5 \cdot 10^{-15}$ cm^3s^{-1} *
25	$H_2 + e \rightarrow H_2^+ + e + e$	$3.8 \cdot 10^{-12}$ cm^3s^{-1} *
26	$H + e \rightarrow H^+ + e + e$	$3.8 \cdot 10^{-12}$ cm^3s^{-1} *
27	$H_2 + e \rightarrow H + H + e$	$1.9 \cdot 10^{-13}$ cm^3s^{-1} *
27	$H_2^+ + e \rightarrow H + H$	$1.39 \cdot 10^{-8}$ cm^3s^{-1} *
28	$H_2 + e \rightarrow H^- + H$	$6.8 \cdot 10^{-11}$ cm^3s^{-1} *
29	$O^- + O_2 \rightarrow O_3 + e$	$5.0 \cdot 10^{-15}$ cm^3s^{-1}
30	$O^- + O_2^+ + M \rightarrow O_3 + M$	$2.0 \cdot 10^{-25}$ cm^6s^{-1}
31	$O + O_2 + M \rightarrow O_3 + M$	$6.9 \cdot 10^{-34}$ cm^6s^{-1}
32	$O^- + O_2(a) \rightarrow O_3 + e$	$5.0 \cdot 10^{-14}$ cm^3s^{-1}
33	$O^- + M^+ \rightarrow O + M$	$8.0 \cdot 10^{-8}$ cm^3s^{-1}
34	$O^- + e \rightarrow O + e + e$	$4.7 \cdot 10^{-8}$ cm^3s^{-1}
35	$O^- + M \rightarrow O + M + e$	$2.0 \cdot 10^{-10}$ cm^3s^{-1}
36	$O^+ + O + M \rightarrow O_2^+ + M$	$1.0 \cdot 10^{-32}$ cm^6s^{-1}
37	$H^- + M^+ \rightarrow H + M$	$8.0 \cdot 10^{-8}$ cm^3s^{-1}
38	$H^- + e \rightarrow H + e + e$	$5.36 \cdot 10^{-10}$ cm^3s^{-1}

38	$H^- + M \rightarrow H + M + e$	$2.8 \cdot 10^{-10}$ $cm^3 s^{-1}$
39	$H^+ + H + M \rightarrow H_2^+ + M$	$1.0 \cdot 10^{-34}$ $cm^6 s^{-1}$
40	$OH^- + M^+ \rightarrow OH + M$	$8.0 \cdot 10^{-8}$ $cm^3 s^{-1}$
41	$O + HO_2 \rightarrow OH + O_2$	$5 \cdot 10^{-11}$ $cm^3 s^{-1}$
42	$H_2O_2 + h\nu \rightarrow OH + OH$	$7.77 \cdot 10^{-6}$ s^{-1}
43	$H_2O_2 + h\nu \rightarrow OH + OH$	$3.87 \cdot 10^{-5}$ s^{-1}
44	$O_3 + h\nu \rightarrow O(d) + O_2(a)$	$9.07 \cdot 10^{-4}$ s^{-1}
45	$O_3 + h\nu \rightarrow O + O_2$	$1.0 \cdot 10^{-4}$ s^{-1}
46	$O + H_2O_2 \rightarrow OH + HO_2$	$1.45 \cdot 10^{-15}$ $cm^3 s^{-1}$
47	$O + H_2O_2 \rightarrow H_2O + O_2$	$1.45 \cdot 10^{-15}$ $cm^3 s^{-1}$
48	$O + OH \rightarrow O_2 + H$	$5.0 \cdot 10^{-11}$ $cm^3 s^{-1}$
49	$H_2O_2 + OH \rightarrow HO_2 + H_2O$	$2.0 \cdot 10^{-12}$ $cm^3 s^{-1}$
50	$O(s) + H_2O \rightarrow O(d) + H_2O$	$3.0 \cdot 10^{-10}$ $cm^3 s^{-1}$
51	$O(s) + H_2O \rightarrow O + H_2O$	$3.0 \cdot 10^{-10}$ $cm^3 s^{-1}$
52	$O(s) + H_2O \rightarrow OH + OH$	$3.0 \cdot 10^{-10}$ $cm^3 s^{-1}$
52	$O(s) + H_2O \rightarrow H_2 + O_2$	$3.0 \cdot 10^{-10}$ $cm^3 s^{-1}$
53	$OH + HO_2 \rightarrow H_2O + O_2$	$7.0 \cdot 10^{-11}$ $cm^3 s^{-1}$
54	$O(d) + O_3 \rightarrow O + O + O_2$	$2.33 \cdot 10^{-10}$ $cm^3 s^{-1}$
55	$O(d) + O_3 \rightarrow O_2 + O_2$	$2.33 \cdot 10^{-10}$ $cm^3 s^{-1}$
56	$O_2(b) + O_3 \rightarrow O + O_2 + O_2$	$1.03 \cdot 10^{-11}$ $cm^3 s^{-1}$
57	$O_2(b) + O_3 \rightarrow O_2(a) + O_3$	$1.03 \cdot 10^{-11}$ $cm^3 s^{-1}$
58	$OH + O_3 \rightarrow HO_2 + O_2$	$7.55 \cdot 10^{-14}$ $cm^3 s^{-1}$
59	$H + HO_2 \rightarrow H_2O_2^*$	$9.0 \cdot 10^{-11}$ $cm^3 s^{-1}$
60	$H + HO_2 \rightarrow OH + OH$	$9.0 \cdot 10^{-11}$ $cm^3 s^{-1}$

*- rates were calculated with the use of electron energy distribution function.

Figure captions.

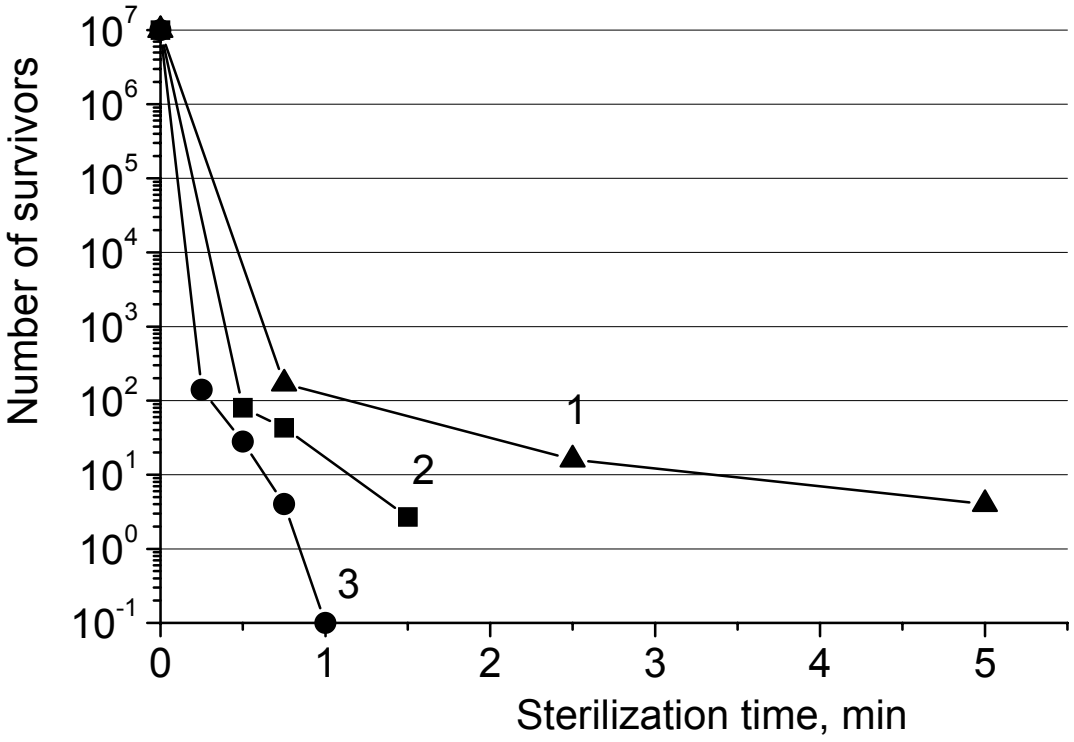
Figure 1. Survival curves for spores Bac. subtilis, obtained by colony count method during the sterilization of open surfaces on test objects using three methods: ultraviolet radiation of the plasma (curve 1), hydrogen peroxide vapor (curve 2), and joint action of discharge plasma and hydrogen peroxide vapor (curve 3). H_2O_2 concentration in the chamber 6.75 mg/l (working medium dose - 0.5 ml 30% hydrogen peroxide), P ~ 0.2 Torr, W_d = 0.003 W/cm^2. Initial bacterial loading 10^7 spores.

Figure 2. Survival curves for spores Bac. subtilis, obtained by colony count method during sterilization of test objects under paper TPT-0260 (Type 1073-B Tyvek), obtained with various doses of 30% H_2O_2 solution injected into the chamber (various hydrogen peroxide concentrations in the chamber): 1 - 0.125 ml (1.7 mg/l), 2 - 0.5 ml (6.75 mg/l), 3 - 1.0 ml (13.5 mg/l). P ~ 0.2 Torr, W_d = 0.007 W/cm^3. Initial bacterial loading 10^7 spores.

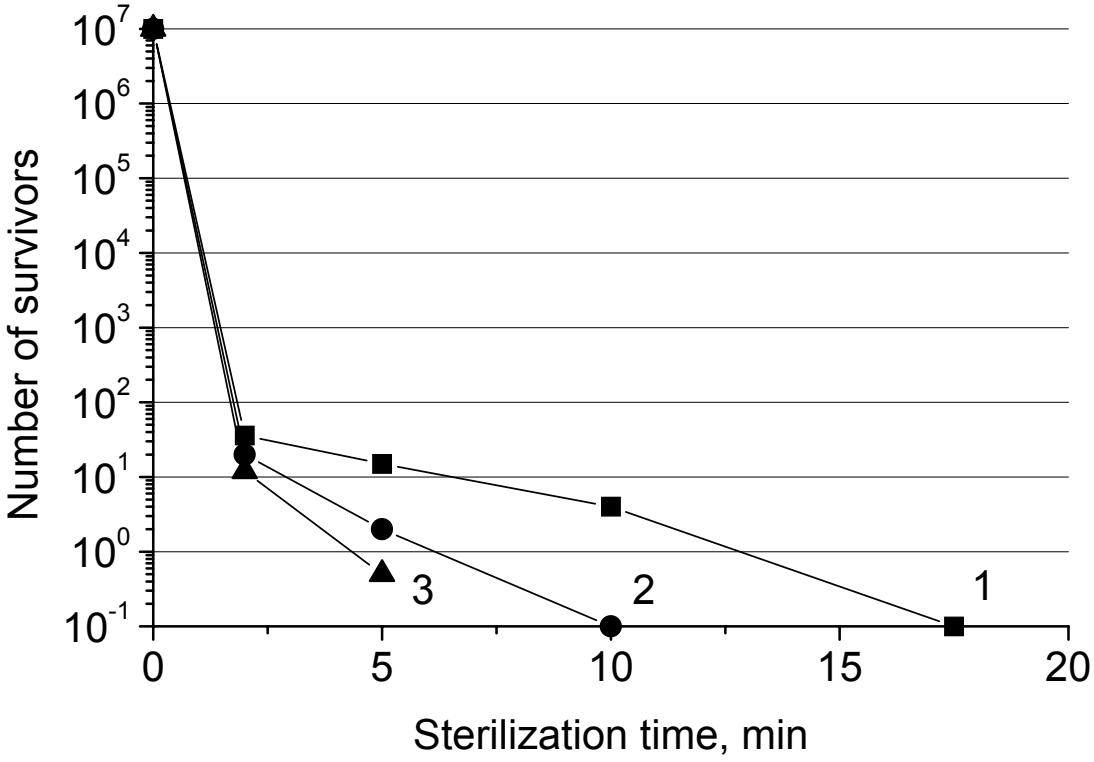
Figure 3. Survival curves for spores Bac. subtilis, obtained by colony count method during sterilization of test objects under paper TPT-0260 (Type 1073-B Tyvek) by oxygen (curve 1) and peroxide (curves 2-5) plasmas at various powers, introduced into the discharge:
1 - W_d = 0.003 W/cc, 2 - W_d = 0.000 W/cc, 3 - W_d = 0.003 W/cc, 4 - W_d = 0.007 W/cc,
5 - W_d = 0.010 W/cc. Working medium dose injected into the chamber - 0.5 ml 30% hydrogen peroxide (6.75 mg/l concentration), P ~ 0.2 Torr. Initial bacterial loading 10^7 spores.

Figure 4. Survival curves for spores Bac. subtilis, obtained by colony count method during sterilization of test objects under paper TPT-0260 (Type 1073-B Tyvek) with injection of 0.25 ml 60% H_2O_2 solution (hydrogen peroxide concentration 7.5 mg/l) for various powers, introduced into the discharge: 1 - W_d = 0.003 W/cc, 2 - W_d = 0.007 W/cc, 3 - W_d = 0.010 W/cc. P ~ 0.2 Torr. Initial bacterial loading 10^7 spores.

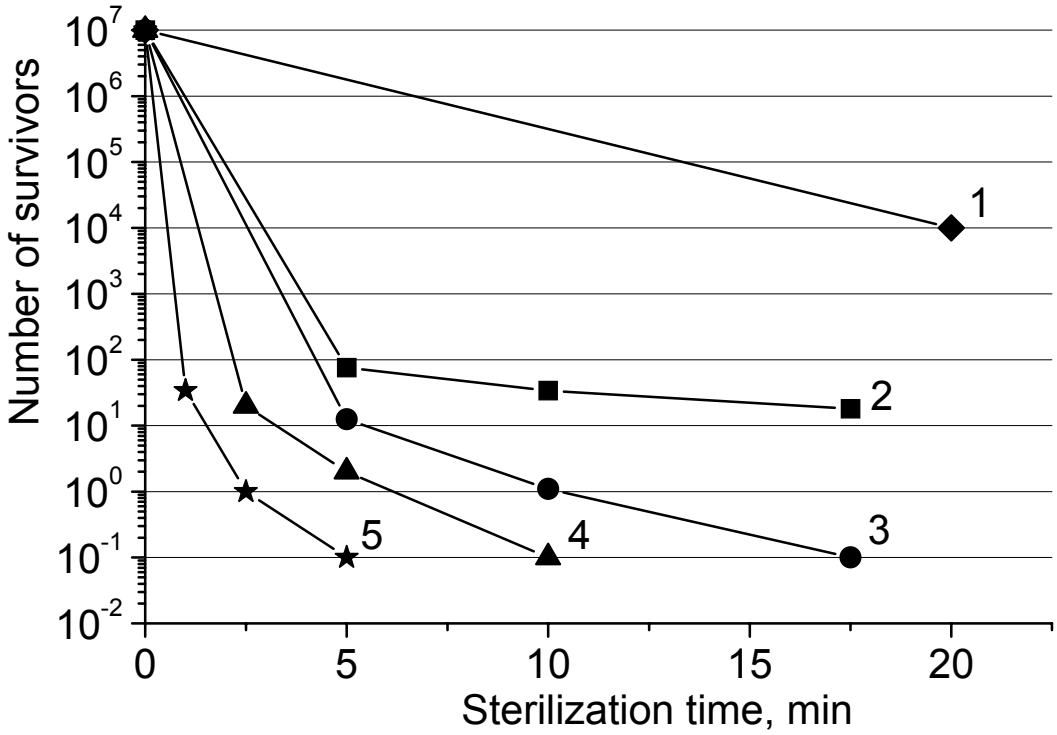
Figure 5. Temporal evolution of neutral plasma components of the discharge in vapor of 60% aqueous solution of hydrogen peroxide; W_d = 0.005 W/cc, P = 0.1 Torr.



I.A.Soloshenko et.al. Figure 1.



I.A.Soloshenko et.al. Figure 2.



I.A.Soloshenko et.al. Figure 3.

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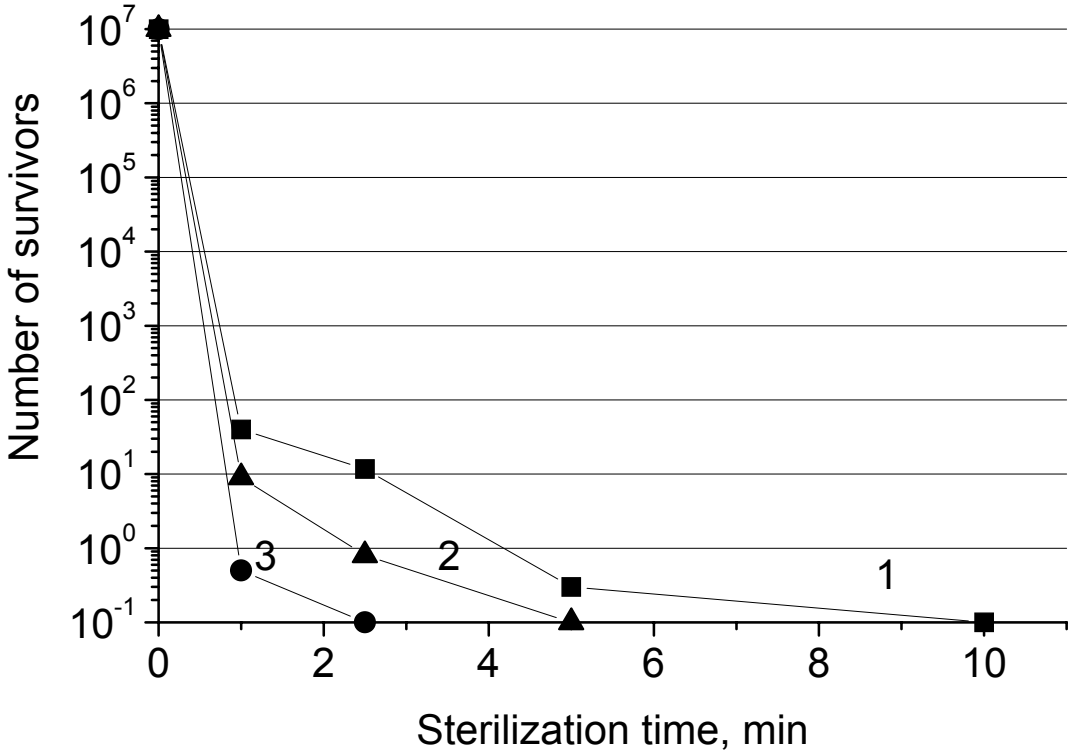
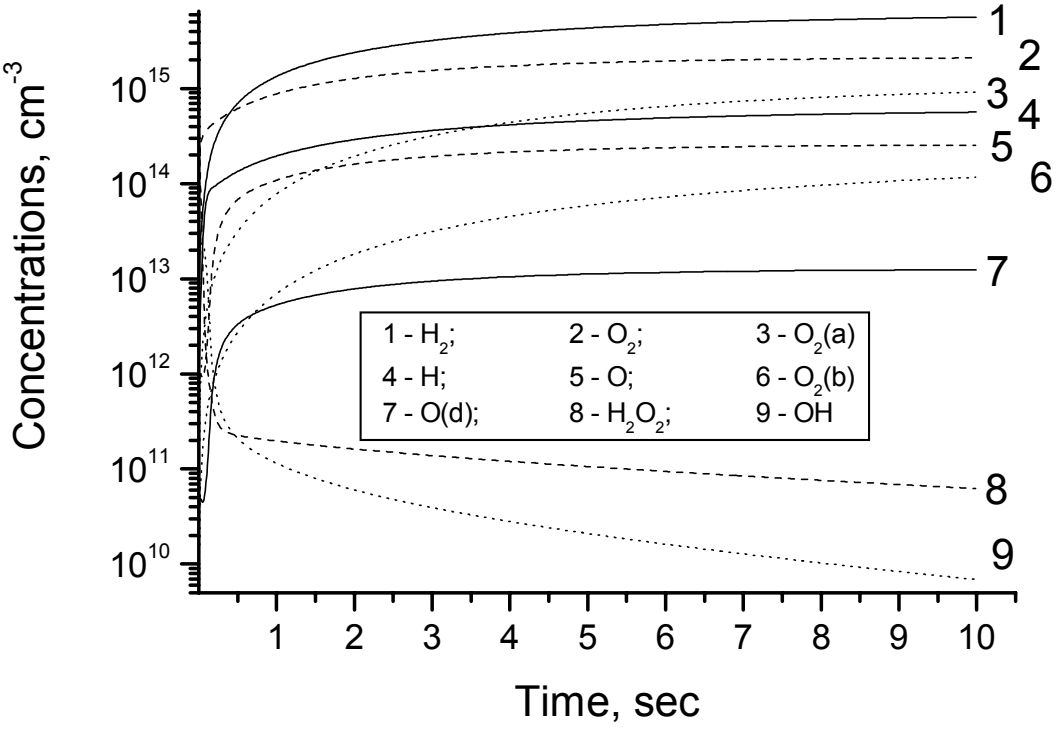


I.A.Soloshenko et.al. Figure 4.



I.A.Soloshenko et.al. Figure 5.