NON-THERMAL PLASMA STERILIZATION IN RF AND MW DISCHARGES*

V. AZHARONOK[1], I. FILATOVA[1], IU. BOSNEAGA[2], M. BOLOGA[2], O. SHEDIKOVA[3]

[1]B.I.Stepanov Institute of Physics NAS Belarus, Nezavisimosti Ave., 68, 220072, Minsk, Belarus,
E-mail: azharonok@imaph.bas-net.by,
Email: filatova@imaph.bas-net.by
[2]Institute of Applied Physics, Academy of Sciences, Academiei Str., 5, Moldova, MD-2028,
E-mail: iubosneaga@gmail.com
[3]The Republican Scientific-Practical Centre of Hygiene, Akademicheskaya str., 19-14 Minsk, Belarus,
E-mail: olgashed@rambler.ru

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The thermodynamical approach is proposed for the optimization of sterilization processes. A comparative study of radio frequency (RF) and microwave (MW) plasmas has been performed, followed by a bactericidal effect investigation for different surfaces contaminated with pathogenic microorganisms. Plasma parameters have been studied by optical spectroscopic methods and microwave dielectric properties' measurements. Perspective directions for the sterilization process improvement are defined.

Key words: RF and microwave plasma, microorganisms' inactivation, survival curves.

1. INTRODUCTION. RATIONALE FOR KEY SOLUTIONS

Due to the world population growth (recently UNESCO announced that total urban population has surpassed the rural one), the acceleration of natural cataclysms and ecological environment degradation (as a result of the powerful anthropogenous influence) – the urgency of microbiological safety maintenance rises steeply. For example, the problem of astronomical quantities of potable water sterilization ranks on the economic importance energy problem, and on the social importance - surpasses it. The population of cities, in many cases, has no access to primary drinking water resources and has to be content with water which is reused many times.

Nowadays non-thermal plasma is considered functionally, energetically and ecologically as the most efficient tool for pathogenic bacteria inactivation - due to high chemical activity of low-temperature energy efficient plasma, short-time treatment and minimal thermolabile materials' destruction. The problem of the

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plasma sterilization process optimization is a multiparametric one, and the thermodynamical approach facilitates the finding of optimal solutions.

The dynamics of organism growth (degradation) at a given temperature and pressure can be characterized by the variation of free energy, accumulated in the organism:

$$\Delta G = \Delta H - T\Delta S\,. \tag{1}$$

The increase of free energy in the organism (due to energy assimilation from the environment) is a mandatory condition for growth; respectively, reduction of free energy means degradation (see Figure 1).

The proper functioning of an organism's growth rate control is the duty of the "biological clock", implanted in every living organism. Programmable disruption of the pathogen's "biological clock" could be extremely useful in sterilization technologies.

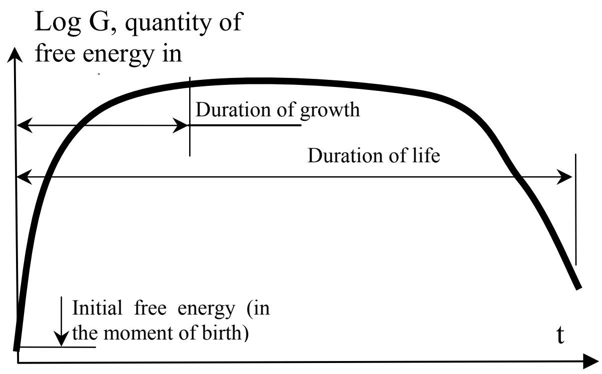

Fig.1 – Typical free energy variation in the organism during life.

Apoptosis ("cellular suicide") is now intensely studied (as a part of the complex self-regulation mechanism - *homeostasis*), with motivated expectations of obtaining a decisive solution for the cancer problem, but at the moment it seems to be far from practical sterilization use.

For the realization of life-maintaining, non-spontaneous endothermic reactions, the variation of free energy must necessarily be positive ($\Delta G > 0$), which means that there is a need of external energy input. A good example of a non-spontaneous endothermic life-symbol reaction is the reaction of photosynthesis, for which absorption of high potential solar energy is an indispensable condition for realization:

$$6CO_2 + 6H_2O \xrightarrow{h\nu} C_6H_{12}O_6 + 6O_2\,. \tag{2}$$

The reverse reaction (3) - of oxidation - is, on the contrary, a spontaneous exothermic one, and it means destruction of organic life. Release of low-potential energy *Q* ("dead" energy) makes this reaction irreversible (respectively $\Delta G < 0$):

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + Q\ . \tag{3}$$

In such spontaneous exothermic reactions of oxidation, the total reduction of free energy ($\Delta G < 0$) is due to both entropy (degree of disorder) increasing, and enthalpy (heat content) decreasing - see formula (1).

In conclusion, the plasma chemical reaction of oxidation is the best method for irreversible sterilization, without any chance of eventual "adaptation" for microorganisms: being the shortest way of obtaining sterility, it transforms finally the organic compounds in gas phase. Most of the physical methods of sterilization (heating, US, UV, and other radiations, etc.) provoke "endothermic" structural destructions ($\Delta G > 0$), and there always remains a certain possibility of reparation, or even creation of new mutations - viable (virulent) forms. So, it is indicated to use the above mentioned methods only as a supplement, together with oxidation. Mutagen components of UV and ionizing radiation cannot be neglected because their carcinogenicity is a widely recognized fact. Another evidence of their "creativity" is the relatively frequent emergence of new virus modifications in the near equatorial regions (we suppose it is due to high tropopause altitudes in these regions).

Choosing the best oxidizing agent can be done using data from Table 1.

Table 1

Constants of atoms (molecules), characterizing the oxidation capacity [1, 2]

Molecule type	**F_2**	**Cl_2**	**O_2**	**O_3**	**N_2**
Bond (dissoc.) mol. energy, *eV (kJ/mol)* &respec.rad.λ (λ_{max} dissociation limit)	1,61 (155,0) (λ_{max}= 772,0 nm) $F_2 + h\nu \rightarrow F + F$	2,52 (242,6) (λ_{max}= 493,3 nm) $Cl_2 + h\nu \rightarrow Cl + Cl$	5,12 (493,6) (λ_{max}= 242,4 nm) $O_2 + h\nu \rightarrow O + O$	1,11 (107,0) (λ_{max}=1117,3 nm) $O_3 + h\nu \rightarrow O_2 + O$	9,81 (945,3) (λ_{max}= 126,6 nm) $N_2 + h\nu \rightarrow N + N$
Electron affinity, *eV*	3,45 (for atom F)	3,61 (for atom Cl)	1,47 (for atom O) 0,44 (for mol.O_2)	2,26 (for mol. O_3)	-0,21 (for atom N)
Ionization energy (for atoms, molecules), *eV*	17,42 ($F + h\nu \rightarrow F^+ + e$) 15,70 ($F_2 + h\nu \rightarrow F_2^+ + e$)	12,96 ($Cl + h\nu \rightarrow Cl^+ + e$) 11,48 ($Cl_2 + h\nu \rightarrow Cl_2^+ + e$)	13,62 ($O + h\nu \rightarrow O^+ + e$) 12,08 ($O_2 + h\nu \rightarrow O_2^+ + e$)	12,52 ($O_3 + h\nu \rightarrow O_3^+ + e$)	14,53 ($N + h\nu \rightarrow N^+ + e$) 15,58 ($N_2 + h\nu \rightarrow N_2^+ + e$)
Electronegativity, atoms(byLPauling)	**3,98**	**3,16**	**3,50**	**3,50**	**3,05**
Covalent radius of atoms, *nm*	0,064	0,099	0,066	0,066	0,074

The data presented show that fluorine is the most powerful oxidizer. Fluorine, however, is so active that any manipulations with it are extremely dangerous (with respect to fluorine, only diamond and some species of glassy carbon are stable). So, oxygen is the most indicated oxidizer. Oxygen is ecologically perfect, being the most abundant element on Earth and also the main component of tissues. (Unfortunately, in many cases, chlorine was chosen as the main sterilizing agent, and this is unhealthy - from many points of view).

Thus, molecular oxygen should be an obligatory component of the plasma sterilizer. Also, a convenient plasma support gas for atomic oxygen (O) generation is the mix of water vapor H_2O with hydrogen peroxide H_2O_2. In addition to the atomic oxygen formed due to hydrogen peroxide decomposition, H_2O molecules dissociation in high-frequency plasma occurs, resulting in the formation of O_2 and H_2O_2. So, the additional continuous replenishment of the mix oxidizing potential occurs (as atomic oxygen is consumed for oxidation). Additionally, a high ionizing potential component is appropriate (one of the noble gases is suitable for this task) - for proper UV-radiation wavelength generation. Unfortunately, UV is not efficient for non-transparent or shaded areas. For parallel UV and atomic oxygen generation - the UV wavelength should be sufficiently short (see Table 1 - the condition for atomic oxygen generation is $\lambda_{UV} < 242{,}4$ nm).

It is easy to observe that atmospheric air largely satisfies the formulated conditions - such components as oxygen, water vapors and argon are relevant. Only nitrogen is out of place - molecules of nitrogen needlessly absorb electromagnetic energy for their dissociation (note from Table 1 - high bond energy of the molecule N_2), with the subsequent formation of undesirable NO_x. Nevertheless, we have accepted atmospheric air as plasma support gas (as the first approximation). The pressure could be less than the atmospheric one - inclusively because in medicine it is important to obtain an additional indicator-marker of the sterilization quality (in this case - a constant low pressure in a hermetical package).

The high level of non-equilibrium is characteristic for high frequency discharge plasma, the energy being pumped predominantly into electrons (respectively $T_e >> T_i$). Chemically active ions and radicals are formed in such plasma mostly due to electron impacts. From the above description comes a special interest for the state of oxygen plasma components. According to the characteristics of applied electromagnetic power, a certain level of dynamic equilibrium occurs in plasma. In Table 1 oxygen-related plasma reactions are presented, which are all non-spontaneous endothermic and reversible - because $\Delta G > 0$ for all of them (they are induced due to the income of electromagnetic energy). All the processes of dissociation, association, ionization and recombination are accompanied by absorption or emission mainly of UV photons (with an exception - in the case of ozone (O_3) dissociation-association do participate IR photons). Also, reverse exothermic spontaneous reactions take place (for which $\Delta G < 0$ - and a part of gained high-potential energy is transformed into heat):

$O_2 + O + M \rightarrow O_3 + M^Q$; $O + O + M \rightarrow O_2 + M^Q$; $O_3 + O \rightarrow 2O_2$ (catalyst - nitric oxide NO, or hydroxyl OH - is necessary); $2O_3 + M \rightarrow 3O_2 + M^Q$; (4)

The picture will be more valid if we also mention the reactions of dissociative recombination (additional to "usual" recombination - at a third body):

$$O_2^+ + e^- \rightarrow O + O, \quad O_3^+ + e^- \rightarrow O_2 + O \tag{5}$$

(Their intensity grows with the decreasing of pressure in the plasma reactor).

Among all the mentioned oxygen forms, it is the allotrope of oxygen - ozone (O_3, trioxygen) that is the most stable (approximately 30 minutes half-life - for atmospheric conditions). Oxygen ions and radicals are very reactive, and they intensely oxidize all organics.

2. EXPERIMENTAL INSTALLATIONS AND TREATMENT RESULTS

At significant similarity of RF and microwave methods of plasma generation (pure electrodeless discharges at a high level of non-equilibrium and relatively low gas temperature) there are also available basic differences. Steady microwave discharge can be maintained at a higher pressure, up to the atmospheric one (especially in the case of the pulse discharge) and at a greater degree of plasma non-equilibrium. RF plasma volume makes a uniform, interdependent electrical circuit with the RF generator, being a relatively open system; this simplifies the design and control. Microwave plasma source is a closed system; microwave loading (plasma volume) practically does not influence the frequency and the power of the generator. Respectively, RF equipment is usually described in terms of lumped-parameter circuits, and the microwave one - in terms of distributed-parameter circuits.

Series of experiments with representative bacteria inactivation were performed in low-pressure plasma of RF and microwave discharges. A capacitively coupled RF discharge was operated between two parallel round copper electrodes with the diameter of 120 mm [3]. Microwave plasma generation was done in regular rectangular waveguides - with the possibility of on-line control of plasma dielectric properties. Ambient air at pressure P = 0.4 – 3.0 Torr was used as a working gas. The gas temperature T_g control was performed using the OES methods: it has been deduced from the rotationally unresolved spectral bands of the second positive (2+) system of N_2 [4]. The measured T_g value did not exceed 35° C under the exposure conditions, so thermolabile materials could not be damaged by overheating.

The strains of Gram-positive bacteria – *Staphylococcus aureus ATCC 6538 (S. aureus)* and Gram-negative bacteria – *Escherichia coli ATCC 8739* (*E. coli*), clinical isolates of *Staphylococcus - 37C1* and *Enterobacteriaceae EB 158*, as well as spores of *B. subtilis ATCC 6633* and fermented *Saccharomyces vini* were chosen as tested species - due to their specific physiological, biochemical and morphological indications and good growth properties.

To study the peculiarities of microorganisms inactivation on different surfaces in plasma, the culture crop emulsion was plotted on a surface of sterile medical products made of various materials: polymers, metals (stainless steel or copper) and capillary-porous (surgical suture) samples. Fat-free sterile samples were contaminated in a glass tube containing a suspension of microorganisms with a concentration of 10^9 cfu/ml prepared according to McFarland turbidity standards. The concentration of microorganisms on spore-inoculated products was varied in the range of 10^5-10^7 cfu/ml, depending on an effective area. The exposure duration was 5, 10, 15 and 20 min. A test of microorganisms survival for treated and control samples was obtained by performing several consecutive dilutions (1:10 in NaCl) of wash-outs from the surface of tested samples (by immersion into a sterile physiological solution). Then a portion of the solution from the most suitable dilution was pipetted into the appropriate culture media and incubated at temperature 37° C for 24 – 48 hours before the examination.

Typical results are shown in Figure 2. The cultivable cell concentration on the tested surfaces decreased at least by 4 – 6 orders of magnitude, in function of the support structure (metallic, polymeric or porous samples) and microorganism genus - after 20 minutes of plasma exposure. In the case of samples with a solid surface (polymer, metal) micro-organisms suspension, plotted by a wash-out, assigned rather evenly on their surface, easily accessible for plasma irradiation. The reason for the decrease of the plasma sterilizing effect in the case of a porous sample treatment is connected apparently with peculiarities of plasma interaction with porous materials. To ensure the effect of homogeneous sterilization of investigated suture throughout their volume, chemically active species must be able to penetrate through the material without losing their modifying ability. Furthermore, it is necessary to consider the distribution of charged particles and electric field distribution on the micro-relief of the treated surface.

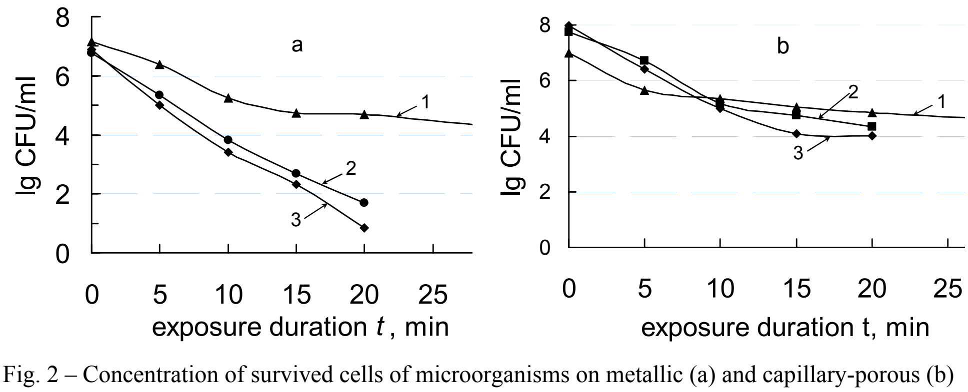

Fig. 2 – Concentration of survived cells of microorganisms on metallic (a) and capillary-porous (b) samples in dependence on time of RF air plasma irradiation: 1 – spore of *B.subtilis* ATCC 6633, 2 and 3 – Gram-positive and Gram-negative bacteria correspondingly.

We have assumed from optical emission spectra, recorded during microorganisms inactivation, that the sterilization effect of air plasma was determined, along with the UV radiation, by oxygen atoms and radicals formed in the discharge due to dissociation of oxygen-containing molecules that belong to ambient air (the presence of excited OI atoms was revealed in spectra, see Figure 3). It is in agreement with the results obtained in [5].

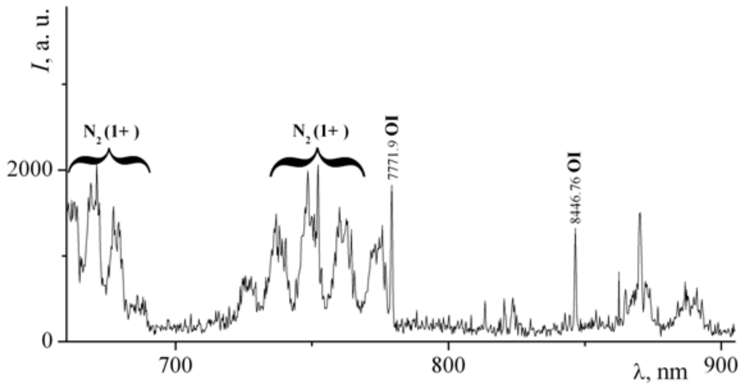

Fig. 3 – Emission spectrum of RF air plasma used for bacteria inactivation.

So, high frequency low-temperature plasma is a flexible and effective decontamination agent for various applications. For each case there are possibilities of plasma characteristics adaptation, inclusively by the choice of RF or microwave discharges.

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