

Comparison of biocidal and sporicidal effects of spark discharge plasma and mercury-vapor low pressure lamp radiations

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Abstract: In this work it was shown that the inactivation micromycets spore (fungi) to hot plasma radiation of self-sustained spark discharge on air is much more effectively than UV-radiation mercury-vapor lamp. Though the discharge electrical power is 15 times and UV-photons flux 430 times more for mercury lamp than for spark discharge. Discussed method inactivation is very simple.

Key words: micromycet inactivation, spore, hot plasma radiation, UV-mercury lamp

I. INTRODUCTION.

The germicidal effect of UV-radiation is well known. It due to photon energy absorption by DNA molecules, as result the chemical reactions are initiated, which damage these molecules [1]. The surface sterilization is important and needful procedure, which is used in medicine, food production and others. One of the methods microorganism inactivation is treatment to UV-radiation. The absorption spectrum of DNA molecules is continues from wavelength 180 up to 300 nm with broad maximum at 190 – 200 and 260 nm [2]. The radiation spectrum of mercury-vapor low pressure lamp (MVLPL) is narrow line 253.7 nm, its wavelength is in region maximum absorption of DNA 260 nm. Therefore the mercury (quartz) lamp has biocidal effect. The source of UV-radiation can be electric discharge plasma [3]. If plasma is cold, it can directly contact with treated surface, don't damage surface. The sterilization effect in this case is achieved by combined action of discharge plasma radiation and active particles, generated in liquid and gas phase (radicals, ions, neutrals) [4].

If plasma is hot, direct contact with treated surface leads to its heat damage, therefore hot discharge plasma must be on safe distance, and germicidal effect is provided by plasma radiation. The plasma cord heated up to high temperature radiates as black body. The radiation spectrum of black body is continuous; the maximum position is determined by cord temperature. This spectrum can be consensual in shape with absorption spectrum of DNA, and then germicidal effect of electric discharge increases.

Continuous arc discharge has limitation on power, as electrodes can melt. Pulse spark discharge allows to heat a plasma cord up to more high temperature without electrode melting, than arc. With temperature increasing the maximum of radiation spectrum move in direction of shorter wavelength. The DNA absorption maximum at 190 – 200 nm frequently is more significant, then at 260 nm [2], therefore the germicidal radiation effect increase with decreasing wavelength. Authors of this work developed the radiation hot plasma generator on the base of self-sustained spark discharge (SD) on air. The aim of this work is to compare the biocidal and sporicidal effects of SD and mercury-vapor low pressure lamp radiations (MVLPL).

II. MATERIALS AND METHODS

2.1. Radiation sources: spark discharge (SD)

Discharger with stainless still rod electrode 2 mm diameter formed self-sustained spark discharge. Length of electrodes was not more than 45 mm. Spark gap was ~3 mm, breakdown voltage of gap was 6 – 6.5 kV. Parallel discharge gap is capacitor 3.3 nF, $V = 10$ kV. Value of capacitor was chosen to provide maximum chemical effects in distilled water. Capacitor was charged via ballast resistor 10 M Ω from power supply 11 kV. For this electrical parameters arose self-supporting spark discharge with frequency 10 Hz, pulse duration 100 μ s, front duration – 0.1 μ s. Pulse energy was $5.9 \cdot 10^{-2}$ J. Spark discharge was source of UV-radiation continuous spectrum with maximum at $\lambda = 220$ nm. Gas, heated in spark gap, radiate as black body, maximum position is determined by spark cord temperature. Maximum of spectrum is dislocated when gas cooling in direction of more long wavelength. Active particles (radicals) are created in water under action of UV-radiation. Photons flux of UV-range on the treated samples (1 cm distance from discharge gap) was $1.26 \cdot 10^{-10}$ M cm⁻²s⁻¹.

2.2. Mercury-vapor low pressure lamp (MVLPL)

Mercury-vapor low pressure lamp DBK-9 was used as UV-radiator, giving monochromatic line 253.7 nm. Arc discharge burnt in lamp, the discharge current was 170 mA. Electric power of lamp was 9 J s^{-1} . The radiation of heated plasma cord, as black body, gives some contribution, but it was negligible. The main role play mercury line 253.7 nm. Photon flux in the place of treated sample (3 cm from lamp) was $5.4 \cdot 10^{-8} \text{ M cm}^{-2} \text{ s}^{-1}$. When compare energetic characteristics sources of radiation we see, power mercury lamp is 15 times more than SD, photon flux 253.7 nm is 430 times more, than UV-photons flux from SD, but average wavelength from SD is essentially shorter than 253.7 nm.

2.3. Biological samples

The experiment was carried on bacterial strains of antibiotic-resistant gram-positive microorganisms of *Staphylococcus aureus* 5913 and gram-negative microorganisms of *Escherichia coli* 775-3. Bacterial strains were taken from the museum of the Department of Microbiology and Immunology, Nizhny Novgorod State Medical Academy. For analyses there were pre-prepared daily culture. Then bacterial cells were resuspended in Hanks' solution up to concentration of $(10 - 15) \cdot 10^6$ cells per 1 ml.

In experiment there was assessed the bacterial cell growth inhibition after plasma irradiation. Microorganism suspension, 4 ml in volume, was exposed to plasma radiation within 2, 5, 10, 15, 30, 60 s. The plating in Petri dishes on stiff agar was carried out. After that the samples were placed in a oven for a day at 37° . Biocidal effect of plasma radiation was estimated by the number of colony-forming units (CFU).

Micromycete cultures (*Alternaria alternata* BKM F-1120, *Aspergillus Niger* BKM F-1119, *Chaetomium Globosum* BKM F-109, *Penicillium Chrysogenum* BKM F-245) were taken from All Russia collection of microorganisms. Spores suspension in water with concentration $(2 - 4) \cdot 10^6$ cells in 1 ml was prepared. Samples in volume 4 ml were treated to SD during 1, 7.5 and 10 minutes, and to MVLPL during 10 minutes. After that the plating in Petri dishes on Czapek Doks media was carried out. Then the samples were placed in a oven for 3 days at 27°C . The number of CFU estimated sporicidal effect of plasma radiation.

The data obtained in the experiment were statistically processed using application program package EXCEL, Statistica 6.0. Statistical significance of the average differences was determined using Mann-Whitney test.

III. RESULTS

Parameters of spark discharge were chosen so as to provide maximum chemical effect in treated distilled water [5]. In works [5, 6, 7] characteristics (yields) of active particles, nascent in water under action of this discharge were determined, and, for comparison, yields the same particles by MVLPL. Initial active particles yields are given in table 1. Main active particles for both irradiators are HO_2^\bullet radicals, yields of which for SD and MVLPL are practically equal. In course of water irradiation by SD take place significant pH decreasing, as by MVLPL irradiation the pH-value change is small. By SD 14 products in water are created: HO_2^\bullet , OH^\bullet , H_2O_2 , O^\bullet , $\text{O}_2^{\bullet-}$, O_3 , N_2O , NO , NO_2 , N_2O_3 , N_2O_4 , HNO_2 , HNO_3 , $\text{ONOO}^{\bullet-}$, for many of it the stationary concentration is in limits $10^{-4} - 10^{-6} \text{ M l}^{-1}$ [7]. Ozone and hydrogen peroxide, playing usually crucial role during sterilization process were determined, but its concentrations are vary small to play significant role.

UV- and IR-spectra of chemicals, produced in water and gas phase were measured [6]. In discharge gas phase a compounds of nitrogen are created, which contain CN- and NH- groups, as well organic compounds with CH- and CC - groups. The more appreciably is nitroamine production. Traces of compounds, containing metals, which could evaporate due to electrode heating, don't detected. Absence of electrode evaporation is result low discharge energy: $5.9 \cdot 10^{-2} \text{ J}$. The survivability of microorganisms after irradiation to SD and MVLPL are investigated. Transformations in lipids and proteins during irradiation in our experiment have an influence on living cell, which consist out of these molecules. Lipids oxidation reduces the protective properties of biological membranes, proteins oxidation and modification destroys cell function. Variation of pH outside the cell provided that intracellular pH value wills the same lead to appearance a difference of potentials, applied to membrane. Value ΔpH according Nernst equation determines difference of red-ox potentials: $\Delta\phi = \phi_0 - \Delta\text{pH} \cdot 60 \text{ (mV)}$. This difference of potentials can destroy membrane functions.

Radiation spectrum of SD is broad and more similar to absorption spectrum of DNA, than spectrum of MVLPL. Pulse mode of SD radiation provides higher instantaneous active particles concentration that enhances the chemical reactions rate under SD as compared with MVLPL.

The comparison of germicidal effect SD and LPML shows in table 2, where presents a number of Colony-Forming Units (CFU) for *S.aureus* и *E.coli* depending on time treatment. It is seen for both kinds of microorganisms the time of 10 times CFU decrease under LPML is about 2 seconds. For SD time of the same CFU decrease is 60 seconds. It is not surprising as photons flux in UV region of MVLPL spectrum is 430 times more than SD spectrum.

Radiation dose required 10 times bacteria inactivation for SD is 30 – 50 times more than for MVLPL, i.e. energy consumption for inactivation *S.aureus* and *E.coli* to SD is essentially more than to MVLPL. Spark discharge radiation is pulse, frequency 10 Hz. Pulse repetition time is 100 ms, but pulse duration is 100 μs . I.e. 99.9% full time SD treatment radiation is absent. During 600 s treatment radiation acts only 0.6 s. Therefore a possible mechanism of energetic efficiency decrease to SD can be adaptation processes in pause between radiation pulses [8].

The deactivation by means of MVLPL for microorganisms spore is inefficient. Microscopic fungi (micromycetes, spores) are one of these microorganisms. Example is in table 3, where is presented a number of CFU in control (not treated) probe and after treatment to SD and MVLPL for 4 kinds of microscopic fungi, micromycetes (A. Alternata, A. Niger, Ch. Globosum, P. Chrysogenum). The count of CFU was made in 3 days incubation at 27°C after sowing and irradiation. It is seen in samples treated with SD during 10 minutes micromycetes colony not found, while after treatment during the same times with MVLPL spore survivability compared to control is still the same. Energy expense for spore 10 times inactivation by means SD is 0.3 – 3.5 J cm⁻² for various spores. This value should compare with MVLPL, when at energy expense 50 J cm⁻² there is no effect.

IV. CONCLUSION.

In this work it was shown the treatment of micromycetes spore to spark discharge give essentially more sporicide effect compared to low pressure mercury-vapor lamp, but energy expense remain considerable. The mechanism of sporicide action is complicated. The realization of method is very simple.

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Table 1. Initial yields of active particles (experiment) [5, 6, 7].

Agent	SD	MVLPL
HO ₂ [•]	(1.2 ± 0.3) 10 ⁻⁶ M (l s) ⁻¹	(1.1 ± 0.5) 10 ⁻⁶ M (l s) ⁻¹
NO ₃ ⁻ + NO ₂ ⁻	(5.8 ± 1.6) 10 ⁻⁷ M (l s) ⁻¹	(3.4 ± 1) 10 ⁻⁹ M (l s) ⁻¹
NH ₄ ⁺	(1.7 ± 0.5) 10 ⁻¹⁰ M (l s) ⁻¹	(2.5 ± 1.5) 10 ⁻⁸ M (l s) ⁻¹

Table 2. Number of colony-forming units (CFU) in control (without treatment) and after treatment during time t (s) to SD and MVLPL.

Time treatment, t, s	Staphylococcus Aureus		Escherichia coli	
	SD	MVLPL	SD	MVLPL
Control	1072 ± 100	1072 ± 100	984 ± 100	984 ± 100
2	1025 ± 100	96 ± 10	984 ± 100	56 ± 6
5	846 ± 90	90 ± 10	980 ± 100	50 ± 6
10	924 ± 90	–	829 ± 90	–
15	986 ± 90	–	805 ± 80	–
30	282 ± 30	–	504 ± 50	–
60	34 ± 5	–	283 ± 30	–
Desactivation dosage in 10 times, mJ cm ⁻²	86**	2.6*	100**	2*

*) – article [3].

**) – this work.

Table 3. Evaluation of sporicidal effects. Number of CFU in control and after irradiation to SD and MVLPL in 3 days incubation.

Kind of micromycete	SD				MVLPL	
	Control	1 min	7.5 min	10 min	Control	10 min
A.Niger	147±7.5	49.3±2.5	27±2	0	40.7±1.5	39.7±1.5
A.Alternata	23±3.4	6±5.6	0	0	19.7±1.5	17.3±1.5
Ch.Globosum	13.3±5.7	0	0	0	30.0±5.6	27.0±2.6
P.Chrysogenum	71.3±4.9	51.3±6.6	0.7±0.5	0	15.7±2.1	10.3±1.5