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THE COMBINED EFFECT OF KrF-LASER IRRADIATION AND COPPER IONS ON THE SURVIVAL OF E.COLI CELLS

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Abstract-The survival of the E.Coli cells under the separated and combined action of the KrF laser ($\lambda = 248$ nm) and copper ions has been studied. The synergistic effect has been observed at the doses of 10 mJ/cm^2 . The evaluations of light reduction and absorption cross-sections for the E.Coli cells and also the probability of the cell inactivation after light quantum absorption are presented.

In the practice of water treatment the chemical methods of water disinfection are of the most wide usage. The physical methods, such as UV irradiation, electric field etc., are more ecologically pure, but their application is limited by high energy demands. The promising results have been obtained when UV irradiation has been used together with the oxidants - ozone, hydrogen peroxide, chlorine and its compounds [1,2].

UV irradiation causes a some of breakdowns in the bacterial cells, the most critical being the damages of nucleic acids and cell membranes. The permeability of the membranes may be increased, in particular for ions [3,4].

In this work the separated and combined action of the UV irradiation (excimer KrF laser, $\lambda = 248$ nm) and copper ions upon the E.Coli cells has been studied.

Bacterial suspension in the physiological solution with the density of 10^{-6} cells/ml has been prepared from the 18-hour culture of E.Coli 1257. Suspension has been placed into the quartz vessel of $10 \times 10 \times 45$ mm. To obtain the homogeneous irradiation and variability of the dose, the laser beam has been broadened with a quartz lens. The large

doses have been obtained by accumulation of a sequence of pulses. The maximal density of energy in a pulse has been equal to $4,7 \text{ mJ/cm}^2$, and the density of power - 315 kW/cm^2 with the pulse width of 15 ns. The directly measured light reduction cross-section (σ_0) of a cell (scattering + absorption) at $\lambda = 248 \text{ nm}$ has been equal to $4 \times 10^{-8} \text{ cm}^2$, thus weakening the light on 1 cm distance at the cell concentration of 10^6 cm^{-3} for 4%.

At the beginning it has been found that in the range of power densities from 20 to 300 kW/cm^2 at the same dose equal to $4,7 \text{ mJ/cm}^2$ the inactivation degree of the E.Coli cells does not change, what qualitatively corresponds to the Ref. [5] conclusions. The E.Coli cells differ in this way from chlorella, yeast and algae cells, for which the dependence of survival on the power of the UV irradiation has a pronounced threshold character [6].

Fig. 1 illustrates the dependence of the cell survival upon the dose

Fig.1. Survival of the E.Coli cells versus the dose of the laser irradiation.

of irradiation obtained in 6 runs of measurements. The results of single run is marked with the crosses. The experimental data is approximated with the mean-square error of 0.3. The slowdown of the inactivation rate at the doses over 10 mJ/cm^2 may be connected with the initial heterogeneity of the population in its resistance to the UV irradiation, with the screening effects, and unhomogeneity of the light beam.

The combined action of the copper ions and the UV irradiation has been studied at the doses from $0,3$ to 25 mJ/cm^2 with the contact time with copper ions equal to 60 minutes. CuSO_4 solution has been added to suspension so that the concentration of CuIII ions reached

1 mg/l. Such a concentration causes 18% inactivation of cells in 60 min. The experiments have been performed in two variants. In the first one copper has been added immediately after the irradiation of the cells, in the second one suspension has been irradiated immediately after copper has been added. To evaluate the results the ratio of calculated part of survivals (T) supposing the independant action of disinfectants against the experimental count ($\%$) has been used [7]. If the combined action is enhanced, then $T/\% > 1$.

The results of the joined action of the UV irradiation and copper ions are presented in the Table.

Dose mJ/cm ²	I		T/%		I		Probability of difference, %
	I	I	I	I	I	I	
	UV + Cu		Cu + UV				
0,3	0,71 ± 0,13		1,69 ± 0,57				> 90
2,9 - 3,4	0,91 ± 0,17		1,55 ± 0,47				> 90
4,5 - 5,3	0,93 ± 0,14		1,43 ± 0,45				> 80
7,7 - 8,4	1,20 ± 0,38		3,49 ± 0,64				> 99
11,5 - 12,2	2,25 ± 0,44		2,75 ± 1,36				< 40
21,7 - 24,7	1,46 ± 0,32		1,56 ± 1,29				< 40

The data is averaged over 3 to 5 measurements. In the first variant (UV+Cu) at the low doses of irradiation the action of disinfectants is additive. The synergistic effect is observed in the first variant at the doses over 7 mJ/cm² and at all the doses in the second variant (Cu+UV). It is interesting, that the both variants show the maximal synergistic effect at a dose of 11-12 mJ/cm².

The absorbance cross-section of an E.Coli cell has been evaluated according to the transmission spectra recorded on the "Specord" spectrometer. At the wavelength of 250 nm this value was of 10⁻⁹ cm² of order.

The linear part of the dose dependence (Fig. 1) corresponds to the exponential dependence of the number of survivals upon the dose: $N_t = N_0 \exp^{(-kE)}$, or in the differential form:

$$dN_t = -N_t \cdot k \cdot dE, \quad (1)$$

where k - is a slope of the linear part and N_0 is the initial number of the cells.

On the other hand, if the concentration of cells does not reduce the light through the thickness of suspension considerably, a number of

light quanta absorbed by the living cells is

$$dn = \frac{\sigma_a N_t}{h\nu} dE, \quad (2)$$

where $h\nu$ is the energy of a quantum.

The ratio

$$\alpha = \left| \frac{dn}{dN_t} \right| = \frac{\sigma_a}{kh\nu} \quad (3)$$

corresponds to the number of quanta, absorbed by the living cell per one event of inactivation. The obtained experimental data show, that $\alpha \sim 10^6$ quanta/cell.

The equation (1) is valid only in a case, when the probability of cell inactivation does not depend on the number of quanta absorbed by the cell for the previous period. That is only in one of 10^6 absorption acts the lethal damage occurs; all the other damages are not lethal and do not affect the resistance of a cell to the UV irradiation. Among such damages there may be short-lived, corrected by the cell repair system. They may assist the copper ions penetration into the cell and/or enhance their bactericidal effect. It is obvious, that the synergistic effect in this case may occur if the repair time is large enough, for instance at high doses of UV irradiation. The effect must be more pronounced if the irradiated culture contains copper ions already.

CuSO_4 solution exhibits a weak absorption in the 248 nm region, so it is possible, that in the second variant the changes of disinfectant have place, thus increasing its disinfecting abilities.

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