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A new bactericidal UV light sources – excilamps

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ABSTRACT

A reliable bactericidal effect on *Escherichia coli* cells irradiation by excilamps has been established. Both on primary and secondary irradiation there exists the reciprocally proportional dependence between irradiation dose (or exposure time) and survived cells number. The microorganisms survived after primary irradiation are shown to have not changed sensitivity to excilamps irradiation. The best results have been obtained during XeBr-excilamp irradiation. Owing to their technical parameters, the excilamps are promising systems for UV-sterilization. Comparison of capacitive discharge excilamp characteristics with other conventional UV light sources is presented.

A comparative study on UV dose effect of a barrier discharge KrCl-excilamp ($\lambda = 222$ nm) on *Staphylococcus aureus* and *Escherichia coli* inactivation was carried out. KrCl-excilamp emission power is 65 W, and emitting area is 0.1 m². It has been demonstrated that *Staphylococcus aureus* cell sensitivity to UV radiation at this wavelength is higher than that of *Escherichia coli*.

Key words: excimer lamp (excilamp), UV-inactivation, *Escherichia coli*, germicidal lamps.

1. INTRODUCTION

Today there are various disinfection means and sterilization of glass-ware, and one of them is UV irradiation. The specificity of biological action of various ranges of UV spectra was established in the end XIX century by Russian scientist A.M. Maklakov¹. In 1942 L.J. Stadler and F.M. Uger have found out that the nuclear acids served as basic target at lethal and mutagenic action of UV radiation². The further researches have shown that germicidal effect of UV light on viruses and bacteria is primarily due to the formation of thymine, thymine-cytosine(pyrimidine) and cytosine dimers in DNA (they are listed in the order of prevalence). The most effective action has the radiation with wavelengths in a range 200-295 nm (so-called *bactericide* or *germicide* range).

For the purpose of creation of an economic germicide light source the uviol mercury-argon low pressure lamps (for example, the domestic lamps series BYB) were developed in 1936-1940. In its spectrum there is a rather intensive resonant line at 253.7 nm appropriate to a maximum of absorption of number of nuclear acids. Besides for the micro-organisms inactivation purposes the powerful high-pressure mercury-quartz lamps (it corresponds domestic lamps series ППК) have received wide acceptance among another sterilisation methods. The spectrum of these lamps is a broadband continuum but in the wavelength range from 200 to 300 nm no more than 20% of emitting power concentrates.

The comparative analysis of conventional bactericide lamp characteristics (see Table 1) has shown that its bactericide efficiency is low (bactericide efficiency is the ration between light power emitting in the wavelength range 200-295 nm and complete watt consumption). The lamps such as BYB provide rather small luminance (not exceed mW/cm²), but the share of bactericide radiation in general radiant flow exceeds 50 %. And vice versa, the luminance of lamps such as ППК at the expense of significant watt consumption can be essentially increased but the share of bactericide radiation in a spectrum is small.

The listed drawbacks of conventional UV-light systems stimulate the search for new ways and modes of UV-processing.. For example, in ⁴ the high-voltage source of radiation with pulse power up to ~ 10 MW was used. The treated bacterial populations of *Escherichia coli*, *Salmonella enteritidis* and *Bacillus cereus* were reduced by ~ 8 log orders after 1000 light-pulses of the higher UV intensity light and fungal counts had a corresponding reduction of 4.5 log orders. In ⁵ for *Bacillus subtilis* inactivation the exciplex KrF-laser ($\lambda=248$ nm) was applied. The energy per pulse was ~ 120 mJ at 10 Hz in an area of ~ 1 by 3 cm². The pulse duration was nominally 14 ns. It was shown, that the dose of laser radiation in a range up to ~

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104 J/cm² renders action similar to action of the same dose from low pressure bactericide lamp. The disadvantages of these UV-light systems ^{4,5} are its relative expensiveness and additional factors of danger in service of the system (for example, the use of high-voltage up to 30-40 kV).

Table 1. Germicidal lamps characteristics ^{1,3}

Lamp model	Demand power, W	Design germicide power, W	Germicidal efficiency, %	Share of bactericidal power in lamp emission, %
BYB-30	30	2	6.6	~ 60
BYB-15	15	0.8	5.3	~ 60
ППК-7	1000	35.0	3.5	< 20
XeBr-JIEPA-5	81	11.4	14	> 85

Attractive alternative to the above-described systems are so-called excilamps, which active study is conducted last 5-10 years. Independently on the excitation discharge type, spectra of these sources consist of narrow bands, and the main contribution into radiation is made by two excited states, namely, $^1\Sigma_u^+$ and $^3\Sigma_u^+$, at transition to repulsive state $^1\Sigma_g^+$ (the so-called band B→X), and the band halfwidth rarely exceeds 8-10 nm. It is remarkable that the band B→X possesses over 80-90 % of the total radiation of lamp. According to a working molecule, the band B→X is located in different places of UV range (see Tab. 1). For brevity, in [12] it was suggested to call such devices (as a *subclass* of discharge lamps) radiating at the expense of decay of excimer or exciplex molecules as *excilamps*. If it is the lamp based on molecules of inert gas R*₂, it is called then *excimer lamp* [1]. Efficiency of such excilamps can achieve 7-30% [9].

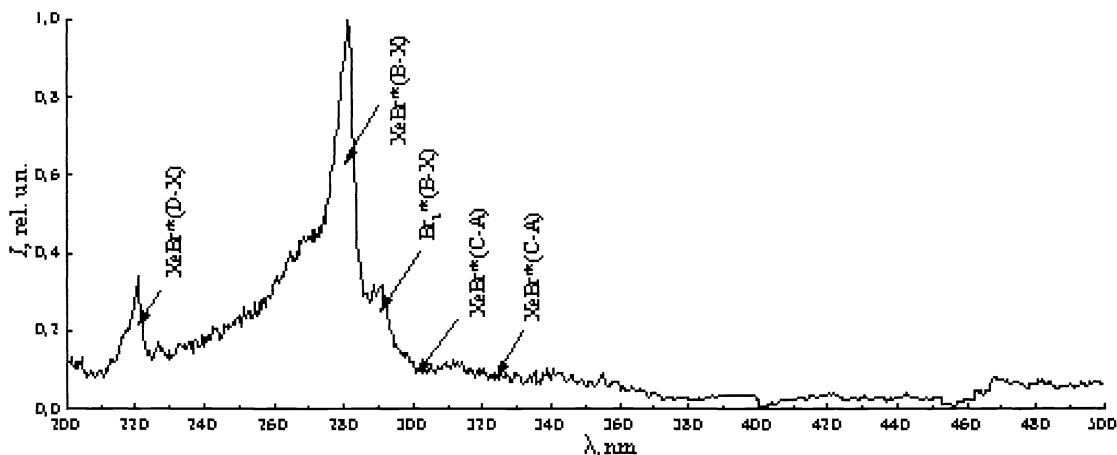


Fig. 1. Capacitive discharge XeBr-excimer lamp spectra.

In comparison with conventional middle-pressure UV-lamps the warming of excilamps bulbs is relatively low, and in western articles it's still named as *cold emission sources*. For example, in ⁶ for the first time were proposed and tested capacitive discharge excilamps with a simple design of bulb and high lifetime suitable due to absence of contact between a working mixture and metal electrodes. The important advantage of the excilamps is the absence of mercury in gas mixture. Owing to this, UV-system becomes safe in operation and removes a problem of expensive recycling of lamp bulbs.

Development of these low-cost, effective light sources promises enormous potential for materials' processing. One of potential application area is disinfection.

This paper is devoted to experimental study of inactivation process of *Escherichia coli* using different capacitive discharge excilamps на *Escherichia coli* under primary and secondary treatment. The second aim of the present work was the comparative study of bactericidal action of barrier discharge KrCl-excimer lamp irradiation on *Escherichia coli* and *Staphylococcus aureus*.

Let us note, that in articles dedicated to UV-sterilisation known for us, for example in ^{4,5}, the estimation of secondary treating of radiation was not carried out.

2. METHODS

2.1. UV lighting sources

Irradiation was performed within various time, irradiation dose, and use various type excilamps developed at Laboratory of Optical Radiation, High Current Electronics Institute SB RAS: KrCl-, XeCl- and XeBr-capacitive discharge cylindrical excilamps^{3,6} with following parameters: discharge gap 20, 20 and 21 cm, tube diameter 3.8, 4.2 and 4.2 cm, wavelength (B–X transition) 308, 222 and 282 nm, specific power density of UV radiation up to 10, 20, and 12 (mW/cm²), efficiency up to 15, 15 and 20 %, correspondingly. The barrier discharge KrCl-excilamp⁷ created at the Laboratory of optical radiation of the Institute of High-Current Electronics Institute SB RAS was also used in experiments. The lamp emission power was 65 W, emitting area as 0.1 m². Figure 2 shows KrCl-excilamp radiation spectrum. Commonly, excilamps have radiation spectrum maximum displaced in direction of short-wave UV radiation being close to the DNA absorption maximum. The last explains the high rate of bactericidal effect of excilamps. Photodetector FEK22-SPU was used in experiments to determine illumination. Irradiation dose (J/cm²) was defined by the product of illumination (W/cm²) and irradiation time (s).

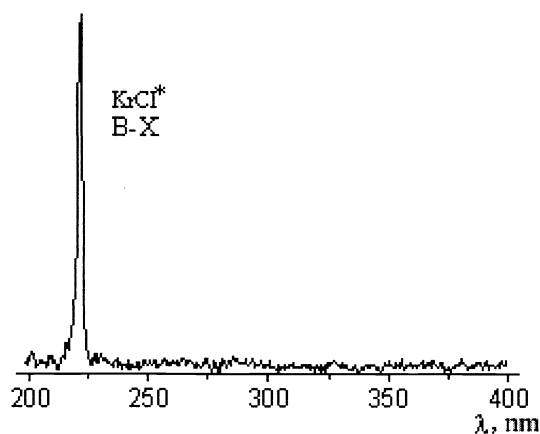


Fig.2. Radiation spectrum of barrier discharge KrCl-excilamp.

2.2. Bacterial cultures

The object of study was the pure culture of *Escherichia coli* and *Staphylococcus aureus*, provided by the Scientific Research Institute of Balneology (Tomsk, Russia). Our selection of colibacillus for study was determined by its belonging to the main species of the enterobacterium group taken for sanitation of disinfection efficiency by UV radiation. Besides that, the *Esch. coli* has one of the most high resistance coefficients among enterobacteria group. The culture *S. aureus* is widely spread test-object in investigations on action of disinfection matters and antibiotics on gram-positive bacteria since this culture is characterised by high adaptability to adverse effects due to its high conjugation between cells.

The bacterial cultures were supported on beef-extract agar (BEA) and kept at the temperature of 4°C. In preliminary study, based on the method of multiple dilutions, the optimal concentration of microbial dredge was found for

experiments.

Primary and secondary irradiation of culture *E. coli* by capacitive discharge excilamps radiation during various periods of time were carried out. Usually, the exposure was made 5 cm distant from the lamp. Thereby the heating effect of substrate was minimised and values of irradiation were kept high. Each exposure was repeated 4-5 times. For control, several dishes were left non-irradiated. The number of the survived micro-organisms was defined by the method of bacterial inoculation in dense media (the Koch method of bacterial inoculation in agarized media in Petri dishes). The inoculates were cultivated at temperature of 20-23°C. The evaluation of the number of survived micro-organisms was made in 72 hours.

The micro-organisms survived after irradiation by a dose leading to inactivation of 99.9 % of *E. coli* allocated in pure culture and subjected to a repeated treatment. The qualitative and quantitative analysis, estimation of bacterial colonies morphology (size, form, consistence, character of edge¹) were conducted.

For statistics of inactivation results, arithmetic average, standard deviation, and error in mean were found using standard program package Statistica 5.0 for Windows, and confidence level was defined by criterion Wilcoxon-Mann-Whitney.

3. RESULTS

3.1. Primary and secondary irradiation of culture *E. coli* by capacitive discharge excilamps

From the experiments performed it may be concluded that all the excilamps can provide pronounced bactericidal effect on testing cultures both to primary and secondary UV-irradiation.

¹ Distinctions from initial culture from other attributes is not marked.

The certainty of bactericide radiation effect on microorganisms number was established by the use of variance analysis (significance value $p < 0.05$).

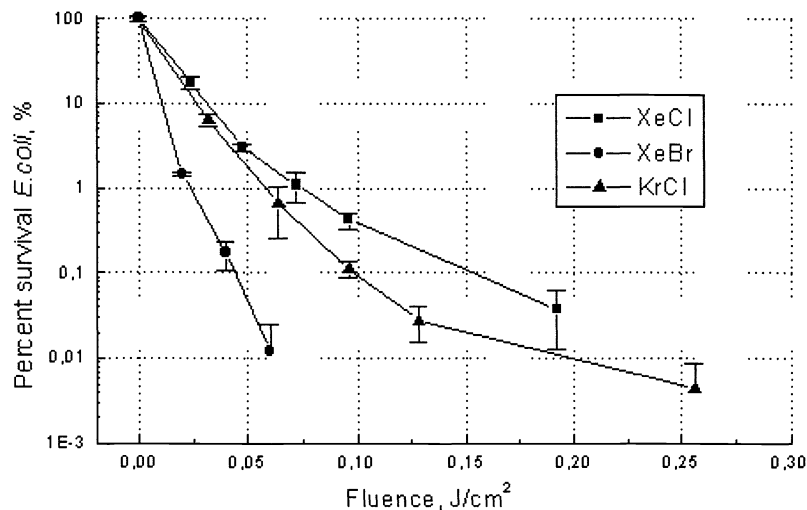


Fig. 3. Inactivation of surface-inoculated *Esch. coli* using different fluences and excilamps under primary irradiation.

primary and secondary irradiation. The number of viable cells is reduced at increase of dose and exposure ($r = -0.846$, $p < 0.05$ at primary influence and $r = -0.876$, $p < 0.05$ at secondary one).

The negative correlation between number of the survived cells *E. coli* and irradiation dose (time) is specific to XeCl-

KrCl-excilamp irradiation leads to inactivation 99.9 % of *E. coli* during 8 seconds of exposure at primary irradiation and during 6 seconds at the secondary irradiation (Fig. 4). With increase of irradiation dose and exposure time the number of microorganisms is reduced. For radiation of KrCl-excilamp ($\lambda \sim 222$ nm) the negative correlation between number of the survived bacteria and irradiation dose (time) was found out. Factor of correlation (r) is equal -0.960 ($p < 0.05$) at primary irradiation, and -0.896 ($p < 0.05$) at secondary one.

Inactivation of 99.9 % of microorganisms at influence of XeBr-excilamp ($\lambda \sim 282$ nm) was provided during 6 seconds both at

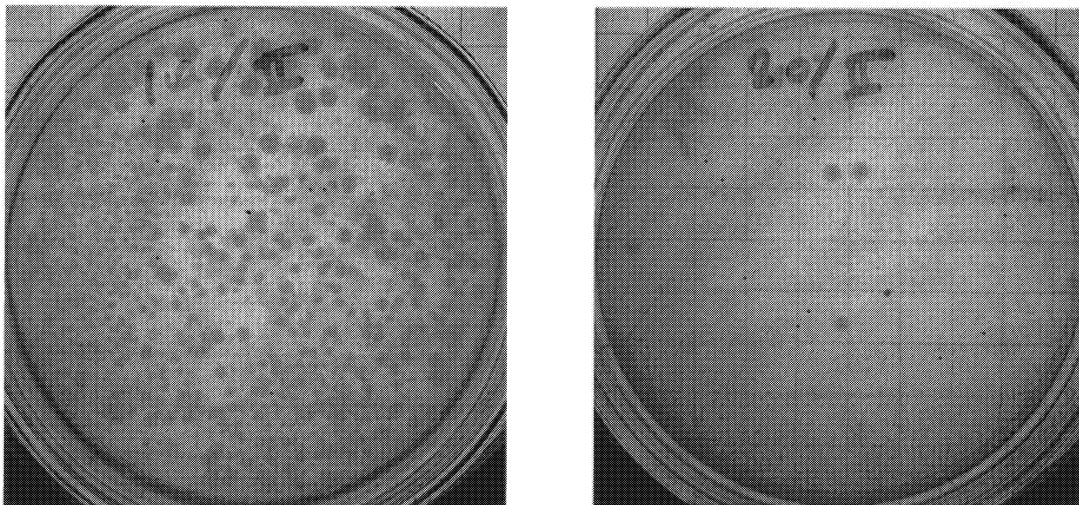


Fig. 4. Photos of survived cultures of *Esch. coli* after 10 seconds (from the left) and 20 seconds (from the right) irradiation by KrCl-excilamp.

excilamp ($\lambda \sim 308$ nm) radiation. Correlation factor at primary irradiation is equal -0.967 ($p < 0.05$) and -0.979 ($p < 0.05$) for the secondary one. Inactivation of 99.9 % of *E. coli* occurs at exposition of 16 seconds at primary treatment and after 32 seconds at the secondary one.

At comparison of efficiency of primary and secondary irradiation the authentic distinctions are not revealed (significance value > 0.05). It suggests that the sensitivity of culture *E. coli*, survived after primary irradiance has not changed.

Comparative study on bactericidal effect of excilamps suggests that the best results can be obtained using XeBr-excilamp under primary as well as secondary irradiation (Fig. 3). This is consistent with photobiology data about nuclear acids radi-

tion absorption². We included the performance parameters of XeBr-excilamp into Table 1. It is evident that this lamp has the best characteristics in respect to power expenses for producing of bactericide radiation.

3.2. The comparative study of bactericidal action of barrier discharge KrCl-excilamp irradiation on *Escherichia coli* and *Staphylococcus aureus*

Inoculation of test-cultures was irradiated by barrier discharge KrCl-excilamp. For each exposure there were three retries. Part of dishes was left non-irradiated for control. Bacterial inoculations were cultivated at room temperature within three days. Then, the number of colonies in dishes was calculated and survival of *E. coli* and *S. aureus* cells was evaluated in percents versus the control samples. On investigation KrCl-excilamp irradiation influence on the cultures gross bactericidal effect has been reached. The percentage of survived cells decreased with UV dose increasing to 21-25 mJ/cm², but with further dose increase the part of colonies increased too from 0.1 to 0.4% in experiments with *E. coli* and from 0.3 to 0.7% in experiments with *S. aureus* (Figs. 4,5). The last may be connected with penetration of unintentional cultures into investigated ones with increase of exposure time.

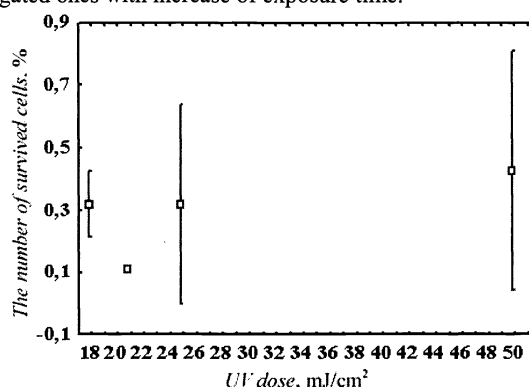


Fig. 4. The number of survived *E. coli* cells.

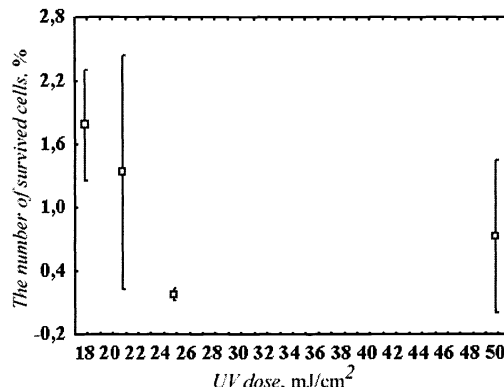


Fig. 5. The number of survived *S. aureus* cells.

CONCLUSION

Thus, a reliable bactericidal effect on *Escherichia coli* cells irradiation by excilamps has been established. Both on primary and secondary irradiation there exists the reciprocally proportional dependence between irradiation dose (or exposure time) and survived cells number. The microorganisms survived after primary irradiation are shown to have not changed sensitivity to excilamps irradiation. The best results have been obtained during XeBr-excilamp irradiation. Owing to their technical parameters, the excilamps are promising systems for UV-sterilization.

During the work performed it was clearly defined that radiation of the barrier discharge KrCl-excilamp exerts strong bactericidal action on the both cultures, namely *E. coli* and *S. aureus*. With increase in UV irradiation dose as 21-25 mJ/cm² the KrCl-excilamp bactericidal effect became stronger. Hence, increase in exposure time results in increasing of the number of non-inactivated cells from 0.1 to 0.3% for *E. coli*, and from 0.4 to 0.7% for *S. aureus*.

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