

Chapter 19

Applications of Excilamps in Microbiological and Medical Investigations

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Abstract In a course long-term and comparative studies it has been shown, that the DBD XeBr-excilamps looks as a good choice for various microorganisms inactivation. The first data about bacteriophage inactivation by XeBr-excilamp has been obtained. Radiant modules for industrial treatment on contaminated water have been developed. The XeCl-excilamp for treatment of skin diseases has been created and tested.

19.1 Introduction

Spontaneous radiation sources, such as the excimer and exciplex lamps (excilamps) find wide applications for science and engineering, in particular in biology and medicine [1–7]. The main reasons are the following:

The great part of excilamp's light energy concentrates in UV or VUV spectral range which depends on gas mixture in a bulb. Photons with energies 5–10 eV (UV and vacuum UV spectral ranges) can initiate and support different chemical, physical, and biological processes. Other advantages of excilamp are their simplicity in comparison with UV and VUV lasers, and have long lifetime.

In the current review the excilamps are described briefly, and the most part of text is dedicated to applications of excilamps in biology and medicine.

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19.2 Study and Development of Excilamps

Since 1992 the researches and development of spontaneous radiation sources (in the first place – excilamps) were carried out in the High Current Electronics Institute. The excilamps filled in pure inert gases or inert gas-halogens mixtures and excites by pulse-periodical or d.c. discharges. Efficient radiation of Ar₂, Kr₂, Xe₂, KrBr*, KrCl*, XeI*, XeBr*, XeCl*, Cl₂* molecules and I atoms was obtained in rare gas or in rare gas – Br₂ (Cl₂, I₂) mixtures. As a rule we use the electrodeless discharge for increasing of excilamp lifetime. Our electrodeless excilamps could be separated into two categories: capacitive discharge (CD) and dielectric barrier discharge (DBD) driven excilamps. In both categories the electrodes in a gas discharge device are covered with dielectric (it is fused quartz in our case) that led to the high lifetime of the working mixture. The dielectric layers have thickness up to several mm and dielectric constant of 3.5–4.

The difference between working pressure values and discharge gap values in typical conditions causes the difference of discharge form and the spectral band width of CD and DBD driven excilamps (Fig. 19.1). For CD driven excilamp is typical the big gap and low pressure values of gas mixtures (up to 10 Torr). As a result the CD excilamp spectra are relatively broad and represent a few optical transitions. For DBD devices the discharge gap is small (up to 10 mm) but a pressure of gas mixtures is relatively higher (up to several hundred Torr). Therefore the spectra become narrowband as we see at Fig. 19.1.

The spectral, temporal, energy and lifetime characteristics of CD and DBD excilamps have been performed. In optimal conditions the radiant power for the just sealed off KrCl ($\lambda \sim 222$ nm) excilamp exceeded 100 W has been obtained. Maximal radiation power density was of 50 mW/cm². UV output power of ~ 75 W and efficiency up to 10%, respectively, at $\lambda \sim 308$ nm (XeCl* excilamp) were obtained under excitation by pulses with frequency of 100 kHz. Application of water cooling allows increasing the radiant power of DBD coaxial excilamps. VUV output power of ~ 120 W at $\lambda \sim 172$ nm was obtained under excitation by pulses with frequency of ~ 100 kHz.

The excilamp radiation efficiency was determined as $\eta = P_{rad}/P_{in}$, where P_{in} is the total power deposited to the lamp (input power), P_{rad} – radiation power.

The lifetime of gas mixtures in small XeCl and KrCl barrier discharge excilamps over 12,000 and 8,000 h was demonstrated (some our results concerning excilamps lifetime see in [5, 8, 9]).

The extraordinary characteristics of excilamps led to a lot of applications, which had been demonstrated in a number of recent studies [1–7]. The example of developed excilamps (BD_X model) is illustrated in Fig. 19.2. There are DBD KrCl- and XeBr-excilamps photo with output window size 13 × 80 cm. The DBD KrCl-excilamp with the radiation maximum at $\lambda = 222$ nm, and XeBr-excilamp with the radiation maximum at $\lambda = 282$ nm have radiant power up to 25 and 30 W, accordingly. Other details were described in [10].

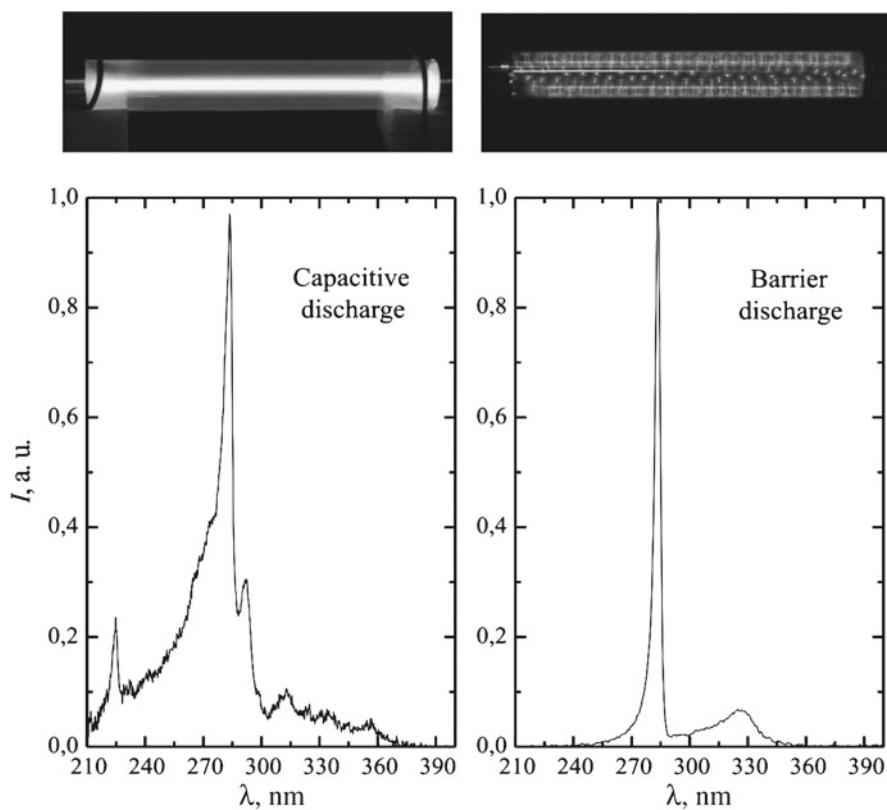


Fig. 19.1 Emission spectrum and general view of lighting of XeBr-excimer lamp excited by CD (*left*) and DBD (*right*). The operating pressure values were 3 and 97 Torr, accordingly



Fig. 19.2 General view of KrCl- and XeBr-excimer lamps (BD_X model)

19.3 Applications

19.3.1 UV Inactivation of Biological Systems by Excilamps

This chapter is devoted to our experimental study of UV inactivation of biological systems. We start these studies in 2001 [11] and now clearly see the advantages of excilamp applications in this field.

UV irradiation has been shown to be a powerful tool in inactivating of both microorganisms and cells such as bacteria, viruses, protozoan parasites, some spores, living cells and subsystems such as enzymes, aminoacids, and lipids (see refs. in [4]).

VUV or UV excilamps appear as an interesting option to conventional light sources for UV disinfection. Thus, one should distinguish between two different disinfection methods: the inactivation of microorganisms by UV irradiation (e.g. by KrCl-, XeBr-, and KrBr-excilamps) or their total VUV-induced photomineralization (by Xe₂-excilamp).

For the first time, the bactericide action of incoherent VUV- and UV-radiation using Xe₂- and KrCl-excilamp flow-through photoreactors (electric input power P_{el} of 150 W) was already manifested by Oppenländer and Baum in 1996 [12].

The comparative analysis of inactivation by excilamps and other means (plasma processing, laser irradiation, LP Hg lamps) has demonstrated that excilamps are the competitive technical systems [13, 14]. In the scientific literature have been prevailed the studies, where the specific excilamp gives inactivation effect on specific microorganisms. So, the inactivation effect of excilamps was demonstrated for a number of microbiological objects (yeasts, heterotrophic bacteria, spores, strains, unicellular organisms, cultures of living cells (see refs in [4])). Therefore we have started to do the comparative studies between inactivation of different microorganisms by different excilamps.

In our first study the *E. coli* microbial samples were exposed by CD KrCl-, XeCl- and XeBr-excilamps. It had been demonstrated that CD XeBr-excilamp is the most efficient light source for inactivation [11]. How we have explained this fact? It is known that the effect of radiation within the bactericidal range is associated primarily with the dimerization processes in the bases of DNA molecules and that the DNA absorption spectrum (Fig. 19.3) has two pronounced peaks near a wavelength of 200 nm and in the band of 250–270 nm. Therefore the action of XeBr-excilamp is determined by its spectrum having a long short-wavelength “tail” of 260–282 nm that covers half of the first DNA absorption peak.

Our later studies had been shown that DBD driven XeBr excilamp is also attractive for inactivation. Let us to give a several results that prove this fact.

In our study [15] the XeBr-excilamp (282 nm) and low-pressure Hg-lamp (253.7 nm) radiation impact on *Escherichia coli* bacterial strain have been presented. The mercury lamps are in wide use owing to their simple power-supply systems and easy maintenance. However, the radiant power of LP Hg lamps is very sensitive to ambient thermal changes, which should be considered in LP Hg disinfection reactors engineering.

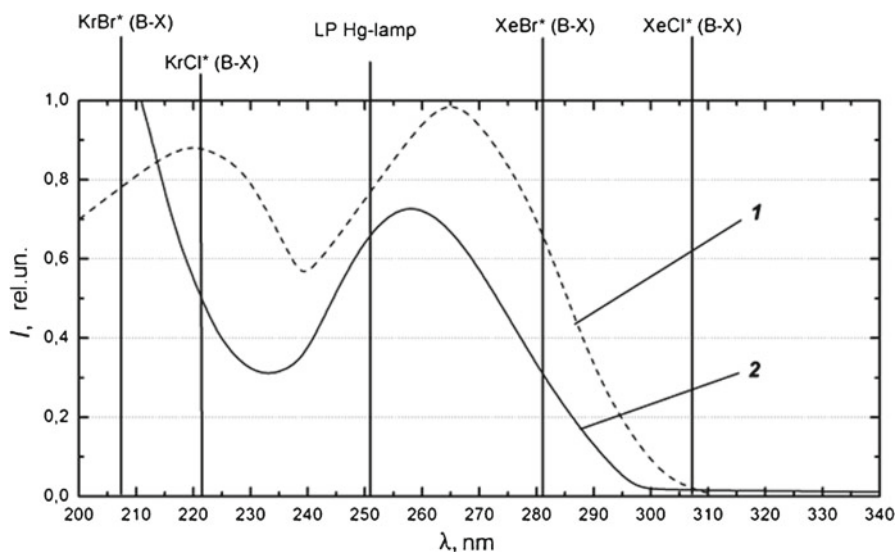


Fig. 19.3 Action spectrum of UV inactivation on *E. coli* (1), DNA absorption spectrum (2) and maxima of radiation bands of various excilamps and low pressure mercury lamp (LP Hg-lamp)

The peak intensity of B-X band of XeBr* molecule (282 nm) is seen to be at about the same distance from the action spectrum maximum, the same as resonance line of a LP Hg-lamp (Fig. 19.4). That is $\Delta\lambda_1 \sim \Delta\lambda_2$. This suggests that the both lamps have comparable bactericidal effect. Note that we do not know any other papers reporting on such direct comparison made. The model of XeBr-excilamp (model XeBr_BD_P, High Current Electronics Institute SB RAS) with the following parameters: discharge gap 0.8 cm, tube diameter 4.2 cm, UV radiant exitance of 3 mW/cm², and a spectrum shown in Fig. 19.4, was used in the experiments.

Another lamp was the conventional germicidal LP Hg-lamp (TUV-15). In the experiments, the lamp was specially furnished with a diaphragm in order to provide the radiation doses comparable in values with the radiation doses of the DBD-driven XeBr-excilamp. As a result, the Hg lamp provided radiant exitance of 2.5 mW/cm². The lamp's radiant power was defined in absolute units by using a C8026 (Hamamatsu Photonics KK) photodetector with the H8025-222 head. Just after the initiation, the XeBr-excilamp achieved its mode, and the LP Hg-lamp needed 2.5 min for its initial heating to provide stable luminous flux.

The object of study was the pure culture of *Escherichia coli* (strain № K12 ATCC 25922) 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 *E. coli* has one of the most high resistance coefficients among enterobacteria group. 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.

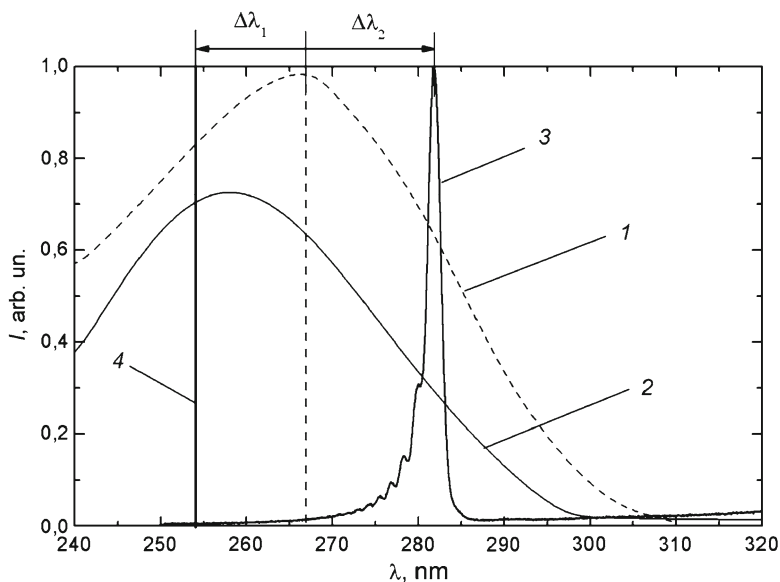


Fig. 19.4 Different spectral characteristics, essential to this investigation: 1 UV action spectra of DNA, 2 the absorption spectrum of DNA, 3 emission spectrum of DBD-driven XeBr-excilamp, 4 resonance line of LP Hg-lamp [15]

Primary and secondary irradiation of *E. coli* via various exposure values were carried out. Usually, the irradiation was made 5 cm distant from the lamp to 10 cm Petri dish with contaminated surface. Thereby the heating effect of substrate was minimized and values of irradiation were kept high. Thus, uniform illumination of contaminated surface was additionally provided. The microorganisms, which survived after irradiation by a dose leading to inactivation of 99.9% of *E. coli*, were allocated in pure culture and subjected to reirradiation. The qualitative and quantitative analysis and estimation of bacterial colonies morphology (size, form, consistency, character of edge) were made. The results of the experiments are shown on Fig. 19.5.

Thus, according to our hypothesis, both types of the light sources provide the similar bactericidal effect due to their radiation in the spectral range where UV action spectra of DNA have comparable values. As compared with traditional mercury bactericidal lamps, the advantages of the modern excilamps, accentuating to their bactericidal application are: (1) high photon flux q_p , extracted from plasma without self-absorption; (2) no elementary mercury in bulbs, which conforms with ecology; (3) extraordinary geometric freedom of bulbs; (4) momentary launching and full radiant power after ignition; (5) variable tuning of photon flux q_p .

As we noted above for the first time we have established bactericidal effect of CD XeBr-excilamps due to their wideband spectra in 2002. Such a spectrum has a short-wave tail from 230 to 282 nm (B-X band), which covers a half of the first maximum of DNA absorption. Besides, the spectrum has a D-X band with a

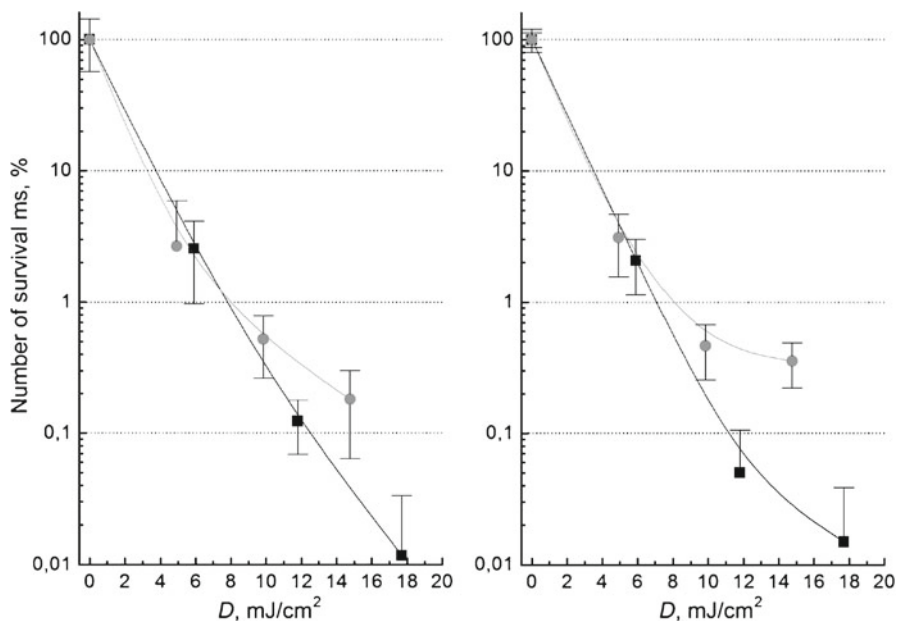


Fig. 19.5 Inactivation of surface inoculated *E. coli*, performed with various UV-doses of XeBr-excilamp (\square) and LP Hg-lamp (o) irradiation. The first bacteria generation results are on the left, and the second bacteria generation results are on the right [15]

maximum at 221 nm, which has a short-wave tail from 210 to 221 nm. It has been shown that the microorganisms, which survived after the first irradiation by that excilamp, kept their radiation susceptibility unchanged [15]. UV resistance protection of microorganisms under the action of wideband UVB irradiation is explained by the fact that biological structure of bacteria bears very many induced failures of biological structure, making it improbable to appear stable mutants to UVB irradiation (the viewpoint validity is being considered in the reviews [16]). At LP Hg-lamp ruled irradiation one might expect appearance of the greater number of UV resistant mutants than at irradiation by a wideband radiation source. Just in this respect maybe be interpreted the fact obtained in the present investigation. At irradiation of bacteria, survived after the first irradiation (their average share was 0.5%), the survival curve obtained for LP Hg-lamp activated bacteria has moves aside from the curve obtained for XeBr-irradiated bacteria. In other words, resistance of *E. coli*, activated by the LP Hg-lamp, is higher in XeBr-excilamp irradiation case.

In [17] the comparison of XeBr-, KrCl- and KrCl+KrBr-excilamps radiation impact on 5 microbiological cultures (*Escherichia coli* (ATCC 25923), *Staphylococcus aureus* (25923) and extracted from human skin representatives *p. Sarcina*, *p. Pseudomonas* and *p. Bacillus*) have been presented.

Emission spectrums of DBD-driven excilamp under using are presented on Fig. 19.6. Data about bactericidal efficiency is assembled to Table 19.1.

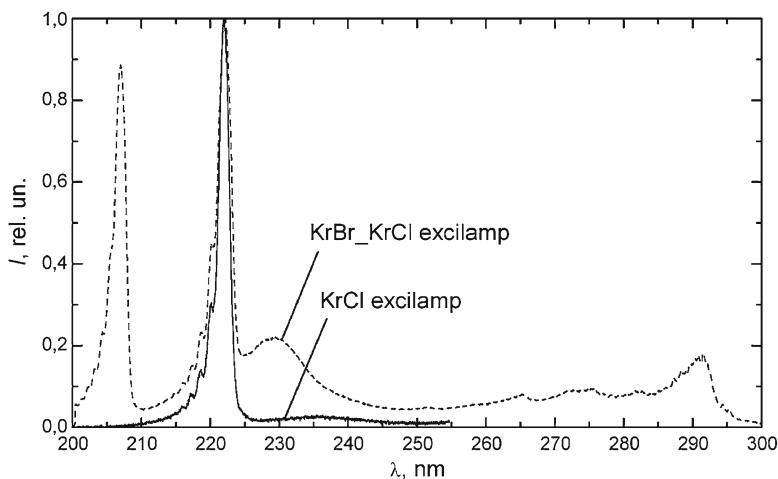


Fig. 19.6 Emission spectrums of DBD-driven KrCl- and KrBr+KrCl-excilamps

Table 19.1 Experimental data of surface irradiation dose which gives 99.9% of bactericidal efficiency for different excilamp [17]

Microorganism	$H_s, \text{J/m}^2$		
	XeBr*	KrCl*	KrCl* + KrBr*
<i>Escherichia coli</i> (ATCC 25923)	60	85	65
<i>Staphylococcus aureus</i> (25923)	150	370	320
p. <i>Sarcina</i>	90	–	120
p. <i>Pseudomonas</i>	110	–	160
p. <i>Bacillus</i>	100	190	130

It is shown that surface irradiation dose which gives 99.9% of bactericidal efficiency for different excilamp and for LP Hg-lamp have a comparable values (if we take into the mind the data from [18]). In the second place the bactericide efficiency are decrease in a row of lamps XeBr (282 nm) > KrCl_KrBr (222 and 206 nm) > KrCl (222 nm).

In 2009–2010 we have studied the sensitivity of hospital infectious agents to UV radiation of excilamp and LP Hg-lamp using the abovementioned cultivation and irradiation methods [19].

The object of study was the pure culture of *Escherichia coli* (strain ATCC 501), *Klebsiella pneumonia* (strain ATCC 2482), *S. aureus* (strain ATCC 209) and two cultures selected from patients of Tomsk Savinich Hospital – *C. albicans* and *P. aeruginosa*. Suspension of daily cultures (with concentration 10^5 CFU/ml in volume 0,1 ml) was inoculated into a meat infusion agar. As the control a suspension of daily cultures in concentration 10^3 CFU/ml was used.

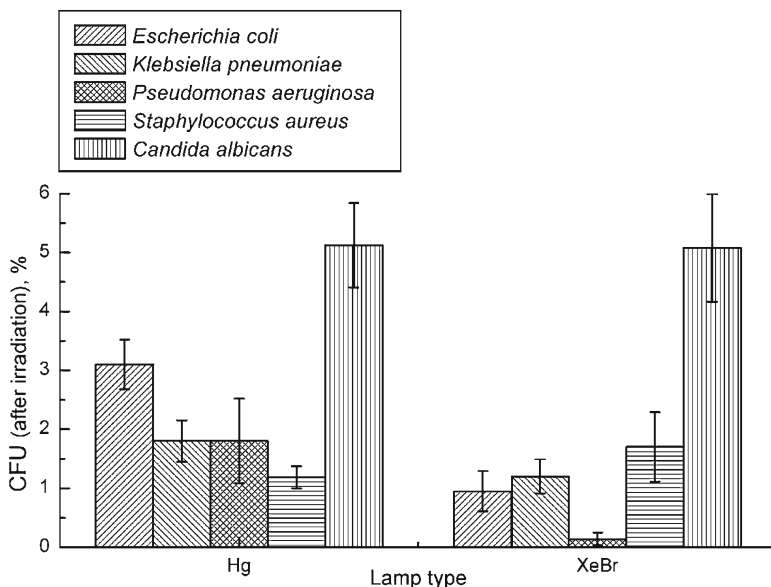


Fig. 19.7 Sensitivity of test cultures to UV radiation of excilamp and LP Hg-lamp after 15-s time exposure (and at the same UV exposure)

The results of our research are illustrated in Fig. 19.7. From this figure we notice that: (1) XeBr-excilamp irradiation gives better germicidal effect for *E. coli*, *P. aeruginosa* cultures; (2) *S. aureus* and *C. albicans* cultures have the same UV-resistance for both light sources.

The low UV-sensitivity of *K. pneumoniae* could be explained by presence of sheath (capsule) in their biological structure. This capsule absorbs a part of radiation flux and decrease the DNA damage frequency.

Our recent results (2010) are dedicated to the sensitivity of *MS2 bacteriophage* under UV radiation of LP Hg-lamp and XeBr-excilamp. The model XeBr_BD_P and LP Hg-lamp (TUV-15) were used in the experiments. The object of study was the *MS2 bacteriophage* (strain PH-1505), which cultivated on *E. coli* K 12 F+ (strain B-3254) after irradiation. Both cultures are from Russian industrial bank of microorganisms. UV sensitivity of bacteriophage has been determined by well-known Gratia method [20]. The exposure value was equal to 45 J/m². Viocidal action of radiation has been estimated by quantity of blank places on Petri dishes (see Fig. 19.8).

The greater viroicidal influence on tested culture has been achieved by means of XeBr-excilamp (Fig. 19.9). We think that it is related to damaging genetic block of bacteriophage as well as protein shell too. Our results testifiers that the excilamps could be use in antiviral applications.

Concluding this chapter, let us note that some microorganisms and cells possess UVA/VIS repair mechanisms (photoreactivation) that substitute or dissociate thymine dimers. Under these circumstances, excilamps as narrow-band emission

Fig. 19.8 Photo of Petri dish after the experiment. Each *dark place* corresponds to the locus where a survived *MS2 bacteriophage* has killed *E. coli*

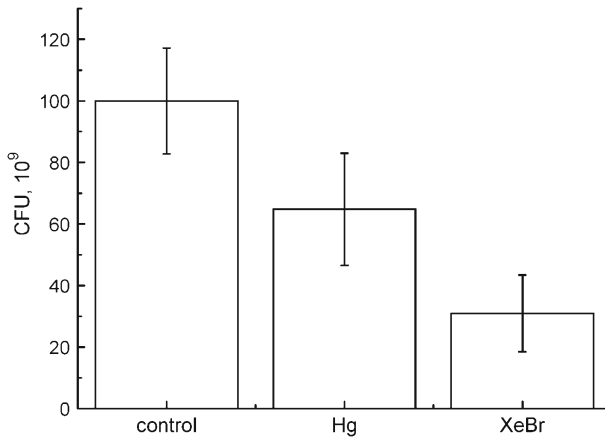
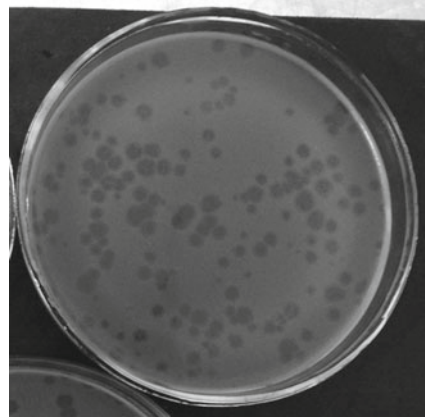


Fig. 19.9 Sensitivity of *MS2 bacteriophage* to UV radiation of XeBr excilamp and LP Hg-lamp at the same UV doses (45 J/m^2) compared with control

sources should be more efficient than wide-band MP Hg lamps. Sure, this problem needs further studies to be done.

19.3.2 UV Phototherapy of Skin Diseases

One of the most effective methods of psoriasis curing is UVB phototherapy. Radiation is absorbed by endogenous chromophores, especially by DNA nucleotides, which lead to suppression of DNA synthesis in epidermal cells, for example in psoriatic plaques. Photochemical reactions of these molecules result in alterations of skin and then lead to the curing effect. Apparently, the DNA damage is the general mechanism at UV curing of skin diseases. Particularly, UV radiation affects



Fig. 19.10 Example of psoriasis curing by the XeCl excilamp (model BD_P, see previous chapter) in Siberian State Medical University (BD_compact model, Optical Radiation Laboratory, Russia, output window square 30 cm², UV photon exitance of 40 mW·cm⁻²): before curing (*left*) and after 10 days at suberythermogenic doses treatment (*right*) [25]

the production of soluble mediators, the expression of cell-surface receptors, to induce apoptosis in pathogenetic relevant cells (cf. [21, 22]).

In 1980, a spectrum of UV radiation effect on psoriasis was obtained and showed that the effective UV radiation spectrum for psoriasis curing lay in the area 296–313 nm [23]. More than 90% of the DBD XeCl-excilamp radiant energy is within the anti-psoriasis action spectrum. Thus, this excilamp is also a good variant for psoriasis curing, which was proposed for the first time in 1994 [24]. For this aim in 2003 we have developed a compact XeCl excilamp for French start-up company “DermOptics SAS”. It was the first prototype of compact device [25, 26]. Now a key idea of this device are used in commercial “Quantel derma” 308 Excimer System. Since 2004 we have tested XeCl-excilamp (Fig. 19.10). The merits of such a therapy method are a good tolerance by patients and the use of suberythermogenic doses. In comparison with a XeCl-laser, the excilamp is cheaper and simpler in use. There are no principal restrictions for XeCl-excilamp radiant area increase, which allows to develop large-scale set-ups both for local and total-body irradiation.

19.4 Conclusion

A study of excilamps applications for researches in photomedicine and photobiology has been carried out. In a course long-term and comparative studies it has been shown, that the CD and DBD XeBr-excilamps are the best choice for various microorganisms inactivation. The first data about bacteriophage inactivation by excilamp has been obtained. We suppose that the further efforts should be concentrated on microorganisms photoreactivation (after excilamps irradiation) studies and

reproducibility of results (because of specific character of microbiological tests). The XeCl-excimer lamp for treatment of skin diseases has been created and tested.

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