UTILIZATION OF ULTRAVIOLET RADIATION OF COLD HOLLOW CATHODE DISCHARGE PLASMA FOR WATER DISINFECTION

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We present the results of experimental studies of the peculiarities of the inactivation of an *Escherichia coli* water suspension by ultraviolet (UV) radiation of the plasma of a glow discharge with hollow cathode in different gaseous media. It is shown that the efficiency of the inactivation by UV of the discharge plasma in oxygen, mixtures of oxygen with deuterium, and water vapor is essentially higher than that of the discharge in air, as well as the discharge in a low-pressure mercury lamp.

1. Introduction

In the last decades, UV radiation attains the growing application as an antimicrobial agent in the disinfection of water and air, sterilization of packing materials for foodstuffs, etc. The inactivation of microorganisms under the action of UV radiation occurs, first of all, due to the damage of DNA as a result of a number of photochemical reactions [1]. The main advantage of the method of sterilization and disinfection by UV as compared to methods using chemical agents (chlorine, hydrogen peroxide, ozone, etc.) consists in that no toxic and/or mutagenic side products are formed on the use of UV radiation.

Modern UV systems, e.g. for water disinfection, use commonly low-pressure mercury lamps generating the radiation with a wavelength of about 254 nm. As an alternative to those lamps, medium pressure mercury lamps generating several wide bands in the spectrum range 200—300 nm are used. The advantages of mercury lamps consist in their high energy efficiency reaching 40—50% for low-pressure lamps and in a long service life reaching 10 thousand hours. However, there is an essential drawback of the use of such lamps due to the high toxicity of a mercury vapor. This results in the extra expenses for the safety assurance in the production and recycling of those lamps. For that reason, a search of the alternative sources of UV radiation providing the high sterilization and/or disinfection efficiency, while containing no high-toxicity substances, is performed in the recent time.

In the experiments on the sterilization of medical articles by gas discharge plasma [2], it has been shown that the efficiency of the sterilization of *Bacillus subtilis* spores by the wide-band (200—300 nm) UV radiation of a cold hollow cathode gas discharge plasma is essentially higher than that by the monochromatic radiation of a low-pressure mercury lamp. Experiments performed with the use of different gases (oxygen, air, nitrogen, and argon) have shown that the highest efficiency is provided by the UV radiation of an oxygen plasma.

Due to these facts, we study the possibility to use the ultraviolet radiation of a hollow cathode discharge plasma for water disinfection. To this end, we have performed the comparative experiments on the influence of ultraviolet radiation of the mentioned discharge plasma, as well as that of a standard low pressure mercury lamp.

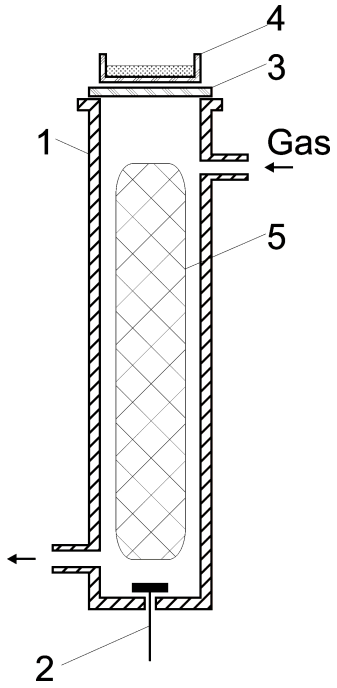

Fig. 1. Scheme of the experimental setup. *1* — chamber-cathode, *2* — anode, *3* — window made of KU-1 quartz, *4* — Petri dish made of KU-1 quartz, *5* — discharge plasma

2. Description of a Setup and Methods of Measurements

The experiments studying the water disinfection efficiency were performed at a setup schematically shown in Fig. 1. The walls of cylindrical chamber *1* having the 400-mm length and 50-mm internal diameter simultaneously served as a discharge cathode. Flat anode *2* 30 mm in diameter was placed at one of the chamber ends. Another end of the chamber was closed by window *3* made of quartz of KU-1 type with 4 mm thickness and the lower bandpass bound of about 175 nm. For avoiding the undesired getting dusty of the window surface, a quartz insert 45 mm in length and 45 mm in internal diameter was placed inside the chamber. At a time of the experiments, a Petri dish made of 2-mm thick KU-1 quartz with the water suspension of test microorganisms was placed on window *3*. Discharge plasma *5* served as a volume source of UV radiation.

The chamber was evacuated by means of a fore pump down to a residual pressure of $(1\div2)\cdot10^{-3}$ Torr. Then the working medium was introduced into the chamber and was pumped through it for 10—20 min prior to the ignition of a discharge. The discharge glowed for 1—2 h before conducting the experiments for the removal of admixtures produced by the chamber walls. Air, oxygen, deuterium, mixture of oxygen and deuterium with various ratios, and water vapor were used as working gases/mixtures. The working pressure in the chamber was varied in range from 0.1 to 1 Torr depending on the type of gas media. A direct current source with steeply falling output characteristic was used for powering the discharge. The power introduced into the discharge was varied in range of 250÷350 W. The plasma density comprised $(5\div10)\cdot10^{11}$ cm^{-3}.

For conducting the experiments with monochromatic UV radiation, we used the device based on low-pressure mercury lamps of DB-30 type which was consistent with requirements described in [3,4]. A mercury lamp was placed in a horizontally situated metal cabinet with the internal surface coated by black paint. A collimating tube with the blacked internal surface having 200-mm length and 40-mm internal diameter was located at the central part of the cabinet perpendicularly to the last one. The top end of the tube was closed by a window made of quartz of KU-1 type of 3 mm in thickness.

Escherichia coli 1257 strain received from the Scientific Research Institute of Standardization and Control of Medical Biological Preparations (Moscow, Russia) served as a test microorganism. (*Escherichia coli* bacterium is one of the common test microorganisms used in microbiology). The culture of *Escherichia coli* bacteria was inoculated from nutrient agar to meat-peptone bouillon and incubated for 18 h at a temperature of 37 °C. After the centrifuging with 2500 g acceleration for 10 min, the sediment was washed by a sterile physiological solution; the procedure was repeated thrice. After the last centrifuging, the sediment was resuspended in distillated water to obtain density of 10^9 CFU/ml (colony forming units/ml). Respective volume of initial suspension was diluted in distillated water down to a density of $10^5 \div 10^6$ CFU/ml. 3 ml of the working suspension was placed into a Petri dish of 32 mm in diameter resulting in the layer thickness of about 3 mm. Measurements by means of a spectrophotometer had shown that, at the used suspension density, the attenuation of UV radiation in the wavelength range of 200—300 nm did not exceed 3—7%. So it could be considered that the suspension was uniformly irradiated over the entire thickness. After the UV irradiation for a predetermined time, the required aliquot of the suspension or its dilution was introduced into the Endo medium, survived bacteria were incubated for 1824 h at a temperature of 37 °C, and the count of colonies was performed. On the basis of obtained data, the bacteria survival curves (the number

of survived bacteria versus the UV radiation dose) were built.

Due to the fact that the experimental studies of the sterilization efficiency of the *Escherichia coli* aqueous suspension were performed with the use of UV radiation with essentially different spectrum shapes (the broad-band radiation of a hollow-cathode gas discharge plasma and the narrow radiation line (λ=254 nm) of a low-pressure mercury lamp), we used the method of determination of the effective irradiation dose for a studied sample. This allowed us to correctly compare the results obtained with the use of the mentioned UV sources. The essence of the method consists in the measurement of the UV irradiation dose integrated over the spectrum in the plane of position of the studied sample by means of a UV-C range radiometer of the DAU-81 type in each experiment on the inactivation of bacteria. The dose determined in such a way was multiplied by the correction factor, whose values were determined for each type of the used UV radiation by "weighing" the spectrum intensity distributions. The method of determination of the correction factors will be described below.

Spectroscopic measurements were performed with the use of an automated setup based on a monochromator of the MDR-23 type. The radiation intensity at the monochromator output was measured by a FEU-39A photomultiplier, and the signal was supplied to the input of a measuring-processing system developed by the authors on a basis of a personal computer with the Intel Celeron 700-MHz processor. The system performed the 16-bit digitizing of an analog signal and its processing with the use of calibration data introduced into the system on the basis of recording the spectrum of a standard deuterium lamp of the DDS-30 type. The output file created by the system contained the spectrum distribution of absolute values of the intensity in the range 200—300 nm expressed in units of $(W/m^2)/nm$.

Let us concentrate on two peculiarities of spectroscopic measurements dealing with the specificity of the used UV radiation sources. The first peculiarity consists in the necessity of choosing the optimum response function of the system which, on the one hand, would enable obtaining the quite detailed spectra and, on the other hand, would exclude possible errors on the averaging of spectra which contain a big number of narrow lines. This choice was realized by setting the widths of the input and output slits of a monochromator to be 0.01 and 1 mm, respectively, (this setting provided about the 1.3-nm response function width and was not changed during all experiments) and by choosing a step of the spectrum recording to be 0.067 nm that is essentially less than the response function width. The second peculiarity was due to the fact that the range of incidence angles of UV radiation onto the bacteria suspension layer placed in the output window plane of the irradiating device comprised from — 30 to + 30 degrees for the hollow-cathode discharge device, and — 10 to + 10 degrees for the device with a mercury lamp, whereas the radiation acceptance angle of a monochromator did not exceed ± 7 degrees. Under such conditions, for the correct measurements of spectrum intensity distributions, the irradiating devices were placed at the optical axis of a monochromator at a distance not less than 400 mm from its input slit, which ensured the correct measurement of illumination in the input slit plane because all possible incidence angles were surely less than the maximum acceptance angle of the monochromator. After that, the ratio of illumination values in the input slit plane and the output window plane of the irradiating device was measured by means of a DAU-81 radiometer with an acceptance angle of ± 80°. This ratio was introduced into the system of automated data processing, so that the output files of the system contained the spectrum distributions of absolute intensity values in the output window plane of the irradiating device, i.e. at the place where the samples were located during medical-biological studies. The validity of such an approach was justified by the additional measurements of spectra, at which the hollow-cathode device was rotated by different angles in the scope of 0—30° in the dispersion plane of the monochromator, while keeping the center of the output window of the discharge device at the optical system axis. As a result, it was found that the shape of the spectrum intensity distribution remained practically unchanged (only the intensity value was changed), and the contribution of radiation with incidence angles of more than 30° was negligible due to the abrupt decrease of the irradiating plasma layer thickness in that direction.

With the use of the mentioned method, the spectrum intensity distributions in the sample irradiation plane were measured experimentally for the entire collection of plasma generating media used in medical-biological studies, at that the discharge parameters were chosen as close as possible to those defined at the irradiation of samples of an *Escherichia coli* water suspension. In the spectroscopic researches, the integral intensity of UV radiation in the sample irradiation plane was measured by a DAU-81 radiometer before and after each record of the spectrum intensity distribution, and the respective

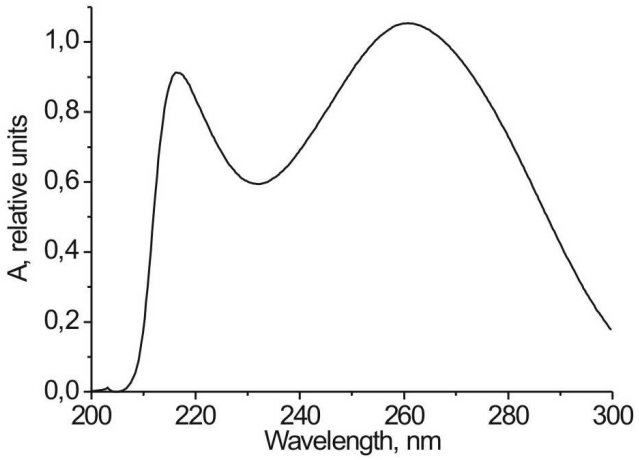

Fig. 2. "Weighing" curve obtained by multiplying the DNA absorption spectra and transmission curve of filter made of BaF_2 of 3 mm in thickness

average values allowed us to link together the collections of experimental data obtained in spectroscopic and medical-biological researches.

On the determination of the correction factor ("weighing" factor), first of all, results of works [5—7] devoted to studies of the DNA molecule absorption and efficiency of bactericidal action of UV radiation on microorganisms in dependence on the radiation frequency were taken into account. It has been shown in [5] that the DNA absorption spectrum in the considered wavelength range ($\approx 200 \div 300$ nm) represents a superposition of broad absorption bands having maxima at about 190 and 260 nm due to the electron excitation of diene and triene fragments of the DNA molecule chain. In experiments with *Bacillus subtilis* spores [6] and *Escherichia coli* bacteria [7], it has been shown that, at long enough wavelengths ($\lambda > 220$ nm), the curves of absorption and bactericidal efficiency versus λ practically coincide, and both reach the maximum at $\lambda \approx 260$ nm. At shorter wavelengths ($\lambda \leq 220$ nm), the dependences are sharply separated: the absorption curve goes up with decrease in the wavelength, whereas the bactericidal efficiency rapidly decreases. (Such a difference is most likely due to the strong absorption of short-wavelength radiation by the structures surrounding DNA — cell walls, plasma membranes, cytoplasm, etc.)

The second circumstance taken into account in the determination of the correction factor was found in the present experiments. It consisted in the fact that the bactericidal action of radiation of a discharge used by us on the studied *Escherichia coli* culture remained completely unchanged at the introduction of an additional filter made of BaF_2 with the low pass boundary $\lambda = 215$ nm. That is, radiation with the wavelength $\lambda \leq 215$ nm provided no essential effect in our case on the vital functions of microorganisms. On the other hand, this fact enabled the expression of a "weighing" function in the form of a product of spectrum dependences of the factors of BaF_2 filter transmission and DNA absorption obtained in [5]. The resulted "weighing" function normalized by 1 at λ=254 nm is presented in Fig. 2. It was actually determined by the factor of filter transmission at short λ and by the factor of DNA absorption at longer λ.

Thus, we used the portion of radiation transmitted by a filter and absorbed by DNA for defining the irradiation dose at the survival curves of microorganisms presented below.

In all experiments, the intensity of UV radiation falling on Petri dishes with an *Escherichia coli* suspension was in the range $0.01 \div 0.04$ mW/cm^2.

3. Experimental Results and Discussion

In Fig. 3, the cold hollow cathode discharge plasma radiation spectra in the wavelength range $200 \div 300$ nm obtained with the use of different working media are presented. It follows from the analysis of these spectra that the main contribution to the UV radiation of the plasma is given by the radiation of the γ system NO ($A^2\sum^+ - X^2\Pi$) in the case of the work in air and by the radiation of the second negative system O_2^+ ($A^2\Pi_u - X^2\Pi_g$) in case of the work in oxygen. One can see from Fig. 3,*c* that the shape of the plasma radiation spectrum for a discharge on the mixture of oxygen and deuterium essentially depends on the ratio of these components. At comparable concentrations of deuterium and oxygen, the radiation of the second negative system O_2^+ ($A^2\Pi_u - X^2\Pi_g$) and the 3064-Å radiation of the system ($A^2\sum^+ - X^2\Pi$)OD are superimposed on the continuous spectrum of the deuterium radiation. With increase in the deuterium content in the mixture, the contribution of the mentioned radiation components decreases. On the work in water vapor (Fig. 3,*d*), the main contribution to the total UV radiation is given by the second negative system O_2^+ ($A^2\Pi_u - X^2\Pi_g$) and the 3064-Å system OH ($A^2\sum^+ - X^2\Pi$).

The "weighted" spectrum distributions of the UV radiation power obtained at the discharge glowing in the same working media are presented in Fig. 4. One can see

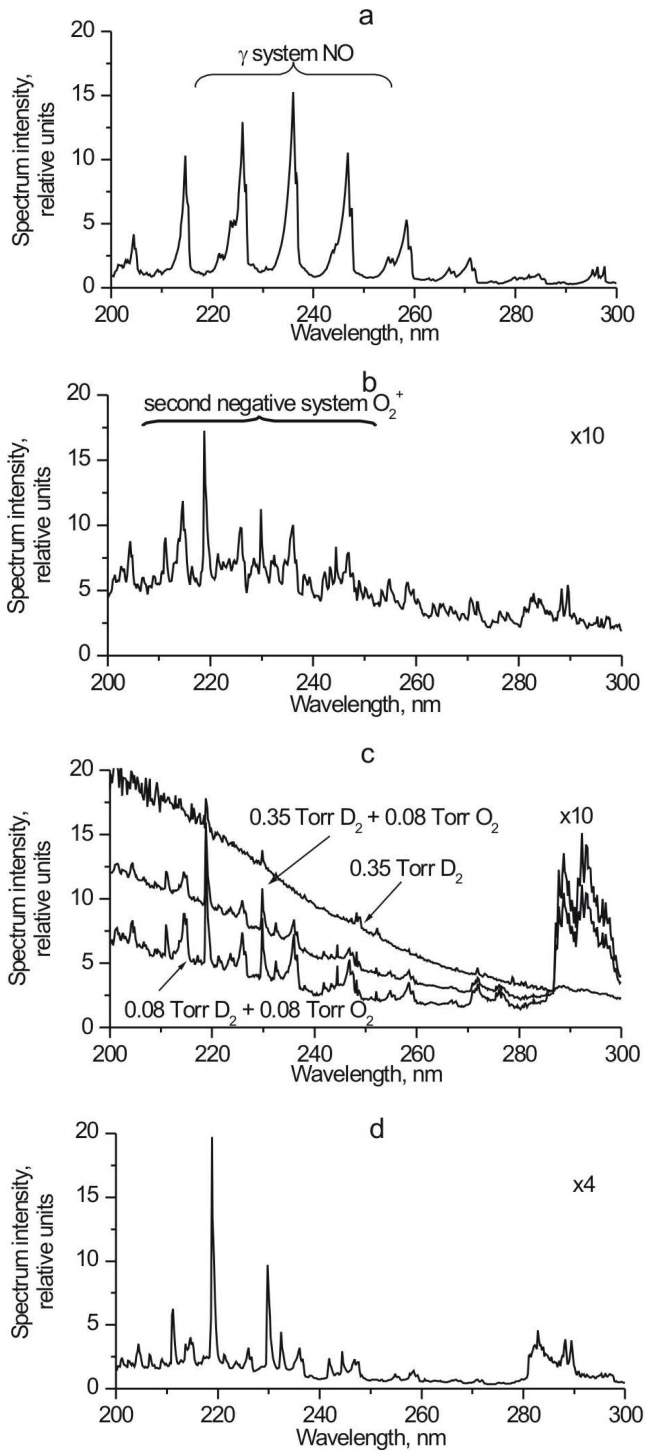

Fig. 3. UV radiation spectra of hollow-cathode discharge plasma on different working media: *a* — air, pressure 0.2 Torr, *b* — oxygen, pressure 0.1 Torr, *c* — mixture of deuterium and oxygen, *d* — water vapor, pressure 0.1 Torr

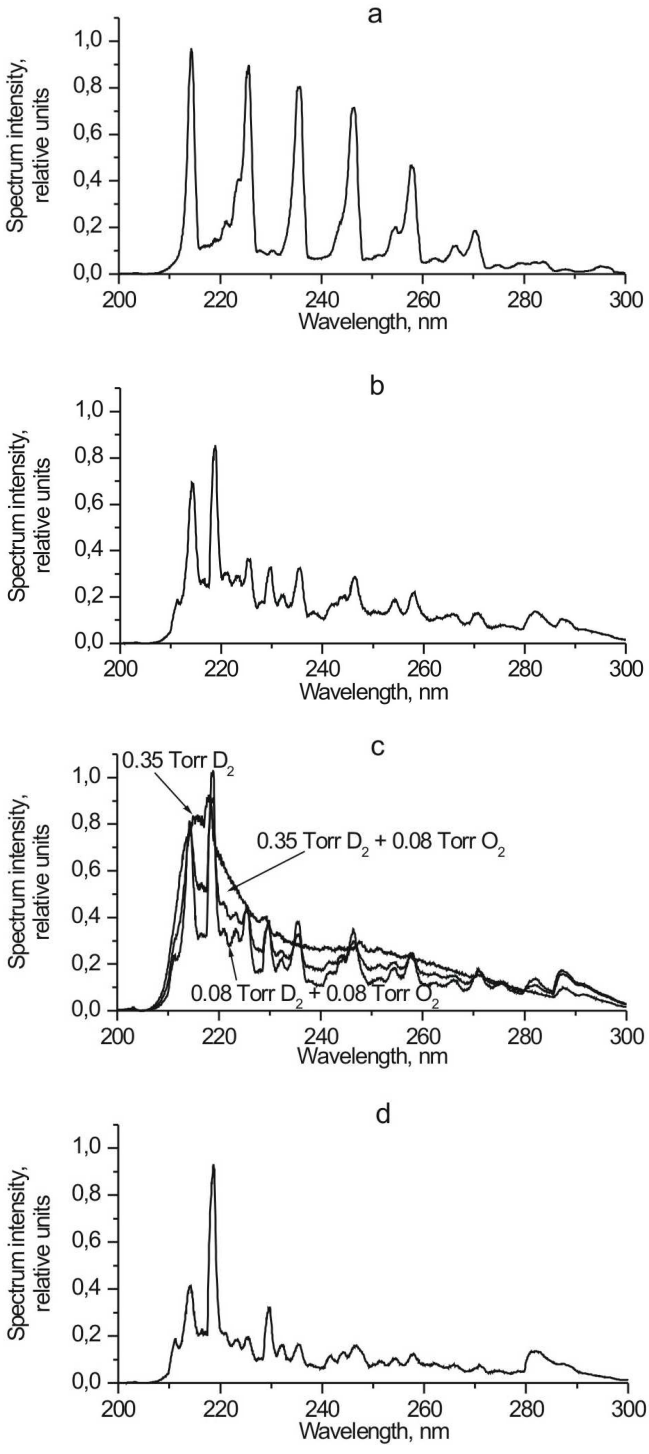

Fig. 4. Spectrum distribution of the "weighed" intensity of UV radiation of the discharge plasma on different working media: *a* — air, pressure 0.2 Torr, *b* — oxygen, pressure 0.1 Torr, *c* — mixture of deuterium and oxygen, *d* — water vapor, pressure 0.1 Torr

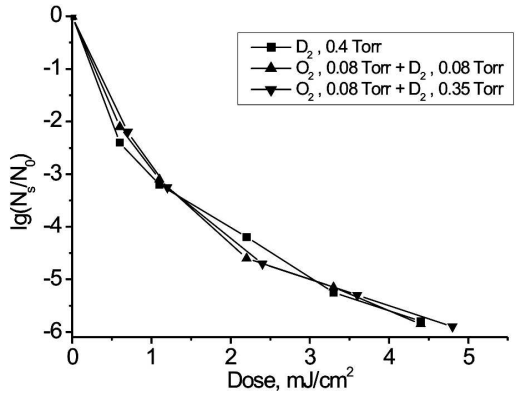

Fig. 5. Bacteria survival curves obtained at the treatment of an *Escherichia coli* suspension with 10^6 CFU/ml density by UV radiation of the discharge plasma on deuterium and mixtures of deuterium with oxygen

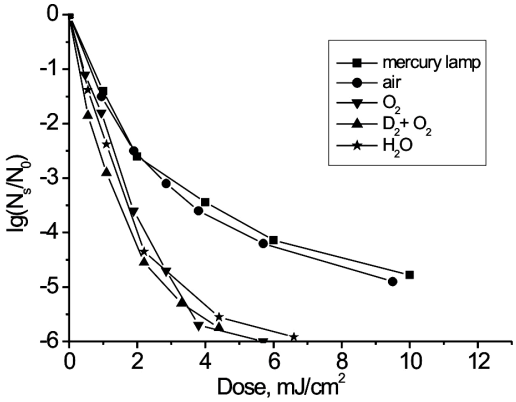

Fig. 6. Bacteria survival curves obtained at the treatment of an *Escherichia coli* suspension with 2×10^6 CFU/ml density by UV radiation of a mercury lamp DB-30 and the radiation of discharges in air, oxygen, water vapor, and mixtures of deuterium with oxygen

from the figure that if oxygen, deuterium, a mixture of deuterium with oxygen, and water vapor are used, the main power of UV radiation is concentrated in the spectrum range $\approx$ 210÷230 nm. But the UV radiation power is spread over several bands in the range 210 ÷ 260 nm in air.

The first stage of the experiments was devoted to the study of bactericidal features of the plasma UV radiation for discharges in deuterium and mixtures of deuterium with oxygen. In Fig. 5, we present the bacteria survival curves which have been obtained at the treatment of an *Escherichia coli* water suspension having density of 10^6 CFU/ml by the plasma UV radiation from discharges on pure deuterium and two mixtures of deuterium with oxygen. (N_0, N_s represent the initial quantity of bacteria and that of survived bacteria, respectively). Each point in the figure represents data averaged for three separate experiments. One can see from the figure that, in spite of a certain difference in the UV spectrum intensity distributions (the continuous radiation spectrum for pure deuterium and a superposition of the deuterium continuous spectrum with the bands of the second negative system of oxygen for the mixtures — see Fig. 4,*c*, the bacteria survival curves practically coincide. However, such a result does not appear for all gaseous media.

In Fig. 6, we present the bacteria survival curves which have been obtained at the treatment of an *Escherichia coli* water suspension with a density of 2×10^6 CFU/ml by the UV radiation of a DB-30 mercury lamp and that of the hollow-cathode discharge plasma in air, oxygen, water vapor, and mixture of deuterium with oxygen. Each point at the figure represents data averaged for three to six separate experiments. (The bacteria survival curve for the D_2+O_2 mixture is obtained by averaging the separate curves for cases of the use of deuterium, the mixture O_2 0.08 Torr + D_2 0.08 Torr, and the mixture O_2 0.08 Torr + D_2 0.35 Torr). One can see from the figure that the bacteria survival curves obtained at the use of the plasma UV radiation from discharges in oxygen, water vapor, and mixtures of deuterium with oxygen practically coincide. Curves obtained at the use of the UV radiation from a mercury lamp and that of the discharge plasma in air are also close to each other. However, they are located above the first set of the curves. Such essential difference in behavior of the curves gives the undoubted evidence to the fact that the bactericidal features of UV radiation in 215—300 nm wavelength range depend not only on a radiation dose absorbed by DNA (as it was observed in the case of monochromatic radiation), but as well on the radiation spectrum shape. The comparison of the spectra of radiation absorbed by DNA for the discharges in different gases (Fig. 4) shows that the maximum efficiency is provided by the broadband radiation having maximum in the 215 to 230 nm spectrum range.

The high efficiency of UV radiation with continuous spectrum having a maximum in the range $\lambda \approx 215 \div 230$ nm is presumably due to:

— difference in the nature of a DNA damage caused by UV radiation in the mentioned wavelength range, as compared to that occurring at the use of radiation with other wavelengths;

— synergetic effect which occurs as a result of the simultaneous action of UV quanta with different energies on DNA;
— stronger damage caused by radiation in the mentioned wavelength range to other biological molecules, particularly, to enzymes which are responsible for the reparation of damaged DNAs [8].

Finally, the obtained results should be compared with those obtained by other authors with respect to the influence of a broadband spectrum on the inactivation efficiency of microorganisms. The mentioned works were performed with different systems and biological media thus introducing an additional uncertainty at comparison of the results. Usually, the experiments with broadband ultraviolet radiation were performed either with continuously operating medium-pressure mercury lamps or pulsed xenon lamps.

The results obtained in those works have different, sometimes contradictory characters. Particularly, at the comparison of results of the experiments performed with medium-pressure mercury lamps (which generate the broadband radiation in the spectrum range $\approx 200 \div 300$ nm) with those using low-pressure mercury lamps (narrow-band radiation, $\lambda \approx 254$ nm), the authors of work [9] who worked with microsporidia *Encephalitozoom intestinalis* spores concluded that the spectrum shape plays no essential role, and the inactivation efficiency is determined only by radiation dose. On the contrary, the authors of works [10,11] who worked with *Bacillus subtilis* spores shown that the broadband UV radiation is more efficient than the narrow-band one (20 nm bandwidth) with a mean wavelength of 254 nm, but it is less efficient than the narrow-band UV radiation with a mean wavelength of 214 nm. In works [12,13], where *Escherichia coli* and *Cryptosporium parvum* were treated, it was shown that the inactivation efficiency of a medium-pressure mercury lamp is significantly higher than that of a low-pressure mercury lamp with small UV irradiation doses (≤ 5 mJ/cm^2). However, unlike our case, with the subsequent increase of UV irradiation dose, the inactivation efficiencies become close, and the total inactivation is achieved at practically equal values of the dose.

At the comparison of results of the experiments with medium-pressure mercury lamps and pulsed xenon lamps, the authors of [14] who worked with *Bacillus subtilis* spores demonstrated that the UV radiation of xenon lamps is more efficient. However, they supposed that the effect is due to peculiarities of the pulsed action of ultraviolet radiation, rather than to those of the xenon lamp spectrum. On a basis of the results obtained in the present work, one can make a contrary conclusion: the main role in this case is also performed by peculiarities of the spectrum. This viewpoint is also confirmed by results of [7, 15], where it was shown that the action efficiency is determined only by the irradiation dose and is independent of the temporal behavior of the action (a pulsed or stationary one) under conditions of identical spectra.

4. Conclusions

On a basis of the obtained results, it is possible to make the following conclusions:

The ultraviolet radiation of hollow-cathode discharge plasma in certain media (oxygen, deuterium, water vapor, and their mixtures) provides a higher bactericidal efficiency in comparison with the radiation of low-pressure mercury lamps and, consequently, can be used for water disinfection.

The high efficiency of the UV radiation of the mentioned discharge plasma is due to peculiarities of the UV radiation spectrum, particularly, to a high radiation intensity in the $\approx (215 \div 230)$ nm wavelength range.

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ВИКОРИСТАННЯ УЛЬТРАФІОЛЕТОВОГО ВИПРОМІНЮВАННЯ ПЛАЗМИ ТЛІЮЧОГО РОЗРЯДУ З ПОРОЖНИСТИМ ХОЛОДНИМ КАТОДОМ ДЛЯ ЗНЕЗАРАЖЕННЯ ВОДИ

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Р е з ю м е

Представлено результати експериментальних досліджень особливостей інактивації водної суспензії *Escherichia coli* ультрафіолетовим випромінюванням плазми тліючого розряду з порожнистим катодом у різних газових середовищах. Показано, що ефективність інактивації ультрафіолетовим випромінюванням плазми розряду в кисні, сумішах кисню з дейтерієм та у водяній парі значно вища, ніж розряду в повітрі, а також розряду у ртутній лампі низького тиску.