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DETERMINING THE EFFECT OF HIGH POWER PULSE ULTRAVIOLET (UV) RADIATION ON *BACILLUS ANTHRACIS*

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The object of this study is the high-power UV radiation at different pulses and its effect on Bacillus anthracis spores. The destruction of pathogenic microorganisms at environmental objects is the key to sustainable epizootological well-being. The results of the study show that the effect of powerful pulses on the destruction of pathogenic microorganisms is achieved during a short series and even single exposures. In this case, a pulsed UV sterilizer based on a magnetoplasma compressor with a pulsed ultraviolet flux power of 3.6 MW with a wavelength of 185–320 nm of ultraviolet radiation was used. The prospects for pulsed sterilization technology have been shown. As a result of laboratory tests, it was established that the elimination degree of spores of the test microorganism Bacillus anthracis CTI, applied to wooden plates, is lg 2.5; applied to plastic plates – lg 3.2, while the degree of death of spores applied to metal plates is lg 4.0. Based on the results, wood and plastic require more attention when choosing disinfection methods due to their lower efficiency in this context. The high resistance of spore microorganisms requires the use of high dose loads to neutralize the anthrax pathogen.

The proposed disinfection methodology is relevant and can be used by veterinary medicine laboratories, livestock farms of various forms of ownership, the scientific community, and industrial enterprises manufacturing technological equipment. Further research should be aimed at devising and improving methods for combating pathogenic microorganisms and ensuring sustainable epizootic well-being

Keywords: bactericidal effect, disinfection, environmental sanitation, anthrax, spores, UV radiation at different pulses

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1. Introduction

The main task of agricultural science and practice is to provide people with high-quality products of animal origin. For this purpose, various means and methods are used in practice to improve the quality of the end products [1, 2]. However, there are still quite a few unresolved issues related to ensuring stable epizootological well-being in groups of productive animals [3], one of which is the resistance of certain pathogenic microorganisms to the action of antimicrobial agents in the environment [4, 5]. Given that a number of pathogens are dangerous not only for animals but also for humans, special attention in this aspect is paid to the anthrax pathogen *Bacillus anthracis*, which has a very high resistance to negative environmental factors [6]. The use of chemical disinfectants to destroy *B. anthracis* is ineffective, so the search for effective effects on this pathogen is quite relevant. Most of the chemical agents used today, along with high antimicrobial properties, have a number of negative characteristics, which in turn reduces their demand in practice [7, 8]. It is reported that the microbiota develops resistance to the action of a number of chemical compounds [9]. The most environmentally friendly and safe are physical agents, which include UV radiation, high temperature, pressure, etc. [10]. The leading task set by practical disinfection is the need to determine the main parameters for using environmentally safe and highly effective physical measures for the purpose of environmental sanitation. Therefore, fundamental research on this topic is modern and relevant for a scientifically based search for new tools for the practical implementation of this issue.

2. Literature review and problem statement

Current classification divides the ultraviolet part of the spectrum into different subbands. Wavelengths shorter than 185 nm undergo significant absorption, which makes it impossible to use them for the purposes of designing sterilization irradiators [11, 12]. It has been shown that the intensity of UV radiation generated by the only natural source – the Sun is almost completely absorbed by the ionosphere, which practically excludes it from the factors of natural sterilization. However, the main route of transmission of respiratory infections, in particular, COVID-19, is recognized as airborne droplets, which are formed in the atmosphere as a result of breathing, sneezing, and coughing of a person who is a carrier of the infection. In addition, according to the modern model of the spread of respiratory infections, the duration of the existence of an aerosol cluster saturated with microflora, extracted by the respiratory system of the carrier in the atmosphere, can reach several hours. Thus, in the surface layer of the atmosphere, in the presence of carriers of infection, a layer of potentially dangerous pathogenic air is formed [13]. In the presence of a stable source of the pathogen, it accumulates in environmental objects, which creates a threat of the spread of infection [14, 15]. The chemical means of disinfection currently available are imperfect from an environmental point of view. Therefore, more and more attention is paid to the introduction of physical means of decontamination.

In the wide palette of modern UV emitters, the main means from those times to the present has been electric discharge lamps based on gas mixtures with mercury vapor. The vast majority of such sources of UV radiation are low-pressure lamps with a power of about 6–30 W, the spectrum of which is concentrated near the line λ=254 nm [16, 17]. Therefore, much attention is paid to designing semiconductor UV emitters, which in a certain way expand the possibilities of producing radiation sterilizers. But issues related to low-power sterilization remained unresolved, including the fundamental impossibility of ensuring complete destruction of pathogenic microflora, as well as the effect of restoring irradiated biomass, which is one of the factors causing nosocomial infections [18, 19].

Analysis of the results of studies on the effect of UV radiation on microorganisms reveals that low-power radiation leads to the implementation of a cumulative mechanism of action, in which the efficiency of pathogen destruction depends exponentially on the radiation dose [20]. However, one can establish effective modes of its use only experimentally.

Analysis of the results of calculations of virus resistance shows that they withstand several tens of mJ/cm^2 but do not exceed 50 mJ/cm^2, which can be taken into account when calculating the duration of UV irradiators to achieve the required standards of disinfection levels. Spore-forming bacteria exhibit significantly higher resistance to UV radiation. Thus, analysis of the results reported in [20] reveals that for *B. anthracis* the threshold values are about 680 mJ/cm^2, i.e., the radiation doses required to obtain a certain level of sterilization efficiency increase by more than an order of magnitude compared to the treatment of viruses.

Thus, the development and creation of highly effective means of combating pathogens and viruses also acquires an economic basis since achieving the required dose loads is associated with significant energy costs [21]. In addition, the time criterion under certain conditions may be decisive, in particular in the case of field surgery and others, when the urgent need to obtain a high level of bactericidal purity comes to the fore.

From analysis of the cumulative mechanism of bactericidal action and sterilization efficiency, it follows that the power of UV radiation plays an important role in calculating the duration of the treatment process since the radiation dose is equal to the power of the irradiator on the object during a certain exposure time. This gave impetus to the study of the bactericidal action of pulsed optical radiation sources and the analysis of the shock mechanism of action of ultrahigh-power electromagnetic radiation [22]. Moreover, the shock mechanism of radiation action leads to the implementation of a fundamental law, according to which an increase in the power of the impact causes a decrease in the integral dose of radiation, which is necessary to ensure the bactericidal effect [23]. However, data on its bactericidal effect on highly resistant microorganisms are absent.

In accordance with this approach, the radiation power, at which the effect of complete destruction of pathogenic microflora is obtained over time, is characterized as follows:

– the shock mechanism of the bactericidal action of pulsed UV radiation has a threshold nature, i.e., the sterilization effect occurs only when the radiation source exceeds the power;

– with increasing radiation power, the time required for complete destruction of pathogens decreases, and the corresponding integral radiation dose tends to a minimum, i.e., it is determined only by the characteristic parameters of the irradiated objects.

As follows from our analysis of those results, the most powerful sources of optical radiation are generators based on plasma radiation formed during the electric discharge of a high-energy accumulator. Such sources include a magnetoplasma compressor (MPC), which is a coaxial end-type plasma accelerator. During experimental studies, a number of experimental emitters were designed [24]. They showed high efficiency in disinfecting surfaces contaminated with pathogenic bacteria (*E. coli*, *S. aureus*) and various viruses (influenza A virus of two subtypes, herpesvirus type 2, the causative agent of viral diarrhea in cattle). The effect of complete sterilization was obtained under the action of two or five pulses [25]. However, such disinfection has not been tested on spore-forming microorganisms, in particular the causative agent of anthrax. All this necessitates a study on determining the effectiveness of using pulsed UV radiation in the disinfection of *Bacillus anthracis* spores.

3. The aim and objectives of the study

The aim of our study is to determine the effectiveness of high-power pulsed ultraviolet radiation in decontamination of surfaces of various types contaminated with anthrax spores. This will contribute to a significant increase in the efficiency of disinfection of environmental objects.

To achieve the goal, the following tasks were set:

– to test high-power pulsed ultraviolet radiation for the purpose of decontamination of surfaces of various compositions contaminated with anthrax spores;

– to determine the effectiveness of the proposed technology in comparison with conventional disinfectants.

4. The study materials and methods

4. 1. The object and hypothesis of the study

The object of our study is high-power ultraviolet radiation and its effect on *Bacillus anthracis* spores.

The main hypothesis of the research assumes that our studies could contribute to the effective decontamination of surfaces of various compositions contaminated with spores of the anthrax pathogen.

4. 2. Research methodology and data processing

A portable pulse sterilizer MPK-300-3 based on a magnetoplasma compressor (Fig. 1), which is a coaxial end-type plasma accelerator manufactured by TOV Triix, Ukraine, was used as a source of pulsed ultraviolet radiation.

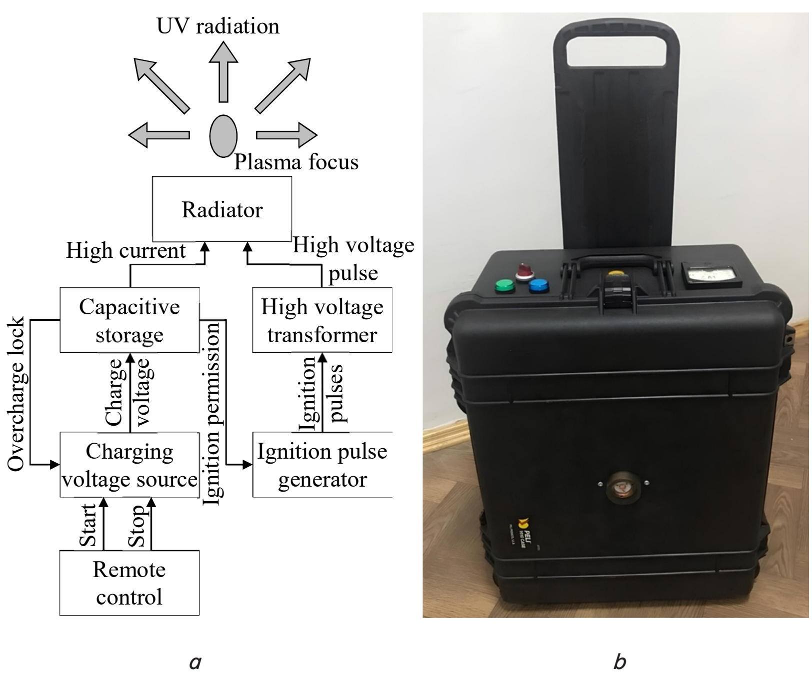

Fig. 1. Experimental setup for generating pulsed optical radiation based on a magnetoplasma compressor: *a* – structural diagram; *b* – general view

Specifications of the device: energy storage capacity – 300 μF; storage charging voltage – 3–5 kV; stored energy – 1350 J; maximum pulse discharge current – 210 kA; discharge pulse duration – 30 μs; radiation temperature – 12000 K; radiation energy in the bactericidal band 185–320 nm – 109.1 J; power – 350 W; pulsed radiation power in the bactericidal radiation band – 3.6–30 MW; operating mode – single/pulse-periodic; pulse generation period – about 30 s.

Bacteria, nutrient media, and reagents. To improve the reproducibility of the methods, commercial dehydrated media were used when preparing nutrient media. When preparing nutrient media, the manufacturer's instructions were strictly followed.

The nutrient media tryptone-soy agar (TSA) (Himedia), tryptone-soy broth (TSB) (Himedia), meat-peptone broth (MPB) (Himedia), meat-peptone agar (MPA) (Himedia) were sterilized in an autoclave at a temperature of (121.0±2.0) °C and a pressure of (1.1±0.2) kgf/cm^2 for 15 min. The pH of the medium was measured at ((20.0–25.0)±1.0) °C.

The spore culture of *Bacillus anthracis* strain CTI, stored in a protective medium at a temperature of (–76–80) °C, was sown on test tubes with MPB and TSB and Petri dishes with MPA and TCA. After 18 hours of cultivation at a temperature of 37.0±0.5 °C, it was checked for purity of growth (visually, making smears, Gram staining, microscopy). A typical broth culture was sown on mattresses with MPA. It was cultivated at a temperature of 37.0±0.5 °C for 5 days. Spore formation was controlled by making smears, Trujillo staining, microscopy, and counting the ratio of spore and rod forms. For further sporulation, the cultures on the mattresses were cultivated at room temperature in a dark place for 4 days. The spore mass was washed with distilled water, heated in a water bath at a temperature of 76 °C for 30 minutes. The washes checked for purity and typical growth were combined.

The collected suspension was purified by centrifugation for 30 min at 3000 rpm. The supernatant was collected, and the precipitate was suspended in distilled water. Washing and centrifugation were repeated 4 times.

The quality of the suspension was controlled by preparing smears on a glass slide, stained according to the spore staining method and viewed under a microscope. The number of remaining vegetative cells did not exceed 20.0 % in the field of view. To prevent contamination, the suspension was heated in a water bath for 30 minutes at a temperature of 76 °C.

The number of bacterial spores in the suspension was adjusted to a concentration of 108 spores/cm^3 by adding sterile distilled water.

Fig. 2 shows the experimental scheme.

Each test surface was coated with one cm^3 of a suspension of the test microorganism *Bacillus anthracis CTI* at a concentration of 1.0×10^8 spores/cm^3, dried, and exposed to high-power pulsed ultraviolet (UV) radiation according to the following scheme: surfaces of three plates of different materials – 1 pulse; 5 pulses; 15 pulses.

To compare the effectiveness of this equipment, disinfectants were used: 5 % sodium hydroxide solution and 0.5 % sodium hypochlorite with exposure of 15, 30, and 60 min. on the corresponding test surfaces.

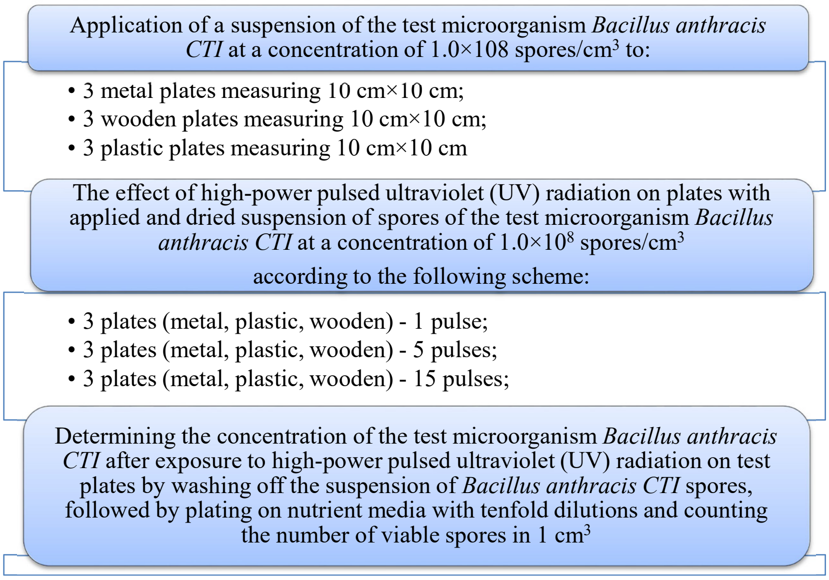

Fig. 2. Experimental setup diagram

5. Results of investigating the effectiveness of high-power pulsed ultraviolet radiation on *Bacillus anthracis* spores

5. 1. Testing of high-power pulsed ultraviolet radiation for the purpose of decontamination of surfaces contaminated with anthrax spores of various compositions

Table 1 gives the results of decontamination of viable *B. anthracis* CTI spores on three different materials: metal, wood, and plastic. The concentration of viable spores before treatment was 1.0×10^8 spores/cm^3 for all materials. The treatment was carried out using different numbers of pulses (1, 5, and 15).

Table 1

Results of decontamination of viable *Bacillus anthracis* spores on surfaces of different composition using high-power pulsed UV radiation

Material type	Number of pulses	Concentration of viable spores before treatment, spores/cm^3	Concentration of viable spores after treatment, spores/cm^3	Reduction, lg
Metal	control	1.0×10^8	–	–
	1		4.0×10^7	0.4
	5		1.2×10^7	1.93
	15		1.0×10^4	4.0
Wood	control		–	–
	1		8.5×10^7	0.1
	5		3.6×10^7	0.3
	15		3.2×10^5	2.5
Plastic	control		–	–
	1		6.5×10^7	0.2
	5		4.9×10^7	1.3
	15		6.0×10^4	3.2

As a result of studies conducted to determine the effect of high-power pulsed UV radiation on *Bacillus anthracis* spores, it was found that the most effective mode, taking into account the various tested surfaces (metal, plastic, and wood), was 15 pulses (Fig. 3–5).

According to our results, the metal surface showed the highest decontamination efficiency under all tested modes. In particular, under the 15-pulse mode, a reduction of 4.0 lg was observed. The degree of death of spores of the test microorganism *Bacillus anthracis* CTI, applied to plastic plates at a concentration of 1.0×10^8 spores/cm^3, was lg 3.2. The treatment of a wooden plate turned out to be the least effective with a maximum reduction of only 2.5 lg under the action of 15 pulses.

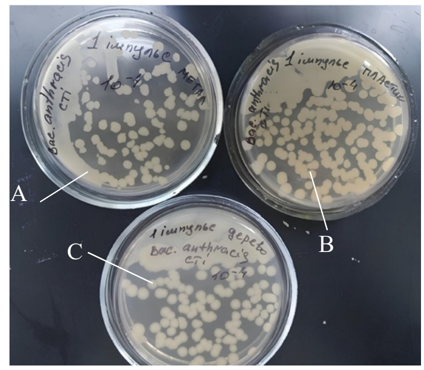

Fig. 3. Recording the growth result of Bacillus anthracis CTI after exposure to a portable pulsed ultraviolet sterilizer MPK-300-3, single pulse mode: A – metal; B – plastic; C – wood

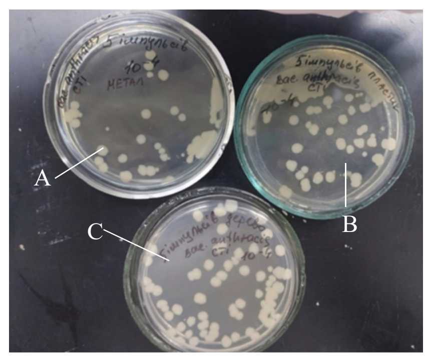

Fig. 4. Recording the growth result of *Bacillus anthracis* CTI after exposure to a portable pulsed ultraviolet sterilizer MPK-300-3, 5-pulse mode: A – metal; B – plastic; C – wood

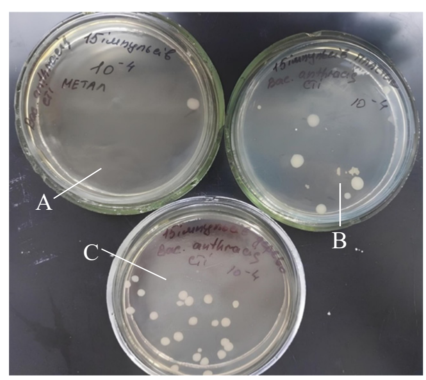

Fig. 5. Recording the growth result of *Bacillus anthracis* CTI after exposure to a portable pulsed ultraviolet sterilizer MPK-300-3, 15-pulse mode: A – metal; B – plastic; C – wood

5. 2. Determining effectiveness of the proposed technology in comparison with conventional disinfectants

In order to compare the proposed methodology of surface decontamination, disinfectant solutions of 5 % sodium hydroxide solution and 0.5 % sodium hypochlorite solution were applied according to different application schemes (Fig. 6).

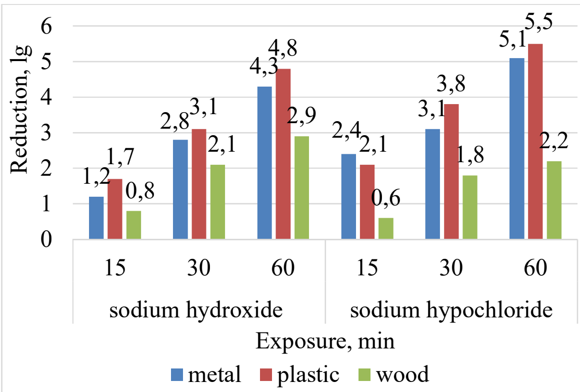

Fig. 6. Results of decontamination of viable *Bacillus anthracis* spores on surfaces of different composition using 5 % sodium hydroxide solution and 0.5 % sodium hypochlorite solution

The data obtained indicate that conventional disinfectants, after 60 min of exposure, reduce the concentration of *Bacillus anthracis* spores on metal and plastic surfaces by almost lg 5. The treated wooden surface – by lg 2.9. Therefore, compared with the data obtained using high-power pulsed ultraviolet radiation, decontamination of viable spores with disinfectants is more effective. However, it also requires a significantly longer exposure time.

6. Discussion of results based on the pulsed UV irradiation test for bacterial spore decontamination

In our studies, we applied high-power UV radiation to *B. anthracis* spores and achieved a positive effect. These results agree with other arguments [26]. Along with this, different degrees of death of spores applied to different test objects were obtained. Thus, a metal surface turned out to be a suitable material for the application of pulsed UV irradiation for bacterial spore decontamination. The degree of spore death was at the level of lg 4.0, while for a wooden surface it was lg 2.5. This is consistent with the results of other studies that indicate a weak disinfection of wood compared to objects of a different physical nature [27]. Based on our results, wood and plastic require more attention when choosing disinfection methods due to their lower efficiency in this context. This result may be due to insufficient energy concentration and inability to penetrate the depth of the material under the tested modes to destroy the pathogen spores specifically on a wooden or plastic surface. The disadvantages of our study are the testing of the pulsed UV irradiator exclusively on the vaccine strain of *B. anthracis*; therefore, in the future it is necessary to take into account the effect of such treatment on virulent field isolates of the pathogen.

The features of the proposed processing technology are the use of an experimental sterilizer capable of forming a high-current discharge in an open atmosphere. With such discharges, the power flow in the bactericidal band of UV radiation significantly exceeds the values that can be realized using conventional UV radiation sources.

The scope of application of our technological advancement potentially includes veterinary medicine laboratories, livestock farms of various forms of ownership, the scientific community, as well as industrial enterprises for the manufacture of technological equipment. The decisive condition for applying the results is the feasibility of designing new devices and determining parameters for the working processes of existing devices for disinfecting environmental objects. The limitation of the proposed processing technology is the impossibility of using the tested device under field conditions.

Further development of the research should be aimed at devising and improving methods for combating pathogenic microorganisms and ensuring sustainable epizootic well-being.

7. Conclusion

1. The mode of a high-power pulsed UV sterilizer has been selected for the treatment of metal, wooden, and plastic surfaces contaminated with spores of the anthrax pathogen at a concentration of 1.0×10^8 spores/cm^3. It was found that under a mode of 15 pulses, a reduction of spores by lg 4.0 was observed on the metal surface. The degree of death of spores of the test microorganism *Bacillus anthracis* CTI, applied to plastic surfaces at a concentration of 1.0×10^8 spores/cm^3, was lg 3.2. The treatment of a wooden plate turned out to be the least effective with a maximum reduction of only lg 2.5 under a mode of 15 pulses.

2. The effectiveness of the proposed decontamination methodology has been proven in comparison with conventional disinfectants, which, after 60 min of exposure, reduces the concentration of *Bacillus anthracis* spores on metal and plastic surfaces by almost lg 5. For the treated wooden surface – by lg 2.9.

Conflicts of interest

The authors declare that they have no conflicts of interest in relation to the current study, including financial, personal, authorship, or any other, that could affect the study, as well as the results reported in this paper.

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Data availability

All data are available, either in numerical or graphical form, in the main text of the manuscript.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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