

## Pulse UV Sterilization of Pathogens

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### ABSTRACT

The results of experimental investigations of the effect of high-intensity pulsed UV radiation on reference strains of opportunistic pathogens *S. aureus* and *E. coli* are presented. The modified pulse UV sterilizer MIIK-300-3 based on an end-face plasma accelerator was used as a radiation source, which provides a power pulsed discharge in an open atmosphere. The high efficiency of inactivation of the pathogens was provided within a short period of time. The possibility of providing urgent 100% sterilization of pathogens has been shown. The prospects for the application of pulse sterilization technology to combat pathogens are considered.

**Keywords:** Sterilization; Pathogens; *Staphylococcus aureus*; *Escherichia coli*

### INTRODUCTION

Research on the mechanism and effectiveness of pulsed UV sterilization is ongoing. As a matter of fact, they did not stop, having started almost 30 years ago [1-6]. Successful tests of the technology of pulse sterilization of viral infection opened the prospect for further research on the effect of UV radiation on microorganisms [7]. The results of all the studies carried out show that the high efficiency of pulse sterilization is provided, first of all, by the high power of UV radiation, which is generated in the pulsed mode. At the same time, generators of optical radiation based on magnetoplasma compressor (MPC) are the most powerful emitting devices. The purpose of these studies was to show the high efficiency of incoherent pulsed UV-radiation generated during the discharge of an end-face coaxial MPC to pathogens and to experimentally confirm the possibility of monopulse sterilization.

### MATERIALS AND METHODS

Studies were performed on reference strains of opportunistic pathogens *Staphylococcus aureus* UCM B-904 (ATCC 25923), *Escherichia coli* UCM B-906 (ATCC 25922), obtained from the Ukrainian collection of microorganisms (UCM, Institute of Microbiology and Virology of NAS of Ukraine) [8].

To determine the sensitivity of microorganisms to UV-radiation, a test culture of bacteria was incubated for 24 hours at 37°C on solid medium LB (Luria-Bertoni). Daily agar cultures were washed with sterile saline, diluted with a suspension according to the

McFarland 1.0 turbidity standard, followed by dilution to  $1 \times 10^6$  CFU (colony-forming units)/ml,  $1 \times 10^4$  CFU/ml,  $1 \times 10^3$  CFU/ml [9]. Volume 0.1 ml bacterial suspension of the appropriate concentration was inoculated by the lawn on Mueller-Hinton agar plates. Source of UV-radiation was placed so that the device was directed downward and placed at a height of  $h=30$  cm from the surface of the laboratory table (Figure 1). On the table under the ultraviolet sterilizer in the operation area the opened Petri cups inoculated with a certain concentration of bacterial suspension were placed, and a single dose of radiation (1 pulse) was exposed. Next, the irradiated cups were closed and set aside, and in their place the next series of inoculated cups were placed and two exposures of radiation (2 pulses) were given. The same principle was used to irradiate 5 and 10 pulses. Petri cups not irradiated with an UV-radiation served as a control. The experiment used three replicates of inoculated cups with different concentrations of bacterial suspension. Irradiated and control cups were incubated in a thermostat at 37°C for 24 hours.

The dishes inoculated with cultures in different concentrations were divided into 3 groups, each of which was irradiated with a certain dose of UV radiation. The dose load on a group of cups was set by the number of pulsed exposures.

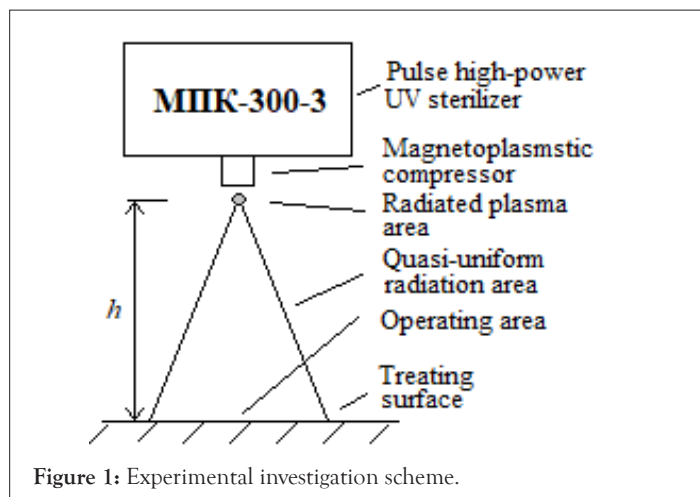
When calculating the results, the number of viable cells was determined, the number of colonies grown on the plates was counted, the cell titer and the survival of irradiated cells as a percentage of control were determined.

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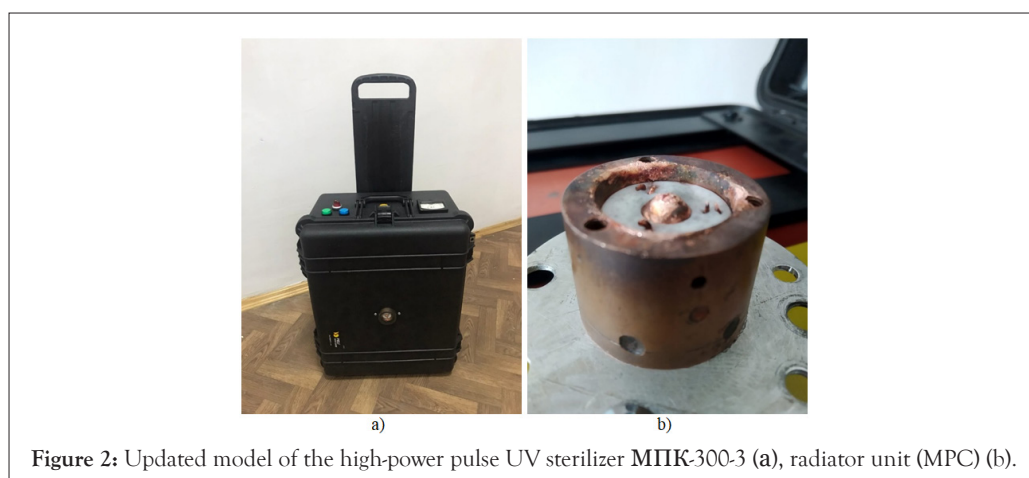
A modified MПК-300-3 sterilizer was used as a source of pulsed UV radiation [6,7]. The circuitry implementation of the main units of the device, the control circuit and the appearance has undergone modernization. The switching elements of the charging-discharge circuit of the electric energy storage have been completely renewed and strengthened. Figure 2 represents updated equipment. Currently, the device provides operation both in the mode of generating single pulses and in the mode of forming a pulse train of a given duration. The use of modern element base has made it possible to increase the stability of the parameters of radiation pulses.

## RESULTS AND DISCUSSION

The results of the study of the effectiveness of the pulsed UV-radiation against reference strains of opportunistic pathogens *S. aureus* and *E. coli* are presented (Tables 1 and 2) (Figures 3-6).

As can be seen from the results of the research, the ultraviolet sterilizer MПК-300-3 showed high bactericidal activity against both test cultures of microorganisms. Already under the action of 1 pulse of the sterilizer MПК-300-3 registered inhibition of growth of *S. aureus* by 99.97% at a concentration of bacterial suspension of  $1 \times 10^6$  CFU/ml, 99.65%- $1 \times 10^4$  CFU/ml, 99.90%- $1 \times 10^3$  CFU/ml. Under the influence of 2 and 5 pulses there is also a high efficiency of the device reaching 99.80%-99.99%, respectively. Under the influence of 10 pulses of the sterilizer, 100% death of *S. aureus* cells is detected in all studied concentrations of bacterial suspension. We also note that at an initial concentration of pathogens equal to  $1 \times 10$  CFU/ml, the microbial residue after a single exposure was only 1 CFU, which is almost equivalent to the effect of monopulse sterilization (Table 3).

Inhibition of *E. coli* cell growth is also registered under the influence of 1 pulse of sterilizer MПК-300-3 by 99.95% at a concentration of bacterial suspension of  $1 \times 10^6$  CFU/ml, 99.32%- $1 \times 10^4$  CFU/ml, 99.00%- $1 \times 10^3$  CFU/ml. Under the influence of 2 pulses, the efficiency of the device reaches 99.80%-99.99%. Under the action of 5 pulses of the sterilizer, 100% death of *E. coli* cells is detected in all studied concentrations of bacterial suspension (Table 2). Thus, *E. coli* bacteria are less resistant to pulsed UV radiation.



**Table 1:** The effectiveness of pulsed UV radiation against *S. aureus*.

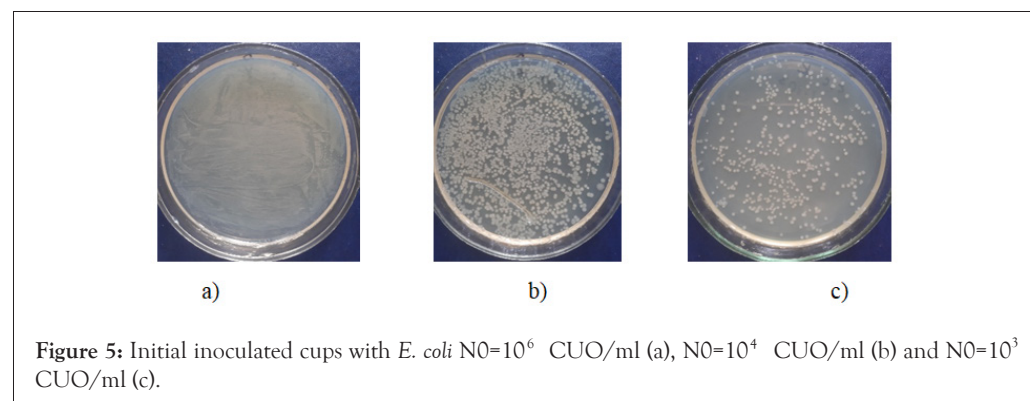
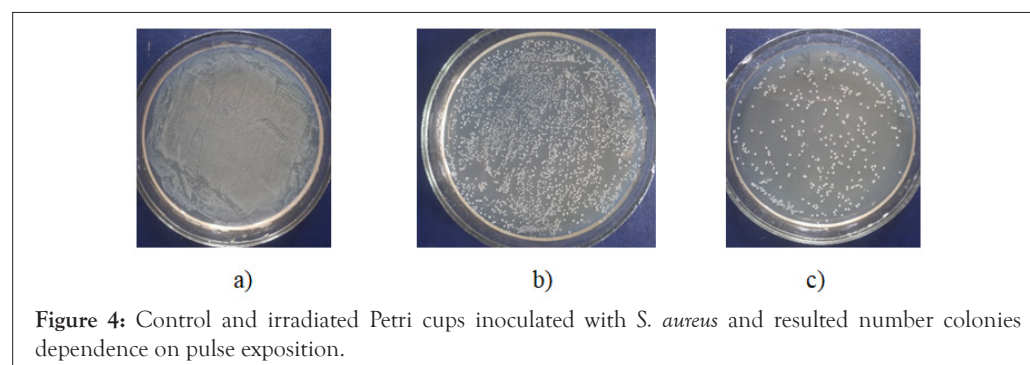
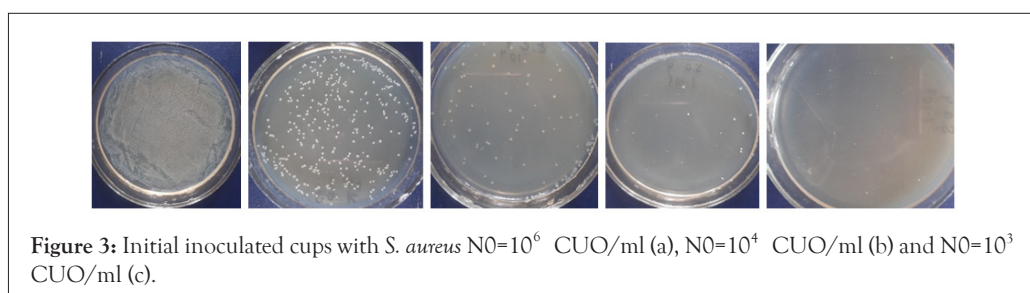
Test-culture	Initial bacterial suspension concentration, control, CFU/ml	Number of pulse exposition	Resulted number of growth colonies	Efficiency %
<i>S. aureus</i>	$10^6$	1	272	99.97
		2	124	99.98
		5	16	99.99
		10	0	100
	$10^4$	1	35	99.65
		2	20	99.8
		5	2	99.98
		10	0	100
	$10^3$	1	1	99.9
		2	1	99.9
5		0	100	
10		0	100	

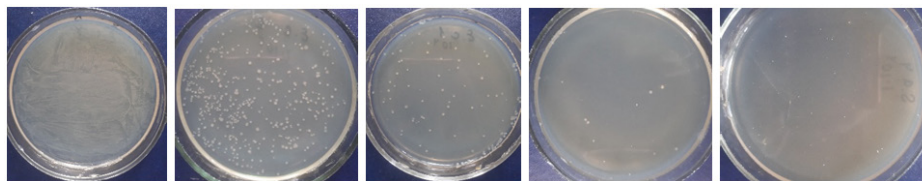
**Table 2:** The effectiveness of pulsed UV-radiation against *E. coli*.

Test-culture	Initial bacterial suspension concentration, control, CFU/ml	Number of pulse exposition	Resulted number of growth colonies	Efficiency %
<i>E. coli</i>	$10^6$	1	468	99.95
		2	71	99.99
		5	13	99.99
		10	0	100
	$10^4$	1	68	99.32
		2	7	99.93
		5	0	100
		10	0	100
	$10^3$	1	10	99
		2	2	99.8
		5	0	100
		10	0	100

**Table 3:** The resulted number of colonies in the tested cups after n number of pulse exposures.

Control inoculated cup	Tested cups resulted number of colonies Nres after n pulse exposures				
$10^6$ CFU/ml	n	1	2	5	10
	Nres	272	124	16	0





**Figure 6:** Control and irradiated Petri cups inoculated with *E. coli* and resulted number colonies dependence on pulse exposition.

Therefore, based on the obtained data, it was found that the portable pulsed ultraviolet sterilizer МПК-300-3 has a high bactericidal effect on the studied opportunistic microorganisms *S. aureus* and *E. coli* with an efficiency of 100%, and can be recommended for use to disinfect surfaces, products, premises of various function.

## CONCLUSIONS

1. A pulsed source of high-power UV radiation has been developed and created for analyzing the bactericidal effect and determining the effectiveness of pulse sterilization.
2. The high bactericidal efficiency of high-power pulsed UV radiation when exposed to pathogens has been experimentally shown.
3. Exposure to pulsed UV radiation results in 100% complete sterilization as a result of irradiation with a finite number of pulsed exposures. Thus, the complete destruction of opportunistic pathogenic microorganisms *S. aureus* is achieved as a result of exposure to 10 pulsed exposures. The complete destruction of *S. coli* bacteria is achieved as a result of exposure to 5 pulses. A decrease in the initial concentration leads to a decrease in the pulsed radiation dose required for complete sterilization.
4. The results obtained determine the fundamental possibility of monopulse sterilization.
5. Results of research open up the prospect of developing and designing means of ensuring air hygiene and breaking the chain of transmission of coronavirus infection between people, which is very important during a pandemic.

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