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Application of electron beam for wastewater disinfection

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Abstract

The objective of the study is to consider the possibility of applying the nanosecond electron beam for disinfecting wastewater. The advantages of this method have been illustrated. The mechanism of influence of ionizing radiation on cells has been described. The microbial suspension has been irradiated with the electron accelerator, and then the effectiveness of the electron beam as a disinfecting agent has been assessed. In most cases there has been a bactericidal effect, while in some cases a bacteriostatic effect has been observed. On the example of *E. coli* culture, it has been shown that the nanosecond electron beam is an effective disinfecting agent for the of wastewater treatment.

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Keywords: Electron beam, bactericidal effect, disinfection, wastewater.

1. Introduction

Nowadays, the problem of clean water shortage is more and more urgent. This is due to the overpopulation of the planet and, as a result, to increased fresh water consumption. The untreated wastewater discharge into water sources leads to microbiological contamination. To solve this problem, chemical methods of disinfection (chlorination, ozonation) are widely applied, but when they are used in water, toxic compounds are produced. Thus, a great alternative to these methods may be nanosecond electron beam disinfection.

Currently, researchers have identified a wide range of nanosecond electron beam for radiochemical sterilization applications. This method can be used for sterilization of medical instruments, glassware for blood preparations, as well as in the food industry for disinfecting bottles[1]. Also, several studies indicate the possibility of using the

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electron beam for the treatment of domestic sewage from organic contaminants, petroleum and textile waste[2, 3]. Based on the above mentioned facts, we can conclude that the prospects of using an electron beam as a sterilizing agent for wastewater disinfection are rather promising.

A bactericidal effect of ionizing radiation (IR) is ensured by its direct (physical) and indirect (chemical) action. When radiation moves through a substance, it gives birth to ionization and excitation; molecular bonds are broken, resulting in damage to biological tissues. The indirect effect of IR is caused by the facts that under its influence free radicals are formed in water that intensely react with each other and with the molecules of the substance. During these reactions hydrogen peroxide may be generated in the cell, which is detrimental to some types of microorganisms[4].Taking into account that we are interested in water suspensions of microorganisms disinfection, this mechanism becomes more important. The biological effect of ionizing radiation is related to the amount of energy that is absorbed by the cell or tissue. In this regard it is important to determine the radiation dose.

For today we know an effective method of disinfecting water with influence of continuous ionizing radiation. However, in this case, irradiation of the whole volume of the treated water leads to an increased power source of ionizing radiation, and it complicates personnel protection[5]. By different groups of researchers it was found that when the nanosecond electron beam is used, sterilization dose is reduced[6], which can significantly reduce the power consumption, and is safer for personnel.

2. Experimental

Identification of the nature of a nanosecond electron beam irradiation on wastewater microorganisms impact was carried out on the example of a typical representative – *Escherichia coli*. The purpose was to determine the basic conditions for successful neutralization of a pure culture *Escherichia coli* in aqueous medium. Irradiation experiments with a culture *Escherichia coli* were repeated three times for averaging the results.

2.1. Materials

A microbial culture of *Escherichia coli* was used for the experiments. The culture was cultivated on the nutrient medium of the following composition: 1 liter of distilled water, agar nutrient for culturing microorganisms, dry 41 g, and 2% glucose (sterilized in a steam autoclave at 120°C, 15 minutes). Sterile distilled water (sterilized in a steam autoclave at 134°C, 20 minutes) was used for preparing samples. 90% ethanol or isopropyl alcohol was used for disinfection. Samples were placed in cuvettes, volume 0.07 ml (Fig. 1).

2.2. Sample preparation

Composite parts of cuvettes were sterilized in a steam autoclave at 134°C, for 20 minutes. The suspension test culture was prepared from a culture *Escherichia coli*, grown on agar medium containing 2% glucose at 37°C for 24 hours. To prepare the bacterial slurry, *E. coli* culture was washed away from the agar by sterile distilled water. The resulting slurry of microbes was used for further manipulation.

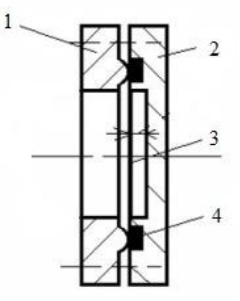

Fig. 1.Diagram of the cuvette: 1 – cover, 2 – frame, 3 – foil, 4 – gaskets.

Using tweezers, calcined in the fire of a spirit lamp, two gaskets (large and small diameter) were placed on the frame of cuvettes for sealing. 60 micro liters of bacterial suspension were placed in cuvettes using a sterile pipette (diameter of an area in which irradiation fluid is localized is 15mm). The slurry was then covered with aluminum foil, disinfected in 90% ethanol (thickness of 150 microns). Thereafter, cuvettes were closed with a lid and tightened by the screws. The cuvettes were placed in a container, pre-treated with 90% ethanol. As a result, 5 cuvettes were prepared. 4 samples were irradiated by an electron beam; one sample was left for control.

2.3. Irradiation of samples

Irradiation of samples was carried out on the accelerator TEU-500[7, 8]. The sample was irradiated by an electron beam (one pulse) with the following characteristics: derived energy up to 90 J., pulse duration 60 nanoseconds, foil thickness 150 mkm, absorbed dose 2,5Mrad per pulse. During irradiation, using additional layers of aluminum foil, the thickness of which depends on the electron energy (Table 1).

Table 1. The thickness of the additional aluminum foils, depending on the energy of the electrons for the TEU-500 (150 micron aluminum)

E_e, keV	200	250	300	350	400	450	500
Thickness, mkm	0	60	120	180	250	330	410

Prints were recorded on a dosimetric film (Fig. 2). Figure 2 shows that the electrons are able to pass only the 180 micron aluminum foil in the reactor – this corresponds to energy of 350 keV. Figure 3 illustrates the oscillograms.

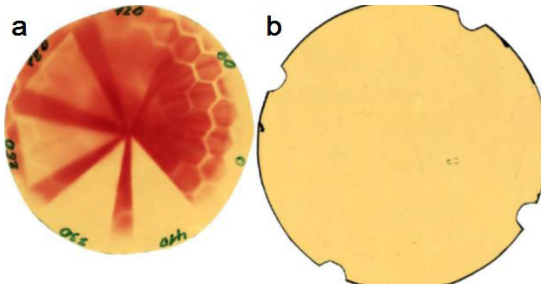

Fig. 2. Imprints on the dosimetric film, accelerator TEU-500; a – 1 pulse per reactor, b – non-irradiated)

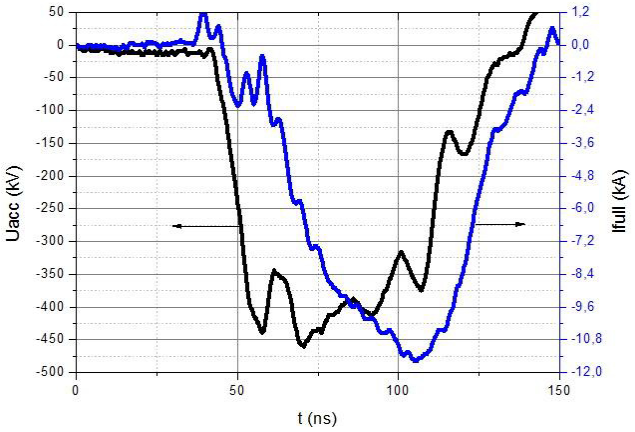

Fig. 3.Oscillograms:Uacc - accelerating voltage; Ifull - the total diode current

2.4. Analysis of the irradiation effectiveness

Irradiated microbial was further seeded suspension in Petri dishes with the fresh nutrient dense medium (agar medium containing 2% glucose). For this, surface of the cuvettes (including foil from the outside) was disinfected with 90% ethanol; microbial suspension was withdrawn from the cuvettes with a sterile syringe application (to this end the foil was punctured) and was seeded in Petri dishes. Concurrently control seeding of a microbial culture and control of medium sterility were made. Materials were placed in an incubator at 37°C, and were observed after 25, 50, 96 hours.

Microscopy was performed on 1000 × magnification with immersion.

Production of preparations for microscopy: fixed smears stained by Gram according to a standard procedure.

3. Results and discussion

3.1. The results of Experiment 1

After 25 hours in a Petri dish with the microbial culture control and unirradiated sample control the confluent growth was observed. Short gram negative coli (corresponding *E. coli*) were observed in the microscope. On other Petri dishes growth was not observed.

After 50 hours on one of Petri dishes the signs of growth in the form of two individual colonies were visible. Similar signs of growth appeared on the Petri dish with the control of medium. After 96 hours, these colonies increased in diameter (Table 2).

3.2. The results of Experiment 2

After 25 and 50 hours the confluent growth in a Petri dish with the microbial culture control and unirradiated sample control was observed. Short gram negative coli (corresponding *E. coli*) were observed in the microscope. On other Petri dishes growth was not observed.

After 96 hours on one of Petri dishes signs of growth in the form of one individual colony were visible.

3.3. The results of Experiment 3

Culture control and unirradiated sample control showed the presence of active microbial growth in 25 hours after seeding, whereas microbial growth on Petri dishes with irradiated samples was not detected after 50 or 96 hours. The nutrient medium remained sterile (Table 4). Short gram negative coli were observed in the microscope.

Table 2. Control *E. coli* culture after irradiation –Experiment 1

№ of sample	The number of impulses / dose Mrad	Microbial growth		
		After 25 hours	After 50 hours	After 96 hours
1	0	+	+	+
2	1/2.5	-	-	-
3	1/2.5	-	+ (2 colonies)	+ (2 colonies)
4	1/2.5	-	-	-
5	1/2.5	-	-	-
E. coli control	0	+	+	+
Sterile of medium control	0	-	+ (1 colony)	+(1 colony)

Table 3. Control *E. coli* culture after irradiation - Experiment 2

№ of sample	The number of impulses / dose Mrad	Microbial growth		
		After 25 hours	After 50 hours	After 96 hours
1	0	+	+	+
2	1/2.5	-	-	-
3	1/2.5	-	-	+(1 colony)
4	1/2.5	-	-	-
5	1/2.5	-	-	-
E. coli control	0	+	+	+
Sterile of medium control	0	-	-	-

Table 4. Control *E. coli* culture after irradiation - Experiment 3

№ of sample	The number of impulses / dose Mrad	Microbial growth		
		After 25 hours	After 50 hours	After 96 hours
1	0	+	+	+
2	1/2.5	-	-	-
3	1/2.5	-	-	-
4	1/2.5	-	-	-
5	1/2.5	-	-	-
E. coli control	0	+	+	+
Sterile of medium control	0	-	-	-

We find that the best results for the decontamination of microbial suspension were achieved in experiment 3. In experiments 1 and 2, the microbial growth appeared in one of the samples after 50 and 96 hours, respectively. This may be due to the electron beam uneven distribution. In case of Experiment 1, it can also be associated with low microbial contamination of medium. In any case, based on the experimental results, we can conclude that there is a bacteriostatic effect of nanosecond electron beam at an absorbed dose of 2.5 Mrad, sufficient for its use as a disinfecting agent in wastewater treatment.

4. Conclusion

Using *Escherichia coli* culture, it has been shown that the nanosecond electron beam is an effective disinfecting agent for wastewater treatment. After irradiation, only in a few cases, under favorable conditions, weak microbial growth was observed after 50-96 hours. In most cases, the irradiation of microbial suspension had a sterilizing effect.

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