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RADIATION SENSITIVITY OF BACTERIA CONTAMINATING FOOD

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Abstract. *Pulsed accelerators with high doses per pulse are currently widely used. The effects of this irradiation on microorganisms are poorly understood. The objectives of our study are: 1) the development of methods to study the effects of pulsed electron beams on microorganisms; 2) the preliminary study of radiation resistance of microorganisms interesting in the context of food safety. In this study, irradiation was conducted on two pulsed accelerators: URT-1 and URT-0.5. Experiments on the dose distribution in the layer of 80 mg/cm² at URT-0.5 and URT-1 showed that the penetration of radiation at URT-1 is greater than at URT-0.5. So, for our experiments, URT-1 is more suitable than URT-0.5.We investigated the effects of pulsed electron beams on the survival of bacterial (Klebsiella, Citrobacter, Staphylococcus aureus, Salmonella) and fungal (Aspergillus) food spoilage agents. The results for Staphylococcus aureus, Salmonella, fungus Aspergillus are similar. 6kGy is the dose at which the survival is reduced by 40-50%, 11 kGy is the dose at which the survival is reduced by 90-100% (11 kGy is upper limit of radappertization). The inactivation of bacteria on the accelerator URT-1 occurred at lower doses compared with the accelerator URT-0.5.*

Key words: *Bacterial inactivation, electron irradiation, nanosecond pulses*

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1. INTRODUCTION

Radiation processing of food products is carried out in order to improve their safety and quality. Food irradiation is used to inactivate pathogens and parasites, for reducing the number of microorganisms that cause food spoilage, for suppressing sprouting bulbs, tubers and roots, for extending the shelf life of products, as well as for the phytosanitary treatment.

To sterilize food, International Atomic Energy Agency (IAEA) special terms are offered:

radicidation (4-6 kGy) –radiation treatment to selectively suppress a specific type of microorganism (e.g., *Salmonella*);

radurization (6-10 kGy) – radiation processing of food products in order to increase storage duration, in doses that lead to the suppression of limited human-pathogenic microorganisms;

radappertization (10-50 kGy) - carried out for industrial sterilization of foods under conditions precluding the repetition of infection by microorganisms.

Currently, the radiation processing of food products involves the following types of ionizing radiation:

1. electron beam with energy up to 10 MeV;
2. γ-radiation radioisotope ^{60}Co ($T_{1/2}$ = 5.27 years, E = 1.25 MeV);
3. γ-radiation radioisotope ^{137}Cs ($T_{1/2}$ = 30.17 years, E = 0.66 MeV);
4. bremsstrahlung generated by electron accelerators with energies up to 5 MeV [1].

The objectives of this study are:

1) development of methods to assess the effects of pulsed electron beams on microorganisms;

2) preliminary study of radiation resistance of microorganisms of interesting in the context of food safety.

The following microorganisms were used as biological models:

a) *Klebsiella* is opportunistic pathogens belonging to the *Enterobacteriaceae* found in human feces, skin and mucous membranes of the respiratory tract, soil, water, fruits and vegetables;
b) *Citrobacter* belonging to the Enterobacteriaceae family, commonly found in the human intestinal microbiota. Pathogenic species are occasionally implicated in food poisoning mainly associated with milk and dairy products, pastries, poultry and meat;
c) *Salmonella*is also a member of the Enterobacteriaceae family that occurs in the human intestine. Pathogenic species can be transmitted by contaminated food, especially eggs, dairy products, poultry and meat;
d) *Staphylococcus aureus* are Gram positive opportunistic pathogens. Although commonly

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occurring in the human skin and oral mucosa, S. aureus is an agent of food intoxication mainly associated to the consumption of milk and dairy products, pastries and ready-to-eat food;

e) *Aspergillus* is a genus of filamentous fungi (molds) that includes several hundred species widely distributed in the environment. Some *Aspergillus* species cause serious diseases in humans and animals. Some pathogenic species like *A. fumigatus* and *A. flavus*, produce aflatoxins which are powerful carcinogens. *Aspergillus* can contaminate food with low water content, such as nuts, seeds and grains [2].

Some previous studies have addressed the effects of electron irradiation on these types of bacteria. For example, at doses up to 2.5 kGy survival of *Salmonella typhi* decreases by 99% under the influence of electron irradiation from a linear accelerator with an energy of 6 MeV [3]. Survival of *Staphylococcus intermedius* decreases by 99% at doses up to 5 kGy [3]. Under the influence of electron irradiation survival of fungus *Aspergillius* decreases by 90% at doses up to 0.2 kGy [4].

2. Irradiation and dosimetry

Irradiation was conducted on two pulsed accelerators, URT-1 and URT-0.5, at the Institute of Electrophysics, the Ural Branch of the Russian Academy of Sciences. Characteristics of the accelerators are shown in Table 1.

Table 1. Characteristics of the accelerators URT-0.5 and URT-1

Characteristics	URT-0,5	URT-1
the accelerating voltage, keV	500	650
the pulse width at half-maximum, ns	50	60
frequency, Hz	200	50
beam size, mm² / beam diameter, mm	120×120	160
beam current density, A / cm²	10	2
the average dose per pulse, kGy / imp.	0,8	1,8

Doses were measured with film dosimeters SOPD(F)R – 5/50 and SOPD(E) – 1/10. The dosimeters are polymer films of single use. They are designed to measure the absorbed dose of photon and electron radiation (copolymer with phenazine dye - SOPD(F)R – 5/50 and copolymer with 4 - dietilaminobenzolovym dye - SOPD(E) – 1/10) The optical density of the film changes during the radiation exposure. We measured the optical density with a spectrophotometer. We determined the absorbed dose using the following formula (1), (2). The formula for dosimeters COPD(F)R - 5/50 is:

$$D = 59{,}71 \cdot A^{1{,}02}, \qquad (1)$$

where D is the absorbed dose of electron radiation in the range of 5 - 50 kGy; A is the optical density measured by a spectrophotometer at a wavelength of λ = 512 nm, relative to a reference sample.

The error in determining the absorbed dose using COPD(F)R - 5/50 does not exceed 12% (P = 0.95) in the working conditions of use.

The formula for dosimeters SOPD(E) – 1/10 is:

$$D = 8{,}78 \cdot A^{0{,}924}, \qquad (2)$$

where D is the absorbed dose of electron radiation in the range of 1 - 10 kGy; A is the optical density measured in a spectrophotometer at a wavelength of λ = 550 nm, relative to a reference sample.

The error in determining the absorbed dose using SOPD(E) – 1/10does not exceed 15% (P = 0.95)in the working conditions of use.

Accelerators URT-0.5 and URT-1 are not directly suitable for radiobiological research. Therefore, some radiation field characteristics were namely: depth dose distribution and area. Irradiation was carried out in a Petri dish and in a plastic bag. As the simulator of biological tissue, we used polyethylene films. Film dosimeters were places between absorber layers. The dose distribution in depth obtained with accelerator URT-0.5 is represented in Figure 1. The dose distribution in depth with accelerator URT-1 is represented in Figure 2.

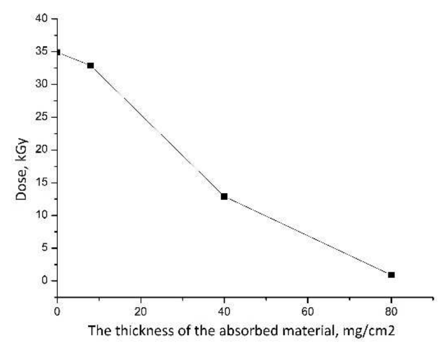

Figure 1. Dose distribution in depth with accelerator URT-0.5

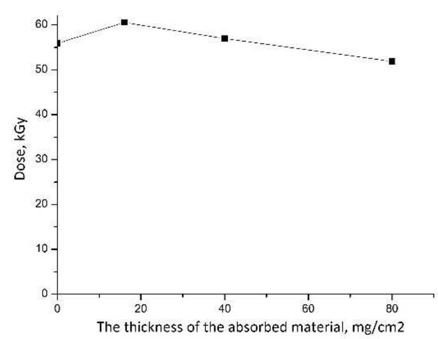

Figure 2. Dose distribution in-depth with accelerator URT-1

A significant decline in the dose was observed with accelerator URT-0.5. Therefore, this accelerator was found for the irradiation of very thin samples. With accelerator URT-1, the dose depth varies only slightly. Consequently, the accelerator URT-1 is more suitable for the use in radiobiological experiments.

Microorganism suspension before irradiation was prepared in a liquid nutrient medium. Then these suspensions were deposited on solid culture medium in Petri dishes and were irradiated. *Klebsiella* and *Citrobacter* were irradiated in the accelerator URT-0.5. We adjusted the dose by changing the number of pulses. The width and pulse frequency were not changed. Irradiation was carried out at the following doses: 5 kGy (8 pulses); 10 kGy (16 pulses); 15 kGy (23 pulses); 20 kGy (31 pulses); 25 kGy (39 pulses); 30 kGy(47 pulses).The plates are placed in an incubator. After 48 hours of incubation, the survival of the bacteria was estimated by counting colony forming units (CFU) per ml. The surviving fraction corresponding to each dose was determined by dividing the number of colonies in irradiated sample on the number of colonies in control sample. Control samples were kept in the same conditions as the other samples, but were not subjected to irradiation. Values of surviving fractions were calculated and approximated by Boltzmann sigmoidal function (3) with two fixed parameters $A_2 = 0$ and $A_1 = 1$

$$f(x) = \left(\frac{(A_1+A_2)}{1+e^{\left(\frac{x-x_0}{dx}\right)}}\right) + A_2. \qquad (3)$$

Here, X_0 = LD50 (the dose at which survival corresponds to 50%) and dx = width of the transition from the survival fraction 1 to fraction = 0 survival.

The results of these experiments are shown in Figure 3.

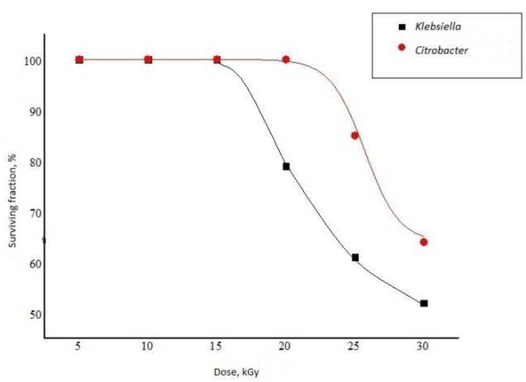

Figure 3. Survival of *Klebsiella* (■) and *Citrobacter* (●) after irradiation with the electron beam accelerator URT-0.5

The LD50, determined by the point values, was 30 kGy for *Klebsiella*and35 kGy for *Citrobacter*.

The thickness of the samples was approximately 80 mg/cm². The dose varies considerably for a given value of the sample thickness on the accelerator-URT 0.5. Therefore, it is likely that the heterogeneity of radiation in depth could affect bacterial survival.

Salmonella, Staphylococcus and Aspergillus were irradiated on the accelerator URT-1. The width and pulse frequency were not changed. We adjusted the dose by changing the number of pulses. Irradiation was carried out at the following doses: 6 kGy (4 pulses); 11 kGy (10 pulses); 15 kGy (22 pulses). Experimental results are presented in Table 2.

At the dose of 6 kGy, the survival of *Staphylococcus* decreased by 40%. The dose at which total inactivation occurred was 11 kGy.

At the dose of 6kGy, the survival of *Salmonella* decreased by 40%, whereas the survival at the dose of 11 kGy decreased by 95%. In addition, the cell colonies changed their shape and color.

We studied the effect of radiation on the hyphae of the fungus *Aspergillus*. The survival of hyphae decreased by 49% at the dose of 6kGy. The dose at which total inactivation occurred was 11 kGy.

The death of the bacteria on the accelerator URT-1 occurred at lower doses compared with the accelerator URT-0.5.

Table 2. Survival of the bacteria after irradiation on the accelerator URT-1 (CFU/mL)

Test/Control	Dose, kGy		
	6	11	15
S. aureus	300	-	-
control	500		
Salmonella	300	27	-
control	500		
Aspergillus	51	-	-
control	100		

3. CONCLUSION

Experiments on the dose distribution in the layer of 80 mg/cm² at URT-0.5 and URT-1 showed that the penetrating ability of radiation at URT-1 is greater than at URT-0.5. Therefore, the accelerator URT-1 is more suitable for the use in radiobiological experiments.

We investigated how nanosecond electron beams affect the survival of the bacteria contaminating food (*Klebsiella, Citrobacter, Staphylococcus aureus, Salmonella*, fungus *Aspergillus*).

Citrobacter and *Klebsiella* cultures were exposed to 500 keV electrons at an average dose of0.8kGy/pulse, with a repetition rate of 200 pulses per second, with a pulse width of 50 ns. The LD50, determined by the point values, was 30 kGy for *Klebsiella* and 35 kGy for *Citrobacter*. Experiments showed that *Klebsiella*is more susceptible to nanosecond electron beams than *Citrobacter*. On the accelerator URT-0.5, the dose varies considerably at a sample thickness of 80 mg/cm². Therefore, it is likely that the heterogeneity of radiation in depth could affect the survival of the bacteria. Thus, we got the bacterial survival for specific experimental conditions. These results cannot be the characteristic of radiosensitivity of these bacteria.

Staphylococcus, *Salmonella* and *Aspergillus* cultures were irradiated with 650 keV electrons, at an average dose of 1.8kGy/pulse with a repetition rate of 50 pulses per second, with a pulse width of 60 ns. We found that the survival of *Staphylococcus* decreased by 40% at the dose of 6kGy. The dose limit at which all the bacteria were destroyed was 11 kGy. The survival of *Salmonella* decreased by 40%at the dose of 6kGy, whereas the survival of *Salmonella* decreased by 95% at the dose of 11 kGy. In addition, the cell colonies changed their shape and color. The survival of *Aspergillus* decreased by 49% at the dose of 6kGy, and fungal hyphae were completely destroyed at the dose of 11 kGy.

The results for the two types of bacteria and fungi are similar.6kGy is the dose at which the survival is reduced by 40-50%, and 11 kGy is the dose at which the survival is reduced by 90-100% (11 kGy is the upper limit for radappertization). The death of the bacteria on the accelerator URT-1 occurred at lower doses compared with the accelerator URT-0.5.

Based on these results, we can conclude that the accelerator URT-1 is optimal for radiobiological studies. All subsequent experiments are planned to be carried out on it. Further, it is necessary to examine how the survival of *Staphylococcus*, *Salmonella* and *Aspergillus* will change at doses up to 11 kGy. In addition, we plan to conduct experiments with the same kinds of bacteria in the linear electron accelerator with the energy of 10 MeV. The preliminary dosimetry for these purposes has already been carried out.

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