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EFFECTS OF ELECTRON BEAM AND OZONE COMBINED PROCESSING ON VARIABILITY OF THE RESIDUAL MICROFLORA

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The problems of the physical-chemical agents' influence on the microbial world and the mutagenesis conditioned by this affect attract one's attention because of the intensive study of the effectiveness of the use of various physical factors and oxidizers for disinfecting natural waters and sewages [1].

It is experimentally proved that dynamics of DNA synthesis in prokaryotic and eukaryotic cells is sensitive to the electron beam and ozone influence [2, 3]. Probably, by applying different amount of energy, these agents can cause damage or just desadaptation in the genetic apparatus. This fact is not only of a general biological interest but there also arises a problem of possible imbalance in ecological microbial interactions, which can conduce to biocenosis' formation suppressing, appearance of bacteria populations characterized by heightened virulent properties, thus have indirect influence on human health. Therefore, in the connection with the problems of water purification from biological objects, one must understand the biological effects produced by the electron beam and ozone.

The goal of the work is to determine the nature of adaptive variability of the sanitary-model microflora and frequency of the mutants' appearance stimulated by the electron beam and ozone influence in various regimes of processing samples of natural water and sewages.

Materials and methods

As the test-objects, we chose representatives of sanitary-model microflora with diametrically opposite ability to resist to the influence of the physical and chemical agents. Our 24 tests indicated that *Escherichia coli* strains (*E.coli* ATCC №25922, *E.coli* B №116), *Citrobacter freundii* (*C.freundii*) №15, *Serratia marcescens* (*S.marcescens*) №184 and *Staphylococcus aureus* (*S.aureus*) ATCC №25923 were the most sensitive to the electron beam and ozone combined influence and similar in their quantitative characteristics. On the contrary, test cultures of *Klebsiella* (*K.pneumoniae* № №32, 159 and *K.oxytoca* №180) were characterized by a heightened resistance to the influence of these agents. The places of sampling natural waters and sewages were defined in the previous reports [4].

Procedure of water disinfection were performed on the laboratory facility developed in National Scientific Center "Kharkov Physical Technical Institute", which consists of the powerful emitter, the high power-saving ozonizer, both flowing and landlocked

system of new type with the ion formation cabinet of disinfection with ion formation [4].

The mutagen properties of antimicrobial agents were examined under the standard conditions with the model samples, samples of test cultures in natural waters and purified sewages.

The model samples were prepared in the form of a microbial suspension of each representative of the sanitary-indicative microflora.

The test cultures' biochemical properties were determined according to "Bergey's Manual of Determinative Bacteriology", 1997 by application of the standard microbiological techniques.

The antilyzocyme and antiinterferon activities were investigated in the cup-plate technique by detection of delayed-action antagonism between the test cultures *Micrococcus luteus* var. *Lysodeiktikus* №2665 and *Corynebacterium xerosis* №181 respectively (test-strains obtained from the Museum of microorganisms of SE «I.I. Mechnikov Institute of Microbiology and Immunology of AMS of Ukraine» collection) [5, 6].

The test-culture anticomplementary activity was determined by the kinetic method proposed by Y.A. Brudastov, which consists in measuring the level of haemolysis in sensitized erythrocytes of a ram [7].

Adhesive properties of bacteria were investigated according to the technique by V.I. Brylis and the co-authors [8]. For estimating the microbe's adhesive properties, we used the criteria of the adhesion average index (AAI), the adhesion coefficient (AC) and the microorganisms' adhesion index (MAI) – the latter is the average number of microbial cells, adhered on one erythrocyte that participates in the adhesion process.

The test cultures' sensitivity to antimicrobial agents was studied by Bauer-Kirbi's method with the use of ready-made commercial disks [9].

The experiments were made three-four times. Statistical analysis was performed using computer programs Microsoft Excel 2003 and "Biostat-4". Parametric tests were used by calculation of mean and its standard deviation. P values of <0,05 were considered significant.

Results and discussion

The choice of objects of the investigations was conditioned by the following circumstances. On the one hand, the given technique stipulates the water disinfection with the help of extremely unstable agents (H^+ , OH^- , H_2 , H_2O_2). On the other hand, combined with the electron beam effect, the activity of heavy radicals is so high that mutagenesis processes can develop among bacteria.

Hitherto there does not exist any universal test-system that could detect all principal types of genetic injuries. All such installations were designed for finding out the adaptive changes. The latter can either heighten the microorganisms' pathogenic potential (generation of toxins, stimulation of ferments' activity) or stimulate the development of new properties, due to which microbial populations would become more resistant to the influence of environment (sensitivity to fags, mobility).

AC, (M±m) %	92,3±2,2	88,4±2,3	89,2±1,5	87,5±1,7	98,0±1,5	82,6±1,7	91,3±1,7	80,3±1,5
AAI, (M±m)	5,7±0,14	5,8±0,09	5,5±0,12	5,4±0,13	6,16±0,7	3,45±0,4*	5,63±0,4	3,2±0,3*
MAI, (M±m)	5,4±0,19	5,12±0,29	5,3±0,15	5,4±0,12	6,04±0,6	2,78±0,3*	5,3±0,3	2,6±0,25*

Notes: AC – adhesion coefficient; AAI – adhesion average index; MAI – microorganisms' adhesion index; C – the check sample; E – the examined sample; * – significant difference from control, (p<0,05).

The obtained results indicate that the given technique of the water disinfection sometimes heightens antilyzocyme, anticomplementary and antiinterferon activity of the test cultures. But, notwithstanding this fact, the method does not stimulate the development of adaptive reactions in bacteria which favour formation of microbial populations with heightened persistent potential. On the contrary, a decrease in the sanitary-model microflora representatives' adhesive features after the sample processing rather indicates the persistent potential reduction.

To estimate a possible after-effects of the given disinfecting technique application on the reservoir microflora, one must know the mode of changes in the sanitary-model bacteria' sensitivity to antimicrobial agents. At present, the problem of formation of pathogenic and opportunistic pathogenic bacteria populations, resistant to antibiotics, has become of special importance all over the world.

As it is known, formation of resistant microbial groups can be stimulated not only by the full-scale application of antimicrobial drugs but also by nonspecific agents influence.

We examined representatives of enterobacteria (*K.pneumoniae* №№ 32, 159; *K.oxytoca* №180; *E.coli* №

№ 3, 158, "B" №116; *C.freundii* №15; *S.marcescens* №184), staphylococcus (*S.aureus* ATCC №25923) and enterococcus (*E.faecalis* №158) strains.

As Table 3 indicates, after the ozonizing during 2 and 4 min, combined with the irradiation with the beam dose 1,6 and 3,2 kGy respectively, the sensitivity of enterobacteria cultures varied at the same frequency in both regimes of processing (34,8-43,5%). In all tests these reactions were identical and characterized by increase in antibiotics susceptibility of enterobacteria, these data were trustworthy from the viewpoint of statistics.

Enterococcus also often (in (56,3±7,2)% of the cases) changed their sensitivity to antibiotics after the laboratory sample processing with the physical and chemical agents (see Table 4). As the data indicate, ozonizing and irradiation stimulated an increase in the test culture sensitivity to antibiotics. This phenomenon in single cases was observed even when the original microbial culture was completely resistant to the antimicrobial agent. In other tests, the enterococci growth was on average 1,6 times delayed in comparison with the check-test values (p<0,01), what testifies that the subpopulation's protective properties were essentially weakened by the physical and chemical agents.

Table 3. Enterobacteria sensitivity to antimicrobial drugs after ozonizing and irradiation with the beam

Regimes of processing	The number of tests	The changes in sensitivity among the tests		An increase or decrease in the sensitivity to antibiotics													
		Number of isolates	%, (M±m)	K		A		G		R		C		S		O	
				I	D	I	D	I	D	I	D	I	D	I	D	I	D
Ozonizing 2 min + irradiation 1,6 κGy	69	30	43,5±5,9	5	0	7	0	9	0	0	0	3	0	6	0	0	0
Ozonizing 4 min + irradiation 3,2 κGy	69	24	34,8±5,7	4	0	5	0	6	0	0	0	4	0	2	0	3	0

The notes: K – Kanamycin; A – Ampicillin; G – Gentamycin; R – Ryphampicine; C – Chloramphenicol; S – Streptomycin; O – Ofloxacin; I – increase in sensitivity; D – decrease in sensitivity.

Table 4. Changes in *E.faecalis* (№158) sensitivity to antimicrobial agents after processing with ozone and the beam.

Antimicrobial means	Growth delay zone diameters before and after the culture processing				
	Check tests	Ozonizing 2 min + irradiation 1,6 κGy		Ozonizing 4 min + irradiation 3,2 κGy	
		M±m	p (compared with check tests)	M±m	p (compared with check tests)
Ampicillin	0	10,3±0,58	0,0001	10,3±0,58	0,0001
Chloramphenicol	14,7±0,58	15,7±0,58	0,1	18,3±0,58	0,001
Tetracycline	14,7±0,58	19,7±0,58	0,0004	20,3±0,58	0,0002

Gentamycin	9,7±0,58	10,7±0,58	0,1	10,7±0,58	0,1
Claritromycin	17,7±0,58	22,3±0,58	0,0004	22,3±0,58	0,0004
Norfloxacin	12,7±0,58	13,3±0,58	0,2	13,3±0,58	0,2
Ofloxacin	9,3±0,58	11,7±0,58	0,01	12,3±0,58	0,002
Cephaclor	12,7±0,58	12,7±0,58	1,0	12,3±0,58	0,6
Ceftriaxon	0	0	0	0	0
Cefepim	0	0	0	0	0

The results obtained at the given stage of detecting the possible phenotypic changes in individual representatives of the sanitary-model microflora in natural water and sewages induced by the electron beam and ozone combined processing permit us to state that the presented technique of the water disinfection does not injure microecology of the environment.

Conclusions

- 360 tests convincingly demonstrate that the frequency of appearance of modifications among the sanitary-model microflora representatives (*K.pneumoniae*, *E.coli*, *C.freundii*, *S.marcescens*, *S.aureus*, *E.faecalis*) depends on the disinfection intensity. After the water sample irradiation in the dose 0,8 kGy and ozonizing during 1 min (ozone concentration makes 15 mg/l) the mutants' specific gravity is about 26%; the dose 1,6 kGy and ozonizing during 2 min bring this parameter close to 56,7%.
- The results of 128 tests testify that the changes lead to the weakening of the modified variants ability to occupy people' biological niches. This conclusion is based on the fact that adhesive properties of test cultures become 1,1 times lower after joint processing of water samples with the beam and ozone ($p < 0,05$).
- Water sample processing with the beam and ozone in the above-mentioned regimes favours the formation of the sanitary-model microflora populations characterized by a heightened sensitivity to antimicrobial agents.

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The article is devoted to study of possible phenotypic changes in individual representatives of the sanitary-model microflora in natural water and sewages induced by the electron beam and ozone combined processing. The results obtained at the given stage permit us to state that the presented technique of the water disinfection does not injure microecology of the environment.

Key words: variability of microorganisms, disinfection of water, ozone, electron beam, sanitary-indicative microorganisms

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**ВЛИЯНИЕ СОВМЕСТНОЙ ОБРАБОТКИ
ОЗОНОМ И ЭЛЕКТОННЫМ ПУЧКОМ НА
ИЗМЕНЧИВОСТЬ ОСТАТОЧНОЙ
МИКРОФЛОРЫ**

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Калиниченко С.В., Семеренская Е.И.**

Статья посвящена изучению возможных вариантов фенотипических изменений, которые происходят после совместного применения озона и электронного пучка у отдельных представителей санитарно-показательной микрофлоры природных и сточных вод. Представленные результаты исследования позволяют на данном этапе изучения положительно оценить предложенную технологию обеззараживания воды с микробиологических позиций.

Ключевые слова: изменчивость микроорганизмов, обеззараживание воды, озон, электронный пучок, санитарно-показательные микроорганизмы

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**ВПЛИВ СУМІСНОЇ ОБРОБКИ ОЗОНОМ ТА
ЕЛЕКТРОННИМ ПУЧКОМ НА МІНЛИВІСТЬ
ЗАЛИШКОВОЇ МІКРОФЛОРИ**

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С.В., Семеренська Е.І.**

Стаття присвячена вивченню можливих варіантів фенотипових змін, що відбуваються після сумісного застосування озону та електронного пучка у окремих представників санітарно-показової мікрофлори природних та стічних вод. Приведені результати досліджень дозволяють на даному етапі вивчення позитивно оцінити запропоновану технологію знезараження води з мікроекологічних позицій.

Ключові слова: мінливість мікроорганізмів, знезараження води, озон, електронний пучок, санітарно-показові мікроорганізми