THE INFLUENCE OF AN ELECTRON BEAM ON THE FREQUENCY OF APPEARANCE OF MODIFICATIONS AND MUTAGENS IN THE RESIDUAL FLORULA

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We have carried out a series of tests for studying an electron beam (E=4 MeV) influence on adaptive mutability test cultures of the sanitary-model microflora in various regimes (0.4…3.2 kGy) of processing samples of natural water and sewages. Mutafacient properties and modifications of the residual microflora stimulated by the electron beam effect are scarcely pronounced. They do not indicate themselves in the regimes that guarantee the water purification up to the indices stipulated by the corresponding standards.

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INTRODUCTION

Biological objects adapt to the negative factors in the environment. This reaction can take the form of appearance of modifications that exist during briefer or longer time intervals. Otherwise, mutagens can be generated. The water disinfection can also stimulate biological changes in the residual microflora so that the latter will essentially differ in its characteristics from the original ones. That is, the influence of electron beam can threaten us with the formation of bacteria' populations, characterized by a high pathogenic potential. Therefore, it is expedient to study of the water disinfection techniques from the viewpoint of a possibility of injuring the environment. For this purpose, one must study changes that can be introduced into biological properties of the microbiological organisms in water media.

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

TECHNICAL APPROACH

We have elaborated the installation on the basis of a resonance accelerator of electrons for investigations of the sewage purification in the running water regime.

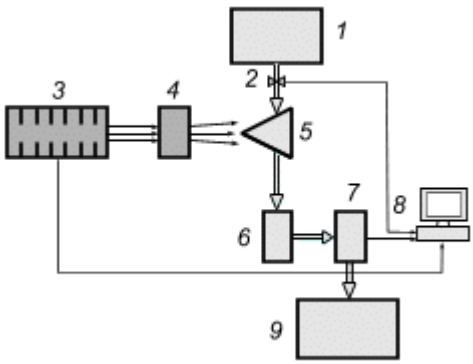

Fig.1. Test bench

1, 6, 9 are water tanks; 2 is a remote-control valve; 3 is the resonance accelerator of electrons; 4 is the beam scanner; 5 is reactor for purification of running water by electron beam; 7 is a pan containing photometric liquid; 8 is a computer.

The reactor (5) is a set of tanks made of stainless steel. Dimensions of the reactor are prescribed by the condition of homogeneity of the irradiation dose absorbed by the working fluid volume. From the side of the beam arrival, a mylar film of the thickness 20 μm is installed to prevent the beam penetration into the water volume. In the reactor bottom- and upper parts, the input and output openings are placed, through which the water under investigation is passing through. Inside the reactor, several plates, made of stainless steel, are inserted under a small angle one to another. Thus, the working fluid is propagating upwards through the irradiation zone in the zigzag-like manner. During the process, the fluid flux is being processed with the electron beam of the energy 4 MeV, the current 0.5 A, the pulse of the duration 2 μs.

The choice of the electron energy is conditioned by the necessity to minimize the energy expenditure and the beam divergence angle during the electron extraction into atmosphere through the titanium foil of the thickness 50 μm. Besides, one has to remove the induced radiation during the processing of the objects. The tilted position of plates permits changing the velocity of running water and avoiding air bubbles in the reactor working volume.

The microbiological objects, intended for the investigations, have been chosen within taxonomic groups of the sanitary-model microflora.

Hitherto there does not exist any universal test-system that could detect all principal types of genetic injuries. All such installations are designed for finding out the adaptive changes. The phenotypic changes are estimated by temporal appearance of microbial cells of untypical forms and sizes, short-term disturbances in the colony morphology and in the test culture biochemical characteristics. The irradiation influence is more minutely investigated by detecting the frequency of appearance of the dissociation of various types (R-forms). Besides, we have examined mutations inherent in one, several and scores of genes: alteration in an individual biochemical feature; disturbance of two or more of the typical characteristics; the bacterium mobility and sensi-

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tivity to fags, respectively. The electron beam influence on the generation of toxins is examined with the help of test cultures of opportunistic pathogenic corynebacteria C.ulcerans, capable of producing exotoxin.

Similar and statistically-identical data are obtained by examining microbiological features of bacteria in samples of natural waters and sewages after their processing with the electron beam in the stationary and running regimes. Therefore, the analysis is given to the results of the investigations in the aggregate.

EXPERIMENTAL RESULTS

In Table, we have submitted the results of the study of phenotypic changes in the residual microflora, detected after irradiating water samples in different regimes.

Phenotypic changes in the residual microflora

Test culture	Modifications (%)		
	0.8 kGy	1.6 kGy	3.2 kGy
K.pneumonia №159	16.6	33.3	50.0
K.pneumonia №32	16.6	41.6	41.6
K.oxytoca №180	25.0	33.3	66.6
E.coli ATCC 25922	25.0	41.6	58.3
E.coli B №116	33.3	58.3	66.6
C.freundii №15	41.6	66.6	66.6
S.marcescens №184	33.3	41.6	50.0
S.aureus ATCC 25923	25.0	50.0	58.3
E.faecalis №183	33.3	41.6	50.0
E.faecalis №185	16.6	50.0	58.3

The frequency of emergence of the induced microbes' modifications depends on the sample processing intensity.

The study of test-cultures' biochemical properties before and after the sample processing indicates that most frequently there arise modifications with the disturbance of just of one of the fermentative feature - 70.2%. Modifications with the disturbance of two fermentative features are detected in 14.9% of the cases.

The water chemical compound can also influence the character of adaptive reactions in the test cultures and frequency of their development. To ascertain the role played by various mineral and organic compounds in the appearance of modifications in the above-mentioned biological objects, we have separately investigated the samples that contain the following substances in high and low concentrations.

Tests have proved that when the chemical compound contents in samples of natural water and sewages are low and high, the frequencies of emergence of modifications make 51.3% and 58.7%, respectively. Changes in test cultures' biological properties concern mainly one or two features of the bacteria' biochemical activity.

Later on, it is suggested to study anti-lyzocyme, anti-complementary and anti-interferon activities of the residual microflora [1, 2]. Such features give the bacteria an advantage in colonization of the man's intestinal tract.

The water samples are processed in such regimes that the representatives of enterobacteria, staphylococcus and enterococcus can survive in large quantities, in certain cases (10.0%) this circumstance stimulates the appearance of modifications with the above-mentioned features. As a rule, such variants are detectable when higher energies (more 1.6 kGy) are spent on the water processing.

The influence on the adhesive properties of different representatives of the sanitary-model microflora is tested on enterobacteria (K.pneumonia #159; E.coli ATCS 25922; C.freundii #116) and staphylococcus (S.aureus ATCS 25923).

The sanitary-model microflora sensitivity to the corresponding bacteriophages should be also regarded as the index of bacterium viability – i.e., the capability of surviving in the environment. An increase in the specific gravity of subpopulations resistant to phages can favor accumulation of bacteria and disturb the ecological balance among many-component associations that belong to the aquatic microflora.

The data of experiments testify that irradiation 1.6 kGy do not affect the test ability to lysis.

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 means. At present, the problem of formation of populations of pathogenic and conditionally pathogenic bacteria, 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 means but also by nonspecific agents.

After irradiation with the beam dose (1.6…3.2 kGy), the sensitivity of enterobacteria' cultures vary at the same frequency in both of the regimes (34.8…43.5%). The results permit determining the tendency in the character of test bacteria' reaction to antibiotics stimulated by the physical and chemical agents. In all the tests these reactions are identical and characterized by an increase in the enterobacteria' sensitivity to antimicrobial means, these data being trustworthy from the viewpoint of statistics.

Enterococcus also often (in 56.3% of the cases) alter their sensitivity to antibiotics after the laboratory sample processing with the electron beam. As the data indicate, the irradiation does stimulate an increase in the test culture sensitivity to antibiotics. This phenomenon is observed even in single cases when the original microbial culture is completely resistant to the antimicrobial agent.

The electron beam induces the staphylococcus modifications of two kinds. 48 of 53 changed isolates (90.6%) are characterized by a higher sensitivity to certain antibiotics. On the contrary, the rest of isolates are more resistant than the original test cultures.

The isolates that preserve changed features induced by the electron beam during ten generations are defined as stable modifications.

A specific form of variability is related to mutant changes. It indicates itself via dissociation of bacteria: there arise two forms of colonies – R (uneven edges) and S (even edges). There are three regimes of processing the

laboratory samples of natural waters and sewages: irradiation 0.8; 1.6; 3.2 kGy.

The data of experiments indicate that enterococci, staphylococci and enterococci statistically do not differ from one another in the frequency of appearance stable modifications among their representatives (within (6.7…9.7)%). Besides, the appearance of R-form has been observed just in certain cases.

It is worth mentioning an increase in the test-cultures' sensitivity to antibiotics (19 isolates), the fermentative activity suppression (5 variants) and decrease in adhesive characteristics (2 modifications) belong to stable changes in the bacteria' characteristics.

The electron beam not only affects the bacteria' properties due to which the population viability in the environment either improves or gets worsen. These factors can be responsible for changes in the bacteria' virulent properties too.

For estimating the given technique of water processing from this viewpoint, it is important to understand the mechanism for the physical-chemical agent influence on the intensity of pathogens' reproduction and production of exotoxin. For these tests we have taken certain cultures of corynebacteria characterized by toxicogenic properties (C.ulcerans, C.diphtheriae gravis №75, C.diphtheriae mitis №2).

In the first series of the experiments, we have studied specificities of the test-cultures' reproduction after the water sample processing in various regimes. The comparative analysis given to kinetics of the development of the check- and processed samples indicates the chosen regimes of the water processing steeply suppress the bacteria' reproduction ability. After irradiation with the doses (0.8 and 1.6) kGy, the number of colony-forming cells has become (4 and 11.5) times as small, correspondingly.

The next step of our investigation was to determine the electron beam influence on one of the most important characteristic of the difteric microbe – the exotoxine generation.

Variants of gravis and mitis have been also subjected to irradiating with the doses (0.8 and 1.6) kGy. After processing in the first regime, the majority of isolates of gravis (85.0%) and mitis (84.6%) lost their ability to produce exotoxins. As regards the other clones of both the types, the exotoxin synthesis intensity was slowed down. After 10-times reseeding on solid selective mediums, just some of isolates (6.0…7.5%) restored their capability to produce toxin.

Thus, the study of electron beam influence on certain toxicogenic representatives of corynebacteria indicates that the given technique of water processing in the regimes, after application of which residual microflora is still detectable, all the same diminishes virulent properties of survived isolates.

CONCLUSIONS

Water sample processing with the electron beam in the above-mentioned regimes favors the formation of the sanitary-model microflora populations characterized by a heightened sensitivity to antimicrobial means. At the same time, this technique does not change the phage cultures' ability to lysis as compared with this property inherent in the corresponding original cultures.

The processes of water sample irradiation with the beam doses (0.8 and 3.2) are not mutafacient with respect to sanitary-model microflora representatives.

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ИЗУЧЕНИЕ ЧАСТОТЫ ПОЯВЛЕНИЯ МОДИФИКАЦИЙ И МУТАГЕННЫХ ВАРИАНТОВ ОСТАТОЧНОЙ МИКРОФЛОРЫ ПОД ВЛИЯНИЕМ ЕЕ ОБРАБОТКИ ЭЛЕКТРОННЫМ ПУЧКОМ

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Проведен цикл экспериментов по влиянию электронного пучка (E=4 MeV) на адаптивную изменчивость тест-культур санитарно-показательной микрофлоры в условиях разных режимов (0,4…3,2 кГр) обработки проб природных и сточных вод. Установлено, что мутагенные свойства и модификация остаточной микрофлоры после воздействия электронного пучка имеют слабо выраженный характер и не проявляются в режимах, которые обеспечивают очистку воды до санитарных норм.

ВИВЧЕННЯ ЧАСТОТИ ПОЯВИ МОДИФІКАЦІЙ ТА МУТАГЕННИХ ВАРІАНТІВ ЗАЛИШКОВОЇ МІКРОФЛОРИ ПІД ВПЛИВОМ ЇЇ ОБРОБКИ ЕЛЕКТРОННИМ ПУЧКОМ

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Проведено цикл експериментів по впливу електронного пучка (E=4 МеВ) на адаптивну мінливість тест-культур санітарно-показової мікрофлори в умовах різних режимів (0,4…3,2 кГр) обробки проб природних та стічних вод. Встановлено, що мутагенні властивості та модифікація залишкової мікрофлори після впливу електронного пучка мають слабко виражений характер і не проявляються в режимах, що забезпечують очистку води до санітарних норм.