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EFFICIENCY OF PURIFICATION OF WATER FROM CYCLIC AROMATIC XENOBIOTICS WITH STRAINS *P. FLUORESCENS* ONU328 AND *P. MALTOPHILIA* ONU329

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Abstract. It has been experimentally established that non-pathogenic strains of bacteria of the genus *Pseudomonas,* identified by the fatty acid composition of their cellular lipids as *P. fluorescens* ONU328, *P. maltophilia* ONU329 (isolated from the marine environment), have oxidizing ability for cyclic aromatic xenobiotics (phenol, N-cetylpyridinium bromide). The high phenol-oxidizing ability of the investigated strains of microorganisms is proved. When water of bacterial cells in the amount of 7.5 × 105 CFU/ ml is introduced into phenol contaminated (at a concentration of 300 mg / l), the degree of water purification from phenol is 100% on day 18 when using as a biodestructor strain *P. maltophilia* ONU329 and 22 days - when using strain *P. fluorescens* ONU328. It has been experimentally confirmed that when the free cells of bacteria of *P. fluorescens* ONU328 or *P. maltophilia* ONU329 strains in the amount of 5.5×10^4 CFU/ ml are introduced once into the contaminated water, the purification rate of water from N-cetylpyridinium bromide at a concentration of 20 mg / l reaches 50.7% for the 4th day and 55.7% for the 6th day, respectively. With the repeated introduction of fresh portions of microorganism-destructors or use of

higher starting doses (5.5×10^8 CFU/ ml), the water purification rate from N-CPB is 98.0-98.5%.

Keywords: purification of water, phenol, N-cetylpyridinium bromide, bacteria of the genus *Pseudomonas*

Introduction. Cyclic aromatic xenobiotics (phenolic and other difficult oxidation compounds) are toxic pollutants of the environment. Microbiological methods of water purification from cyclic aromatic xenobiotics, which also include water-soluble salts widespread N-cetylpyridinium limited. This is due to their high antimicrobial activity against fungi, gram-positive and gram-negative microorganisms. N-cetylpyridinium halides are cationic surfactants which have high surface activity and the disinfecting effect; are the basis of most disinfectants: chlorhexidine, a diocid. Diocid is a disinfectant combination agent based on a mixture of N-cetylpyridinium chloride or bromide (N-CPC or N-CPB) and ethanol merkuryl chloride (active mercury antiseptic) in the ratio (2:1). The widespread use of N-cetylpyridinium halides only as the basis of disinfectants leads to their accumulation in effluent production of pharmaceutical preparations, in effluent of medical institutions, etc. Getting into water bodies, these surface-active substances like surface-active substances of anionic and non-ionic nature, participate in the processes of redistribution and transformation of other pollutants, sometimes translating them into a more toxic form [1]. Information on the ability of microorganisms to decompose synthetic surface-active substances of cationic nature on the example of "biologically rigid" N-CPC are presented in [2]. The authors found that the ability to destroy surfactants varies widely among microorganisms, even among members of the same genus. For example, the degree of biodegradation of N-CPC at a concentration of 20 mg / l in the presence of strains of Staphylococcus warneri A and Staphylococcus warneri T is 94% and 45%, respectively, on the 5th day; and in the presence of *Bacillus anthracis, Bacillus licheniformis, Bacillus amyloliquefaciens* - respectively 84.8%, 60.7% and 52.3% (on the 6th day). When achieving high results on biodegradation of N-CPC, the following should be attributed to the shortcomings of the above-mentioned microbiological method: the

duration of the process of cultivation of the most biochemically active destructor strains (isolated from glass-fiber waste water) - from a week to a month with a small cell titer of bacterial strains (10^3- 10^4 CFU/ml) that were added to the surfactant solution; lack of information on the resistance of isolated destructor strains to high concentrations of other cyclic aromatic xenobiotics, including highly toxic phenol (phenol, like N-CPC, is fixed in pharmaceutical effluent, medical facilities); the pathogenicity of the most active strains-destructors has not been evaluated, which is necessary for their wide use in biotechnology of water purification from cyclic aromatic xenobiotics.

The aim of the work is to propose an efficient method of water purification from cyclic aromatic xenobiotics (for example phenol, "biologically rigid" cationic surfactant N-cetylpyridinium bromide) when using some non-pathogenic bacteria of the genus *Pseudomonas* as biodestructors.

Materials and methods. To conduct the study used two strains of bacteria of the genus *Pseudomonas* spp., Which have been previously isolated from marine environment and set of morphological, cultural, physiological and biochemical traits identified using classical bacteriological methods and test systems AER 50 START Medium (bioMerieux, France) are assigned to the species *P. fluorescens* ONU-328 and *P. maltophilia* ONU-329. Additionally, by fatty acid composition, whose spectra were obtained on a Agilent 7890 gas chromatograph (Agilent Technologies, USA), and decoded using a library database program RTSBA6 6.21 MIDI Sherlock, Investigated strains with a high index of similarity (Sim Index> 0,72) identified as *P fluorescens* ONU328, *P. maltophilia* ONU329.

Assessment of the KPAR and phenol-destructive capacity of free cells of strains *P. fluorescens* ONU328 and *P. maltophilia* ONU329 was performed according to the degree of water purification from cyclic aromatic xenobiotics (phenol, N-CPB)

$$\alpha = [(C_0\text{-}C) / C_0] \times 100\%,$$

where C_0 and C are the concentrations of a particular xenobiotic before and after treatment.

After the biodegradation process, all samples were analyzed for the residual concentration of N-cetylpyridinium bromide (N-CPB) in them. Determination of the residual concentration of N-CPB in control and test samples was carried out using an extraction-colorimetric method based on the interaction of N-CPB with methylorange to form a chloroform-soluble yellow complex [3]. The intensity of the color of the chloroform extract is proportional to the concentration of the methyl-a-cationic surfactant complex. Chloroform extracts (containing a certain amount of N-CPB) were photographed at $\lambda = 415$ nm with respect to pure chloroform.

The experiments were performed in triplicate. Statistical processing of the research results was carried out using the computer program "Microsoft Office Excel 2007".

Results and discussions. Our studies have shown that strains of *P. fluorescens* ONU328, *P. maltophilia* ONU329 are a promising basis for biotechnology biopreparations: they are non-pathogenic for humans and have a high oxidative potential for phenolic compounds and cationic surfactants of cyclic structure. The choice of these nonpathogenic strains of microorganisms as possible destructors of "biologically rigid" cationic surfactants of cyclic structure was due to the effective result of their oxidative action against high concentrations of highly toxic phenolic compounds, patented in [4].

The results of water purification from cyclic aromatic xenobiotics - N-cetylpyridinium bromide (N-CPB) in the presence of strains of *P. fluorescens* ONU328 and *P. maltophilia* ONU329 are presented in the table. The method was carried out as follows. The microorganisms were cultured on a shaker incubator New Brunswick Scientific Incubator Shaker INNOVA 43R in vials of 100 ml medium at 150 rpm for 24 hours at 30 °C. Sowing of nutrient medium was carried out by daily culture, grown on MPA in stationary conditions (thermostat). The seed volume was 1.0% of the volume of the medium. Non-pathogenic strains of *P. fluorescens* ONUU328, *P. maltophilia* ONU329 were suspended in the M-9 mineral medium and in an amount of 5.5 × 10 4 cfu / ml were introduced into a storage tank where the water purification process from the cationic surfactant was performed. A day later,

the concentration of N-cetylpyridinium bromide decreased from 20 mg / l to 11.2 ± 0.15 mg / l and 12.8 ± 0.45 mg / l when used as biodestructors of *P. fluorescens* ONU328 and *P. maltophilia* ONU329 respectively (the degree of water purification was 36-44%).

Table

Efficiency of microbiological water purification from a difficult-oxidizing cyclic surfactant of cationic type - N-cetylpyridinium bromide

Strain	Concentration of N-CPB after purification, mg / l	Degree of destruction of N-CPB,%
Exposition - 1 days		
P. fluorescens ONU328	11,2±0,15	44,0
P. maltophilia ONU329	12,8±0,45	36,0
Control	20,0±0,45	0,0
Exposition - 4 days		
P. fluorescens ONU328	9,9±0,20	50,7
P. maltophilia ONU329	10,5±0,15	47,5
Control	20,0±0,45	0,0
Exposition - 6 days		
P. fluorescens ONU328	9,7±0,15	50,6
P. maltophilia ONU329	8,9±0,12	55,7
Control	19,7±0,40	1,5

Note: * concentration of N-CPB in water - 20 mg/l; the concentration of bacterial cells with their one-time introduction into the treated water is 5.5×10^4 cells/ml.

It has been experimentally established that when the free cells of the strain of *P. fluorescens* ONU328 or *P. maltophilia* ONU329 in an amount of 5.5×10^4 CFU/ ml are introduced once into the contaminated water, the purification rate of water from N-cetylpyridinium bromide at a concentration of 20 mg / l reaches 50.7% for the 4th day and 55.7% for the 6th day, respectively (table). If it is necessary to achieve a

higher degree of purification (98.0-98.5%) from N-CPB, fresh portions of microorganisms-destructors can be re-introduced or higher starting doses can be used. The residual concentration of N-CPB in water corresponds to the norm for traditional wastewater treatment plants with activated sludge, that is, the wastewater that has been purified by the microbiological method can be discharged into the municipal sewage system.

Conclusions

It has been experimentally established that the following conditions are optimal for the effective purification of water from phenol, N-cetylpyridinium bromide by microbiological method using the strains of *P. fluorescens* ONU328 or *P. maltophilia* ONU329 as cyclic aromatic xenobiotics destructors: 30 ^{0}C; the culture concentration is (7.5-5.5) × 10^5 CFU/ ml - and the degree of water purification from phenol (with an initial concentration of 300 mg / l) is 100% on the 18-22 day, and the N-CPB is 50.7 -55.7% for single-dose administration of bacterial cultures (from the initial concentration of cationic surfactant 20 mg / l) for 4-6 days. When the fresh portions of microorganism-destructors are re-introduced in the amount of 5.5 × 10^5 CFU/ ml or using their higher starting doses (5.5 × 108 cfu / ml), the water purification rate from N-CPB for a week is 98.0- 98.5%.

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