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DESTRUCTION OF PHENOL AT THE FORMATION OF POLYVIDOUS BIOFILM ON NATURAL AND SYNTHETIC CARRIERS IN THE BIOFILTER

Aim. *To determine the effectiveness of the process of water purification fromphenol by microorganisms-destructors in the formation of a polyvidiofilmbiofilm on natural and synthetic carriers in a biofilter.* ***Methods.*** *An associationof phenol bacterium destructors – Aeromonas ichthiosmia ONU552, Basillus subtilis ONU551, Pseudomonas maltophilia ONU329, Pseudomonas fluorescensONU328, Pseudomonas cepacia ONU327 was used. Stained biofilms were stained with 1% acridine orange solution. Microscopy of the samples was carried out under a Carl Zeiss fluorescence microscope and a Carl Zeiss, Primo Star light microscope with photo fixation. The concentration of phenol in water was determined by the extraction-photometric method using 4-aminoantipyrin.* ***Results.*** *Using fluorescence microscopy, it was confirmed that bacteria used for purification of water from phenol - destructors formed a biofilm in a biofilter on carriers ofdifferent nature – ceramic tubes, mussel valves, peat, zeolite, activated carbon, synthetic media such as "VIYA", sand. In laboratory conditions, the effectiveness of the operation of a column-based biofilter of flow-up-stage type with layer-by-layer complex loading of sorbents during purification of phenol-containing water (initial concentration of phenol – 300 mg/l) was confirmed. After 2 hours of the biofilter operation, the degree of water purification was 40% (residual phenol concentration in water 180±17.2 mg/ l), which was associated with phenol sorption on carriers; during biodegradation, it reached 90% (residual phenol concentration in water – 29.5±2.8 mg/l) on the 6th day. In the following days, the efficiency of the biofilter with continuous intake of phenol-contaminated water was at the level of 50–75%, and in the stationary-cyclic mode reached 80–90% (the concentration of phenol in the water varied from 29.5±2.8 to 60±5.7 mg/l).* ***Conclusion.*** *The new microbial consortium forms a biofilm on natural and synthetic filter carriers, which contributes to the effective purification of water from phenol and the flow-through biofilter operation time (up to 2 months) without additional regeneration.*

Key words: water purification, bacteria – phenol destructors, biofilter, biofilms, fluorescence microscopy

Today essential pollutants of water ecosystems are phenol and its derivatives as by-products of the enterprises of a petrochemical complex, coal and chemical industry, production of pharmaceutical drugs, in connection with their toxicity, stability and ability to collect in the environment [8]. Sources of phenols in natural water objects are drains of production of pharmaceutical drugs, dyes, pesticides, phenol formaldehyde pitches and nonionic surface-active substances, enterprises of a petrochemical complex, coal industry, mechanical engineering, chemical industry [13].

For prevention of negative impact and environment protection against pollution by toxic aromatic connections, including phenolic, apply a biotechnology method with use of the phenol microorganisms destructors attached to different carriers [9, 10]. Advantages of the immobilized microorganisms destructors in biotechnology processes of water purification from organic pollyutant are in detail described [12, 15]. Development of biocatalytic systems is attractive that the immobilization has no stressful impact on cells the carrier protects them from direct influence of toxics and adverse factors of the environment, the oxidizing activity of microbic cells and efficiency of sewage treatment increases [5, 14].

Microorganisms, exchanging with each other substances, energy, and as all live organisms on our planet, submit to laws of thermodynamics which sense comes down to high orderliness of the components for the purpose of preservation of a certain level of energy for oppositions of entropy, i.e. to irreversible dispersion. Therefore they enter in various symbiotic bonds allowing them to survive in different conditions. An example of such bonds is formation of biofilms (biofilms) on different surfaces. Biofilms represent communities of cells microorganisms which are formed on limit of the section of phases. Cells as a part of biofilms are put into a polymeric matrix which part are all classes of biopolymers, with prevalence of various polysaccharides [6].

There is rather large number of the methods allowing to carry out visualization of the created bacterial films [2, 3]. To methods which visualize ultrastructure of microbic communities, it is possible to carry a submicroscopy and confocal laser the scanning microscopy (CLSM). Other methods are based on sorption of molecules of specific dye on structures of a biofilm, with the subsequent them washing (desorption). Such way of indication of biofilms most often it is used in static methods of cultivation of microbic biofilms also allows to give conditional quantitative characteristic to formed to microbic communities, i.e. than the matrix of a biofilm, subjects is formed more more dye is occluded on its surface [11].

The work purpose – to define efficiency of process of water purification from phenol microorganisms destructors when forming polyspecific biofilms on natural and synthetic carriers in the biofilter.

Materials and methods

In work used association from 5 strains of bacteria destructors of phenol: *Aeromonas ichthiosmia* ONU552 and *Bacillus subtilis* ONU551 (are allocated from waste water of the pharmaceutical plant), *Pseudomonas maltophilia* ONU329 and *Pseudomonas fluorescens* ONU328 (are allocated from sea water), *Pseudomonas cepacia* ONU327 (it is allocated from the soil).

For loading of the filter used readily available, cheap natural sorbents – zeolite, shutters of mussels, sand, activated carbon, peatriding; and synthetic sorbents – ceramic tubes, the synthetic carrier of the VIY type TU995990 [16]. For experiments used zeolite with a size of granules of 0.3–0.7 cm; shutters of mussels with a size of plates 0.5–1.0 cm: size of granules of activated carbon of 3–4 mm; sizes of ceramic tubes: diameter is 8 mm, length is 10 mm, wall thickness is 1.5 mm.

All sorbents for loading of the filter and a further immobilization on them associations of microorganisms – destructors of phenol prepared as appropriate. The synthetic carrier of the VIY type and peat riding sterilized within 30 min. in the autoclave at 1 atm. Zeolite, sand river, shutters of mussels at first washed from a fine phase, then sterilized in a spherical case at a temperature of 180 °C; shutters of mussels processed 250–300 °C at more high temperature for burning out of an organic phase.

For inoculation of loading of the biofilter of a bacterium destructors cultivated at a temperature of 28 ºC within 2 days on following M9 environment structure (g/l): Na2HPO4 – 6; KH2PO4 – 3; NH4Cl – 1; NaCl – 0.5–2; peptone 10.0; glucose – 0.2.

The immobilization of bacteria – destructors on carriers in the filter of columnar type was carried out at a temperature of 28 ºC within 2 days, later what residues of bacterial suspension merged, and washed out loading of the biofilter from not attached cells of bacteria mineral M9 environment three times without addition of peptone and glucose.

All used sorbents placed in one filter. Multilevel distribution of sorbents (zeolite, shutters of mussels, synthetic carrier VIY type, activated carbon, peat, ceramic tubes, sand) in the columnar filter promoted a fast gain of microbiological communities.

Before process of water purification from phenol in the biofilter concentration of biomass of 1×10^9 C/g of the carrier was reached. The biofilter was inoculated within 2 days, passing through it a bacterial suspenzniya (speed of a channel of 3 ml/min.) with concentration of 1×10^{12} C/ml. After the termination of inoculation quantity of the remained cells in an inoculum made 10×10^3 C/ml, thus on carriers of the biofilter 1×10^9 C/g were adsorbed. The portion volume of the water polluted by phenol, passing through the biofilter of 500 ml, made 230 ml.

After 10 days of use of the flowing biofilter for cleaning the fenolsoderzhashchikh of waters carriers were removed from the reactor and 96% by ethanol for fixation of biofilms are processed. Then carriers painted immersion in 1% solution acridic orange (a 3.6-acridinediamine-N, N,N', N'-tetramethyl) for 4 minutes, washed with water and dried on slide plates [1].

The microscopy of samples was carried out with use of fluorescent microscope of Carl Zeiss and light microscope of Primo Star PC at increase 10×40, photographed with use of the Olympus DCM camera (3.0 M pixels). As control served the sterile carriers and the fixed smears of above-mentioned strains of bacteria processed by 1% acridic orange.

The efficiency of process of water purification from phenol the biofilter of flowing type was estimated on the equation:

$$\alpha = [(C_0 - C) / C_0] \times 100\%, \quad (1)$$

where C_0 and C – concentration of phenol in water to (300 mg/l) and after processing.

Concentration of phenol was determined by a photocolorimetric method, the painted compounds of phenol with 4 aminoantipyrine based on education in the presence of hexacyanoferrate (III) at pH = 10.0 ± 0.2 [7].

Carried out three series of tests, n=3. Reliability of distinctions between determined by average values of residual concentration of phenol in water on to Styyudent's criterion at level of significance not less than 95% ($p \leq 0.05$). Data processing carried out with use of the program "Microsoft Office Excel 2003".

Results and their discussion

As a result of researches it is established that on various carriers the biofilm is formed in different volumes. Comparative analysis showed, that on sand and the synthetic carrier of VIY type [16] immobilization bacteria-destructors of *A. ichthiosmia* ONU552 phenol, *V. of subtilis* ONU551, *P. maltophilia* ONU329, *P. fluorescens* ONU328, *P. cepacia* ONU327 was slight, the biofilm was not formed.

The most active formation of a biofilm was noted on shutters mussels, peat and ceramic tubes. On the ceramic tubes entering in structure of complex loading of the operating bioreactor, it was formed continuous layer of the developed biofilm of association of bacteria (Fig. 1).

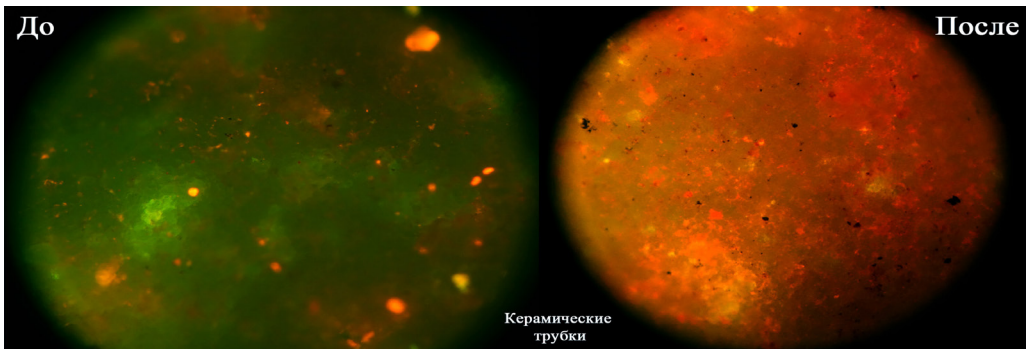

Fig. 1. Biofilm of bacteria – the destructors of phenol on the ceramic tubes before and after inoculation of the biofilter. Coloring with acridine orange, the increase of 10x40

In the course of 10-day use of the biofilter for water purification from phenol adhesion of bacteria and formation of a polyspecific biofilm was registered also on peat, zeolite and activated carbon.

Formation of a polyspecific bacterial biofilm on carriers in the biofilter promoted increase in efficiency of its work throughout a long time (2 months) without additional regeneration and re-peated inoculation bac-teria destructors of complex loading (Fig. 2). Maintaining a specific variety of the used association strains of microorganisms destructors of phenol at a final stage operation of the biofilter it is confirmed with seeding on the agarizovanny environment on morphological-cultural and biochemical signs. Numbern-reached each of strains upon termination of operation of the biofilter about 5×10^4 C/g of a sorbent.

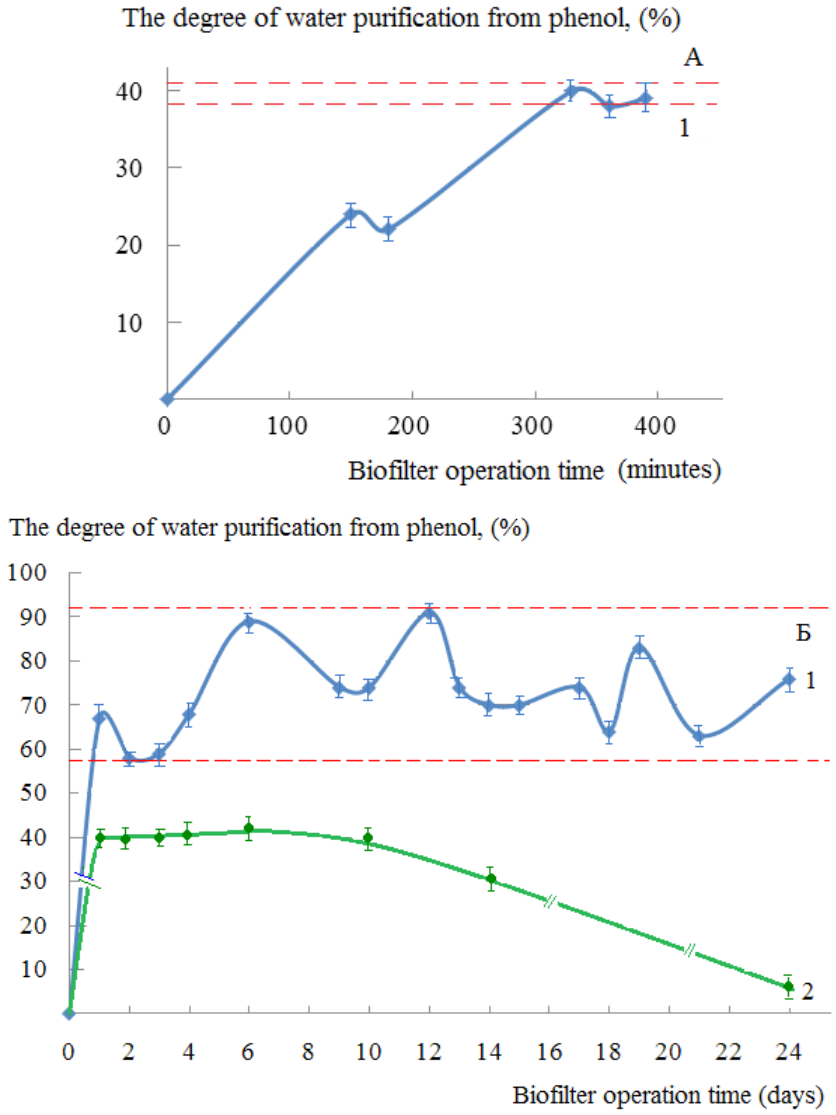

Fig. 2. The degree of purification of phenol-containing water in the filter with immobilized bacteria - destructors (1); native sorbents (2)
Note: the initial concentration of phenol in water is 300 mg / l

In the laboratory investigated the efficiency of the biofilter column of periodic action with a flow-rising type with layer-by-layer modified complex loading at purification of phenol-containing water (initial concentration of phenol 300 mg / l).

After 2 hours of operation of the biofilter at a rate of 10 ml / min. water purification from phenol in the dynamic mode was 40% (residual concentration of phenol in water 180±17.2 mg / l), which was associated with sorption of phenol in the media (Fig. 2, A). After a day of operation of the biofilter in a stationary cyclic mode, the degree of water purification increased to 67% (residual phenol concentration in water – 100±8.5 mg / l), and on the 6th day reached the maximum value is 90% (the residual concentration of phenol in water – 29.5±2.8 mg / l) (Fig. 2, B).

In the following days the efficiency of the biofilter in dynamic mode with a continuous flow of water contaminated with phenol was at 50–75%, and in a stationary-cyclic mode reached 80–90%, phenol concentration in water ranged from 29.5±2.8 mg / l to 60±5.7 mg / l (Fig. 2, B).

The findings suggest that the main mechanism removal of phenol from contaminated water is its biodegradation by immobilized microorganisms (*A. ichthiosmia* ONU552, *B. subtilis* ONU551, *P. maltophilia* ONU329, *P. fluorescens* ONU328, *P. cepacia* ONU327).

By fluorescence microscopy using a dye acridine orange it is confirmed that the phenol destructor bacteria used in the experiments formed a biofilm on carriers of different nature. At the first stage there was adhesion of vegetative forms of bacteria, in subsequently-the formation of the intercellular matrix of biofilms. Can it is believed that in the period of time between 0.5 and 6 h in the biofilm culture there was a transition of the cell adhesion stage to the beginning of the formation of the extracellular matrix of the biofilm. When using ceramic tubes in the form of rings, peat fiber structures biofilm, filled loop-like structures, increases its working surface, acquires two-way contact with purified water, which increases efficiency water purification from phenol.

The results of our studies are consistent with the data of the work [4], the authors of which observed the growth of microorganisms on the powder-fiber loading of the biofilter. They also conducted a study of modified download of the biofilter in order to intensify the cleaning of local waste water and it is shown that in loop-like structures, a bacterial film is formed it forms bridges, which indicates the directed growth of a community of microorganisms forming a matrix to the opposite side of the loop. Such the distribution of bacteria in the biofilm matrix on the polymer loop-like material, promotes RNA synthesis, and therefore leads to an increase in the metabolism of the biofilm as a whole.

Thus, the microbial consortium (*A. ichthiosmia* ONU552, *B. subtilis* ONU551, *P. maltophilia* ONU329, *P. fluorescens* ONU328, *P. cepacia* ONU327) forms a biofilm on natural and synthetic filter media, which improves the efficiency of water purification from phenol (75–90%) and increases the duration of the flow-type biofilter (up to 2 months) without additional regeneration.

By fluorescence microscopy using a dye acridine orange confirmed that the bacteria used in the experiments (*A. ichthiosmia* ONU552, *B. subtilis* ONU551, *P. maltophilia* ONU329, *P. fluorescens* ONU328, *P. cepacia* ONU327) – phenol destructors formed biofilms on carriers of different nature.

In laboratory conditions, the efficiency of the column biofilter of periodic flow-ascending type C was confirmed layer-by-layer modified complex loading at purification of phenol-containing water (initial concentration of phenol-300 mg/l). After 2 hours the degree of purification of water from phenol was 40% (residual concentration of phenol in water 180±17.2 mg / l), a day later-67% (concentration of phenol in water-100±8.5 mg/l) and reached the maximum value 90% (phenol concentration in water 29.5±2.8 mg/l) on the 6th day of treatment.

Association of bacteria *A. ichthiosmia* ONU552, *B. subtilis* ONU551, *P. maltophilia* ONU329, *P. fluorescens* ONU328, *P. cepacia* ONU327 may be it is used for purification of phenol-containing wastewater in a biofilter.

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Summary

Aim. *To determine the effectiveness of the process of water purification from phenol by microorganisms-destructors in the formation of a polyvidiofilm biofilm on natural and synthetic carriers in a biofilter.* ***Methods.*** *An association of phenol bacterium destructors — Aeromonas ichthiosmia ONU552, Basillus subtilis ONU551, Pseudomonas maltophilia ONU329, Pseudomonas fluorescens ONU328, Pseudomonas cepacia ONU327 was used. Stained biofilms were stained with 1% acridine orange solution. Microscopy of the samples was carried out under a Carl Zeiss fluorescence microscope and a Carl Zeiss, Primo Star light microscope with photo fixation. The concentration of phenol in water was determined by the extraction-photometric method using 4-aminoantipyrin.* ***Results.*** *Using fluorescence microscopy, it was confirmed that bacteria used for purification of water from phenol - destructors formed a biofilm in a biofilter on carriers of different nature – ceramic tubes, mussel valves, peat, zeolite, activated carbon, synthetic media such as "VIYA", sand. In laboratory conditions, the effectiveness of the operation of a column-based biofilter of flow-up-stage type with layer-by-layer complex loading of sorbents during purification of phenol-containing water (initial concentration of phenol – 300 mg/l) was confirmed. After 2 hours of the biofilter operation, the degree of water purification was 40% (residual phenol concentration in water 180±17.2 mg/ l), which was associated with phenol sorption on carriers; during biodegradation, it reached 90% (residual phenol concentration in water – 29.5±2.8 mg/l) on the 6th day. In the following days, the efficiency of the biofilter with continuous intake of phenol-contaminated water was at the level of 50–75%, and in the stationary-cyclic mode reached 80–90% (the concentration of phenol in the water varied from 29.5±2.8 to 60±5.7 mg/l).* ***Conclusion.*** *The new microbial consortium forms a biofilm on natural and synthetic filter carriers, which contributes to the effective purification of water from phenol and the flow-through biofilter operation time (up to 2 months) without additional regeneration.*

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References

1. Bioplenki: Biofilms: basic research methods: teaching aid / Mardanova AM. s soavt. Kazan': K(P)FU, 2016: 42 [in Russian].

2. Erohin PS, Kuznecov OS, Konnov NP, Vidjaeva NA, Utkin DV. An integrated approach to the study of microbial biofilms by atomic force microscopy. Izvestija Saratovskogo universiteta. Novaja serija. Serija: Fizika. 2012;12(1):42–46 [in Russian].

3. Pavlova IB, Tolmacheva GM, Lenchenko EM. Experimental study of the formation of biofilms Pseudomonas aeruginosa, Staphylococcus aureus (light and scanning electron microscopy). Problemy veterinarnoj sanitarii, gigieny i jekologii. 2016;4(20):65-73 [in Russian].

4. Pukemo MM, Simakov JuG, Alekseev EV. Fractal growth of microorganisms on the powder-fiber loading of the biofilter and intensification of wastewater treatment Innovacii i investicii. 2015;6:181–185 [in Russian].

5. Cerebrennikova MK. Biodegradation of petroleum hydrocarbons by immobilized rhodococci in a column bioreactor. Avtoref. dis. kand. biol. nauk: 03.02.03 – mikrobiologija, Perm' 2014:23. [in Russian].

6. Sokolova TN. Microbial biofilms and methods for their detection Zhurnal Grodnenskogo gosudarstvennogo medicinskogo universiteta. 2014;4:12–15. [in Russian].

7. Fomin GS. Voda. Kontrol' himicheskoj, bakteriologicheskoj i radiacionnoj bezopasnosti po mezhdunarodnym standartam: Jenciklopedicheskij spravochnik. 3-izd. M., 2000:839. [in Russian].

8. Chasova JeV, Ivchuk VV. Ecological and chemical characteristics and methods of environmental protection from phenol. Vestnik Krivorozhskogo nacional'nogo universiteta. 2013;34(1):209–213. [in Russian].

9. Aromatic hydrocarbons removal by immobilized bacteria *(Pseudomonas sp., Staphylococcus sp.)* in fluidized bed bioreactor / J. Taoufok [et al]. Annals of Microbiology. 2004;54(2):189–200.

10. Biodegradation of phenol in synthetic and industrial wastewater by *Rhodococcus erythropolis* UPV-1 immobilized in an air-stirred reactor with clarifier / MB. Prieto [et al]. Applied Microbiology and Biotechnology. 2002a;58(6):853–859.

11. Camargo AC, Pizzolitto EL. Biofilm formation on catheters used after cesarean section as observed by scanning electron microscopy. International J. of Gynecology and Obstetrics. 2005;90:148–149.

12. Degradation of phenol by *Rhodococcus erythropolis* UPV-1 immobilized on Biolite in packed-bed reactor / MB. Prieto [et al]. Journal of Biotechnology. 2002b;97(1):1–11.

13. Gudzenko Tatyana, Wolodymyr Iwanycja, Olga Woljuwacz, Boris Galkin, Olga Zuk, Elena Gorszkowa. Biodegradacja fenoli i nnych cyklicznych związków aromatycznych. - Publisher: GlobeEdit is a trademark of International Book Market Service Ltd., member of OmniScriptum Publishing Group, 17 Meldrum Street, Beau Bassin 71504, Mauritius. 2018:85.

14. Nor Suhaila Yaacob, Rosfarizan Mohamad, Siti Aqlima Ahmad . The influence of different modes of bioreactor operation on the efficiency of phenol degradation by *Rhodococcus* UKMP-5M. Rendiconti Lincei. 2016;27(4):749–760.

15. Songwen Tan, Xuncai Chen, Chunzhi Cui, Yang Hou, Weiguo Li, Hong You. Biodegradation of saline phenolic wastewater in a biological contact oxidation reactor with immobilized cells of *Oceanimonas* sp. Biotechnology Letters. 2017;39(1):91–96.

16. Patent of Ukrai'ny №97747. Method of aerobic biological treatment of sewage / Gvozdyak PI, Globa LI, Sablij LA, Kaparnyk AI, Borysenko OO, Zhukova VS. Opubl. 12.03.2012. Byul. № 5 [in Ukrainian].

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