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# MICROBIOLOGICAL METHOD OF PURIFICATION OF WATER FROM PHENOL ASSOCIATION OF NON-PATHOGENIC BACTERIA STRAPS OF THE GENUS *PSEUDOMONAS*

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Abstract

A microbiological method for purifying water from phenol has been developed, which consists in using phenol-bacterial association of strains of non-pathogenic bacteria of the genus *Pseudomonas: P. cepacia* ONU-327 and *P.* fluorescens ONU-328, taken in a volume ratio of 1: 1. When using a biopreparation on the basis of the association of the bacterial strains under study, the oxidation of phenol is accelerated 2.2 times as compared to the treatment of phenol-containing water with monocultures. Deep purification of water from phenol with a concentration of 300 mg/l occurs within 10 days with a single injection of bacterial cells in an amount of $7.5\times10^5$ CFU/ml, pH 7, a temperature of 28-30 °C. It has been established that the bacterial association of strains of *P. cepacia* ONU-327 and *P. fluorescens* ONU-328 is capable not only of effective destruction of phenol, but also possesses a high sorption-accumulating potential for ions of heavy metals [Pb (II), Zn (II) , Cu (II), Cr (V)], which reveals the prospects of its use in the biopreparation for the purification of multicomponent pharmacies, with the predominant content of phenolic contaminants.

*Keywords:* purification of water, phenol, association of destructors, *P. cepacia* ONU-327, *P. fluorescens* ONU-328

## Introduction

Phenol is a toxic environmental pollutant. Its maximum permissible concentration (MPC) in the water of reservoirs used for fishery purposes is small and is 0.001 mg/l [1]. Therefore, the problem of finding an effective method for purifying water from phenol is topical.

To date, the microbiological method of water purification from phenolic and other cyclic aromatic compounds, based on the use of the potential of heterotrophic biochemically active microorganisms, capable of consuming a wide range of organic compounds, including aromatic xenobiotics as a substrate for growth and development, is the most environmentally friendly, reliable , in a non-volatile way in comparison with the existing physicochemical methods; does not create secondary hazardous waste and does not require the use of chemical reagents, additional oxidants.

There is information about the mineralization of phenol to simple compounds during its biodegradation with microorganisms *Aspergillus niger* [2], the Indian strain Staphylococcus aureus isolated from Amla Khadi, Ankleshwar [3], halophilic fungi (*Aspergillus, Pencillium, Fusarium*) isolated from sediments along the Gulf of Suez and sediments of the Red Sea [4].

At achievement of high results on water purification from phenol the main disadvantage of the above-mentioned microbiological methods is the use of microorganisms-destructors possessing pathogenic or conditionally pathogenic properties, and therefore are not recommended for use in biotechnologies for cleaning the environment from phenol.

The aim of the work is to offer an association of non-pathogenic strains of bacteria of the genus *Pseudomonas* with increased biochemical activity against phenol.

**Materials and methods**

Two biochemically active non-pathogenic strains of microorganisms were used as the objects of the study. By fatty acid composition, whose spectra were obtained on a Agilent 7890 gas chromatograph and decrypted using the library database program RTSBA6 6.21 MIDI Sherlock, Investigated strains with a high index of similarity (Sim Index ≥ 0,72) identified as *Pseudomonas cepacia* (isolated from soils) and *Pseudomonas fluorescens* (isolated from the marine environment). Strains *Pseudomonas* spp. stored in the collection of microorganisms of the Department of Microbiology, Virology and Biotechnology of Odessa I.I. Mechnikov National University: *Pseudomonas ceracia* ONU-327, *Pseudomonas fluorescens* ONU-328.

The concentration of phenol in water samples was determined by a photometric method based on the formation of colored phenol compounds with 4-aminoantipyrine in the presence of potassium hexacyanoferrate (III) at pH 10.0. The experiments were performed in five replicates. The results are processed using MS Excel 2003.

**Results and discussions**

The results of microbiological purification of water from phenol in the presence of individual strains of microorganisms *P. cepacia* ONU-327, *P. fluorescens* ONU-328 and their associations obtained at 30 °C are shown in fig. 1.

The effectiveness of the microbiological method of water purification from phenol has been experimentally confirmed. In laboratory conditions it has been established that the strains of microorganisms used are resistant to high concentrations of phenol (300 mg/l) and have a phenol-oxidizing ability, especially in association.

The proposed method is that the water containing phenol is purified by bacterial association of *P. cepacia* ONU-327 strains and *P. fluorescens* ONU-328 (1: 1 by volume), the bacteria are cultured for 24 hours at 30 ° C for MPA, then the bacterial cells are suspended in M-9 medium containing up to 300 mg / l of phenol and kept for 10 days (fig. 1). With the introduction of contaminated water into the strain *P. cepacia* ONU-327 in an amount of $7.5\times10^5$ CFU/ml, the degree of water purification from phenol on day 10 reached ~ 45%. The use of strain *P. fluorescens*

ONU-328 increased the efficiency of water dephenolization up to 78% on the 10th day (fig. 1).

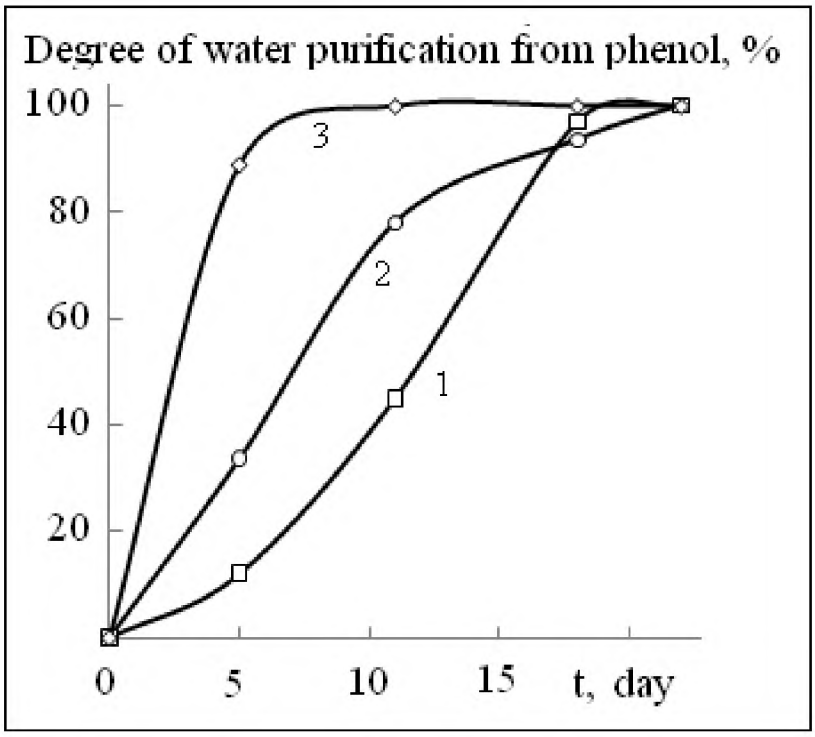


**Fig 1. Degree of water purification from phenol (%) for a time (t, day) in the presence of strains of *P. cepacia* ONU-327 (1); *P. fluorescens* ONU-328 (2) and their associations of 1:1 by volume (3). Note: *initial concentration of phenol is 300 mg/l; the concentration of bacterial cells is 7.5 × 105 CFU/ml**

It has been experimentally confirmed that the use of bacterial association of strains of *P. cepacia* ONU-327 and *P. fluorescens* ONU-328 (1: 1 by volume) for the same period (10 days) promotes deep purification of water from phenol - by 100%. The proposed method compared with [5, 6] allows to accelerate the process of water dephenolization 2.2 times.

In order to develop recommendations for the use of a method for purifying effluents from the production of pharmaceutical preparations that contain a large amount of phenolic contaminants, as well as heavy metal ions, the biotechnological properties of the strains studied and their associations with respect to inorganic pollutants were additionally tested. It has been established that strains of microorganisms possess a high sorption-accumulating ability with respect to metal

ions in solution in cationic form Pb (II), Cu (II), Zn (II); the synergistic effect on Cr (VI) is observed with the use of immobilized bacterial cells in the composition of biofloquial cells of the association of strains of microorganisms *P. cepacia* ONU-327, *P. fluorescens* ONU-328 (taken in a volume ratio of 1: 1). Thus, the bacterial association of strains *P. cepacia* ONU-327, *P. fluorescens* ONU-328 has a wide spectrum of biotechnological action: high destructive potential for phenolic compounds and sorption-accumulating for ions of heavy metals, which reveals the prospects of using bacterial association of microorganisms in the composition biopreparation for the purification of multi-component effluents, with a predominant content of phenolic contaminants.

**Conclusions**

Microbiological method for purifying water from phenol has been developed, which consists in using phenol-bacterial association of strains of non-pathogenic bacteria of the genus *Pseudomonas*: *P. cepacia* ONU-327 and *P. fluorescens* ONU-328, taken in a volume ratio of 1: 1. When using a biopreparation on the basis of the association of the bacterial strains under study, the oxidation of phenol is accelerated 2.2 times as compared to the treatment of phenol-containing water with monocultures. Deep purification of water from phenol with a concentration of 300 mg/l occurs within 10 days with a one-time injection of bacterial cells in an amount of $7.5 \times 10^5$ CFU/ml, pH 7, a temperature of 28-30 °C. The proposed method is suitable for widespread use in biotechnology of effluent treatment of pharmaceutical preparations containing, in addition to phenolic compounds and ions of heavy metals, due to the high phenol-oxidizing capacity of the bacterial association and its storage-accumulating effect on heavy metal ions.

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