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**BIODESTRUCTION OF PHENOL BY NON-PATHOGENIC
BACTERIA OF THE GENUS *PSEUDOMONAS***

Abstract

It has been experimentally established that two strains of bacteria genus *Pseudomonas*, isolated from the marine environment and identified by the fatty acid composition of their cellular lipids as *P. fluorescens* ONU328, *P. maltophilia* ONU329, are not pathogenic and have a high phenol-oxidizing ability. Bacteria are cultured for two days at a temperature of 30 °C on MPA, further bacterial cells are suspended in the mineral medium M-9, and in the optimum amount is introduced into the contaminated water containing up to 300 mg/l phenol. It was established that the strain of *P. maltophilia* possessed a greater phenol-oxidizing activity than the strain *P. fluorescens*. When water was treated with strain *P. maltophilia* with a culture concentration of 3.5×10^4 CFU/ml, deep purification from phenol was observed on 34 day, and with an increase in the culture concentration to 7.5×10^5 CFU /ml on 18 day, $t=30$ °C.

Key words: biodegradation, phenol, bacteria of the genus *Pseudomonas*

Introduction

The modern development of chemical, medical and pharmaceutical production causes a powerful release into the environment of phenolic compounds (PC) that have a toxic effect. Energy dependence and high cost of physical and chemical technologies for neutralizing phenol-containing wastewater stimulates the need for widespread introduction of effective, ecosafety and non-volatile biological methods in the purification of such effluents. A method has been developed for the oxidation of PC using tyrosinase of *Agaricus bisporus* fungi [1]. There are data on the complete mineralization of PC in the process of their biodegradation by pathogenic 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 the sediments of the Red Sea [4].



The aim of the work is to investigate the destruction of phenol by nonpathogenic bacteria of the genus *Pseudomonas*.

Materials and methods

Biochemically active strains of bacteria of the genus *Pseudomonas* spp. The strains were previously isolated from the marine environment and, in the aggregate of morphological, cultural and physiological-biochemical features determined using classical bacteriological methods and the API 50 CHB Medium test system (bioMerieux, France), are classified as *P. fluorescens* ONU328 and *P. maltophilia* ONU329. Additionally, the fatty acid composition, whose spectra were obtained on an Agilent 7890 gas chromatograph and deciphered using the RTSBA6 6.21 library of the MIDI Sherlock program, investigated strains with a high similarity index (Sim Index ≥ 0.72) were identified as *P. fluorescens* ONU328, *P. maltophilia* ONU329. They are not pathogenic and are currently stored in the collection of microorganisms of the Department of microbiology, virology and biotechnology of the Odessa I.I. Mechnikov National University.

To carry out the microbiological method of water purification from phenol, the bacteria were cultivated at a temperature of $28 \pm 1^\circ\text{C}$, in a nutrient medium of the composition (g/l): KH_2PO_4 – 1.5; Na_2HPO_4 – 3.0; NaCl – 5.0; NH_4Cl – 1.0; peptone – 10.0; glucose – 2.0; yeast extract – 5.0 (pH = 7). Biomass build-up was carried out for 48 hours before reaching a culture density of at least 5 g/l in dry biomass. The concentration of phenol in water samples was determined by a photometric method based on the formation of stained phenol compounds with 4-aminoantipyrine in the presence of potassium hexacyanoferrate (III) at pH 10.0 ± 0.1 [5]. The measurements were made on a photoelectric colorimeter at a wavelength of 540 nm. The experiments were performed in five replicates. Statistical processing of the results of the studies was carried out using standard methods of variation statistics using the program «Microsoft Office Excel 2003» with the definition of Student's t-test. The difference was statistically significant for $p < 0.05$.

Results and discussions

Results on the biodegradation of phenol in the presence of marine microorganisms *P. fluorescens* ONU328 and *P. maltophilia* ONU329, obtained at different temperatures, are shown in Fig. 1. It has been experimentally established that the strains of microorganisms used have a high phenol-oxidizing ability at temperatures of 18 and 30°C . The strain of *P. maltophilia* ONU329 as compared to strain *P. fluorescens* ONU328 possessed a greater biochemical activity with respect to phenol with a concentration of 300 mg/l.

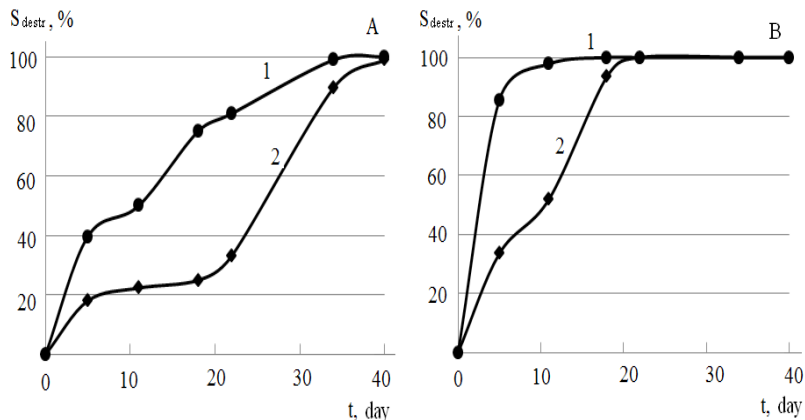


Fig. 1. Kinetic curve of the degree of destruction of phenol* ($S_{destr}, \%$) in the presence of *P. maltophilia* ONU329 (curve 1), *P. fluorescens* ONU328 (curve 2) in an amount of 7.5×10^5 CFU/ml at a temperature: A $-18\text{ }^{\circ}\text{C}$; B $30\text{ }^{\circ}\text{C}$.

Note: * The initial concentration of phenol in water is 300 mg/l.

When water was treated with strain *P. maltophilia* ONU329 with a culture concentration of 3.5×10^4 CFU/ml, deep purification from phenol was observed on day 34, and with an increase in the culture concentration to 7.5×10^5 CFU/ml on day 18 at a temperature of $30\text{ }^{\circ}\text{C}$.

Conclusions

It has been experimentally established that the following conditions are optimal for the deep purification of water from phenol by a microbiological method using the phenol of *Pseudomonas* genus as the destructors: $30\text{ }^{\circ}\text{C}$; culture concentration - 7.5×10^5 CFU/ml; The contact time of microorganisms with phenol-containing water is from 18 to 22 days using the strain *P. maltophilia* ONU329 and *P. fluorescens* ONU328, respectively.

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