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## **FORMATION OF THE MULTISPECIES BIOFILM OF PHENOL BACTERIA-DESTRUCTORS ON NATURAL AND SYNTHETIC CARRIERS IN A BIOFILTER**

### **Abstract**

*Fluorescent microscopy using acridine orange dye confirmed that bacteria phenol-destructors used for water purification formed biofilm on the biofilter media of different nature – zeolite, flaps mussels, synthetic carrier type VII, charcoal, peat, ceramic tubes, sand.*

*Keywords: phenol, purification of water, bacteria -destructors, biofilms, biofilter.*

### **Introduction**

Today priority pollutants of aquatic ecosystems are phenol and its derivatives as by-products of petrochemical enterprises, coal industry, chemical industry, pharmaceutical production, due to their toxicity, ability to accumulate in the environment and sustainability [1].

Sources of phenols in natural waters are drains of petrochemical enterprises, coal industry, mechanical engineering, chemical industry, household drains and drains of pharmaceuticals, dyes, pesticides, phenol-formaldehyde resins and non-ionic surfactants [2].

To prevent negative effects and protect the environment from pollution with phenolic compounds, a biotechnological method is applied using phenol destructors attached to different carriers [3, 4].

The aim of the study was to determine the presence of the bacteria destructors biofilm on carriers of different origin in the biofilter using a fluorescent dye.

### **Materials and methods**

To study the formation of biofilm by museum strains of bacteria:

- *Aeromonas ichthiosmia* ONU552
- *Bacillus subtilis* ONU551
- *Pseudomonas maltophilia* ONU329
- *Pseudomonas fluorescens* ONU328
- *Pseudomonas cepacia* ONU327

on carriers of natural origin (zeolite, ceramic tubes, mussel doors, peat, coal, sand) and synthetic fibers (VII) fluorescent dye acridine orange was used [5].

All carriers were removed from the flow filter after 10 days of operation and treated with 96% ethanol for 15 minutes, after the carriers were stained by immersion in 1% acridine orange solution for 4 minutes. Then all the carriers were washed with water and dried on slides.



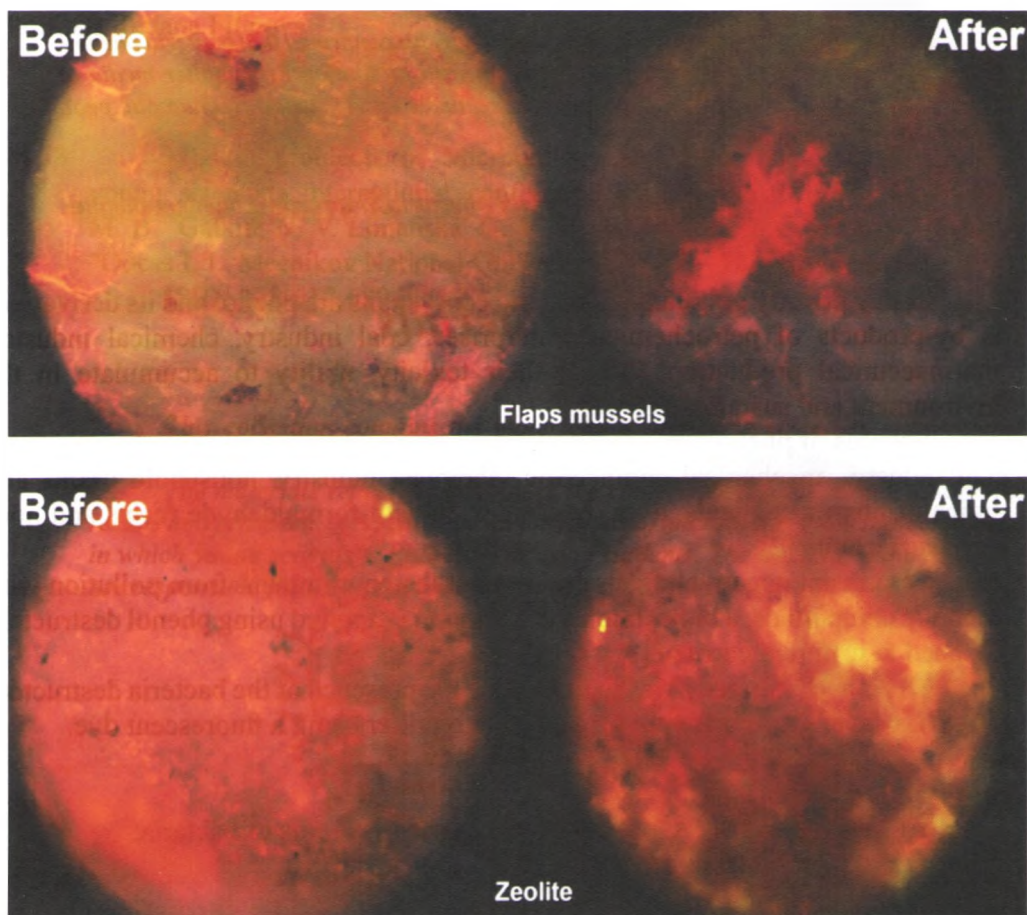
The samples were analyzed under a Carl Zeiss fluorescence microscope and a Carl Zeiss, Primo Star light microscope with photo-fixation.

Sterile carriers and fixed smears of the above strains treated with 1% acridine orange served as controls.

### Results

The study of the formation of biofilm by strains destructors on carriers showed that on each carrier a biofilm is formed in different ways and in different volumes.

A visual comparative analysis showed (Fig. 1) that mussels, peat, zeolite, peep and ceramic tubes have the formation of a clearly visible biofilm.



**Fig. 1. Photographs of the microbial strains association biofilms on mussel valves and zeolite obtained by fluorescence microscopy after staining with acridine orange**

During visual analysis of activated carbon surface under the light and fluorescent microscope the formation of biofilms was not observed. Only single cells in cracks and pores are observed when analyzed on a fluorescence microscope. On synthetic VII carriers no changes were detected after treatment with a fluorescent dye.



## References

1. Часова Э. В. Эколого-химические характеристики и методы защиты окружающей среды от фенола / Э. В. Часова, В. В. Ивчук // Вестник Криворожского национального университета. – 2013. - №34(1). – С. 209–213
2. 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. (ISBN: 978-613-8-25347-1). – 85 p.
3. 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). – P. 749-760.
4. Songwen Tan, Xuncaï 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. – Vol. 39, Issue 1, P. 91–96
5. Биопленки: основные методы исследования: учебно-методическое пособие / Марданова А.М. с соавт. – Казань: К(П)ФУ, 2016, – 42 с.