

## A BIODEGRADATION ACTIVITY OF MICROBIAL ASSOCIATIONS

Ignat Abrashev<sup>1\*</sup>, Rajcho Rachev<sup>1</sup>, Todor Donev<sup>2</sup> and Mariana Petrova<sup>1</sup>

<sup>1</sup>Institute of Microbiology, Bulgarian Academy of Sciences, "Acad. G. Bonchev" st., bl. 26, 1113 Sofia, Bulgaria; <sup>2</sup>National Bank for Industrial Microorganisms and Cell Cultures, 1113 Sofia, P. O. Box 239, Bulgaria

### Summary

*A microbiological and chromatographic analysis of water samples from 10 oxidizing lakes was performed. These lakes were artificially created in order to prevent the ecological pollution of the area of refinery Plama Ltd, Pleven. The chromatographic analysis detected 20.4 mg/L average concentration of the decomposed oil in the lakes. Inversely proportional correlation between the amount of the bacterial cells and the concentration and the structure of the oil hydrocarbons was observed.*

### Introduction

The presence of crude or refined oil in the water basins stimulates the growth of those microorganisms, which can adapt to the changed living conditions. Some of these microorganisms can decompose oil products. The process of selection of such microorganisms is especially important due to their potential application in cases of accidents with oil-transporting tankers, etc. The main components of the oil are the alkanes (in almost all kinds of oil they represent 20-60 %) [3, 12, 15]; the cycloalkanes - cyclopentol and cyclohexane hydrocarbons, pentalone, hyndrin-dane, decalin, norbornane, adamantane, hole-spene, lupane; the arenas - benzol, diphenol [16], naphthalene [2, 7], diphenylmethane, pyrene [8, 4], phenanthrene [10], fluoranthene [15, 10], anthracene [5], chrysene; sulphur-, oxygen-

and nitrogen-containing compounds and some inorganic substances.

The mechanisms of the microbial influence on the oil products are complicated. They depend on both the external factors and some genetically determined properties of the microorganisms. The ones with mon-oxygenase enzyme system can oxidize the paraffins to form the corresponding alcohols (*Methylococcus caosulphatus*, a range of species from the genus *Pseudomonas*) [6, 14]. In the aerobic methanotrophs the scheme of biological oxidation methane-methanol-carbon dioxide is catalyzed by the methane mon-oxygenase. The alkanes are oxidized to their corresponding 1,2-epoxides [6]. Some microorganisms decompose the aromatic and cyclic alkanes by attacking their aromatic ring

or their side alkaline chain, for example the cyclopropane is oxidized to cyclopropanol, toluene – to benzene alcohol and cresol. The oxygenase enzymes (monooxygenases and dioxygenases) catalyze the decomposition of the aromatic ring [18]. The growth coefficients for phenol, chlorine- and methyl-substituted derivatives and their corresponding phenoxalkanoic acids show assimilation in the ortho-pathway, which is with 9-23 % higher than in the meta-pathway. In the presence of benzol *Ps. putidae* accumulates cis-1, 2-dihydrobenzol. The microorganisms have the necessary enzyme capacity for oxidation of the polycyclic aromatic hydrocarbons (PAH) from

naphthalene to nanspyrene [5]. The aromatic hydrocarbons with two or three rings are more easily oxidized than PAH. Some bacteria form cis-dihydrodiols. Yeasts and fungi metabolize the aromatic hydrocarbons to catechols by means of monooxygenase and epoxide hydrolase. The intermediate stages of the process include the formation of epoxide and transdiol [13]. Some lignolytic fungi oxidize PAH to hynones and this reaction is catalyzed by lignin peroxidases. The aromatic and the naphthalene fractions are oxidized with lower speed [8]. The oxidation of the paraffin hydrocarbons of medium molecular mass is the fastest.

## Materials and Methods

The samples for the microbiological and chemical analysis were taken from 3-10 points at a different depth from 10 oxidizing lakes. The lakes were situated in the region of the oil refinery Plama Ltd, Pleven. The general microbial number and amount were determined according to the Koch's method on solid nutrient media: nutrient agar, beer agar, Krasilnikov's glucose-nitrate medium (CP-1) [9].

"Erba Science" gas chromatograph was used for the analysis of the oil hydrocarbons from mean water samples according to a modified method of EPA (Environmental Protection Agency). The water samples were transferred through a column full of Amberlite XAD-2 macroporous resin. The procedure was followed by washing with distilled water and eluting with acetone. The extracts were evaporated under vacuum in a rotary evaporator until dry mass was received. The residual substance was weighted and dis-

solved in tetrachlormethane. 1 µl from this solution was introduced in the gas chromatograph, which was supplied with capillary column DB 5 (25 m x 0.22 mm). Standard samples from C<sub>5</sub>-C<sub>40</sub> paraffins and PAH (Macherey Nagel, Düren, Germany) were used for identification of the components. By extraction of the samples with diethyl ether, followed by drying with sodium sulphate and evaporation in a vacuum evaporator, the residual oil products from the active slime were separated.

Thin layer chromatography was applied for PAH identification. 0.25 mm thin silica gel (Macherey Nagel,) was used as a sorbent. The experiment was carried out for approximately 0.5 h in disolvent hexan-benzene in conditions of darkness. Bands representing PAH were identified in the sample extracts by comparison to PAH standards (Macherey Nagel) under UV light.

## Results and Discussion

Gas chromatography was applied for qualitative and quantitative analysis of the oil products (Fig. 1). This analysis revealed that normal C<sub>18</sub>-C<sub>30</sub> paraffins were presented in the investiga-

ted lakes. Some peaks were caused by olefins or paraffins with branched chains. A compound with retention time 36.8 min was found in the samples. It was suggested to be 3,4-benzpyrene.

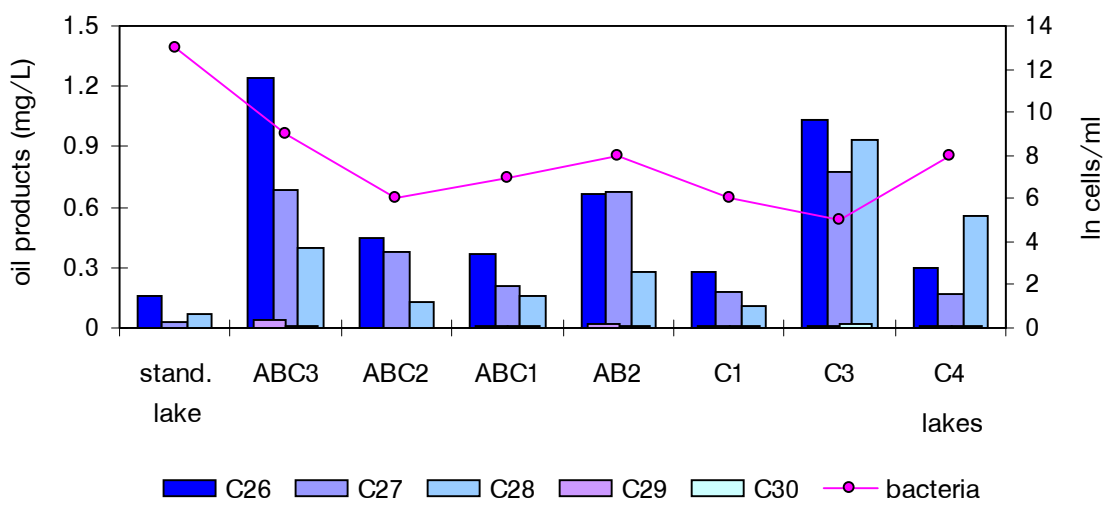
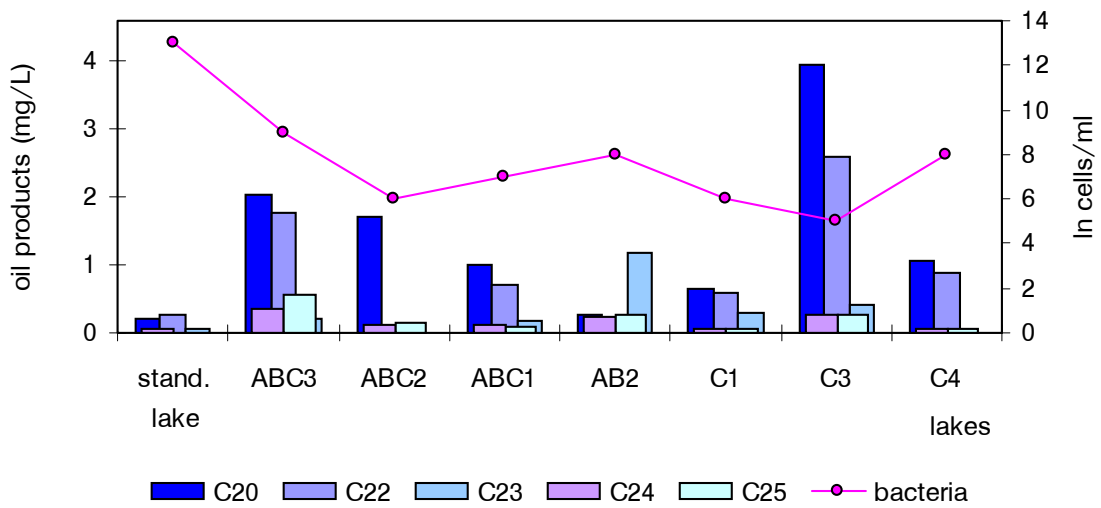
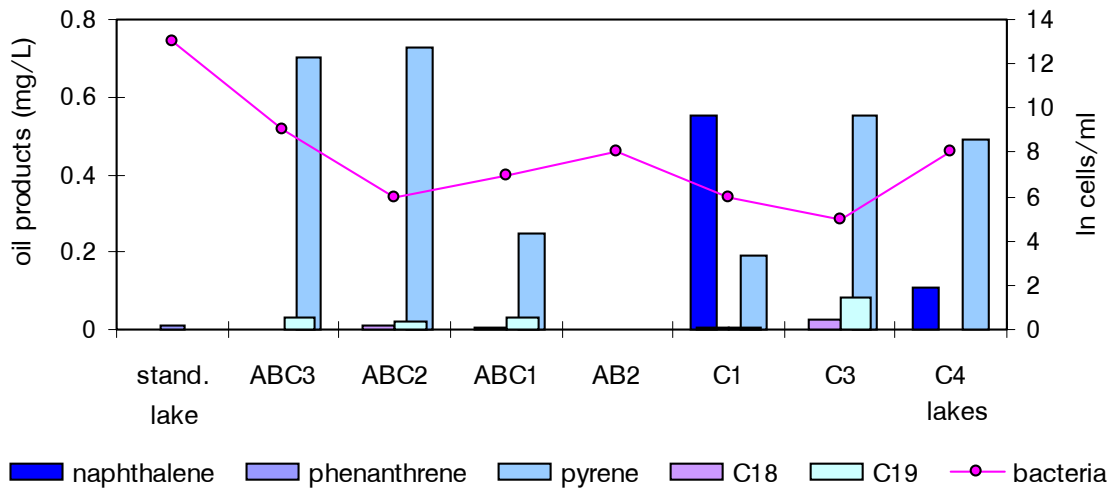


Fig. 1. Oil hydrocarbons and bacterial amount in the investigated lakes.

The thin layer chromatography confirmed the presence of 3,4 benzpyrene in the samples. PAH such as naphthalene, phenanthrene and pyrene were also detected in the samples. The chromatograms of the extracts proved that undecomposed oil was presented in high concentration in the investigated lakes. These chromatograms were identical with the ones of the standard crude oil. At the same time they considerably differed with the ones of the water samples taken at the lake outflows due to the presence of naphthalene fraction (lakes C1, C4).

The results indicated that the active slime from the oxidizing lakes was characterized by concentration of the oil products 20 times as high as the one in the standard lake (up to 1.5-2.0 g/L). The microbiological analysis revealed that the general microbial number on nutrient agar was  $3.6 \times 10^4 - 4.7 \times 10^5$  cells per ml. In comparison with the other investigated taxonomic groups the bacteria from the lakes showed the best adaptive po-

tential (Fig.1). The representatives of the genera *Pseudomonas* and *Bacillus* showed high biodegradative ability.

The standard lake was characterized by low concentration of chrysene, phenanthrene, C<sub>22</sub>-C<sub>30</sub> hydrocarbons and lack of naphthalene, pyrene and C<sub>21</sub>. The highest bacterial amount was detected there. The microbial amount decreased due to the presence of pyrene (0.73 mg/L), C<sub>24</sub>-C<sub>26</sub> hydrocarbons (0.19 mg/L), C<sub>22</sub>-C<sub>26</sub> hydrocarbons (0.55 mg/L) in lakes ABC2, C1 and C3, respectively

The performed investigation indicates that the high concentration of hydrocarbons in the water causes decrease in the microbial number and variety. The bacteria from the genera *Pseudomonas* and *Bacillus* are characterized by high adaptive and degradative potential. They can be introduced in future bioremedial technologies for purification of the oxidizing lakes.

## References

1. Abrashev, I., R. Rachev, T. Donev, 2000-2002. *J. Culture Collections*, **3**, 38-42.
2. Auger, R. A., J. Jacobson, M. M. Domachq, 1995. *J. Hazard. Mat.*, **43**, 263-269.
3. Berecaa, M., A. Steinbuchel, 2000. *Appl. Environ. Microbiol.*, **66**, 4465-4467.
4. Boldrin, B., A. Tiehm, C. Fritsche, 1993. *Appl. Environ. Microbiol.*, **59**, 1927-1930 .
5. Bouchez, M, D. Blanchet, J. P. Vandecasteele, 1995. *Appl. Microbiol. Biotechnol.*, **42**, 156-164.
6. Colby, J., D. I. Stirling, H. Dalton, 1977. *Biochem. J.*, **165**, 395-402.
7. Davies, J. A., W. E. Evans, 1964. *Biochem. J.*, **91**, 251-261.
8. Ghoshal, S., R.G. Luthy, 1998. *Biothechn. Bioeng.*, **57** (3), 356-366.
9. Gushterov, G., P. Andonov, C. Todorov, L. Kominikov, M. Gintcheva-Starcheva, 1977. *Practice in Microbiology and Virology*, Sofia: Nauka i izkustvo (in Bulgarian).
10. Hammel, K. E., W. Z. Gai, B. Green, M. A. Moen, 1992. *Appl. Environ. Microbiol.*, **58**, 1832-1838.
11. Mueller, J. G., P. J. Chapman, B. O. Blattman, P. H. Pritchard, 1990. *Appl. Environ. Microbiol.*, **56**, 1079-1086.
12. Schroder, E., H. J. Rehm, 1981. *Appl. Environ. Microbiol.*, **12**, 36-38.
13. Sheril, L. M., T. Stowu, 1980. *J. Bacteriol.*, **17**, 209-215.
14. Tong, G. M., C. J. Knoweles, D. E. F. Harrison, I. J. Higgins, 1975. *FEBS Lett.*, **44**, 106-110.
15. Van Eyk, J., T. J. Bartels, 1968. *J. Bacteriol.*, **34**, 671-676.
16. Weissenfels, W. D., M. Beyer, J. Klein, H. J. Rehm, 1991. *Appl. Microbiol. Biotechnol.*, **34**, 528-535.
17. Woodzinski, R. S., D. Larocca, 1997. *Appl. Environ. Microbiol.*, **33**, 660-665.
18. Wunder, T., S. Kremer, O. Sterner, H. Anke, 1994. *Appl. Microbiol. Biotechnol.*, **42**, 536-641.

## **БИОДЕГРАДАЦИОННА СПОСОБНОСТ НА МИКРОБНИ АСОЦИАЦИИ**

Игнат Абрашев<sup>1\*</sup>, Райчо Рачев<sup>1</sup>, Тодор Донеv<sup>2</sup>, Мариана Петрова<sup>1</sup>

### **Резюме**

*Извършен е микробиологичен и хроматографски анализ на водни проби от 10 окислителни езера (изкуствени езера, създадени с цел предпазване от екологично замърсяване) от района на "Плама АД", Плевен. Хроматографският анализ показва, че концентрацията на неразградения нефт в тези езера е средно 20.4 мг/л за всяко езеро. Установена е обратно пропорционална зависимост между количеството на бактериалните клетки и концентрацията и структурата на наличните в езерата нефтени въглеродороди.*