

INVESTIGATION OF THE MICROBIAL ASSOCIATIONS IN WATER BASINS POLLUTED WITH OIL

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Summary

Microbial associations from 10 oxidizing lakes, situated in the region of the oil refinery Plama Ltd, were investigated. The general number of microorganisms for each taxonomic group was determined. The isolated oil-assimilating strains of bacteria, fungi and actinomycetes belonged mostly to the genera Pseudomonas, Bacillus, Penicillium, sections Aureus and Cynereus. The inhibiting effect of the oil products and the ability of the isolated microorganisms to assimilate oil were studied.

Introduction

The process of decomposition of oil and oil products in the natural and artificial water basins is realized mainly by the autochthonic microflora. It includes bacteria, actinomycetes, filamentous fungi and yeasts. These microorganisms are adapted to the specific conditions of the environment (such as pH, humidity, temperature, chemical composition, concentration of oxygen, nitrogen and phosphorus). The rich in organic compounds active slime is a favorable medium for the oil-assimilating microorganisms [16]. In the pure water basins the oil-decomposing microorganisms represent 1 % from the whole heterotrophic population. In waters polluted with oil their amount reaches 10 %.

The following main groups and representatives of groups of oil-assimilating micro-

organisms are distributed in nature. They can live independently or in syntrophy with other species:

- Bacteria from the family *Pseudomonaceae* - genus *Pseudomonas* (*Ps. aeruginosa* [13], *Ps. stutzeri*, *Ps. putida*, *Ps. paucimobilis* [10] - its representatives can assimilate alkanes, aromatic and polycyclic aromatic hydrocarbons), genus *Xanthomonas* and genus *Zoogloea*; family *Enterobacteriaceae* - genera *Escherichia*, *Enterobacter* and *Proteus*; family *Bacillaceae* - genera *Bacillus* (*B. sphaericus*, *B. licheniformis* [8,11], *B. coagulans*) and *Clostridium*; genus *Micrococcus*; genus *Corynebacterium* (*C. flavescens*); genus *Achromobacter*, genus *Alcaligenes* (*A. faecalis*); genus *Mycobacterium* (*M. fortuitum*, *M. ratisbonense* - its representatives assimilate normal and

isoalkanes with high molecular mass and oil hydrocarbons with more complicated structure [2]); methanotrophic bacteria from the genera *Methylomicrobium*, *Methylosinus*, *Methylocystis*, *Methylococcus*, *Methylobacter* [5,18].

- Yeasts from the genera *Saccharomyces*, *Candida*, *Rhodotorula*, *Torulopsis*, *Trichosporon* [6]. They assimilate mainly normal alkanes with medium molecular mass (C₁₂ - C₂₂).

- Actinomycetes from the genus *Streptomyces* [14].

- Fungi from the class *Deuteromycetes* - *Aspergillus niger*, *Penicillium janthinellum*, *Crinipellis stipitaria*, *Phanerochaete chrysosporium*, *Rhizoctonia solani*, *Pleorotus ostreatus* [12].

Strains from the same species (genus *Pseudomonas*) and from different species (*Stenotrophomonas maltophilia*, *P. janthinellum*) can realize co-metabolic processes. This way they can perform low effective assimilation [17].

Materials and Methods

The samples were taken from ten oxidizing lakes within the period July – October 1995. The lakes were situated in the region of the oil refinery Plama Ltd., Pleven. The samples were taken from 3 to 10 points at a different depth. They include water from the lakes, bottom (active) slime and fuller's earth polluted with oil. The control samples were taken from a standard lake. It was characterized by very low concentration of the available oil products. The general number of microorganisms (cells per ml or g) was determined on agar media - nutrient agar (NA), beer agar (BA), Krasilnikov's medium (CP-1) and Erochin medium (EP) [3,4]. The period for bacterial incubation lasted for 24-48 hours at 37°C. Yeasts and fungi were incubated for 48 to 240 hours at 28°C, actinomycetes -

for 249 to 280 hours at 28°C.

The methods for proving the microbial ability to assimilate oil products were based on the application of the salt-containing media CP-1 and EP. They contained minimal amount from a carbon source and 1.5 % crude oil. Two methods for selection of oil-decomposing microorganisms were applied: a test on a solid nutrient medium and a test on a liquid one [15].

The identification of the isolated strains was performed according to standard procedures [1, 3, 7].

After the extraction with diethyl ether, drying with sodium sulphate and evaporation in a rotary vacuum evaporator the amount of the oil products was determined.

Results and Discussion

The chemical analysis revealed that the concentration of the oil products in the active slime of the standard lake was 8 times as high as the one at its outflow. For the lake ABC3 the concentration of the oil products at the lake outflow was 23.6 times as high as in its active slime. For the lake ABC1 it was 10 times as high, for the lake AB1 - 9.6 times.

The results from the microbiological investigations with the four selective nutrient media showed that the general number of microorganisms in the lakes was within the interval 3.6×10^4 - 4.7×10^5 (Fig. 1).

Fig. 2 presents the microbial distribution in systematic groups and in dependence on the concentration of the residual oil in each lake.

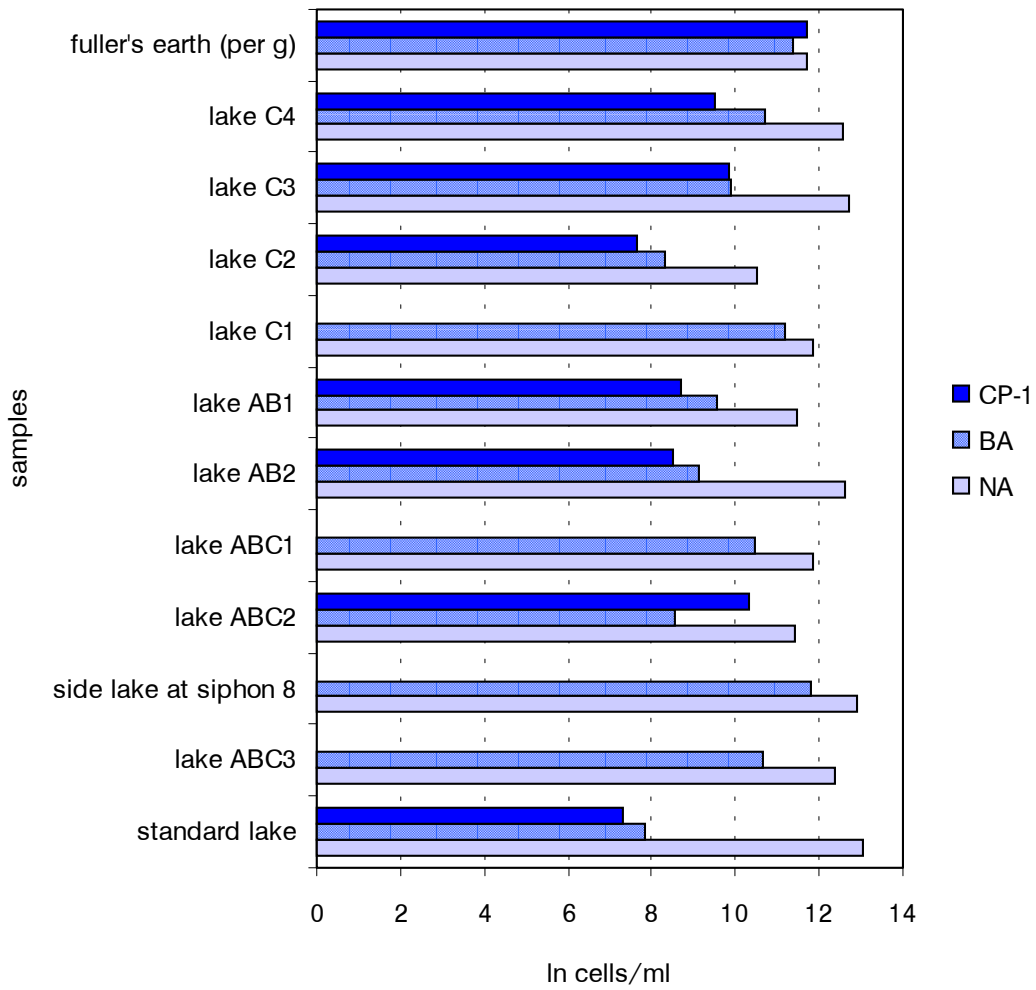


Fig. 1. The microbial amount in the investigated lakes.

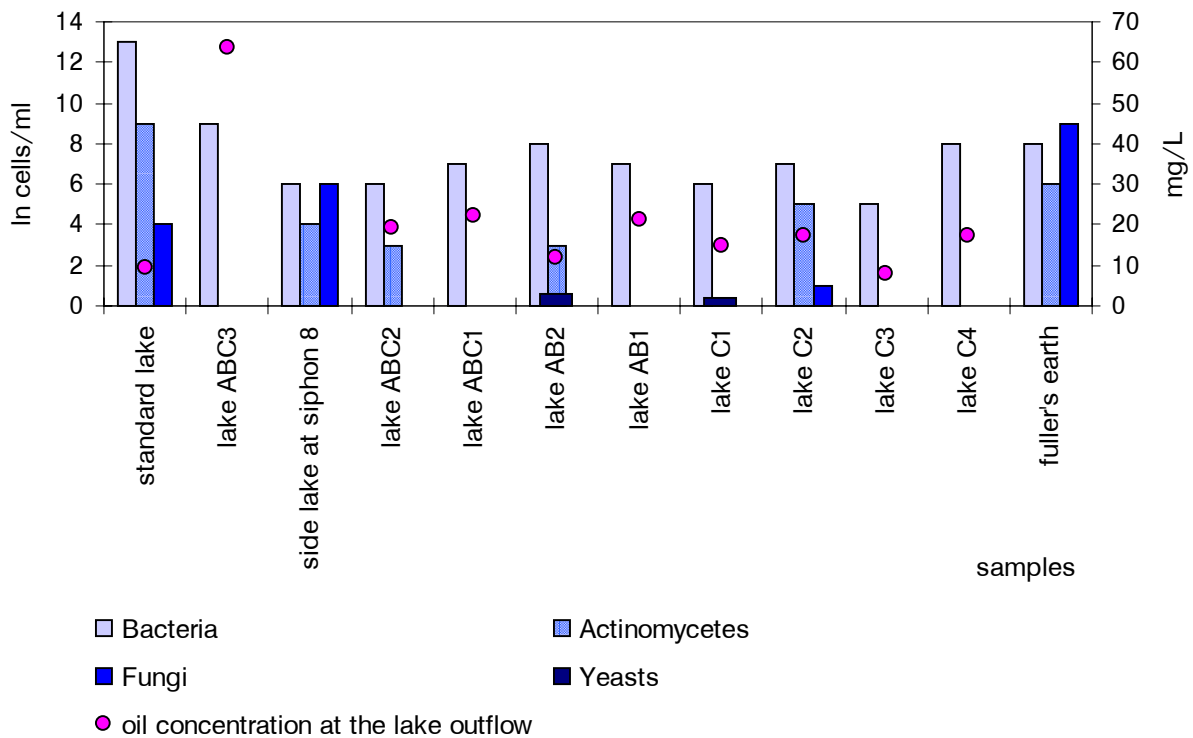


Fig. 2. The amount of the oil products and the number of the investigated microorganisms.

Bacteria were the predominant inhabitants in all the lakes. They were the main participants in the processes of decomposition of the oil products. The presence of residual amounts of oil products inhibited the development of fungi, actinomycetes and yeasts. They were well distributed in the standard lake as well as in the lakes with low concentration of the oil products. In the investigated lakes their number showed a decrease - only bacteria were isolated from ABC3, ABC1, AB1, C3 and C4. The lake ABC3 was characterized by the highest concentration of oil products - 63.8 mg/ml. The bacterial amount in it was 1.4 fold lower than in the standard lake. The lower concentration of oil products at the outflows of the lakes ABC1, AB1, C3 and C4 (about 4.9 fold lower) was accompanied by 1.2-fold increase in the bacterial number.

The rod-like, spore-producing Gram-positive bacteria predominated among the 90 isolated strains. According to their cultural, morphological and biochemical properties they

were referred to the genera *Pseudomonas*, *Micrococcus*, *Mycobacterium* and *Sarcina*. According to Gauze's guide and based on their complete characterization the actinomycetes were defined as representatives of the genus *Streptomyces*, sections *Aureus*, *Cynereus*, *Hevoloflavus* and *Albus*.

Fungi from the genera *Penicillium*, *Mucor* and *Aspergillus* predominated in the samples (the data are not shown in details).

The results presented in Fig. 2 indicated that the relatively high concentrations of oil products in the water and in the active slime inhibited the development of actinomycetes, yeasts and fungi. Among the investigated taxonomic groups from the lakes bacteria showed the best adaptive potential for decomposition of oil products.

Based on this study we defined bacteria as the main oil-decomposing genera in the lakes. The obtained results necessitate a further investigation of the chemical composition of the oil products and their influence on the microbial population in the lakes.

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ИЗСЛЕДВАНЕ НА МИКРОБНИ АСОЦИАЦИИ В ЗАМЪРСЕНИ С НЕФТ ВОДИ

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Резюме

*Изследвани са микробни асоциации, изолирани от 10 окислителни езера в района на нефтопреработвателната рафинерия "Плама" АД, Плевен. Определен е общият брой микроорганизми поотделно за всяка таксономична група. Изолираните щамове нефт-усвояващи бактерии, плесени и актиномицети принадлежат главно към родовете *Pseudomonas*, *Bacillus* и *Penicillium*, секции *Aureus* и *Supereus*. Проучени са инхибиращият ефект, който оказват присъстващите в средата нефтопродукти, и нефт-усвояващата способност на изолираните микроорганизми.*