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Aromatic Hydrocarbons Decomposition by Neustonic Bacteria
Part II - Policyclic Aromatic Hydrocarbons Biodegradation

M. Walczak[1], W. Donderski[1], Z. Mudryk[2], P. Skorczewski[2]

[1] Department of Water Microbiology and Biotechnology, Institute of Ecology and Environmental Protection, Nicholas Copernicus University, 87 -100 Torun, Poland University of Education, Department of Experimental Biology, 76-200 Słupsk, Poland

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Abstract

The present paper provides results of a study on heterotrophic bacteria occurrence and quantity in the biofilm and subsurface water of lake Jeziorak Maty. Degradation of several polycyclic aromatic hydrocarbons (PAH's) (anthracene, naphthalene) by neustonic and planktonic bacteria have been examined. It has been discovered that some aromatic hydrocarbons were better decomposed by neustonic bacteria than by planktonic ones. Both Total Number of bacteria (TN) and heterotrophic bacteria number (TVC) were higher in the surface microlayer than in the subsurface water.

Keywords! neustonic bacteria, planktonic bacteria, polycyclic aromatic hydrocarbons, surface microlayer

Introduction

The surface of water accompanied by the organic layer formed on the water by chemical and biological components, together form a unique environment for micro-organisms. According to the definition used by Norkrans [1], organic layers have been called a surface microlyaer. Adhesive power resulting from inter-molecular attraction between two phases: water and air contribute to the occurrence of the surface biofilm. Physical stability of the film is provided by the force of surface tension. On the other hand, however, due to extreme temperature conditions, or sunlight amount, and salinity rate it is not very stable as compared to the depths of water [2].

Nutritional conditions here are most favourable for chemoautotrophs as the carbon dioxide, reduced mineral compounds and heterotrophs resulting from a thickened accumulation of organic compounds are within easy reach [3].

Aromatic hydrocarbons are among the chemical factors that most heavily affect ecosystems. Fritsche [4] revealed that about 20% of microorganisms have the facility to decompose hydrocarbons by way of metabolism. Among them were such bacterial strains as *Pseudomonas, Achromobacter, Alcaligenes, Flavobacterium,* and *Micrococcus.* The most frequently occurring are micro-organisms that are able to decompose aliphatic hydrocarbons; but few have the facility to use aromatic hydrocarbons or cycloalcanes. The objective of this study is to measure the degradation extent of polycyclic aromatic hydrocarbons (anthracene and naphthalene) by micro-organisms inhabiting the surface microlayer and the deep water of lake Jeziorak Mały.

Materials and Methods

Study Area

The study was carried out in lake Jeziorak Maty, situated within the city of Hawa and forming a part of the Ilawskie Lake District. This lake has no inflows nor outflows; it is linked only to lake Jeziorak by a narrow and shallow connection at its northern end (1.5 m). Surface area is 26 ha, maximum depth 6.4 m.

Sampling

The water was sampled in June (20th June), July (17th July) and August (21st Aug.) 1999 from three different stations (Fig. 1). It was sampled from the surface microlayer using of four techniques:

1) a glass plate collecting 100 µm thick water layers
2) a plexi-glass plate collecting 150 µm thick water layers
3) Garret's net 1 of 65 µm mesh collecting 250 µm thick water layers
4) Garret's net 2 of 200 µm mesh collecting 300 µm thick water layers

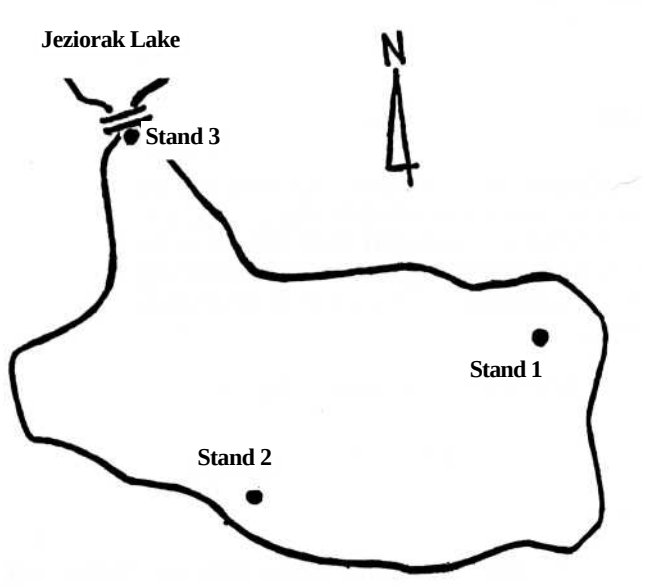


Fig. 1. Outline of lake Jeziorak Maty.

Subsurface water samples were raised from the depth of 20 cm by means of a sterile glass pipette with the use of a Pippet-boy (De Ville) pump. The microlayer and subsurface samples were whisked into sterile glass containers. They were then transported to the laboratory in a thermoinsulated vessel containing ice at ~ 7°C. The time lapse between sampling and analysis was less than 6 hours.

Total Number of Bacteria Counting

Total number (TN) of sampled bacteria was determined by direct counting on 0.22 µm membrane filter (Millipore), under an epifluorescence microscope (Carl Zeiss Jena) in specimens stained with orange acridine [5].

Heterotrophic Bacteria Number

Heterotrophic bacteria number (TVC) was calculated on spread plates. Sterile buffered water was used for diluting [6]. Samples of 0.1 ml volume were then inoculated on Plate Count Agar (Difco) culture. After 6 days of cultivating at 20°C, the heterotrophic bacteria colonies were counted and then, at random, transferred onto iron-peptone semiliquid medium [7]. Having checked the culture purity in Gram preparations, the strains were stored in a refrigerator for further experiments, and transplanted onto a fresh semiliquid medium every two months.

Polycyclic Aromatic Hydrocarbons Degradation Extent Measurement

In order to determine the extent of carbohydrate degradation, the following mineral medium was provided: K_2HPO_4 - 1.0 g; KNO_3 - 0.5 g; $MgSO_4$ x $7H_2O$ - 0.4 g; $CaCl_2$ - 0.2 g; NaCl - 0.1 g; $FeCl_3$ - 0.01 g; glucose - 1.0 g; bacto-peptone - 1.0 g; $H_2O_{(distilled)}$ - 1000 ml; pH 6.8 - 7.2. Portions of 100 ml of the medium were poured into Erlenmayer flasks, sterilsed at 121°C for 20 min and then a particular type of polycyclic aromatic hydrocarbon (anthracene or naphthalene) was added. Target PAH concentration in the medium was 5 mg/1. The media were then inoculated with 1 ml of liquid culture of the studied strain, which was multiplied on the liquid medium (see above). Inoculum was made of bacteria suspension of optic density of 0.2, measured on Spekol spectrophotometer at 565 nm wave length. The inoculated flasks were incubated in a rotation shaker for 72 h at 20°C.

Following the incubation process, the remaining amount of PAH was extracted out of the medium and its concentration was determined after Hermanowicz [8] with the use of a Marcel Pro Eko spectrophotometer. The percentage of hydrocarbon degradation was calculated based on the results obtained.

Results

Table 1 presents the data on Bacteria Total Number (TN) and Heterotrophic Bacteria Abundance (CFU) from the analysed samples. It turns out that TN and TVC in the surface microlayer reached their maximum in July. In May and August the results were a little lower but, on average, similar. In the subsurface water the TN maximum occurred in May, TVC maximum -in August.

The obtained surface microlayer result analysis obtained by different sampling techniques effected in

Table 1. Number of bacteria in the surface microlayer and subsurface water in lake Jeziorak Mały.

Sampling data	Sampling method					Average		E
	a	b	c	d	e	SM	SUB	SM/SUB
June 20.06.99	32.51* 169.1**	27.50 371.0	24.33 79.8	20.4 8	15.67 14.3	26.21 171.3	15.6 7	1.67 11.9
July 17.07.99	48.31 123.3	47.56 353.3	18.12 183.2	41.30 99.9	10.80 19.2	38.82 189.9	10.80 19.2	3.5 9
August 21.08.99	28.52 43.3	46.91 79.3	19.59 200.0	16.31 120.0	10.39 30.0	27.83 110.6	10.39 30.0	2.68 3.6

Explanations: a - sampling by glass plate, b - Plexi-glass plate sampling, c - Garret's net 1 sampling, d - Garret's net 2 sampling, e - subsurface water, SM - surface microlayer, SUB - subsurface water, E - enrichment coefficient, * - Total Bacteria Number x 10^6, ** - heterotrophic bacteria number x 10^3.

a greater bacteria number was collected with the Perplex plate sampling while the least - by Garret' s net 2 sampling.

The subsurface water samples always contained much less bacteria than those recovered from the biofilm.

Figs. 2 and 3 present degradation extent of anthracene and naphthalene. Mean value analyses

Fig. 2. Extent of anthracene degradation [%] by neustonic and planktonic bacteria in Lake Jeziorak Maly.

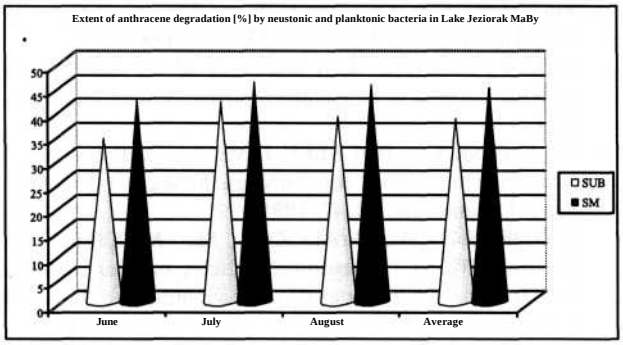
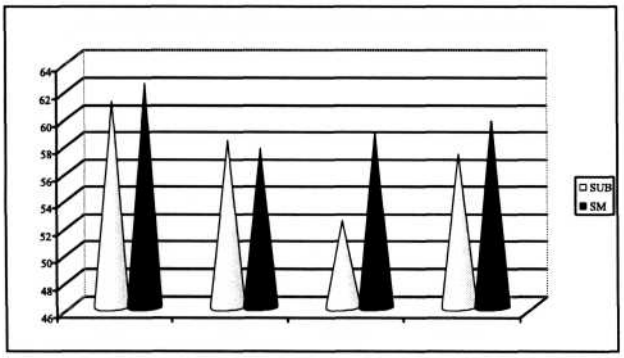


Fig.3. Extent of naphtalene degradation [%] by neustonic and planktonic bacteria in Lake Jeziorak Maty.



(Tab. 2) show that the PAH used in the present study were better decomposed by neustonic bacteria than by planktonic ones. Strains isolated from the biofilm degraded anthracene in 4% on average, subsurface bacteria - 38.2%. What is more, the process of anthracene degradation was most intense among the strains isolated in July (Fig. 2). In the case of naphthalene, it was decomposed by 59.6% neustonic strains on average, and 57.2% planktonic strains (Tab. 2). The greatest decomposition rate was found at bacteria isolated in June (neuston-62.4%, plankton - 61.1%) (Fig.2).

Table 2. Anthracene and naphthalene degraded by neustonic and planktonic bacteria in lake Jeziorak Maly (mean values)

Hydrocarbon	SM	SUB
Anthracene (5 mg/1)	44.4*	38.2
Naphthalene (5 mg/1)	59.6	57.2

SM - surface microlayer; SUB - subsurface water * - percent of degradation.

Discussion

In the 1 mm surface microlayer there occurs a clear gradient of particular chemical components. It is well reflected in number, biomass and species composition of the bacteria inhabiting that environment. The data on Bacteria Total Number (TN) and heterotrophic bacteria number (TVC) presented in this paper go well together with the studies by Niewolak [9]. The Bacteria Total Number in the surface microlayer was, as a rule, 2-3 times greater than in the subsurface water at the depth of 10 cm. The following scientists presented similar data confirming bacterioneuston number overgrowing plankton bacteria in deeper water layers: [10, 11]. Niewolak [12] gave reasons for the greater number of bacteria in the subsurface water, ascribing it to the selective role of UV rays. However, later studies [13, 14] have revealed that pigmentation and plasmids present in cells (with a coded UV resistance) protect bacterioneuston against the harmful influence of those rays. A greater number of heterotrophic bacteria in neuston is somehow a logical

effect of the generally greater total number of bacteria in that water layer. Besides, a greater abundance of organic compounds accumulating in the biofilm positively affects the development of heterotrophic bacterioneuston [15]. Apart from nutrients, in biofilm there occur also substances harmful to bacterioneuston, aromatic hydrocarbon including of both natural and anthropogenic sources.

The study on anthracene and naphthalene showed that microbes were generally able to use those hydrocarbons, and the biodegradation of those compounds was much more extensive when done through the biofilm strains than the strains originating from subsurface water. It may be accounted for by aromatic hydrocarbons' poor solubility in water, yet a perfect solubility in organic solvents and lipids which accumulate in the surface microlayer. Besides, a better PAH degradation in the biofilm may result from the greater amount and variety of microorganisms occurring in that layer than in the surface layer, which had been proven by earlier studies [16]. A lower percentage of lipid decomposing bacteria [17] (along with PAH dissolved in it) in subsurface water causes greater hydrocarbon degradation in the biofilm.

The data obtained in the course of the present study demonstrates that in many cases the same strains decomposed both anthracene and naphthalene. Gosh and Mishara [18] showed that, despite structural differences in substrate construction, decomposition of two and even three polycyclic hydrocarbons by the same strain of bacteria is quite possible. Such a process is usually run by the same enzymatic systems, but enzymes responsible for particular reshaping may occur in various concentrations.

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