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Aromatic Hydrocarbon Decomposition by Neustonic Bacteria

Part I. Single-ring Hydrocarbon Biodegradation

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Abstract

The present paper provides an account of a study on benzene, toluene and xylene degradation by neustonic and planktonic bacteria in lake Jeziorak Maty. Aromatic hydrocarbons were better decomposed by neustonic bacteria than planktonic bacteria. Moreover, the obtained data point to the total number of bacteria and heterotrophic bacteria number being greater in surface microlayer than in subsurface water.

Keywords: Neustonic bacteria, planktonic bacteria, aromatic hydrocarbons, surface microlayer

Introduction

A surface microlayer is formed by accumulating organic compounds, fats, proteins and sugars in particular [1]. Due to polar construction of these compounds they form a stratified film on the surface of water bodies [2, 3]. That layer is a very stable environment for microorganisms in terms of nutritional substances abundance. On the other hand, however, due to extreme temperature conditions, or sunlight, it is not favourable for microbe growth and development as compared to the depths of water. Bacteria inhabiting that extreme environment are affected by many negative physical and chemical factors which determine taxonomic and physiological differences between the bacteria in the biofilm and those at the depth.

Aromatic hydrocarbons are among the chemical factors that most heavily affect ecosystems. Fritsche [4] revealed that about 20% of micro-organisms have the facility to decompose hydrocarbons by way of metabolism. Among them were such bacterial strains as: *Pseudomonas, Achromobacter, Alcaligenes, Flavobacterium, 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 the study was to measure the degradation extent of various aromatic hydrocarbons by micro-organisms inhabiting the surface microlayer and the deep water of lake Jeziorak Maiy.

Material and Methods

The Study Area

The study was carried out in lake Jeziorak Maly. It is situated within the city of Ilawa and makes part of the Ilawskie Lake District. This lake has no tributaries nor outflows; it is only by a narrow and shallow connection at its northern end (1.5 m) that it is linked to lake Jeziorak. The water body surface is 26 ha, the maximum depth 6.4 m.

Sampling

The water was sampled in June (20th June), July (17th July) and August (21st Aug.) 1998 from three different stations (Fig. 1). It was sampled from the surface microlayer by use of four techniques: 1) a glass dish collecting

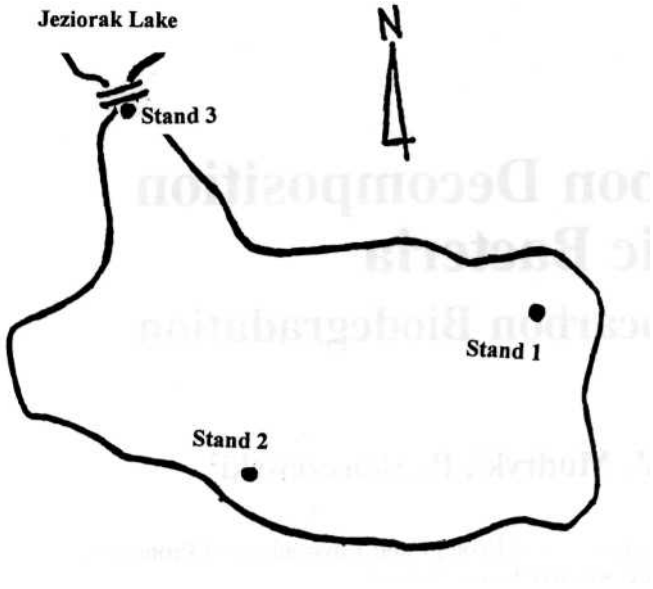

Fig. 1. Outline of lake Jeziorak Maty.

100 µ^i thick water layers, 2) a plexi-glass dish 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.

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 samples were whisked into sterile glass bottles. They were then transported to the laboratory in a thermoinsulated vessel containing ice at ~7°C. Usually the time between the sampling and analysis did not exceed 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 orange acridine stained specimens [5].

Heterotrophic Bacteria Number

Heterotrophic bacteria number (CFU) was calculated on spread plate count method. Sterile buffered water was used for diluting [6]. 0.1 ml volumes of samples were then inoculated on Plate Count Agar (Difco) medium. After 6 days of cultivating at 20°C, the heterotrophic bacteria were counted and then, at random, transferred onto a semisolid medium. The strains were stored in a refrigerator for further experiments, and transplanted onto a fresh semisolid medium every two months.

Hydrocarbon Degradation Extent Measurement

The following mineral medium was prepared for measuring the hydrocarbon degradation extent: 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. Then 100 ml medium doses were placed in Erlenmayer flasks to be sterilized and a specific type of aromatic hydrocarbon was added to them (benzene, toluene or xylene). The eventual benzene concentration in the medium was 5 mg/1, and that of toluene or xylene - 10 mg/1. Thus, the prepared medium was inoculated by 1 ml of the strain's liquid culture, which was multiplied on liquid medium (as quoted above). A bacterial suspension made the inoculum which had optical density of 0.3, as measured at 565 nm wave length on Spekol spectrophotometer. The inoculated flasks were incubated at 20°C in a rotation shaker for 72 hours.

On terminating the incubation, the remaining part of hydrocarbon was split out of the medium and its concentration was determined by means of colorimetric method after Hermanowicz [7], where a Marcel Pro Eko spectrophotometer was employed.

Results

Table 1 presents the data on bacteria total number (TN) and heterotrophic bacteria abundance (CFU) from the analyzed samples. It turns out that TN and CFU in

Table 1. Bacteria number in the surface microlayer and subsurface water in lake Jeziorak Maty. (Number/1).

Date of sampling	Sampling method					Average		E
	a	b	c	d	e	SM	SUB	SM/SUB
June (20.05.96)	32.51* 169.1**	27.50 371.0	24.33 79.8	20.48 65.3	15.67 14.3	26.21 171.3	15.67 14.3	1.67 11.9
July (16.07.96)	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	3.59 9.8
August (21.08.96)	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 – glass plate sampling, 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.

the surface microlayer reached their maximum in July. In June and August the results were a little lower but, on average, similar. In subsurface water the TN and CFU were lower than in the surface microlayer. Maximum TN occurred in June and CFU maximum occurred in August.

Table 2 and Figures 2, 3 and 4 present data on degradation of particular aromatic hydrocarbons. Mean values analysis (Tab. 2) points to the fact that the hydrocarbons used for the tests were more extensively used by neustonic bacteria than the planktonic ones. Benzene was degraded by surface microlayer strains in 65 %, while in the case of subsurface water bacteria degraded in 57.2%. The most intensive benzene degradation occurred with strains isolated in June (Fig. 2). Toluene was best degraded by bacteria isolated in July, both from microlayer (72.7%) and subsurface water (63.2%). On the whole, in the case of toluene, there was a distinct difference between degradation extent done by strains isolated from microlayer (70%) and those isolated from subsurface water (47.1%). Similarly, xylene was easier degraded by neustonic bacteria (69%) than by deep water ones (33.6%). But, at the same time, the greatest percentage of xylene degraded by microlayer bacteria was recorded with the strains isolated in June (Fig. 4).

Table 2. Degradation of hydrocarbons by neustonic and planktonic bacteria in lake Jeziorak Maly.

Name of hydrocarbon	Bacteria	
	neustonic	planktonic
Benzene	65.0*	57.2
Toluene	70.0	47.1
Xylene	69.0	33.6

Explanations: * – values average in percent.

Discussion

Some hundreds of micrometers inside the microlayer and still deeper, physical and chemical factors change, which obviously determines microbe development and biochemical transformation kinetics. This 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 (CFU) presented in this paper go well together with other studies [8]. The bacteria total number in the surface microlayer was, as a rule, 2-3 times greater than in the subsurface water at a depth of 20 cm. The following scientists presented similar data confirming bacterioneuston number overgrowing plankton bacteria in deeper water layers [9, 10]. Niewolak [11] gave reasons for the greater number of bacteria in the subsurface water, ascribing it to the selective role of the UV rays. However, later studies [12, 13] 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 logic effect of the generally greater total

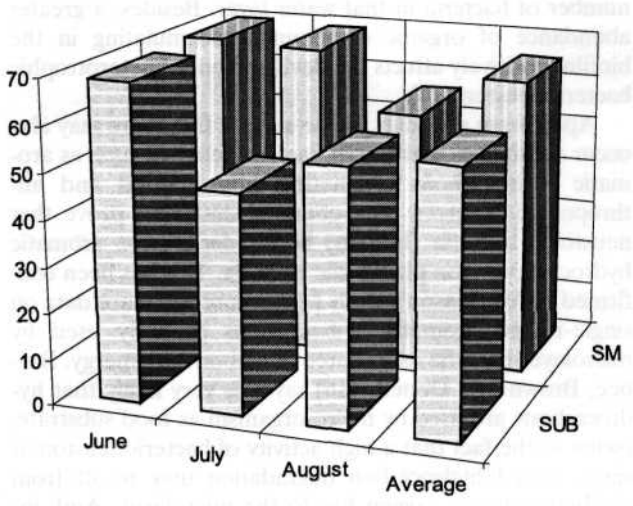

Fig. 2. Benzene degradation extent by neustonic and planktonic bacteria in lake Jeziorak Maly (expressed in %).

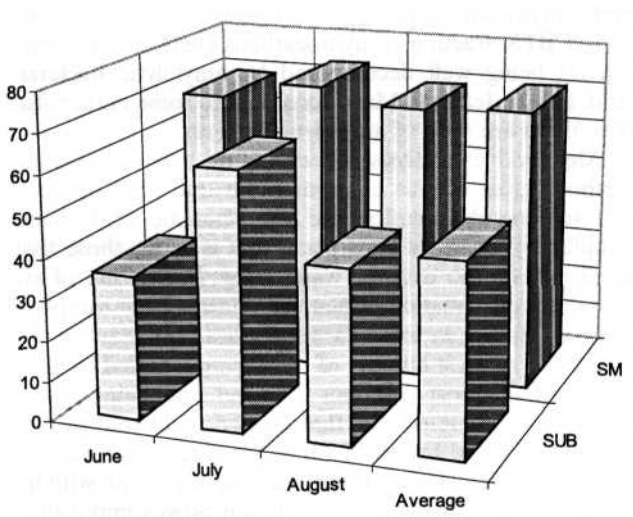

Fig. 3. Toluene degradation extent by neustonic and planktonic bacteria in lake Jeziorak Maly (expressed in %).

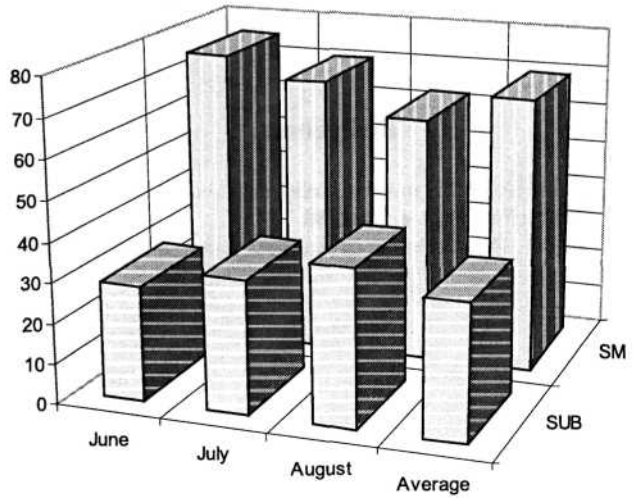

Fig. 4. Xylene degradation extent by neustonic and planktonic bacteria in lake Jeziorak Maty (expressed in %).

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 [14].

Apart from nutrients, in the surface film there may also occur substances harmful to bacterioneuston, such as aromatic hydrocarbons originating from natural and anthropogenic sources. The obtained data best prove that neustonic bacteria (biofilm) better decompose aromatic hydrocarbons than planktonic bacteria. This has been confirmed by Watkinson's study [15], where there are data on single-ringed aromatic hydrocarbons properly used by microlayer bacteria as a source of carbon and energy. Barbee, Brown and Donelly [16] say it is very likely that hydrocarbons are used by micro-organism as food substrate, owing to the fact that a high activity of bacterioneuston at single-ringed hydrocarbon degradation may result from the better access oxygen has to the microlayer. And, indeed, oxygen is indispensable at the primary activation of hydrocarbon particles which takes attaching two oxygen atoms to those particles, and this reaction is catalyzed by a dioxygenasis which is characteristic of prokaryotic enzymatic organism arrangements. Johanson et al. [17] observed BTX fraction of hydrocarbons (benzene, toluene, xylene) being well decomposed by amylolytic bacteria. And, in lake Jeziorak Maty, according to some earlier studies, there are 48% of amylolytic bacteria.

Mean values analysis concerning microlayer sampling methods gives way to an assumption that high benzene and toluene degradation are characteristic of bacteria sampled by means of glass plate, that is to say those that dwell in the most external water layer. In the case of xylene the same situation occurs with Garret 1 net sampling method. Having in mind earlier studies [18] showing the fact of the greatest number of rods being sampled by glass plates and the most abundant at Garret 1 net being bacilli, one may risk a statement that benzene and toluene are best decomposed by bacilli while rods are better decomposed by xylene. However, those are merely a hint; with no other papers available a confrontation proves impossible. None but a few, as Bossert [19] and Pike and Carrington [20] presented data on *Bacillus, Pseudomonas, Achromobacter* and *Alcaligens* genera being active at oil related substances decomposition. Thus, those issues are open to discussion and call for more thorough insight.

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