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Original Research

Neustonic Bacteria Number, Biomass and Taxonomy

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Abstract

This paper presents results of research concerning bacteria inhabiting the surface microlayer and subsurface water of the eutrophic lake Jeziorak Maty. Total number, biomass and share of heterotrophic bacteria was higher in surface microlayer than in subsurface water. Identification shows significant differences between genera of bacteria inhabiting surface biofilm and subsurface water.

Keywords: Neuston, surface microlayer, biofilm, taxonomy.

Introduction

A surface microlayer is formed by accummulating organic compounds, fats, proteins and sugars in particular [17]. Due to a polar construction of these compounds they form a stratified film on the surface of water bodies [9]. That layer makes a very stable environment for microorganisms in terms of nutritional substances abundance. On the other hand, due to extreme temperature conditions, or sunlight amount, it is not very favourable for their growth and development as compared to the depths of water.

Organisms dwelling in that extreme environment are affected by many negative physical and chemical factors which determine taxonomic and physiologic differences between the bacteria in the biofilm and the deep ones.

From the taxonomic point of view the bacterioneuston has been little investigated, but analyses by some scientists have revealed its peculiar species composition. Identification of bacteria strains isolated from the surface biofilm reveal that most of them represented the genera *Bacterium, Chromobacterium, Pseudomonas, Flavobacterium, Corynebacterium* of the *Vibrio-Aeromonas* group and the *Enterobacteriaceae* family [3, 7, 15, 19].

The aim of the present paper was to investigate the differences in number, biomass and taxonomy of surface microlayer and deep water microorganisms in the lake Jeziorak Maly.

Materials and Methods

The Study Area

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

Sampling

The water was sampled on 20th May, 17th July and 21st August 1996 from three different stations (Fig. 1). It was sampled from the surface microlayer by use of four techniques:

1) a glass plate collecting water layers 100 μm thick
2) a Perplex plate collecting water layers 150 μ^i thick
3) Garret's net 1 of 65 μm mesh collecting water layers 250 μm thick
4) Garret's net 2 of 200 μm mesh collecting water lay ers 300 μm thick

Subsurface water samples were raised from the depth of 10-20 cm by means of a sterile glass pipette with the use of a Pippet-boy (De Ville) device. The samples were whisked

into sterile glass containers. They were then transported to the laboratory in a thermoinsulated vessel containing glass at a temperature of ~ 7°C. The time elapse between sampling and analyzing was less than 6 hours.

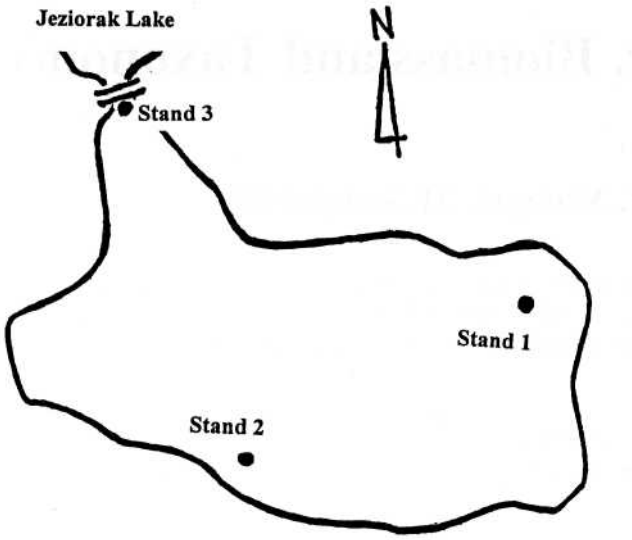

Fig. 1. Outline of lake Jeziorak Mały.

Total Number of Bacteria and Biomass Counting

Total number (TN) of sampled bacteria was determined by direct counting on membrane filter (Millipore), under an epifluorescence microscope in specimens stained with orange acridine [20].

Bacteria cell number in the samples was calculated by multiplying the bacteria total number by permanent contents of organic carbon for one cell, i.e. about 20 fgC_{org} [12].

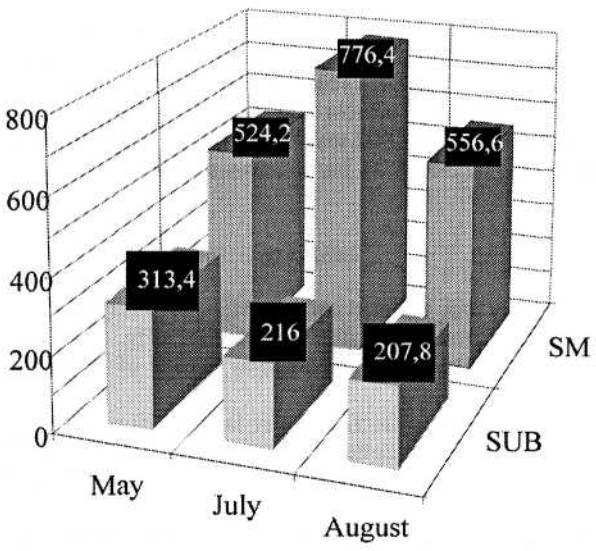

Fig. 2. Biomass of bacteria in surface microlayer and subsurface water of lake Jeziorak Mały (μg C_{org}/L).
Explanations: SM - surface microlayer, SUB - subsurface water

Heterotrophic Bacteria Number

Heterotrophic bacteria number (CFU) was calculated on spread plates methods. Sterile buffer water was used for dilluting [4]. 0.1 ml volumes of samples were then inoculated on Plate Count Agar (Difco) surface and spread by glass stroking rods. After 6 days of cultivating at 20°C, the heterotrophic bacteria were counted and then, at random, transferred onto a semiliquid medium. The strains were stored in a refrigerator for further experiments, and transplanted onto a fresh semiliquid medium every two weeks.

The isolated strains identification was carried out according to an Inland Aquaenvironment Diagnostic Chart for bacteria [1].

The identification covered the following tests:

1) bacteria morphology,
2) mobility,
3) fluorescein production,
4) cytochrome oxidasis production,
5) arginin dihydrolasis production,
6) indol production,
7) phosphatasis production,
8) ornitine decarboxylasis production,
9) citrate decomposition,
10) cazein decomposition,
11) starch decomposition,
12) Tween decomposition,
13) RNA decomposition,
14) maltosis decomposition,
15) sodium formate decomposition,
16) lecithin decomposition,
17) esculin decomposition,
18) buffer red-water reaction,
19) Voges-Poskauer reaction,
20) t-stimulated growth = 37°C,
21) t-stimulated growth = 4°C,
22) growth in a 4% NaCl solution,
23) growth in a 6% NaCl solution,
24) growth on McConkey medium,
25) growth on TCBS medium,
26) gelatine hydrolysis,
27) L-arabinosis usage, and
28) gluconian oxidizing.

Results

Table 1 presents the data on bacteria total number (TN) and heterotrophic bacteria abundance (CFU) from the analyzed samples. It turned out that TN and CFU in the surface microlayer reached their maximum in July. In May and August the results were a little lower but, on the average, similar. In the subsurface water the TN maximum occurred in May, CFU maximum in August.

The obtained surface microlayer result analysis obtained by different sampling techniques effected in a similar bacteria number regardless of the glass or Perplex plate sampling. An analogous situation happened in the case of Garret's net 1 and 2 sampling. The subsurface water samples always contained much less bacteria than the surface water samples.

Bacteria biomass in the analyzed water samples corresponded to the total bacteria number (Fig. 2). As the Figure

Table 1. Number of bacteria in the surface microlayer and subsurface water in lake Jeziorak Maly. (Number/1L).

Date of sampling		Sampling methods					Average		E
		a	b	c	d	e	SM	SUB	SM/SUB
May (20.05.96)	*	32.51	27.50	24.33	20.48	15.67	26.21	15.67	1.67
	**	169.1	371.0	79.8	65.3	14.3	171.3	14.3	11.9
July (16.07.96)	*	48.31	47.56	18.12	41.30	10.80	38.82	10.80	3.59
	**	123.3	353.3	183.2	99.9	19.2	189.9	19.2	9.8
August (21.08.96)	*	28.52	46.91	19.59	16.31	10.39	27.83	10.39	2.68
	**	43.3	79.3	200.0	120.0	30.0	110.6	30.0	3.6

Explanations: a - sampling by glass plate, b - Perplex 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.

shows, the bacteria biomass values oscillated between 207.8 µm C_{org}/L (August, subsurface water) and 776.4 µg C_{ors}/L (July, surface microlayer).

Table 2 presents investigation results dealing with heterotrophic aerobic bacteria taxonomy in the surface microlayer (biofilm) and with the subsurface water. It is evident that in the biofilm bacteria of the *Aeromonas* (24.8%) genus were most abundant, they belonged to the following species: *Aeromonas hydrophila* 11.9%, *Aeromonas salmonicida* 10%, *Aeromonas sp.* 2.9%. In July this genus maximum was found in surface microlayer (30.4%), and in subsurface layer in May (15.8%). In the subsurface water the *Aeromonas* genus made up about 9.5% of heterotrophic bacteria. The second largest bacteria group occurring in the microlayer was the family of *Enterobacteriaceae* - 24.6% on the average. At the same time, that family bacteria were most abundant in the subsurface water - 25.1% on average.

Within the *Enterobacteriaceae* family the following bacteria species were noted: *Enterobacter aerogens, Erwinia steward, Serratia marcescens, Serratia sp.* A heavy abundance of *Enterobacter aerogens* occurred both in the biofilm (23.4% on the average) and in the subsurface water (23.9% on the average). The highest number of those bacteria in the biofilm was encountered in May (28.2%) and in the subsurface water in July (28.6%). The *Erwinia stewartii* occurrence in the biofilm was not recorded, and in the subsurface water it appeared only in July (3.6%).

The bacteria of *Serratia* genus were noted only in the surface film; the *Serratia marcescens* in May and *Serratia sp.* in May and July.

The genus *Bacillus* was the one that frequently occurred in the water samples. In the subsurface water the bacteria were more abundant than in the surface film (22.1% and 8.7% respectively). Within the genus the following species were determined *Bacillus, cereus, B. firmus, B. megaterium.* Similar relations were found with the bacteria of the genus *Staphylococcus.* On the average they made 16.7% of all the strains in the subsurface water and in the microlayer - 5.6 %.

Generally speaking, the genus *Micrococcus* was more abundant in the subsurface water than in the microlayer (6.5% and 5.8% respectively). However, *Micrococcus varians* was encountered only in the surface microlayer in the May and July samples. Interesting results were obtained in reference to the species of *Cytophaga salmonicolor, Flavobacterium sp.* and *Flexibacter aggregans.* Strains contained in the above mentioned taxons were isolated only from the surface film, and the species *Cytophaga salmonicolor* occurred quite abundantly (8.5% on average), with its maximum in August - 20.7%.

Spiral-shaped cells were represented by *Vibrio fluvialis,* which were more frequent in the surface film than in the subsurface water (5.4% and 1.8% respectively). The number maximum in the microlayer was noted in May - 11.5%. The bacteria number dropped in subsequent months.

The obtained data gave an image of differences existing between the microflora from the microlayer and the microflora from the subsurface water. In the surface microlayer bacteria of the genera *Aeromonas, Cytophaga salmonicolor, Flavobacterium sp., Flexibacter aggregans, Pseudomonas, Serratia* and *Vibrio* genus were represented most abundantly. Conversely, in the subsurface more abundant were the bacteria of the *Enterobacteriacae* family and the genera *Bacillus, Staphylococcus* and *Micrococcus.* All other identified taxons did not show significant differences between surface and the subsurface water.

Discussion

Some hundreds of micrometers inside the microlayer and still deeper lie the physical and chemical factors which obviously determine microbe development and the biochemical transformations kinetics. 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 (CFU) presented in this paper remains in line with the studies by Niewolak [16]. The bacteria total number in the surface microlayer was, as a rule, 2-3 times higher than in the subsurface water at a depth of 10 cm. The following papers presented similar data confirming bacterioneuston number overgrowing plankton bacteria in deeper water layers [2, 7]. Niewolak [15] 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 [8, 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 number of bacteria in that water layer. Besides, a greater abundance of organic compounds accummulating in the biofilm positively affects the development of heterotrophic bacterioneuston [5].

Many papers so far have presented data on a greater bacterial biomass occurring in surface microlayer than in

Table 2. Taxonomy of bacteria isolated from surface microlayer (SM) and subsurface water (SUB) of lake Jeziorak Maly (values given in %).

Bacteria	Occurence							
	May		July		August		Average	
	SM	SUB	SM	SUB	SM	SUB	SM	SUB
Genus Aeromonas	26.9	15.8	30.3	3.6	17.2	9.1	24.8	9.5
Aeromonas sp.	6.4	5.3	2.3	0.0	0.0	0.0	2.9	1.7
Aeromonas hydrophila	14.1	10.5	12.4	3.6	9.2	0.0	11.9	4.7
Aeromonas salmonicida	6.4	0.0	15.7	0.0	8.1	9.1	10.0	3.0
Genus Alcaligenes	2.6	10.5	6.1	0.0	4.5	3.6	4.4	4.7
Alcaligenes sp.	2.6	10.5	4.9	0.0	1.1	0.0	2.8	3.5
Alcaligenes denitrificans	0.0	0.0	0.0	0.0	2.6	3.6	0.8	1.2
Alcaligenes faecalis	0.0	0.0	1.2	0.0	1.1	0.0	0.8	0.0
Genus Bacillus	9.0	21.1	9.0	17.9	8.1	27.3	8.7	22.1
Bacillus cereus	0.0	5.3	0.0	10.7	2.3	2.0	0.8	6.0
Bacillus firmus	0.0	0.0	0.0	10.7	2.3	9.1	0.8	6.6
Bacillus megaterium	1.3	0.0	0.0	0.0	2.3	1.8	1.2	0.6
Bordetella bronchiseptica	0.0	0.0	0.0	0.0	1.2	0.0	0.4	0.0
Cytophaga salmonicolor	2.6	0.0	2.3	0.0	20.7	0.0	8.5	0.0
Enterobacter aerogenes	28.2	15.8	22.5	28.6	19.5	27.3	23.4	23.9
Erwinia stewartii	0.0	0.0	0.0	3.6	0.0	0.0	0.0	1.2
Enterobacteriaceae	30.8	15.8	23.6	32.2	19.5	27.3	24.6	25.1
Flavobacterium sp.	2.6	0.0	1.1	0.0	1.2	0.0	1.6	0.0
Flexibacter aggregans	0.0	0.0	1.1	0.0	1.2	0.0	0.8	0.0
Flexibacter-Cytophaga	0.0	0.0	0.0	0.0	10.3	9.1	3.4	3.0
Genus Micrococcus	2.6	10.5	9.0	0.0	5.8	9.1	5.8	6.5
Micrococcus sp.	0.0	10.5	0.0	0.0	5.8	9.1	1.9	6.5
Micrococcus roseus	0.0	0.0	1.1	0.0	0.0	0.0	0.4	0.0
Micrococcus varians	2.6	0.0	7.9	0.0	0.0	0.0	3.5	0.0
Pseudomonas fluorescens	0.0	0.0	3.4	3.6	4.6	0.0	2.7	1.2
Genus Serratia	2.6	0.0	1.1	0.0	0.0	0.0	1.2	0.0
Serratia sp.	1.3	0.0	1.1	0.0	0.0	0.0	0.8	0.0
Serratia marcescens	1.3	0.0	0.0	0.0	0.0	0.0	0.4	0.0
Genus Staphylococcus	8.9	10.5	5.6	21.4	2.3	18.2	5.6	16.7
Staphylococcus sp.	7.6	10.5	5.6	21.4	2.3	0.0	5.2	10.6
Staphylococcus epidermidis	1.3	0.0	0.0	0.0	0.0	18.2	0.4	6.1
Vibrio fluvialis	11.5	5.3	3.4	0.0	1.2	0.0	5.4	1.8

subsurface water [3, 10, 14]. This paper presents similar results, although in a sample taken in May at station 2 a quite contrary phenomenon was recorded. The situation might have been caused by a disturbance in biofilm structure due to water masses mixed by wind, which happened before sampling.

The differences in the distribution of bacteria types between the microlayer and the subsurface water, recorded by the present paper, stress the separation of those two environments. Among strains isolated from surface microlayers the most abundant was the genus of *Aeromonas*, often affiliated with the group of *Aeromonas-Vibrio* along with *Vibrio* [15]. The majority of scientists have given evidence of great abundance of *Aeromonas* genus in the surface biofilm; it was only Fehon and Oliver [7] who did not record any representatives of this genus in the surface microlayer. But, according to them the isolated tribes number was not representative enough. A greater *Vibrio* bacteria number in the surface film was in conformity with the data by Kaneko and Colwell [11], which proved a common occurrence of the bacteria of that genus in the neuston layer in all water bodies.

This paper and [7, 16] recorded bacteria of the *Flavobacterium* genus only in the surface layer which shows that these microbes are better adapted to insolation conditions. According to [3, 15] the *Alcaligens* genus occurred abundantly in the biofilm, but Fehon and Oliver [7] maintained the opinion that there were more of those bacteria in the subsurface water. This paper, on the other hand, has given evidence of the genus occurring in both environments in even quantities (4.4% and 4.7%, respectively). The bacteria of the *Alcaligens* genus might be changing their environments more often than other bacteria, physical and chemical conditions depending.

Bacteria of the *Cytophaga* genus occurred in the surface microlayer in fairly big numbers although they were not recorded in the subsurface water. A similar situation happened in the case of *Flexibacter aggregans* species and the *Serratia* genus. Unfortunately, lack of information concerning their distribution made it impossible to state whether they were more abundant in the biofilm or in deeper parts. The data provided by the present paper indicate that after all the mentioned bacteria occurred in neuston more often than in the deep water, at least because of their pigmentation.

The genus *Pseudomonas* were represented only by *Pseudomonas fluorescens* in the microlayer seems very strange as it commonly occurred both in the biofilm and in deep water according to [6, 3, 15]. It might have resulted from inappropriate media and growth conditions adopted in the early phase of the research, which triggered partial elimination of bacteria.

The obtained data generally confirmed that of previous scientists pointing to differences in both environment types. The many stressing factors affecting the bacterioneuston determine the development of its biologic life and biochemical transformations kinetics in the surface biofilm in all the water bodies. Therefore, knowledge of the laws controlling the succession of organism inhabiting the surface microlayer is of crucial importance in understanding the significant, yet not very appreciated relationships, of water bodies.

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