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Metabolic Activity of Heterotrophic Bacteria in the Presence of Humic Substances and Their Fractions

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Abstract

Metabolic activity of heterotrophic bacteria isolated from lakes Stegwica, Jeziorak and Jasne were investigated. A humic substances of commercial preparation by Aldrich-Chemie, as well as natural humic substances, fulvic and humin acids isolated from lake Stegwica were used as a source of energy. The results indicate that 70-80% of bacteria strains are able to utilize the humus matter. The greatest metabolic activity was recorded in bacteria which were supported by natural humic substances isolated from lake Stegwica; whereas the lowest was noted in the presence of humin acids.

Keywords: humic substances, fulvic acid, humin acid, metabolic activity, heterotrophic bacteria.

Introduction

Humic substances make a group of organic compounds occurring in various environments: in the soil, in marine and fresh waters and their sediments as well as in peat-bogs and in ground water [1, 2]. In every one of those environments they have their own properties which result from the way they are formed, their chemical structure, and the role they play.

It has recently been discovered that humic substances are made of a composition of polyelectrolytes-spherocolloids. They are formed as a result of very complex processes of biochemical condensation and polymerization of decomposition products of plant, animal and microorganism metabolites [3].

The classical methodology distinguishes the following fractions of humic substances: humin acids (soluble in alkaline environment and coagulating in the process of acidifying alkali extracts, with pH < 2), fulvic acids (soluble in both alkali and acids) and humins (persisting extraction when treated with diluted alkali or acids) [4]. The above-mentioned humic substance fractions have similar colloidal aliphatic-aromatic structure, yet they are different in terms of molecular mass, chemical structure and various contributions of function groups.

The accumulation of Earth's organic carbon in the form of humic substances is significant. According to Moran and Hodson [5] humic substances constitute 25-26% of dissolved organic carbon in surface waters, and up 90% in swamps and peat-bogs. Despite the fact that humic substances have been focused upon in many research studies and investigations, knowledge of the role played by microorganisms in forming and decomposing them, as well as how they use it, is still insufficient. The chemical composition and energetic volume they offer make humic substances a very "attractive" source of energy, carbon and nitrogen for microorganisms.

Material and Methods

Study Area

The material was sampled from water and bottom sediments of lakes Jeziorak, Jasne and Stegwica. All the lakes make part of Ilawa Lakeland.

Lake Jeziorak is the sixth greatest lake in Poland with a surface of 3219.4 ha. Maximum length is 27.4 km and width - 2.4 km, and mean depth is 4.8 m. Lake Jeziorak is eutrophic.

Lake Jasne is a small oligo-mesotrophic lake situated among the woodland. Its surface is 10.5 ha and its mean depth is 8.13 m. The lake is distinguished by high water transparency (9-11 m) and a low water pH ranging from 4.0-4.8.

Lake Stegwica is a shallow water body (mean depth 1.0-1.5 m) located in an agricultural and forest area. It is a highly eutrophic lake with symptoms of hypertrophy, which places it among alloiotrophic water bodies. The water is dark brown which results from high humic substance contents, which in that case makes so called "sweet humus".

Sampling

Sampling was carried out in the spring (24th May), summer (27th July) and autumn (22nd Oct.) of 1997 in the pelagic zone of the investigated lakes. The samples were recovered by means of a Ruttner sampler at 1 m depth and then the sampled water was transferred to sterile glass flasks. The water for humic substance isola tion was scooped into polyethylene containers of 25 1 to be eventually acidified by concentrated HC1 (36.5%) un til pH=2.0 was reached. Bottom sediments were sampled by means of a self-constructed tube sampler. A 20 cm upper layer of sediments was removed and transferred into sterile glass flasks. Then samples of water and bot tom sediments were transported in a container filled with ice (about 7ºC) to a laboratory for microbiological exam ination.

Isolating Bacterial Strains

Bacteria undergoing examination were isolated by means of the spread plate method on an iron-peptone agar medium ace. to Ferrer, Stapert and Sokolski [6]. Following 10-day incubation at 20ºC, 25 bacterial colonies were randomly isolated and transferred onto a semi-solid iron-peptone medium. The strains were then checked for culture purity and stored for further examinations at 4ºC.

Isolating Humic Substances

For the isolation of humic substances from water the method described by Thurman and Malcolm [7] was used. The water was first filtered through a nitro-cellulose filter with pore size 0.45 µm (Sartorius) and then passed through an "Amberlite XAD-8" ion-exchangeable resin filled column (Fluka). Humic substances assimilated by sorbent were then rinsed by 0.25 M NH_4OH. The obtained eluate was then thickened in a vacuum evaporator at 45°C to a volume of 100 cm^3. In order to isolate humic substances from bottom sediments the samples underwent a 16 hour extraction of 0.1 M NaOH at 40°C. The sediment was then centrifuged for 10 min at 1500 x g. Thus obtained supernatant made a humus matter solution which occurs in bottom sediments, and then was thickened in the same way as described above.

Humic Substance Fraction Separation

To the solution of humic substances alkaline fraction 2 N NH_3PO_4 was added until the residue precipitated, then it was centrifuged in order to separate fractions of fulvic acid solution (supernatant) and humin acid (residue). Humin acid was dissolved in 0.01 M phosphate buffer with pH=7.2. In order to purify the fulvic acid, the supernatant was passed through a sorption column filled with "Amberlite XAD-8" ion-exchangable resin (Fluka). This process was followed by eluation of the adsorbed fulvic acids by 0.25 M NH_4OH.

Humic Substance Concentration Marking

Concentrations of humic substances and their fractions in the obtained solutions were marked by the gravimetric method. Eluate samples underwent evaporation to reach solid mass at 105°C.

Measurement of Bacteria Metabolic Activity in the Presence of Humic Substances

Metabolic activity of the examined bacteria was determined by respirometric method with the use of a Warburg apparatus. The following substrates were used:

a) a natural preparation of humic substances isolated from the water of lake Stegwica;
b) commercial preparation of humic substances by Aldrich-Chemie (Germany);
c) humin acids isolated from the water of lake Stegwica;
d) humin acids isolated from bottom sediments of lake Stegwica;
e) fulvic acids isolated from the water of lake Stegwica;
f) fulvic acids isolated from bottom sediments of lake Stegwica.

The concentration of all the examined substrates was 5 mg/cm^3. Oxygen absorption was measured for a period of 3 hours at 26"C and at constant vessel shaking (120 cycle/min). Metabolic activity of the bacteria investigated was expressed in µl of used oxygen and was calculated according to the following formula:

$$O_2\ uptake = \frac{\Delta h \cdot k}{t} \left[\frac{\mu l O_2}{h}\right]$$

where:

Δh - pressure change in mm of Brody liquid
k - vessel constant
t - measurement duration

Results

Table 1 and Figures 1, 2 present bacteria respirometric data of those isolated from water of lakes Jasne, Jeziorak and Stegwica. According to the data in the Table and Figure 1, bacteria displayed higher metabolic activity when natural humic substances isolated from lake

Table 1. Utilization of humic substances and their fractions as metabolic substrate by bacteria isolated from the investigated lakes.

Lake	Metabolic substrate	Strains consuming the examined substrate (%)	Oxygen uptake ($\mu l\ O_2/h$)		Number of examined strains
			Value range	Mean	
Stęgwica	Humic substances (natural)	70	0 – 28.0	9.94	10
	Humic substances (commercial preparation)	80	0 – 8.4	3.22	10
	Humin acids (sediment)	40	0 – 8.4	1.82	10
	Fulvic acids (sediment)	70	0 – 16.8	6.44	10
	Humin acids (water)	20	0 – 5.6	0.56	10
	Fulvic acids (water)	40	0 – 11.2	1.68	10
Jasne	Humic substances (natural)	73	0 – 32.2	10.82	11
	Humic substances (commercial preparation)	73	0 – 8.4	2.67	11
	Humin acids (sediment)	36	0 – 7.0	1.65	11
	Fulvic acids (sediment)	82	0 – 19.6	10.31	11
	Humin acids (water)	67	0 – 2.8	1.87	11
	Fulvic acids (water)	67	0 – 15.4	7.93	11
Jeziorak	Humic substances (natural)	75	0 – 29.4	9.22	12
	Humic substances (commercial preparation)	75	0 – 21.0	5.25	12
	Humin acids (sediment)	33	0 – 8.4	1.75	12
	Fulvic acids (sediment)	75	0 – 19.6	7.23	12
	Humin acids (water)	0	0	0	12
	Fulvic acids (water)	33	0 – 21.0	7.0	12

Stegwica were present as opposed to the situation when the process was going in the presence of a commercial preparation by Aldrich. The strains isolated from lake Jasne displayed a greater activity in using the natural humic substances whereas the Jeziorak strains were the least active. However, the Jeziorak strains were the most active ones in using the commercial substance.

Figure 2 presents average consumption of oxygen by the investigated bacteria when accompanied by various fractions of humic substances. The data provide information on the Jeziorak bacteria being most active in using the humin acids isolated from bottom sediments, while humin acids isolated from water were most attractive for the lake Jasne bacteria. Lake Jasne bacteria strains were most active in using fulvic acids isolated from bottom sediments while fulvic acids isolated from water were most demanded by the lake Jeziorak bacteria.

The data collected in Table 1 and Figures 1 and 2 show clearly that on the whole, the bacteria isolated from the investigated lakes were more active in respect to fulvic and humin acids isolated from bottom sediments of lake Stegwica than in the case of the same compounds isolated from water. Overall oxygen consumption was greater in the presence of fulvic acids than humin acids. Humin acids isolated from water were of interest to only a few strains isolated from lakes Jasne and Stegwica.

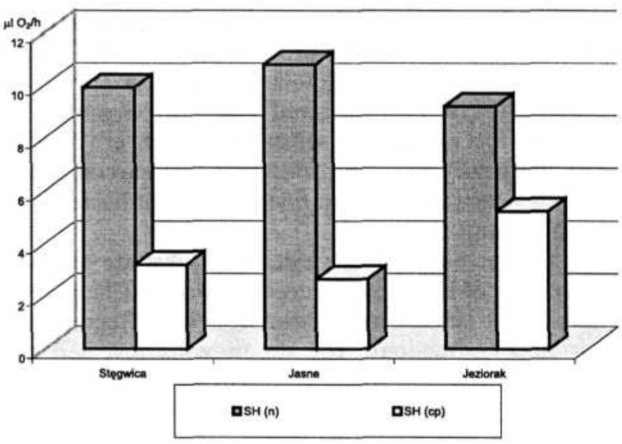

Fig. 1. Oxygen uptake by bacteria in the presence of humic substances (average). Explanations:

SHn - natural preparation of humic substances isolated from lake Stegwica.

SHcp - commercial preparation of humic substances by Aldrich-Chemie

Discussion

Heterotrophic bacteria make one of the most important groups among all organisms participating in energy transformation and metabolism in nature. In water bodies they are responsible for the mineralization of over 50% of organic matter [8]. The substrate uptake and consumption abilities of particular microorganisms vary greatly. Under natural conditions, heterotrophic bacteria are active in the metabolism of mainly labile substrates of native primary production origin. Nevertheless, it seems obvious that in polyhumic lakes higher contents of dissolved organic matter (DOM) consisting of humic components [9, 10] cause an increase of bacteria growth and development rate [11, 12]. According to Hessen [13], organic carbon of the photosynthesis origin going on in humic lakes does not play a significant role in bacterial mass production in terms of quantity. Thus, one

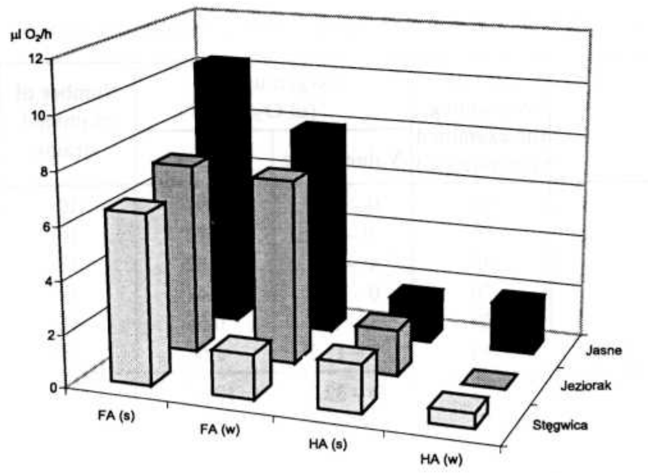

Fig. 2. Oxygen uptake by bacteria in the presence of various fractions of humic substances (average).
Explanations:
FA (s) - fulvic acids isolated from bottom sediments of lake Stegwica
FA (w) - fulvic acids isolated from water of lake Stegwica
HA (s) - humin acids isolated from bottom sediments of lake Stegwica
HA (w) - humin acids isolated from water of lake Stegwica.

may anticipate that humic substances make the major carbon source for bacteria providing 90% of the carbon which is necessary to maintain the bacteria growth recorded. Tranvik [14] observed that microorganism increase at the expense of substances which are hard to decompose goes at a slower rate than when accompanied by labile substrates; yet the final amount of bacterial biomass is comparable.

In the present study the metabolic substrate used was sodium salt of humic substances by Aldrich-Chemie and origin humic substances isolated from lake Stegwica and their various fractions. The experiments revealed that humic substances, of whatever origin were consumed by 70-80% of the bacteria strains. Data presented by Visser [15] suggest that humic substances not be available for bacteria. However, later experiments by Tranvik and Sieburth [16], Moran and Hodson [5], and Donderski and Wodkowska [17] pointed to the biological importance of humic substances as a food substrate for bacteria.

The supply of natural humic substances made the bacteria more active in respiration than when supplied with the commercial substance. That phenomenon may have been caused by the fact that, depending on its origin, the humic substances are not homogenous and are composed of various fractions occurring in different quantity ratios. The importance and availability of the fractions to bacteria is differentiated [18]. The very extraction techniques used are also of some importance to humic substances compliance. The extraction procedures used may either cause its partial split and make it prone to microbiological decomposition or make it totally unavailable and not assimilable.

The present data have shown that fulvic acids are better assimilated by the bacteria of the examined lakes. In the presence of those acids both the percentage of strains able to assimilate them as metabolic substrate and bacterial metabolic activity accompanied by that fraction were greater than in the case of humin acids. According to Kononova [19], fulvic acids have a lower molecular mass and are better soluble in water, which affects their being assimilated by bacteria. The data provided by Tulonen, Salonen and Arvol [20] fractions of small molecular mass better enter bacterial metabolism although bacterial growth rate is greater in the company of high molecular mass components. Also Munster and Chrost [21] maintain that bacteria use lower molecular mass fractions to a smaller extent for their growth and to a greater extent for their energetic needs.

The bacteria used for the present experiment were recovered from environments of varied trophies and different humic substance contents. What had been expected was that bacteria isolated from an alloitrophic lake of rich humus (Stegwica) would better assimilate the humic substances than the strains isolated from lakes Jasne and Jeziorak. That was, however, not the case. Bacteria isolated from lakes Jasne and Jeziorak on many occasions displayed a greater metabolic activity when accompanied by humic substances and their fractions than the bacteria isolated from lake Stegwica. Experiments done by Tranvik and Hofle [22] also revealed that humic substances undergo degradation by oligo- and dystrophic lakes' bacteria.

Investigations carried out by a number of authors [5, 9, 14, 15, 16] show that the influence of humic substances on microorganisms has many various aspects. The present paper is meant to be a contribution to a better and more comprehensive understanding assimilation of the humic substances by bacteria.

Conclusions

1. The examined bacteria displayed a greater metabolic activity in the presence of natural humic substances than the commercial preparation of humic substances sodium salt (manufactured by Aldrich-Chemie Germany);
2. Oxygen consumption by bacteria in the presence of fulvic acids and humin acids isolated from bottom sediments of lake Stegwica was greater than when accompanied by the same compounds isolated from water of the same lake.
3. The bacteria isolated from the lakes under investigation showed a greater metabolic activity in the presence of fulvic acids than humin acids.

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