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Decomposition and Utilization of Particulate Organic Matter by Bacteria in Lakes of Different Trophic Status

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Abstract

Enzymatic decomposition and bacterial utilization of various types of particulate and dissolved substrates was studied during spring-summer period in four lakes of Mazurian Lake District (Northern Poland). We found that seston particles, similarly as dissolved organic matter (DOM), undergo intensive decomposition processes in lake water, but only after their previous colonization by bacteria. In lakes of low or moderate trophic status free-living microorganisms predominated. They preferentially utilized low molecular weight, dissolved organic compounds. Increases in particulate organic matter (POM) content in these environments caused rapid change of substrate exploitation strategy and adaptation of these bacteria to live in particle-attached forms. In lakes of POM and colloidal DOM (CDOM) abundant particle-attached microheterotrophs, although less metabolically active than free-living bacteria, were mainly responsible for secondary production and POM mineralization A mechanisms that permit effective POM exploitation by seston-attached bacteria was overproduction of relatively low active (high Km) enzymes (e.g. aminopeptidase) and/or synthesis of the enzymes (e.g. β-glucosidase or glucosaminidase) that were optimally adapted (low Km) to the environment.

Keywords: lakes, particulate organic matter, dissolved organic matter, bacteria, ectoenzymes

Introduction

Organic matter in aquatic environments (autochtonous or allochtonous) is composed of two basic pools of constituents: dissolved organic matter (DOM) – fraction smaller that 0.2 μm, and particulate organic matter (POM) – fraction that is retained on 0.2 μm filters [1]. DOM concentration in natural waters depends upon their trophic status and varies from 1 mg C/l (oligotrophic waters) to 50 mg C/l (highly eutrophic waters). POM usually contributes less than 10% of the total organic matter and its concentration does not exceed 0.2 mg C/l in oligotrophic and 1 – 2 mg C/l in eutrophic environments [2].

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It is generally believed that the low-molecular-weight organic compounds (i.e. amino acids, short chain peptides, fatty acids, monosaccharides) can be directly assimilated (withought their preliminary decomposition outside the cell) and metabolized by bacteria. These readily utilizable constituents of DOM (UDOM) are basic sources of nutrients and energy for aquatic microheterotrophs. UDOM fraction is intensively utilized by bacteria in the course of their secondary production and respiration processes. However, it constitutes less than 5% of total DOM and 2-3% of total organic matter content in lake water [3, 4]. Several studies suggest that UDOM fraction is permanently supplemented by the POM solubilization [5] and algal extracellular release processes [6, 7] in aquatic environments.

Although the POM in lake water amounts to only

10% of the total organic matter concentration, it seems to be equally important for the bacteria as the DOM fraction. The studies carried out by Riley [8] showed that the POM was mainly composed of proteins (about 50%), polysaccharides (25%) and chemicelluloses (25%). Chitin, uric acid and lipids were generally less abundant [8, 9]. High content of relatively easy utilizable proteins and polysaccharides by bacteria causes that particulate organic matter may be rapidly colonized and degraded by aquatic microhetherotrophs. Moreover, large surfaces of seston particles strongly adsorb a variety of nutrients and dissolved organic substrates. It causes that an influx of autochthonous or allochthonous POM to lake water, stimulates growth of heterotrophic microorganisms [12] and increases bacterial hydrolytic enzyme activity [13, 14]. It is well documented that from 88 to 99% of POM generated in the photic zone can be decomposed within the water column of the lake [10, 11].

Numerous studies have shown that the activity of various bacterial hydrolases is primarilly responsible for decomposition and utilization of organic matter in aquatic ecosystems. These hydrolases are located on the external surfaces of cell membranes and/or in the periplasmatic space of bacterial cells (ectoenzymes) or they are liberated by microplankton into the environment, as free (extracellular) enzymes [15]. A variety of ecto- and extracellular enzymes participate in decomposition of DOM and POM (i.e. peptidase, endo- and exonucleases, 5'-nucleotidase, lipase, α- and β-glucosidase and alkaline phosphatase). Most of them are adaptative enzymes and their synthesis and activity in lake water is regulated by complicated, often multistep, induction/derepression mechanisms inside the cell. Ecto- and extracellular enzyme activity is also strongly affected by various physico-chemical factors of the environment [5, 16]. The role of these enzymes in DOM decomposition processes is relatively well documented [5, 17]. However, knowledge on mechanisms of POM colonization and enzymatic degradation by bacteria is still fragmentary and inadequate.

The main purpose of this report is to analyze some aspects of the mechanisms of seston colonization by bacteria in lakes of different trophic status and to discuss the role of bacterial ectoenzymes in POM and DOM decomposition and utilization processes.

Materials and Methods

Study Area and Sampling

The investigations were carried out during a spring-summer period in four lakes (Kuc, Ryńskie, Szymon, Smolak) of the Mazurian Lake District (Poland) that differed in their trophic status and organic matter content (Table 1). Water samples (3 l) were collected under non-sterile conditions into polypropylene bottles from the surface layer (1 m) in the pelagic zone of the studied lakes.

Basic Physico-Chemical and Biological Parameters

Basic physico-chemical and biological parameters of water samples in the studied lakes were determined by standard limnological methods. Temperature and dissolved oxygen was measured with YSI Oxygen Meter (model 54, Yellow Spring Instr.). Water transparency (WT) was determined using Secchi disc; seston content was estimated as dry weight of the particles (SDW) collected on GF/F Whatmann filters. Chlorophyll$_a$ (Chl$_a$) was extracted from the seston with 96% ethanol and analysed spectrofotometrically [18]. The number of bacteria in samples (TVC) was estimated by epifluorescence microscopy after 4,6-diamidino-2-phenylindole (DAPI) staining [19]. Bacterial secondary production (BSP) was determined by the [3H-methyl]tymidine incorporation method [20]. Amounts of assimilated [3H-methyl]tymidine were transformed to bacterial organic carbon production with the use of the conversion factor 19.8 fg C cell^{-1} [21]. Trophic state index (TSI) of the studied lakes was calculated according to Carlson [22].

Enzyme Assay

Rates of hydrolysis (v) of soluble substrates by aminopeptidase (AMP), β-glucosidase (β-Gluc), glucosaminidase (Gluc-ami) and esterase (EST) were determined fluorometrically according to Chróst et al. [23]. Increase in the fluorescence of the tested water samples

Table 1. Basic environmental parameters of surface waters (1 m depth) of the studied lakes.

Lake	Temp. (°C)	O_2 (mg/l)	WT (m)	SDW (mg/l)	Chl$_a$ (μg/l)	Chl$_a$/SDW	TVC (x 10^6 cells/ml)	BSP (μg C/l h)
Kuc	16.4 (4.0-22.5)	10.2 (9.2-13.0)	5.9 (4.3-7.5)	1.25 (0.9-1.5)	2.58 (1.4-4.5)	2.06 (1.2-3.0)	1.85 (0.91-2.73)	1.91 (1.67-2.65)
Ryńskie	15.9 (5.0-21.5)	11.1 (7.8-14.0)	1.33 (0.8-1.8)	6.67 (4.5-7.3)	34.82 (25.1-41.2)	5.22 (4.1-6.3)	2.77 (2.1-3.4)	4.22 (0.94-11.56)
Szymon	16.5 (4.5-22.5)	13.2 (9.3-15.6)	0.75 (0.6-1.3)	13.46 (8.1-20.9)	81.16 (43.7-149.4)	6.03 (3.6-14.9)	3.0 (17.0-37.2)	12.82 (2.2-33.2)
Smolak	16.3 (6.0-22.5)	9.1 (7.7-10.0)	0.60 (0.5-0.7)	5.23 (3.3-8.4)	33.52 (25.6-42.3)	6.41 (4.8-12.7)	2.45 (1.4-2.9)	22.24 (15.1-34.7)

Table contains mean values calculated from six determinations. In parentheses – range of the value.

was measured with Shimadzu RF 1501 Spectrofluorimeter. Concentration of enzymatically liberated, fluorescent products was calculated from the standard curves. Further details of enzyme assay are given in Table 2. Kinetic parameters of the studied enzyme-substrate interactions were calculated from the direct plot of reaction velocity (v) versus substrate concentration [S] using the IBM PC computer software program "Enzpack" (Elsevier-Biosoft, U.K.).

Hydrolysis rates of particulate substrates Sigma: Hide Powder Azure (HPA), Cellulose Azure (CA) and Chitin Azure (ChA), by peptidase, cellulase and chitinase was determined spectro-colorimetrically (Table 2). To separate dissolved products of enzymatic reaction from non-hydrolyzed particulate substrate, samples were filtered through GF/F Whatmann glass-fiber filters after incubation. Absorbance of liberated, dissolved dye (proportional to the activity of investigated enzyme) was measured with a Shimadzu UV-1202 Spectrophotometer.

Results

Comparison of average values of indicators of POM content (WT and SDW) with selected parameters characterizing aboundance and activity of microorganisms (Chla, TVC and BSP) in surface waters of the studied lakes (Table 1) showed that mesotrophic Lake Kuc was about 5 times less POM abundant than Lake Ryńskie and Lake Smolak. The concentration of seston in hyper-eutrophic Lake Szymon was distinctly (2-6 times) higher than in other lakes, but its water transparency was similar to Lake Ryńskie and Lake Smolak. Generally, contents of microbial biomass in the seston, which was presumably proportional to the chl_a/SDW ratio, increased with the trophic status of the studied lakes. Only in Lake Smolak, despite its moderate trophic status (TSI = 52.2), amount of "living" POM was relatively high (Chla/SDW = 6.41). The mean number of bacteria (2.5 – 3.0 x 10^6 cells/ml) was comparable in the surface waters of all eutrophic lakes. In mesotrophic Lake Kuc, TVC was apparently lower (1.8 x 10^6 cells/ml). Mean rates of bacterial secondary production (BSP) varied from 1.91 μg C l^{-1} h^{-1} (Lake Kuc) to 22.2 μg C l^{-1} h^{-1} (Lake Smolak). Mean BSP of bacteria was about two times higher than particle-attached bacteria in Lake Kuc and Lake Ryńskie (Fig. 1A). Contribution of free-living and particle-attached bacteria to total bacterial secondary production was almost equal in Lake Szymon. In polyhumic Lake Smolak particle-attached bacteria were mainly responsible for high BSP rates. The participation of particle-attached bacteria to total BSP generally increased with the POM concentration in surface waters of meso- and eutrophic lakes

Table 2. Enzyme assays.

Enzyme (Substrate)	Sample (ml)	Substrate		Buffer	Control	Incubation (h)	Detection of the product		
							Fluorometric		Colorimetric
		(ml) *(mg)	Final conc. (μM) *(mg/ml)				Ex. (nm)	Em. (nm)	Abs. (nm)
AMP (L-leucin-7-amido-4-methyl-coumarin)	3.9	0.1	0.1 - 25.0	-	Flourescence measured at T_o time.	0.5 - 2.0	380	440	
EST (Fluorescein diacetate)	3.8	0.1	0.1 - 25.0	0.1 ml 1M Tris-HCl pH 7.2	Fluorescence measured at T_o time.	0.3 - 1.0	489	510	
β-Gluc (MUF-β-d-glucopyranoside) **and Gluc-ami** (MUF-N-acetyl-β-d-lucosaminide)	3.8	0.1	10.0 - 250.0	-	**Fluorescence of the sample fixed with 0.1 ml 1M glycine-NaOH buffer, pH 11.2	12.0 - 48.0	365	460	
Peptidase (Hide Powder Azzure)	20.0	*10.0	*0.5		Sample without particulate substrate	24.0 - 72.0 mixing	-	-	595
Cellulase (Cellulose Azzure)									575
Chitinase (Chitin Azzure)									565

* particulate substrates

** Glycine-NaOH buffer that inactivated enzyme was added at the beginning (controls) or at the end (samples) of incubation. Controls were supplemented with the substrate at the end of incubation. Non enzymatic release of MUF from β-Gluc and Gluc-ami substrates in samples during incubation was negligible and did not affect obtained results.

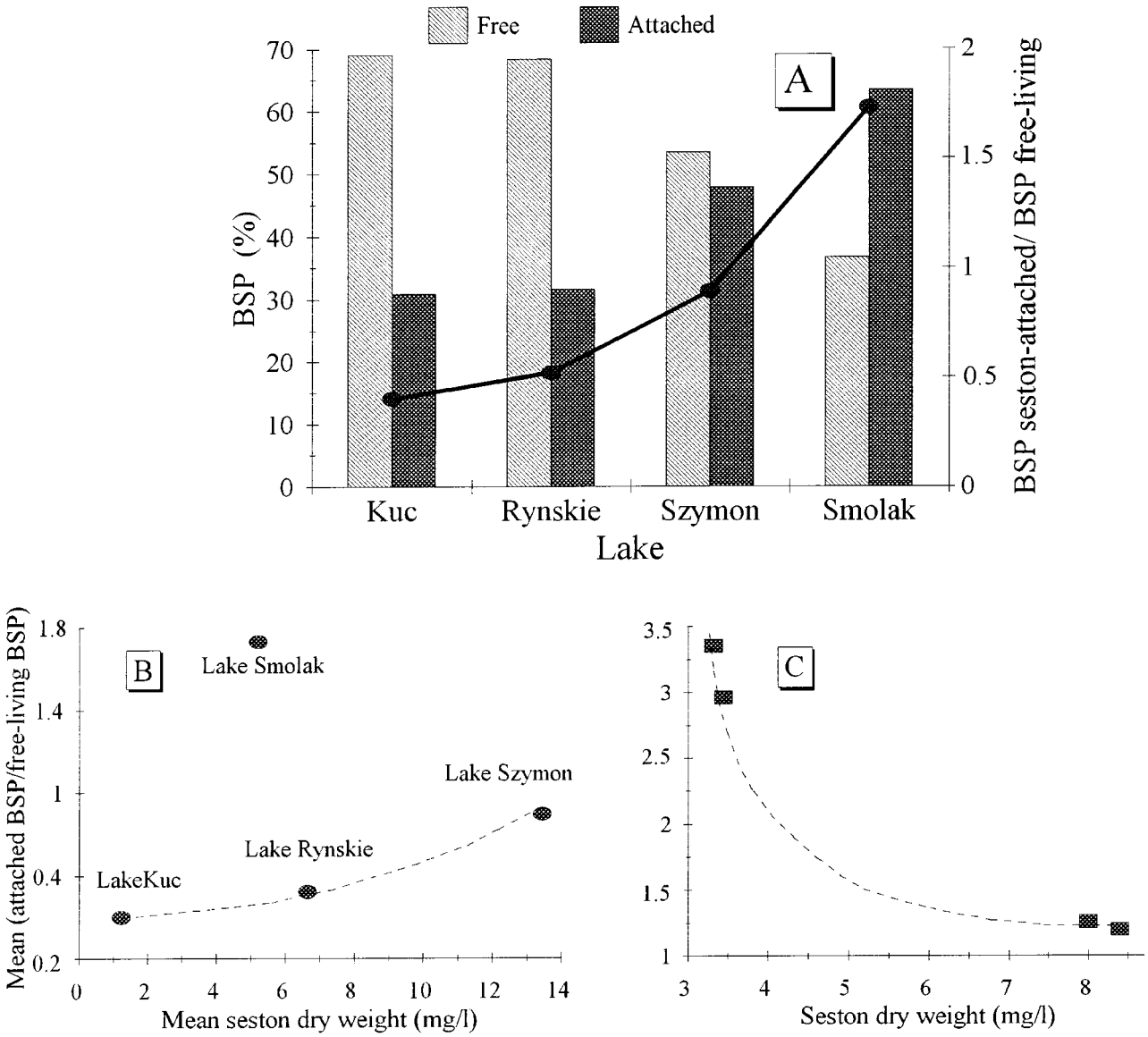

Fig. 1. Participation of free-living and seston-attached bacteria to total BSP (A) and relationship between SDW and secondary production of free-living and seston-attached bacteria in surface waters of all studied lakes (B) and in Lake Smolak (C). Bars and circles exhibit mean values calculated for five measurements made during spring – summer period (April-October 1997), squares – results of single measurement.

(Fig. 1B). Inversely, in Lake Smolak seston particles and colloidal humic substances probably stimulated BSP of free-living bacteria (Fig. 1C).

Amount of soluble products liberated during degradation processes from standard particulate substrates (HPA, CA and ChA) was strongly dependent on concentration of these substrates in the sample (Fig. 2A), incubation time (Fig. 2B) and temperature (Fig. 2C). The presence of other particulate and soluble enzyme substrates and their degradation products also altered decomposition of particulate substrates (Fig. 3). Hydrolysis rates of particulate substrates, determined in non-fixed containing living microorganisms samples, were dependent on their colonization rates by bacteria (Fig. 2D). Increase of particulate substrate (HPA) degradation products with incubation time was similar to typical bacterial growth curve (Fig. 4).

Mean activities of the POM hydrolyzing enzymes (i.e., rates of POM colonization by bacteria) in the studied lakes presents Table 3. In Lake Kuc, Lake Ryńskie and Lake Szymon, bacteria preferentially degraded particulate proteins (HPA). Cellulose and chitin was colonized 2 and 4 times slower, respectively. In Lake Smolak, bacteria most effectively utilized cellulose (CA). Mean rate of substrate colonization for all tested particulate substrates was similar in the first 3 studied lakes (HPA: 0.070-0.088 abs. units, CA: 0.032 – 0.042 abs. units and ChA: 0.017 – 0.022 abs. units, respectively). In Lake

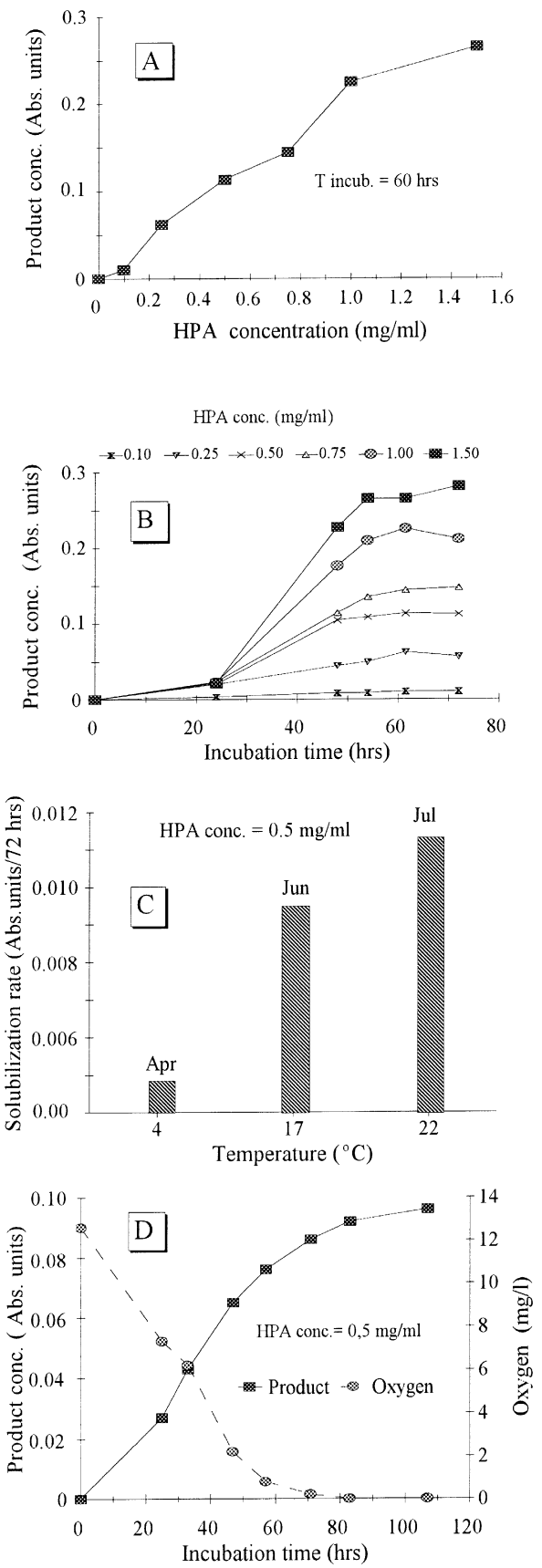

Fig. 2. Solubilization of particulate substrate (HPA) as a function of concentration (A), incubation time (B), temperature (C) and oxygen consumption by bacteria (D).

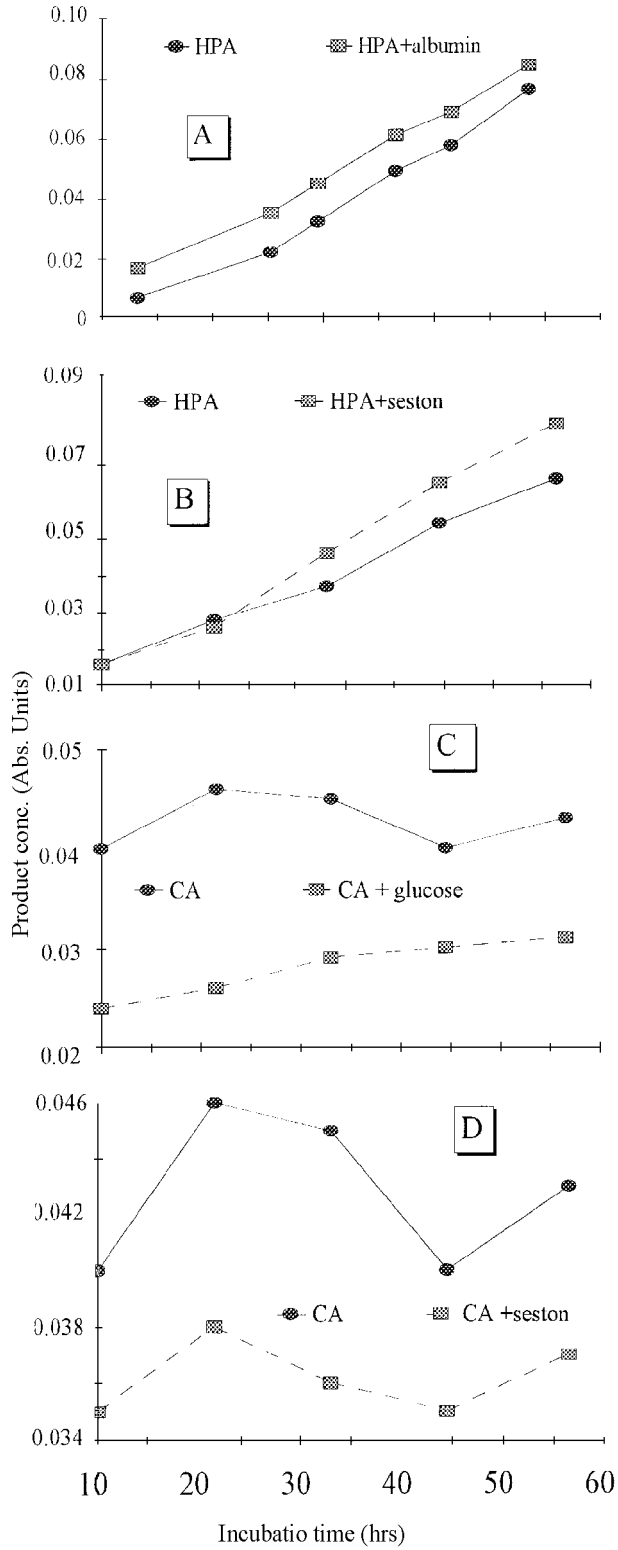

Fig. 3. The influence of dissolved and particulate organic matter on solubilization rates of HPA (A, B) and CA (C, D) in the surface water (1m depth) of Lake Ryńskie. Albumin and glucose conc. – 200 mgl^{-1}. Final conc. of "low-molecular-weight compounds free seston" – 3.7 mgl^{-1}. "Low-molecular-weight compounds free seston" was obtained from natural seston collected on 0.2 μm Nuclepore filters, autoclaved (17 min., 120°C), washed three times with distilled water for initial separation of dissolved organics and dialyzed 6 hrs using 100,000 D cut off membrane.

Table. 3. Mean rates of particulate substrate hydrolysis by selected bacterial enzymes from the surface water (1m depth) of then studied lakes.

Substrate (Enzyme) / Lake	Hydrolysis rate (Abs. units/ 72 hrs)		
	HPA (Protease)	CA (Cellulase)	ChA (chitinase)
Kuc	0.070 (0.044-0.087)	0.042 (0.030-0.056)	0.020 (0.011-0.029)
Ryńskie	0.088 (0.030-0.197)	0.040 (0.028-0.060)	0.017 (0.004-0.030)
Szymon	0.083 (0.044-0.095)	0.032 (0.014-0.040)	0.022 (0.012-0.033)
Smolak	0.012 (0.002-0.024)	0.064 (0.045-0.111)	0.003 (0.00-0.004)

Averages were calculated from five determinations. In parentheses – range of the mean values.

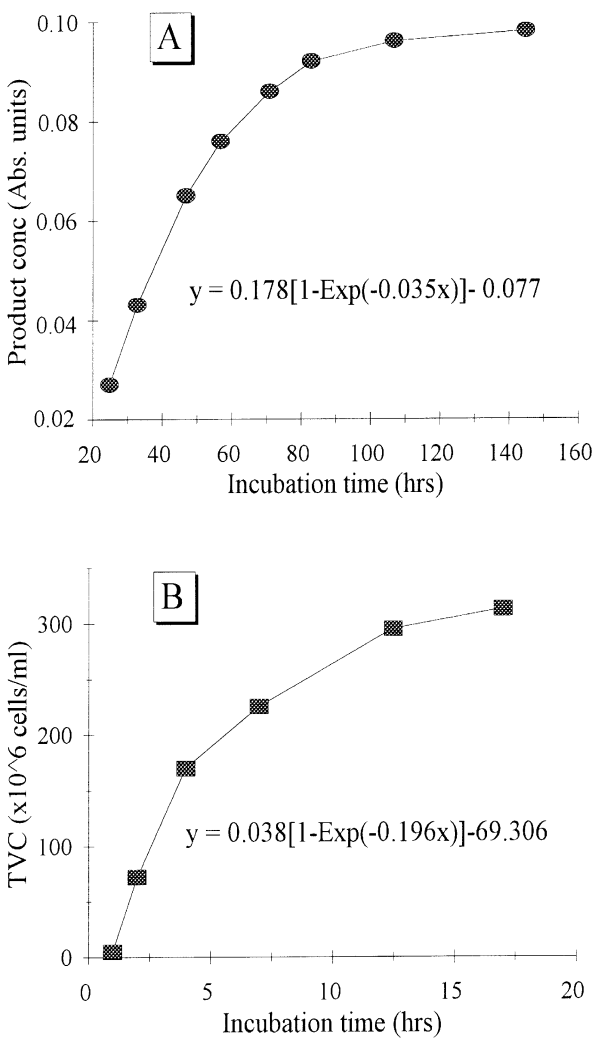

Fig. 4. Increase in particulate protein degradation products during incubation of lake water sample enriched with 0.5 mg ml^{-1} HPA – (A), and growth curve of aquatic bacteria cultivated on liquid medium [Wcisło unpubl.] – (B).

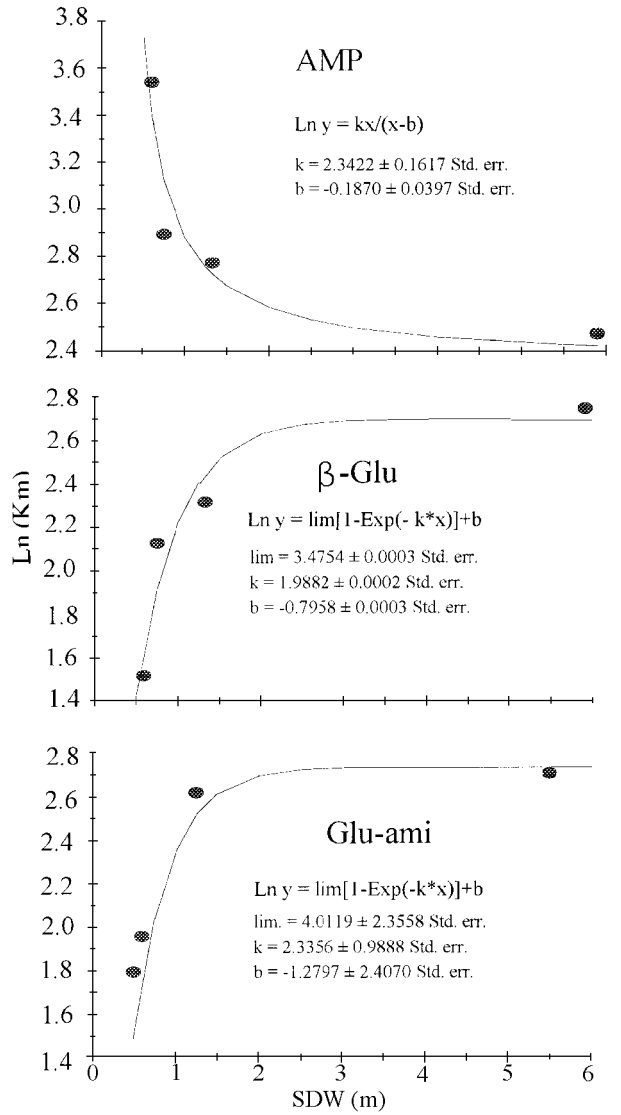

Fig. 5. The relationship between water transparency and affinity (Km) of the investigated enzymes to their substrates, in surface water of the studied lakes. For calculation of regressions mean values obtained from five determinations made during spring-summer period (April-October 1997) were used.

Smolak, except cellulose, particulate substrates (HPA and ChA) were colonized 5-6 times slower.

Analysis of mean activities (Vmax) of the studied enzymes decomposing DOM, and the turnover time of their substrates (Tt) (Table 4) showed that proteins and low-molecular-weight organic esters were the most important carbon and energy sources for aquatic bacteria. Observed AMP and EST activities were 1-2 orders of magnitude higher than enzyme activity responsible for hydrolysis of polysaccharides (β-glucosidase and glucosaminidase). Specific activities (Vmax/bacterial cell) of all studied ectoenzymes in polyhumic Lake Smolak were 10 to 15 times higher than in surface waters of other investigated lakes. The largest differences were observed for enzymes decomposing polysaccharides; smaller variations were found for enzymes hydrolyzing proteins and

Table 4. Kinetic parameters of hydrolysis of soluble substrates by the studied bacterial ectoenzymes in the surface water (1m depth) of the studied lakes.

Parameter	Lake	Enzyme			
		AMP	β-Gluc	Gluc-ami	EST
Vmax μmol l^{-1} h^{-1}	Kuc	73.2 (59.1-83.1)	8.7 (4.6-15.7)	3.9 (1.9-6.7)	33.6 (25.5-40.8)
	Ryńskie	681.3 (487.7-824.9)	17.3 (7.9-30.5)	16.7 (8.9-35.6)	168.5 (91.7-239.9)
	Szymon	1430 (634.7-2506.5)	54.0 (38.9-74.4)	26.4 (21.7-32.2)	119.3 (57.7-191.2)
	Smolak	4478.5 (3443.7-6794.7)	652.7 (225.6-960.5)	520.9 (167.7-729.1)	754.0 (536.4-1062.4)
$Vmax_{spec.}$ amol h^{-1} $cell^{-1}$	Kuc	4.0 (2.2-7.8)	0.5 (0.2-0.3)	0.2 (0.1-0.3)	1.8 (1.5-2.8)
	Ryńskie	24.6 (17.0-33.0)	0.6 (0.5-1.5)	0.6 (0.3-1.2)	4.3 (1.2-8.7)
	Szymon	47.5 (23.0-92.0)	1.8 (1.5-7.0)	0.9 (0.7-1.6)	5.6 (2.1-6.8)
	Smolak	190.8 (61.0-307.0)	27.8 (7.7-53.0)	22.2 (5.7-39.0)	32.1 (20.0-52.0)
Km μM	Kuc	11.8 (5.9-18.4)	15.5 (0.2-26.1)	14.9 (0.1-43.8)	4.6 (2.3-6.4)
	Ryńskie	16.0 (11.8-22.6)	10.1 (0.2-44.1)	13.8 (0.1-59.3)	3.3 (1.8-4.3)
	Szymon	18.0 (9.4-38.2)	8.4 (0.6-25.0)	7.1 (0.6-26.1)	4.5 (2.3-5.9)
	Smolak	34.5 (0.5-70.5)	4.6 (0.8-8.8)	6.0 (1.5-7.8)	3.0 (1.7-5.2)
Tt $days^{-1}$	Kuc	6.7 (4.2-10.1)	69.1 (1.1-233.3)	145.8 (1.9-158.3)	5.8 (3.0-8.0)
	Ryńskie	1.0 (07-1.2)	51.2 (0.6-232.0)	19.6 (0.4-69.4)	0.9 (0.5-1.3)
	Szymon	0.4 (0.3-0.6)	6.5 (0.6-18.4)	10.0 (0.9-33.8)	1.8 (0.9-2.0)
	Smolak	0.3 (0.02-0.9)	0.3 (0.05-0.6)	0.5 (0.1-0.9)	0.17 (0.1-0.2)

Table contains averages calculated from five determinations. In parentheses — range of values.

Table 5. Correlations between the rates of enzymatic solubilization of particulate substrates (HPA, CA and ChA) and the rates of decompostion of their soluble products (oligopeptides, β-glucosides and glucosaminides) by heterotrophic bacteria in surface waters (1 m depth) of the studied lakes.

Correlated parameters	Lake			
	Kuc	Ryńskie	Szymon	Smolak
Peptidase* vs AMP**	r=0.22, n=5, nonsign.	r=0.25, n=5, nonsign.	r=-0.87, n=5, p<0.05 $y=-3.7\cdot10^{-5}x + 9.8\cdot10^{-2}$	r=-0.33, n=5, nonsign.
Cellulase* vs β-Gluc.**	r=-0.75, n=5, nonsign. $y=1.6\cdot10^{-3}x + 5.5\cdot10^{-2}$	r=0.76, n=5, nonsign. $y=1.2\cdot10^{-3} x +2\cdot10^{-2}$	r=0.33, n=5, nonsign.	r=0.90, n=5, p<0.05 $y=-7.9\cdot10^{-5} x +0.1$
Chitinase* vs Gluc-ami**	r=-0.91, n=5, p<0.05 $y=-3.3\cdot10^{-3}x + 3.3\cdot10^{-2}$	r=-0.78, n=5, nonsign. $y=-6.9\cdot10^{-4}x +2.8\cdot10^{-2}$	r=-0.54, n=5, nonsign.	r=-0.75, n=5, nonsign.

* Determined using particulate substrates.
** Determined using soluble substrates.

various esters. The enzyme-substrate affinity was dependent on the kind of the enzyme and concentration of the seston in water samples of the studied lakes. In the case of AMP, Km increased when water transparency decreased (Fig. 5 A). Inversely, Km calculated for β-glucosidase and glucosaminidase increased logarithmically with the increase of water transparency (Fig. 5 B, C). Relatively short Tt of standard soluble substrates (Table 4) indicated that in polyhumic Lake Smolak enzymatic activity of heterotrophic bacteria was extremely high. Mean Tt (for each enzyme-substrate system) did not exceed 0.3 day. For comparison, in Lake Kuc the shortest Tt observed for esterase was 5.8 days.

Comparison of the activity of peptidase towards soluble and particulate substrate (Fig. 6 A, B, C, D) and calculations of the correlation between soluble and particulate substrate utilization by bacteria in the surface waters of the studied lakes (Fig. 6 C) showed that rates of their enzymatic hydrolysis were significantly correlated ($r = -0.62$, $p < 0.001$, $n = 20$). More detailed statistical analysis showed (Table 5) that these preferences were especially significant for samples with high seston contents e.g. from Lake Szymon ($r = -0.87$, $p < 0.05$, $n = 5$). In mesotrophic Lake Kuc, heterotrophic bacteria were probably less specialized and they simultaneously utilized both particulate and dissolved protein substrates. Similar calculations were done for chitinase – glucosaminase and cellulase – β-glucosidase systems (Table 5). Celulolytic bacteria, opposite to proteolytic bacteria, distinctly preferred only one type (particulate or dissolved) substrate

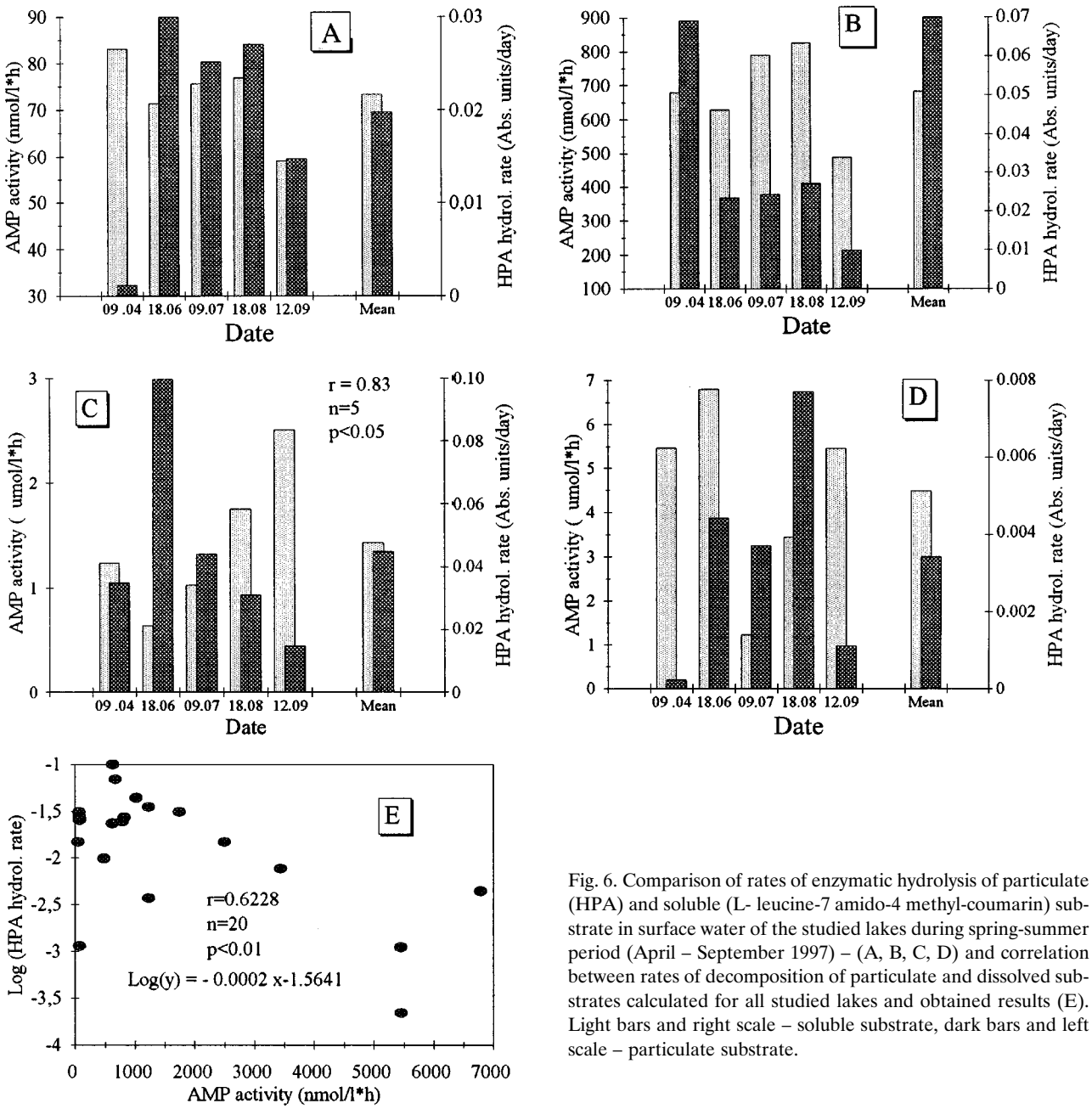

Fig. 6. Comparison of rates of enzymatic hydrolysis of particulate (HPA) and soluble (L- leucine-7 amido-4 methyl-coumarin) substrate in surface water of the studied lakes during spring-summer period (April – September 1997) – (A, B, C, D) and correlation between rates of decomposition of particulate and dissolved substrates calculated for all studied lakes and obtained results (E). Light bars and right scale – soluble substrate, dark bars and left scale – particulate substrate.

in lakes with relatively low seston concentration (e.g. Lake Kuc and Lake Smolak). In eutrophic lakes, with high content of seston (Lake Szymon and Lake Ryńskie), no preference for substrate was observed. In the case of chitinolytic enzymes, the presence of dissolved products of chitin degradation (glucosamines) decreased the rate of chitin polymer solubilization.

The results of the studies on the influence of enzymatic activity (degrading particulate and dissolved substrates) on secondary production of free-living and seston-attached bacteria are presented in Figs. 7 and 8. Generally, specific activities of all tested enzymes decomposing dissolved substrates were significantly higher in lakes where production of particle-attached bacteria predominated (Fig. 7 A, B, C, D). Particle-attached bacteria exhibited higher activity (synthesis rate) of each tested enzyme in comparison to free-living bacteria. This was the most distinct in polihumic Lake Smolak and less evident in seston rich Lake Szymon. In Lake Kuc and Lake Ryńskie, where free-living bacteria mainly participated in total secondary production, activities of all studied enzymes were similar.

The measurement of real rates of decomposition of particulate substrates was difficult. This made it impossible to analyze more precisely the relationship between the specific activity of the enzymes participating in these processes and secondary production of each (particle-attached and free-living) group of aquatic bacteria. However, arrangement of data points in Fig. 8. suggests that the activities of the studied enzymes distinctly affected secondary

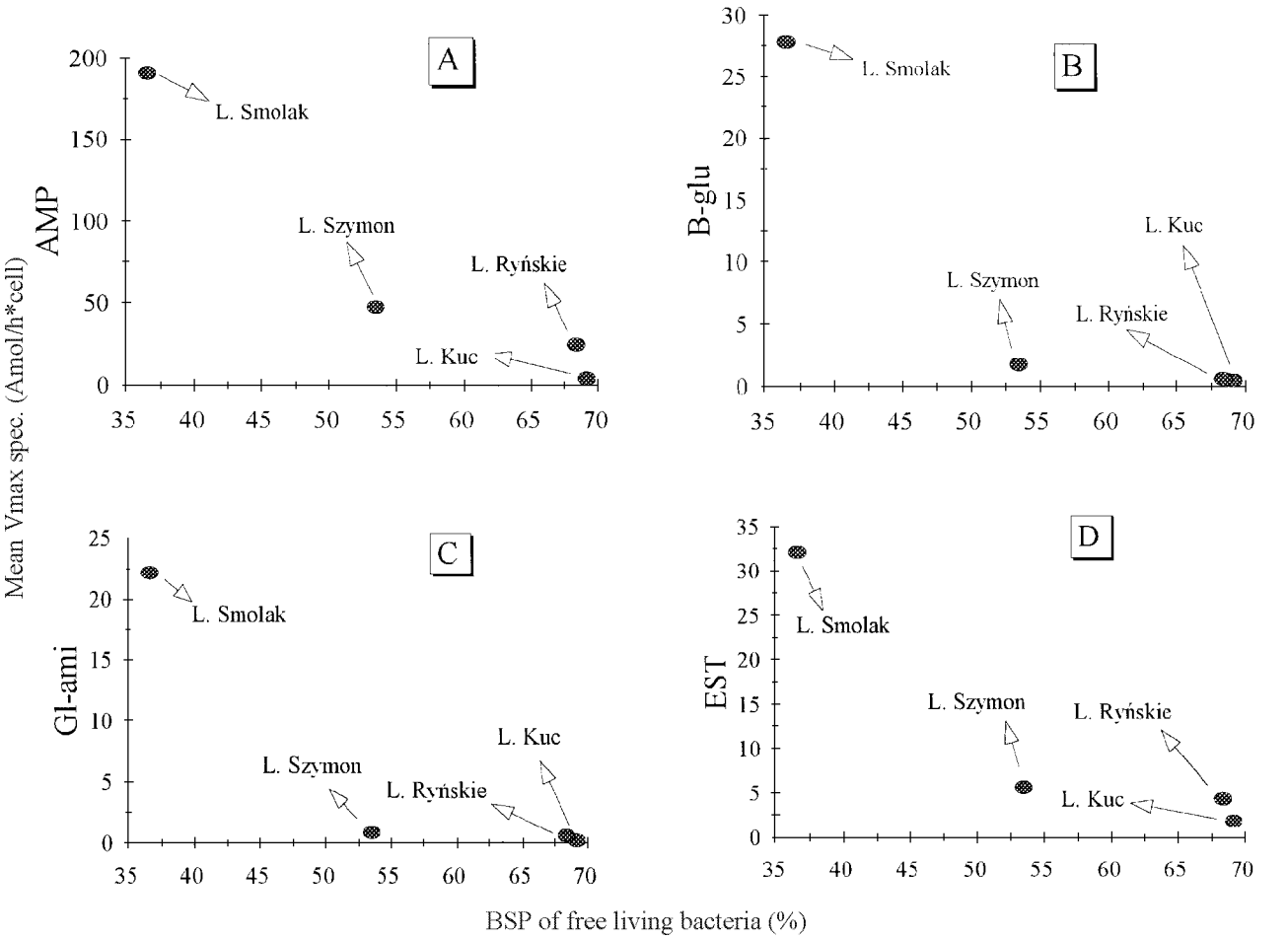

Fig. 7. Relationship between mean specific activity (Vmax spec.) of aminopeptidase (A) – glucosidase (B), glucosaminidase (C), esterase (D) and secondary production of free-living bacteria. Mean values were calculated for five measurements made during spring-summer period (April – October 1997).

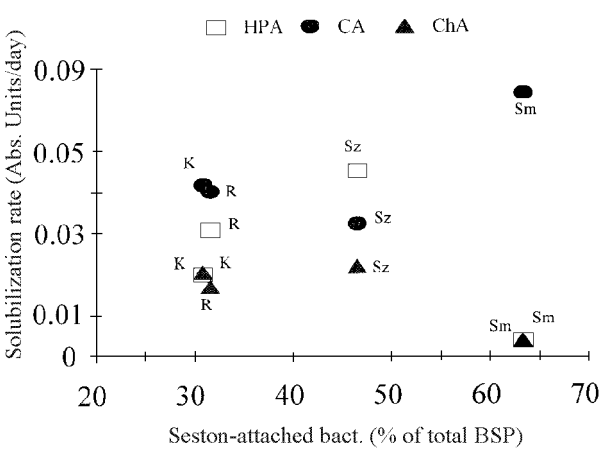

Fig. 8. Correlation between rates of solubilization of proteins (HPA) cellulose (CA) and chitin and secondary production of attached bacteria in the surface water of the studied lakes. Each point exhibits mean value calculated for 5 measurements made during spring-summer period (April – October 1997).

production neither free-living nor particle-attached bacteria. Otherwise, secondary production of both bacterial groups was dependent equally on activity of enzymes decomposing both dissolved and particulate substrates.

Discussion

The use of enzymatic techniques for qualitative description of transformation and decomposition of organic matter in lake water is not questioned [5]. However, it should be pointed out that applying the obtained data for the quantitative characterization of enzymatic hydrolysis of various DOM and POM components in aquatic environment is difficult. Application of the results of laboratory experiments to natural environments is seriously complicated by the fact that the methods of enzyme activity determination are based on the use of single, "artificial" substrate (which has chemical bound identical to natural substrates) that does not exist in natural environment. Moreover, the measurements of enzyme activity in water samples are usually performed in suboptimal and partially non-controlled conditions (including red- ox potential, presence of natural enzyme inhibitors and activators). However, the importance of some of these limitations can be minimized, e.g. by using additional "controls", standardized incubation conditions and saturating enzyme substrate concentrations. The conclusions drawn from obtained results should still be treated with great caution. The experiments described in this report were constructed to obtain information about enzymatic

decomposition of proteins and polysaccharides, two major groups of organic constituents in the pool of organic matter in aquatic environments. For this purpose, two groups of enzymes were investigated. Activity of these enzymes (proteases, cellulases and chitinases) that were responsible for preliminary POM decomposition (solubilization) was determined using particulate substrates (HPA CA and ChA). For measurement of the non-specific enzymes that catalyze further hydrolysis of oligomers liberated from particulate proteins and polysaccharides (aminopeptidase, β-glucosidase, glucosaminidase and esterase) soluble substrates (L-leucin-7-amido-4-methyl-coumarin, MUF-β-d-glucopyranoside, MUF-N-acetyl-β-d-glucosaminide and fluorescein diacetate, respectively) were used.

POM and DOM – Carbon and Energy Source for Aquatic Bacteria

According to commonly accepted opinion dissolved organic matter is the major source of substrates for heterotrophic microorganisms in all aquatic environments. The predominant role of DOM for carbon and energy cycling in aquatic environments is well known. However, it is not so obvious if one recognize that only small part of the low-molecular-weight compounds existing in lake water are free (really dissolved) molecules. Although the pool of "really dissolved" DOM is continuously supplemented by algal extracellular release, cell damage (autolysis or phage lysis) and POM solubilization processes, the majority of dissolved organic compounds in aquatic ecosystems is still, more or less strongly, bound to surfaces of dead and living seston particles. They may also create a so-called "organic overcoat" of large mineral and organic colloid molecules [24]. Lake Smolak is a good example of a lake where POM and colloidal fractions of DOM (CDOM) are equal, or even more important as DOM for overall effect of organic matter decomposition processes. High concentration of humic substances in this lake probably resulted in permanent deficiency of the low-molecular-weight compounds in *stricte* liquid phase and simultaneously, in abundance of these substances adsorbed to colloidal and particulate material. According to Munster [25], concentration of the low-molecular-weight organic compounds, which are easily utilizable by bacteria (UDOM) does not exceed 0.1 – 1.0% of total DOC content in polyhumic lake. In spite of apparent deficiency of UDOM, activity of all studied enzymes, as well as bacterial secondary production in Lake Smolak were extremely high. The significance of POM and colloidal DOM for bacterial metabolism was also confirmed by our further studies [26]. An effect of sorption and immobilization processes in lakes containing large amounts of natural sorbents (i.e. seston particles and/or mineral and organic colloids) resulted in overproduction of hydrolytic enzymes which appeared as an increase in enzyme activity exhibited by single bacterial cell (specific activity). We found that mean specific activity of the studied enzymes, calculated for each examined lake, was always proportional to POM concentration. Similar phenomenon caused by mineral colloids was observed by Jansson [27] in acidified Swedish lake.

In mesotrophic Lake Kuc, bacterioplankton mainly utilized UDOM, because POM concentration even during summer stratification period was commonly low. Relatively high extracellular release of photosynthetic products was the most important source of organic substrates supporting growth of heterotrophic bacteria [20]. Contrary to polyhumic Lake Smolak, in Lake Kuc secondary production of free-living bacteria predominated. In typical eutrophic lakes like Lake Ryńskie the significance of POM for the whole processes of organic matter decomposition was probably dependent on the time of year. Particulate organic matter was presumably less important in spring and early summer but more significant during autumn.

Actual nutrients and energy requirement strongly affect the synthesis rates, structure and efficiency of microbial ectoenzymes. On the other hand, resultant activity of ectoenzymes determines the sequence and rates of utilization of various DOM and POM constituents by microorganisms. Studies on activity of ectoenzymes in natural (untreated) water samples and in samples enriched with POM showed that proteins and phosphate esters were firstly utilized by aquatic bacteria. Enzymatic decomposition of other low-molecular-weight esters, cellulose and chitin began later and was generally slower. Therefore, during periods when the concentration of P and N containing compounds in DOM pool did not fulfill bacterial demand, microorganisms intensified enzymatic mechanisms to utilize substrates from the POM pool.

Observations on enzymatic hydrolysis of POM substrates in Lake Smolak are especially interesting, although they are not fully understood. Despite high AMP activity noted in this lake (that suggested a large amino acids requirement of bacteria), mean rates of HPA solubilization were 4 times lower than hydrolysis rates of cellulose. It seems that two alternative hypotheses can explain this phenomenon. First, in lakes with high potential sorption of organic suspension bacteria utilize primarily soluble proteins adsorbed on sestonic and colloidal particles. This leads to overproduction of non-specialized enzymes with both high specific Vmax and Km. Exponential decrease affinity of AMP to its substrate was noted in water samples with increasing amounts of the seston (Fig. 5). On the other hand, the decrease in affinity of the enzyme produced by particle-attached bacteria to its substrate could also be an artifact, because Km estimated experimentally was in fact a sum of Michaelis constant of AMP for L-leucyl-4-methyl-7-coumarinylamine (standard substrate added to the samples) and the concentration of natural aminopeptidase substrates present in the lake water. Assuming this, one can argue that the observed increase in Km value in seston-rich water samples was only an effect of competitive inhibition of hydrolysis of standard substrate by soluble proteins liberated from the particles of POM suspended in the environment.

Participation of Particle-Attached and Free-Living Bacteria in Processes of POM and DOM Decomposition

The technique of direct counting under the epifluorescence microscope has many limitations for quantitative determination of free-living and especially for

counting of seston-attached bacteria. Focusing field depth of a greatly magnified picture (1000x) caused by large (in comparison to bacterial cells) dimensions of the seston particles, made difficult precise enumeration of bacteria in water samples from seston-rich lakes. Therefore, for description of the role and importance of both groups of bacteria, we have been forced to use secondary production (BSP) and enzymatic activity toward dissolved and particulate substrates.

Similarly to other studies [28, 29, 30], our observations showed that free-living bacteria mainly contributed to total BSP in a majority of lakes. However, we found that in lakes with high CDOM and/or POM content (Lake Szymon and Lake Smolak) BSP and respiration of seston-attached bacteria predominated (Chróst unpubl.). According to Tranvik [1] the growth yield of free-living microorganisms exceed 25% and is about 2-times higher than that of particle-attached bacteria. This may be explained probably by relatively good availability of a great variety of nutrients and easily utilizable organic compounds that are dissolved in liquid phase. In microenvironments created by solid particles food sources are more abundant but qualitatively less variable and generally hardly available.

The results of our studies on efficiency of enzymatic mineralization of organic matter by both groups of bacteria indicated that in lakes with low (Lake Kuc) and moderate (Lake Ryńskie) POM content, where secondary production of free-living bacteria dominated, decomposition of organic matter was slower (longer Tt and higher Km) than in lakes abundant with colloidal DOM and POM, where particle-attached bacteria predominated in BSP (Lake Smolak and Lake Szymon). Our findings are contradictory to the conclusions of Tranvik [1] and to results obtained by Marshall & Bitton [31] and Paerl [32]. These reports suggested that the majority of free-living bacteria were attached to seston particles when POM concentration in lake water increased. We hypothesize that the presence of DOM stimulates an increase of bacterial biomass, whereas high content of POM in lake water accelerates respiratory processes.

One of the most interesting aspects of the role of free-living and particle-attached bacteria in organic matter decomposition processes is the question of whether permanently high POM or colloidal DOM concentration induces the selection of bacterial populations that utilize preferentially seston or DOM adsorbed on large colloidal particles as the major nutrient source. It is not completely clear whether bacteria "prefer" to be free-living or attached to the surface of solid particles. Observations of Marshall and Bitton [31], and Paerl [32] that free-living bacteria colonize the seston during POM abundance periods suggests that their tight coupling with POM particles can be advantageous and give them a distinct superiority over free-living bacteria. Consequently, in spite of organic seston particles hardly available as food and energy sources for aquatic bacteria, one can presume that both POM and colloidal DOM may be attractive equally as DOM for aquatic microheterotrphs in some ecological circumstances. Results obtained during our investigations suggested that bacterial populations might be, to some extent, specialized to utilize the same types of substrate from both POM and DOM fractions. In lakes rich with particulate substrates, bacteria commonly preferred one of two types protein substrates (dissolved or particulate), whereas particulate cellulose and dissolved β-glucosides were used simultaneously. In less POM abundant Lake Kuc distinct preferences were observed in the case of saccharides, while both forms of the protein substrates were utilized with equal efficiency.

The problem of specialization and adaptation of bacteria for utilizing both DOM and POM in different types of lakes has not yet been resolved. The only one, moderately consistent model of organic particle colonization by bacteria in aquatic environments was proposed for protein substrates [33]. It was shown that some species of bacteria (e.g. *Flavobacterium sp.*) with relatively constant proteolytic activity were especially well adapted to colonization of particulate proteins when concentration of dissolved proteins was low. Afterwards, when proteolytic activity of the first group of microorganisms increased the concentration of dissolved proteins in the environment, other strains of bacteria that were specialized in decomposition of dissolved proteins (e.g. *Pseudomonas sp*.) appeared. Conclusions of our studies are partially consistent with the above statements. However, it is still unclear whether bacteria producing constitutive enzymes are responsible for preliminary steps of POM solubilization and whether the same bacteria (with similar ectoenzyme staff) participate in decomposition of particulate and soluble substrates. Literature data [33] and the fact that standard particulate substrates (HPA, CA and Cha) were resistant for degradation by free (extracellular) enzymes support the first possibility. The second hypothesis confirms facts that seston particles may be colonized by free-living bacteria and that solubilization rates of particulate substrates may be controlled by a catabolic repression mechanism that is characteristic for ectoenzymes. We observed this phenomenon in the case of cellulose. CA hydrolysis was strongly affected by the presence of glucose, which is a well known repressor of β-glucosidase, i. e. enzyme which hydrolyse cellulose degradation products (cellobiose) [5].

Conclusions

Results of our studies indicated that POM and colloidal DOM fractions are both the significant nutrient and energy sources for aquatic bacteria. Seston particles in lake water, similar to DOM, undergo intensive enzyme degradation processes after their previous colonization by microorganisms. Increased influx of DOM (particularly UDOM) to the environment increases the number of heterotrophic bacteria, whereas the presence of POM and colloidal DOM intensify commonly bacterial respiration, and thus the overall organic matter decomposition rates in aquatic environments. Free-living bacteria, that preferentially utilize UDOM, predominate in lakes of low or moderate trophic status. Increase in POM content in these environments causes rapid changes in substrate exploitation strategy resulting in adaptation of bacteria to live in particle-attached stage. In lakes where POM and colloidal DOM are abundant seston-attached bacteria are responsible mainly for secondary production and

probably for POM mineralization. Moreover, one can presume that they also utilize preferentially UDOM adsorbed to the seston particles and subsequently highly polymerized, *stricte* particulate POM. Possible mechanisms that permit effective POM degradation by particle-attached bacteria are: overproduction of enzymes (e.g. aminopeptidase) with relatively low affinity (high Km values) and/or changes in their enzyme systems (increased synthesis of the isoenzymes with high affinity, e.g. β-glucosidase or glucosaminidase) that are optimal for specific environmental conditions.

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