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Extracellular enzymes in a polyhumic lake: important regulators in detritus processing

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Abstract

Eight extracellular enzymes and their corresponding natural substrates were studied in an acid polyhumic lake. Highest activities were found for phosphatases (100–150 nmol l^{-1} h^{-1}), glucosidase (70–120 nmol l^{-1} h^{-1}) and aminopeptidases (20–30 nmol l^{-1} h^{-1}). Significant relationships were found for natural polymeric substrate composition, variation and enzyme activities. Identified carbohydrates and amino acids contributed 1–5% to the DOC pool and are assumed to undergo significant processing by microbial glycosidases and aminopeptidases. Measured enzymes are partially modified in their activity by lake water acidity, temperature and humic matter. Extracellular enzymes are regarded as important regulators in microbial detritus processing and substrate utilization.

Introduction

In many aquatic environments a major part of dissolved (DOM) and particulate organic matter (POM) is channelled through microheterotrophs to higher trophic levels (Sorokin, 1977; Williams, 1981; Pomeroy, 1974; Pomeroy & Wiebe, 1988). This 'microbial loop' has been now accepted as an important part of the food web in aquatic ecosystems (Azam *et al.*, 1983; Fenchel, 1987).

DOM contains many different solutes from which only 5–10% can be easily assimilated and metabolized by micro-heterotrophs (Overbeck, 1979). Of those solutes, the most important and frequently studied are dissolved free amino acids (DFAA) and carbohydrates (DFCHO), which comprise mostly monomeric and partially oligomeric compounds (Münster, 1984; Münster & Chróst, 1990). The other part of the DOM (80–90%) is polymeric in natura, e.g. polypeptides, polysaccharides, lipids, polynucleotides, other organophosphorus compounds, and recalcitrant polyphenolic humic substances (HS), (Münster, 1985; Steinberg & Münster, 1985; Münster & Chróst, 1990; Thurman, 1985).

The ratio between monomeric to polymeric substrates is in the range of 1:10 to 1:100 (Wetzel, 1983). Therefore, the polymeric compounds are the dominant organic solutes in the DOM pool. Monomeric, easily utilizable dissolved organic matter (UDOM) comprises predominately DFAA and DFCHO pools, and concentrations are in the nMOL to μMol range (Thurman, 1985; Jørgensen, 1987; Münster & Chróst, 1990). Because of their efficient uptake systems, aquatic bacteria assimilate these monomeric solutes rapidly from the UDOM pool for energy, carbon, phosphorus and nitrogen requirements (Over-

beck, 1979; Jørgensen, 1982; Azam & Cho, 1987). With respect to microbial growth, these monomers are rapidly exhausted and represents a first threshold concentration in the DOM pool (Jannasch, 1970; Sieburth, 1979). Bacterial growth also depends therefore, on the availability and utilization of polymeric substrates. Microplankton is known to be an efficient user of different kinds of organic matter, e.g. bacteria can synthesize extracellular enzymes for cleavage of polymeric substrates into monomeric and oligomeric solutes, with subsequent rapid uptake (Pollock, 1962; Rogers, 1961; Priest, 1984; Chróst *et al.*, 1989; Chróst, 1990; Münster, 1991). The ecological significance of such extracellular enzyme activities has been shown already in many aquatic environments (Hoppe, 1983; Hollibaugh & Azam, 1983; Ammerman & Azam, 1985; Billén, 1984; Meyer-Reil, 1986; Hoppe *et al.*, 1988; Chróst, 1989; Chróst *et al.*, 1989; Münster *et al.*, 1989; Münster, 1991). In some of these papers, correlations were found between heterotrophic uptake of [^{14}C]-glucose or [^{3}H]-leucine, bacterial production and exoenzyme activities (Hoppe, 1983; Hoppe *et al.*, 1988; Billén, 1984; Chróst *et al.*, 1989; Somville, 1984; Meyer-Reil, 1986). In most of these papers, the relations between added artificial, synthetic substrates and extracellular enzyme activities were tested. Fewer investigations have been made of the relation between natural substrates (e.g. polysaccharides, polypeptides) and the corresponding enzymes on the one hand and the relation between the enzymatic hydrolysis products (monomers) and the extracellular enzyme activities on the other hand (Chróst *et al.*, 1989; Münster, 1991).

We hypothesize that in most aquatic environments there should be a significant relationship between extracellular enzyme activities, their corresponding substrates (polymers) and their hydrolysis products (monmers). The validity of this hypothesis has already been verified in the eutrophic lake Plußsee (Chróst *et al.*, 1989). We intend to test this hypothesis also in a lake with high DOM and Humic Substance (HS) content, but probably lower UDOM content and we expect that polyphenolic compounds may modify enzyme activities partially, and environmental processes may affect their distribution patterns and activities. We consider these enzyme-substrate systems as important regulators for detritus processing and also for microbial food chain processes.

Study site

Mekkojärvi is a head water lake, located in the Evo forest area in southern Finland (Fig. 1), near Lammi Biological Station. In this area (about 100 km^2) more then 100 lakes with different trophic state and HS content can be found. Most lakes are located in bedrock areas of Diorit and Gneis, covered by morainic sediments. Like other lakes in this area, Mekkojärvi is surrounded by forest with spruce and scots pine and the littoral zone is in close contact with 0.5 to 1 m thick peat layers.

Mekkojärvi contains high amounts of allochthonous detritus. The water is brown coloured

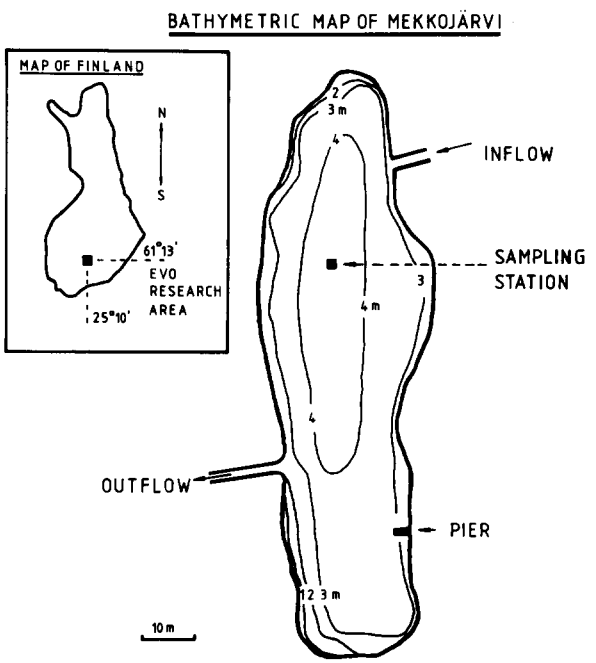

Fig. 1. Location of studies and bathymetric map of Mekkojärvi with sampling station, modified from Münster *et al.* (1989).

(300–600 mg Pt units l^{-1}) and after ice break the water warms rapidly. This water colour influences the light environment and compresses the trophogenic layer to a depth of 0.5–1 m. Rapid and steep light, nutrient, oxygen and temperature gradients can be measured (Jones & Arvola, 1984; Salonen, 1984). Because of this thin trophogenic zone, the most important nutrients (PO_4^{3-}, NO_3^-) are already rapidly depleted during spring bloom development (Arvola & Rask, 1984). The most important physical and chemical parameters are summarized in Table 1.

The dominant algae in polyhumic lakes like Mekkojärvi are motile, mostly flagellates like *Chlorophytes* and *Chrysophytes* (Ilmavirta, 1988). These can comprise more than 45% of total phytoplankton biomass in Mekkojärvi (Ilmavirta, 1983; Arvola, 1986). Intensive grazing by heterotrophic nanoflagellates (HNF) may also be expected. Salonen & Jokinen (1988) measured grazing rates by HNF in Mekkojärvi up to 40 bacteria cells $cell^{-1} h^{-1}$. Phagotrophic species like *Ochromonas*, *Chromulina*, *Chrysococcus* and *Uroglena* may contribute up to 30–80% of phytoplankton biomass (Salonen & Jokinen, 1988).

Methods

Lake water was sampled weekly from May until August 1986 and 1987 with a 30 cm long Patalas sampler in the trophogenic layer (0.2–1 m depth). Lake water was immediately filtered through a 100 μm plankton net (to remove larger zooplank-

Table 1. Basic limnological data of lake Mekkojärvi*, modified from Münster *et al.* (1991).

Parameter		Range	Average
Surface area	(ha)	–	0.35
Max. depth	(m)	–	0.35
Mean depth	(m)	–	2.2
Secchi depth	(m)	0.3–0.5	0.4
Mixing depth	(m)	0.5–0.9	0.7
Water color	(mg Pt l^{-1})	300–600	450
Conductivity	(μS cm^{-1})	2–10	6
pH		4.2–6.6	5.4
P_{tot}	(μg P l^{-1})	10–25	17
$P\text{-}PO_4^{3-}$	(μg P l^{-1})	2–12	7
N_{tot}	(μ N l^{-1})	500–1500	1000
$N\text{-}NO_3^-$	(μg N l^{-1})	20–80	50
$N\text{-}NH_4^+$	(μg N l^{-1})	10–70	40
DIC (diss. inorg. carbon)	(mg C l^{-1})	0.1–0.5	0.3
DOC (diss. organ. carbon)	(mg C l^{-1})	15–35	25
DFAA (diss. free amino acids)	(μg C l^{-1})	3–110	56
DFAA % C of DOC		0.02–0.6	0.3
DCAA (diss. combined amino acids)	(μg C l^{-1})	160–850	505
DCAA % C of DOC		0.85–3.2	2.2
DFCHO (diss. free carbohydrates)	(μg C l^{-1})	1–50	25
DFCHO % C of DOC		0.01–0.1	0.05
DCCHO (diss. combined carbohydrates)	(μg C l^{-1})	0.5–100	50
DCCHO % C of DOC		0.02–0.5	0.25
Chlorophyll-a	(μg l^{-1})	5–30	18
Bacteria numbers (AFDC)	(10^6 cells ml^{-1})	1–6	3
Bacteria biomass	(μg C l^{-1})	10–40	25
Heterotrophic activity	(μg glucose $l^{-1} h^{-1}$)	0.3–0.05	0.16

* Most physical and chemical data were taken from surface water (0–1 m) in 1986/87.

ton) into autoclaved plastic flasks (Nalgene Inc. USA), which were kept in coolers during sampling and transportation to the laboratory.

Nutrient concentrations ($P\text{-}PO_4^{3-}$, $N\text{-}NO_3^-$, P_{tot}, N_{tot}) were measured by standard methods as described by Arvola & Rask (1984) with an Akea auto analyzer. Dissolved organic carbon (DOC) was measured with a carbon analyzer (model Tocamaster, Beckman Inc. USA) with total combustion and IR-detection after lake water filtration through precombusted Whatman GF/F and prewashed Nuclepore 0.2 μm polycarbonate filters.

For chlorophyll measurements, lake water was filtered through precombusted glass fiber filter (Whatman GF/F). Filters were extracted with 10 ml of acetone for 10 hours at 4 °C in a refrigerator. Extracted chlorophylls were measured fluorimetrically according to Wetzel & Likens (1979), before and after acidification and corrected for phaeophytin. Chlorophyll concentrations were calculated from chlorophyll-*a* and phaeophytin-*a* calibration curves prepared with purified chlorophyll-*a*/phaeophytin-*a* from *Anacystis nidulans* (Sigma Chemie, FRG).

For bacterial counting, lake water was filtered through 0.2 μm Nuclepore filters, stained with acriflavine and counted by epifluorescence (Bergström *et al.*, 1986). According to this procedure, bacterial numbers are expressed as acriflavine direct counts (AFDC).

Extracellular enzyme assays were prepared according to Hoppe (1983) with small modifications as described by Münster *et al.* (1989). From freshly prepared 4-methylumbelliferyl (4-MUF) substrates and leucine-4-methyl-coumarinyl-7-amide (leu-MCA) stock solutions (10 mMol), 100 μl of 4-MUF- and leu-MCA substrate were added to 9.9 ml freshly sampled and 100 μm prefiltered lake water and incubated at *in-situ* temperature and lake water pH. Extracellular enzyme activities were followed fluorimetrically from enzymatic release of fluorescent 4-MUF and MCA from added non-fluorescent 4-MUF- and leu-MCA substrates. 4-MUF and MCA concentrations were calculated from freshly prepared 4-MUF and MCA calibration curves in filtered and preautoclaved lake water, to consider potential quenching or interference of 4-MUF- and MCA-fluorescence with HS (Münster *et al.*, 1989). Differentiation between acid- and alkaline phosphatase activities were achieved by measurements at two different pH values (pH = 5.5 and 8.2, adjusted with 0.5 mol borate buffer) in lake water.

The effect of polyvinylpyrrolidone (PVP) on enzyme activity was tested by adding soluble polyvinylpyrrolidone-15 (Serva Feinbiochemica, Heidelberg, FRG) to a final concentration of 3% (w/v) to lake water. After one hour of preincubation with PVP, enzyme activity was measured as described above.

Substrate analysis

Delicate flagellates are common in the plankton of Mekkojärvi, so 10–20 ml lake water were filtered through precombusted Whatman GF/F and subsequently through prewashed Nuclepore polycarbonate filters (0.2 μm) without vacuum. This pre-tested procedure minimized probable cell leakage or disruption of flagellates during filtration. Filtered lake water was kept frozen (−25 °C) until analysis.

Carbohydrates were analyzed with gas liquid chromatography (glc) according to Münster (1985) before and after hydrolysis with 1 N HCl at 110 °C for 10 h under N_2 atmosphere in precombusted glass ampoules. Using this procedure, two carbohydrate fractions were analyzed: dissolved free carbohydrates (DFCHO) and dissolved combined carbohydrates (DCCHO). Amino acids were analyzed as ortho-phthaldialdehyde (OPA)-derivatives according to Lindroth & Mopper (1979) on a Spherisorb ODS-2 column (5 μm, 4.3 × 250 mm) with a Spectra-Physics HPLC system, model SP 8700, connected to a SP 4100 integrator. The solvent system was prepared from solvent-A (methanol, LiChrosolve, Merck) and solvent-B (0.03 Mol Na-Acetate, pH:6.55, with 10% methanol and 0.5% THF v/v). Amino acids were detected fluorimetrically with a Kratos SF 970 fluorescence detector, with wavelength settings of 225 nm at excitation and

410 nm band path filter at the emission side. With these wavelength settings, we have obtained higher sensitivities combined with lower background noise, according to HS interference on the HPLC column. Amino acids were analyzed before and after hydrolysis with freshly three-fold distilled 6 N HCl at 110 °C under nitrogen atmosphere in precombusted and sealed glass ampoules. By this procedure, we could differentiate between dissolved free (DFAA) and dissolved combined amino acids (DCAA).

Results

Biomass indicators

Chlorophyll concentration varied between 0.5–60 $\mu g\, l^{-1}$ (Fig. 2). This is in the upper range of more than 50 different lakes studied in this area (Arvola, 1986). Highest concentrations were found just after ice melting, which suggest that nutrient availability enables highest biomass production.

Number of bacteria (AFDC) varied between 1–7 10^6 cells ml^{-1} (Fig. 2), but bacteria were small, with a mean volume of 0.04 μm^3. Calculated biomass varied from 10–40 $\mu g\, C\, l^{-1}$. Significant covariance between chlorophyll and number of bacteria (AFDC) was observed (Fig. 2).

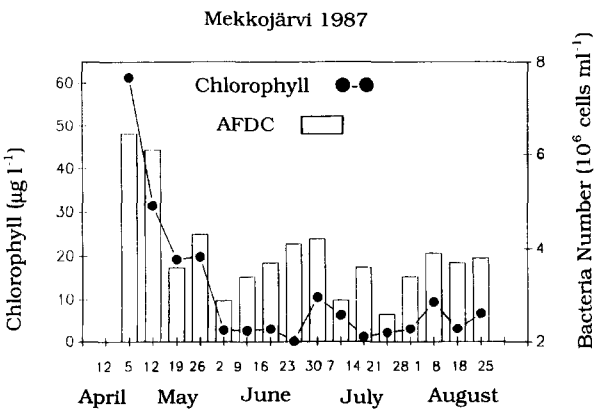

Fig. 2. Distribution of chlorophyll (filled circles) and bacterial number (AFDC, bars); modified from Münster (1991).

Composition of dissolved organic matter (DOM)

Because of the large drainage basin and the close contact of the littoral with peat land, Mekkojärvi water is rich in HS, Dissolved organic carbon content (DOC) is therefore high and varies between 8–25 mg $C\, l^{-1}$ (Tab. 1). But the content of UDOM, such as dissolved free amino acids (DFAA) and carbohydrates (DFCHO), is low (Table 1). The carbon content of DFAA comprise only 0.02–0.6% of the DOC and the DFCHO even lower (0.01–0.1% of DOC, Table 1). This is less then the remaining part of DOC. The dissolved combined amino acids (DCAA) and carbohydrates (DCCHO) were found in a range of 0.3–3.2% (mean 2.2%) and 0.02–0.5% (mean 0.3%) of the DOC pool, respectively. We found consistently that the UDOM pool in Mekkojärvi was extremely small compared to eutrophic alkaline lakes (Münster, 1984, 1985; Münster & Chróst, 1990). Consequently the amount of protein comprised only 0.92–3.8% of DOC from Mekkojärvi. From these data it can be assumed that utilizable polymeric solutes like DCAA and DCHO in the DOM are also less available for heterotrophic assimilation.

Extracellular enzyme activity, variation and distribution

Because of the low concentration of UDOM solutes, bacterial growth may also depend on the utilization and availability of polymeric substrates like DCAA and DCCHO. However, polymeric substrates have to be cleaved into monomers and oligomers by extracellular enzymes before they can be assimilated (Rogers, 1961). Only small molecules can enter microbial membranes and make contact with transport systems. Extracellular enzymatic substrate depolymerization may therefor become a rate-limiting step for growth of microheterotrophs.

We have followed eight different enzymes in Mekkojärvi at 0–1 depth (Table 2). Of these, phosphatases, glycosidases and aminopeptidase displayed the highest activities (Fig. 3). Highest

Table 2. Measured enzymes in Mekkojärvi.

Enzyme	Substrate	Enzyme activity (nmol l^{-1} h^{-1})	
		Range	Mean (± SD)
Phosphatase	MUF*–phosphate	51–162	50.4 ± 28.2
Leucine-aminopeptidase	MCA**–leucine	6.5– 26	15.0 ± 7.5
α-glucosidase	MUF-α-glucose	0.1– 90	6.7 ± 6.1
β-glucosidase	MUF-β-glucose	4.4– 68	23.6 ± 13.5
α-galactosidase	MUF-α-galactose	0.1–117	11.1 – 4.5
β-galactosidase	MUF-βgalactose	0.1– 65	8.7 ± 10.6
α-mannosidase	MUF-α-mannose	0.1–102	6.9 ± 3.9
β-mannosidose	MUF-β-mannose	0.1– 81	6.2 ± 3.6

* MUF: 4-methylumbelliferyl-
** MCA: 4-methyl-coumarinyl-7-amide-

activities (100–150 nmol l^{-1} h^{-1}) were measured for phosphatases and glycosidases in June and July. Glycosidase activity had maxima (70–120 nmol l^{-1} h^{-1}) in July and August. Aminopeptidase measurements started later and fluctuated between 20–30 nmol l^{-1} h^{-1}. Generally, a significant decrease of enzyme activity was measured from June to August. Among studied glycosidases (Table 3) β-glucosidase gave the highest activity. For all measured glycosidases the β-form displayed always higher values than α-form (Table 2). At present, we have no clear explanation for this result, and preliminary results from carbohydrate analysis in the DOM also gave no clear explanations. For all identified hexoses (glucose, galactose, mannose) only in 10–40% of measurements were the β-form of hexoses higher than the α-form. Because of analytical limitations with elucidating the complete structure of DOM and the included carbohydrates, we cannot clarify this phenomenon, but we assume that already during the analytical procedure (i.e. during the hydrolysis procedure) anomerization can occur at the C-1 position of analyzed hexoses (Pigman &

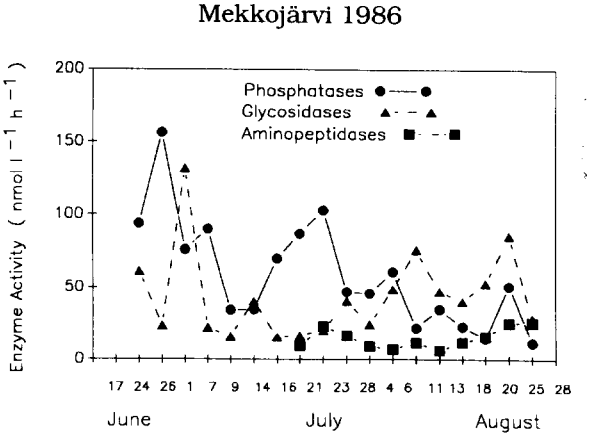

Fig. 3. Distribution of the three most active extracellular enzyme systems in Mekkojärvi (0–1 m depth): phosphatases (filled circles), glycosidases (filled triangles) and aminopeptidases (filled squares), modified from Münster (1991).

Table 3. α:β ratio of glycosidases activities in lake Mekkojärvi, 0–1 m depth, after Münster (1991).

Sampling	α:β-ratio		
Date	Glucosidase	Galactosidase	Mannosidase
1–7–86	0.31	0.14	3.00
7–7–86	0.01	0.01	0.01
9–7–86	0.13	0.01	0.01
21–7–86	0.57	0.17	0.10
28–7–86	0.10	–	–
4–8–86	0.20	–	–
6–8–86	0.32	0.08	–
11–8–86	0.18	0.71	–
13–8–86	0.54	–	1.71
18–8–86	0.79	1.77	0.35
20–8–86	0.79	0.95	2.95
25–8–86	0.62	1.83	0.01
28–8–86	0.47	0.83	1.32

Anet, 1972). This means that physical-chemical reactions (hydrolysis and anomerization) can modify the structural composition (α:β ratio) of enzymatically and chemically cleaved polymeric carbohydrates.

Substrate-enzyme interactions in Mekkojärvi

The significance of extracellular enzyme-substrate interactions in natural aquatic habitats has already been demonstrated (Hoppe, 1983; Chróst *et al.*, 1989). However in polyhumic lakes with high HS content and partially acid character, such studies are scarce (Münster *et al.*, 1989; Münster, 1991). We assume that extracellular substrate depolymerization can contribute to the availability and dynamics of UDOM solutes. We selected two enzyme-substrate systems to evaluate their participation in DOM processing.

The leucine-aminopeptidase-DFAA/DCAA system
Although we incubated only one fluorogenic substrate (leucine-MCA) for measuring aminopeptidase activities, we expected a reasonable relationship to the DFAA and DCAA pool sizes and variations. The DFAA pool varied less than the DCAA pool size (Fig. 4) and the ratio of DFAA to DCAA concentration varies between 1:10 to 1:5, but with significantly higher dynamics and variation of the DCAA pool size. Both substrate concentrations are certainly regulated by release and uptake by microplankton. There is no significant relation between concentration of DFAA and measured aminopeptidase activity (Fig. 5a). However, the DCAA concentration was significantly correlated to aminopeptidase activity (Fig. 5b). A further detailed analysis of the relation of measured aminopeptidase activity and the composition of identified AA in the DFAA and DCAA pools in 1987 revealed that with this aminopeptidase enzyme assay, we have not measured a highly specific cleaving mechanism, but

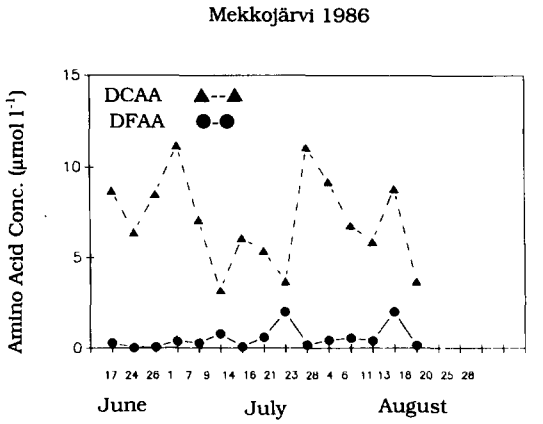

Fig. 4. Distribution and variation of dissolved free (DFAA, filled circles) and dissolved combined (DCAA, filled triangles) amino acids in Mekkojärvi (0–1 m depth).

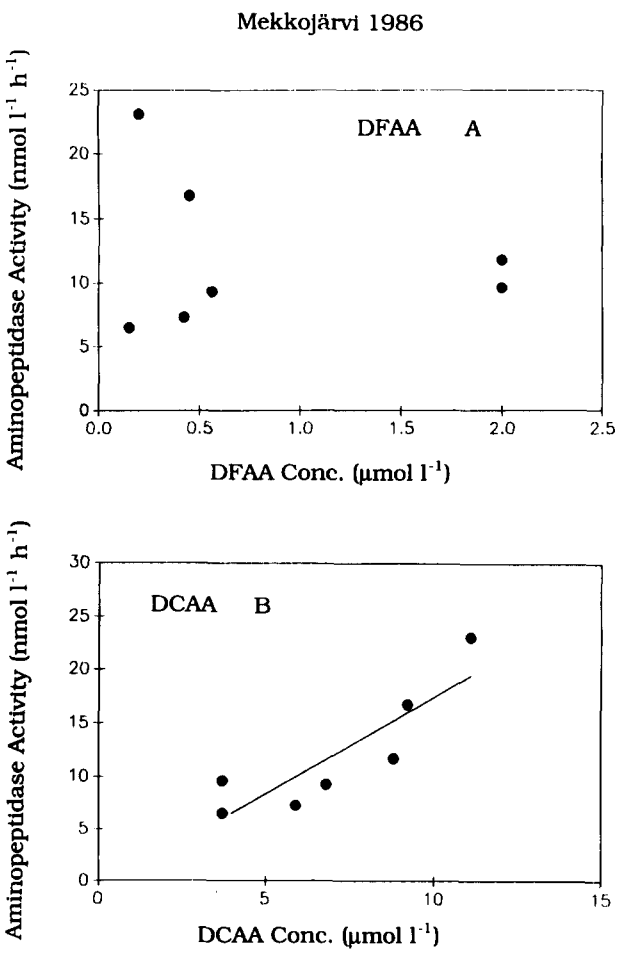

Fig. 5. Relationship between amino acids and aminopeptidase activity in Mekkojärvi water (0–1 m depth). A. Dissolved free amino acids (DFAA); B. Dissolved combined amino acids (DCAA, linear regression plot $y = 1.8257X - 0.7751$, $r^2 = 0.75459$, $n = 7$, $p < 0.0001$), after Münster (1991).

Table 4. Coefficients of correlations (r^2) between leucine-aminopeptidase and dissolved amino acids (daa) in lake Mekkojärvi, 1987, 0–1 m depth, after Münster (1991). $n = 7$, $p < 0.01$ in all cases.

No.	DAA*	Correlation (r^2)	
		DFAA**	DCAA***
1	Asp	−0.70	+0.87
2	Glu	−0.56	+0.87
3	Asn	−0.50	+0.88
4	Ser	−o.59	+0.81
5	Gly	−0.79	+0.78
6	Thr	−0.78	+0.87
7	Arg	−0.62	+0.77
8	Tyr	−0.77	+0.85
9	Val	−0.77	+0.65
10	Meth	−0.53	+0.61
11	Phe	−0.61	+0.81
12	Ile	−0.73	+0.78
13	Leu	−0.74	+0.93

* DAA : Dissolved amino acids.
** DFAA: Dissolved free amino acids.
*** DCAA: Dissolved combined amino acids.

rather unspecific peptide-cleaving mechanisms. Simple linear regression of DCAA with leucine-aminopeptidase activity from 1987 (Table 4) gave significant positive relationships for all DCAA (Münster, 1991). Measured aminopeptidase activity displayed, therefore, a less specific peptide-bond cleaving reaction in Mekkojärvi water.

The glucosidase-DFCHO/DCCHO system

From all measured carbohydrases in Mekkojärvi, about 50–70% of activity is represented by glucosidase activity. Because of the structural diversity of polysaccharides, we have measured α- and β-glucosidases. There is a significant decrease of enzyme activity from June to August and the β-form is predominant except in June (Fig. 6). Maxima of activity (90–60 nmol l^{-1} h^{-1}) were observed in June and July. As with the leucine-aminopeptidases system, we expected a positive relationship to natural substrate composition. The relation of DF-glucose to DC-glucose and their variation is shown in Fig. 7. Concentration of DF-glucose varied between 1–250 nmol l^{-1} and surpassed (in some cases) that of DC-glucose,

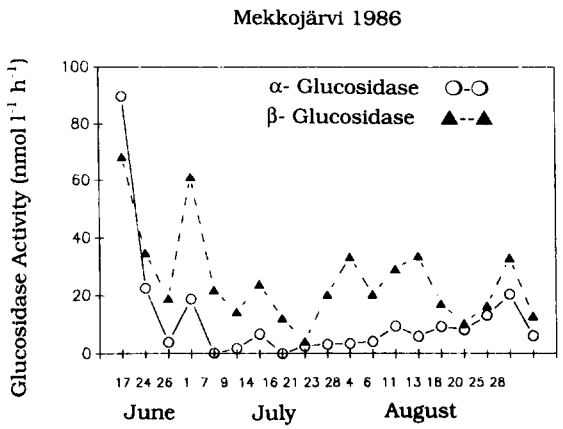

Fig. 6. Distribution and variation of glucosidase activity (α-glucosidase open circles, β-glucosidase filled triangles) in Mekkojärvi water (0–1 m depth).

which varied between 50–500 nmol l^{-1}. It can be clearly seen that DF-glucose and DC-glucose maxima and minima are oscillating in an opposite pattern. The mechanisms and explanations for such patterns might be important in relation to the glucosidase activities. Therefore, the relationship of extracellular glucosidase activity to DF- and DC-glucose is plotted in Fig. 8. Surprisingly, a significant clear relationship exists between both glucose pools and the glucosidase activity. DF-glucose has a significant negative correlation and

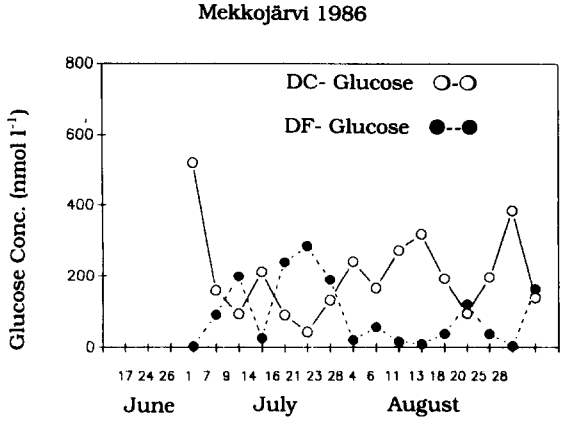

Fig. 7. Distribution and variation of dissolved free (DF-glucose, filled circles) and dissolved combined (DC-glucose, open circles) glucose concentration in Mekkojärvi water (0–1 m depth).

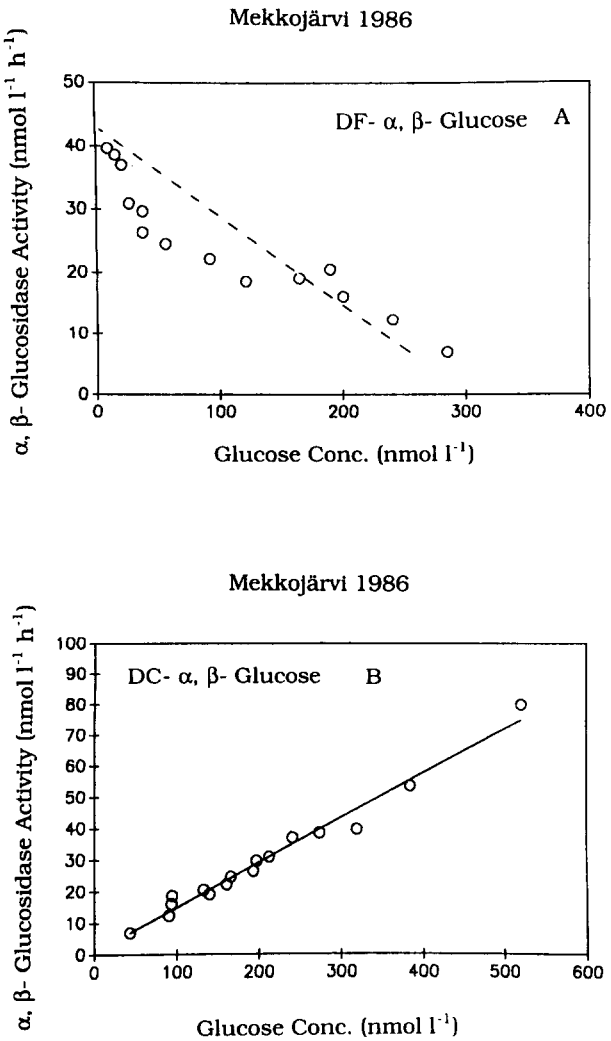

Fig. 8. Relationship between glucose and glucosidase activity in Mekkojärvi water (0–1 m depth). A. Dissolved free glucose (DFCHO) and glucosidase (linear regression plot, $y = -1.4237 + 43.155$, $r^2 = 0.56015$, $p < 0.0001$, $n = 16$) B. Dissolved combined glucose (DCCHO) and glucosidase (linear regression plot, $y = 1.4264X + 0.6897$, $r^2 = 0.9762$, $n = 16$, $p < 0.0001$), after Münster (1991).

DC-glucose a positive, similar to that found between DCAA and aminopeptidase. However, in contrast to the DFAA-aminopeptidase system DF-glucose gave a clear relation to the glucosidase activity. This deviation between both enzyme-substrate systems is not clearly understood, but it might be reasonable to suggest that glucosidases have a more specific reaction or cleaving mechanism, whereas leucine-aminopeptidase reacts unspecifically with many peptide bonds. It is not specifically inhibited by enzyme reaction products like DFAA (Table 4), whereas DF-glucose can inactivate the glucose-glucosidase system (Fig. 8a). We conclude that glucosidase activity is significantly regulated by product inhibition mechanisms, whereas leucine aminopeptidase was not significantly inhibited by the DFAA pool.

Environmental influences on extracellular enzyme activities

Our knowledge about hydrolytic enzyme activities and their regulation in natural habitats is still rather limited. Enzyme characterization has mostly been done with purified enzymes. Such enzyme assays are recommended and useful for biochemical studies, but enzyme studies in an ecological context have to consider environmental aspects, temporal and spatial variability of activity, external influences like temperature, acidity, inhibition and immobilization reactions. This means that measurements have to be made under '*in-situ*' conditions. Therefore, we have studied extracellular enzyme activities under natural conditions, and have taken into account temporal and spatial variability of activity, effects of temperature, acidity, ion composition, inhibition and immobilization (Figs. 3, 6, 9).

Temporal and spatial variation

Enzymes clearly fluctuate in a temporal manner (Figs. 3, 6). We also expect spatial variation. This fluctuation is partially due to the oscillation of the whole lake metabolism. Many complex metabolic processes are responsible for such distribution patterns. The aim of such studies was to evaluate the different regulating parameters, which influence extracellular enzyme activities.

Lake water acidity

Acidity (pH) in small head water lakes may change according to the nature and composition of allochthonous material and may therefore in-

fluence the activity of extracellular enzymes. Five measured enzymes showed different activities at changing pH in lake water (Fig. 9). Phosphatases, and all three glycosidases (α-, β-glycosidases, exo-cellobiohydrolases) have their relative maxima of activity at pH = 5.4, which was the mean pH of Mekkojärvi water during the 1987 sampling season. The leucine-aminopeptidases had their maxima of activity at pH = 7.8. For phosphatases we measured two maxima, the larger in the acid region (pH = 5.4) and the smaller in the slight alkaline region (pH = 8.5). We have not followed specifically the contribution of the alkaline phosphatases to the processing of organic phosphorus compounds in Mekkojärvi, but we estimated from laboratory experiments that about 5–10% of total phosphohydrolases is represented by alkaline phosphatase in Mekkojärvi water.

Temperature effects

Studies on temperature dependence of extracellular enzymes in Mekkojärvi revealed that, in comparison with the pH dependency, the enzymes do not operate under optimal temperature conditions. For all measured enzymes, we found temperature optima at 35–38 °C (data not shown!). This is far from '*in situ*' conditions, but not very different from laboratory studies on purified enzymes.

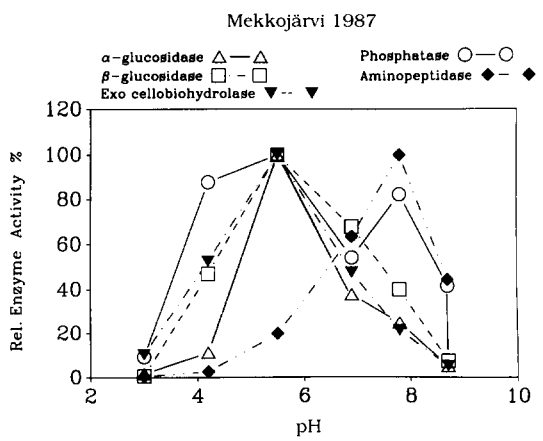

Fig. 9. pH dependence of phosphatases (open circles), amino-peptidases (filled diamonds), exo-cellobiohydrolases (filled inverse triangles), α-glucosidases (open triangles) and β-glucosidases (open squares) in Mekkojärvi water (0–1 m depth), after Münster (1991).

Immobilization by HS

Besides temperature and acidity, enzyme activities can be further modified and influenced by co-occurring organic or inorganic solutes. Because of the high amount of HS in Mekkojärvi water, we have tested the influence of HS on extracellular enzyme activities. According to Cannell *et al.* (1987, 1988a), microalgae can also produce protease and glycosidase inhibitors. These inhibitors have been characterized and identified as pentagalloylglucose derivatives (Cannell *et al.*, 1988b). The inhibitory mechanisms can be described as phenol-protein interactions (McManus *et al.*, 1985; Haslam, 1974). As HS contains polyphenolic compounds (Christman & Gjessing, 1983), such interactions may also occur in Mekkojärvi water. We have tried to evaluate such inhibitory interactions in the water samples by adding polyvinylpyrrolidone (PVP). PVP has been shown to react significantly with polyphenols via hydrogen bridge bonding (Olsson & Samuelson, 1974; Münster, 1985), which bind to the polyphenolic cores. If PVP is added to polyphenols, it immobilizes phenolic compounds. Such immobilized polyphenols no longer interact with proteins and decrease the inhibition of enzyme activities (Cannell *et al.*, 1988b). After adding PVP to Mekkojärvi water, enzyme assays displayed 10–23% higher activities when compared to untreated enzyme assays (Table 5). These results confirm our hypothesis that extracellular enzymes in natural habitats are partially modified in their activities.

Table 5. Extracellular enzyme activities before and after Polyvinylpyrrolidone (PVP) addition.

Name of enzyme	Enzyme activity (nmol l^{-1} h^{-1})		
	Control	+ PVP	% increase
Phosphatase	185.2	212.9	14.9
Leucine-aminopoptidase	5.5	6.9	25.6
β-glucosidase	52.8	57.8	9.5

Discussion

Studies on the role of extracellular enzymes in aquatic food web processes are relatively new and have only recently emphasized their importance in connection with microbial activities (Hoppe, 1983; Ammerman & Azam, 1985; Billén, 1984; Chróst, 1990; Wetzel, 1991; Münster, 1991). In recent studies on food chain structures in dystrophic lakes, it has been shown that heterotrophic activity may exceed that of autotrophy (Salonen, 1981; Winberg, 1980; Tranvik, 1989). The higher community respiration in polyhumic lakes has been attributed to additional DOM utilization by bacterioplankton (Salonen *et al.*, 1983). Allochthonous DOM, especially HS, was regarded as an additional carbon and energy source for bacterial growth (Salonen *et al.*, 1983; Salonen & Hammar, 1986; Johansson, 1983; Tranvik & Höfle, 1987; Tranvik, 1989). Simple UDOM solutes like radiolabelled monomeric sugars, amino acids, and also radiolabelled phenol have been tested as potential substrates for bacterial growth (Tranvik & Höfle, 1987), but these papers do not explain how the mechanisms operate or how rapidly bacterioplankton utilize natural polymeric and refractory substrates. Because of the complexity and dynamics of DOM in general and of HS in particular, there is still a need for information about the mechanisms and regulation of detritus processing (Wetzel, 1984; Münster & Chróst, 1990; Münster, 1991). UDOM solutes in Mekkojärvi do not comprise more than 0.01–0.35% of DOC (Table 1). Also, polymeric substrates like DCAA and DCCHO do not comprise more than 0.1% to 4% of DOC (Table 1) or 1–5% of dissolved organic nitrogen (DON). This is much lower in comparison to eutrophic lake water (Münster, 1985; Münster & Chróst, 1990; Jørgensen, 1987). We have to assume that bacterioplankton in Mekkojärvi are substrate-limited. Degradation and utilization of recalcitrant DOM, like HS, may contribute only slowly to the carbon demand of bacterioplankton. There is convincing evidence that natural bacterial assemblages in polyhumic lakes may have higher metabolic potentials for degradation of aromatic and polyphenolic compounds than those from clear water lakes (De Haan, 1974, 1977; Tranvik & Höfle, 1987). According to Schütt (1988), such metabolic potential for degradation of recalcitrant DOM may be even genetically localized (extrachromosomal DNA; i.e. plasmids). Different microbial communities may be responsible for HS degradation (Wetzel, 1984). Carbon dating have revealed that formation and degradation of HS proceed slowly (Stuermer *et al.*, 1978). Therefore, the supply of energy and carbon via HS degradation to the rapid dynamic processes in aquatic microbial loops may only partially support the required merits for nutrients, carbon and energy.

[^{14}C]-glucose uptake experiments in Mekkojärvi water revealed that uptake velocity of glucose varied between 0.1–3.5 nmol l^{-1} h^{-1} (mean 1.65 nmol l^{-1} h^{-1}); these values are in a similar range to those recorded in the eutrophic lake Plußsee (Chróst & Overbeck, 1989). Since UDOM pool sizes of DOM in Mekkojärvi are at least 5 to 10 times lower, and the bacterial biomass is in a similar range as in eutrophic lakes (e.g. lake Plußsee), a rapid turn-over of UDOM substrates is assumed. Unfortunately, we have not measured uptake kinetics to calculate the actual turnover e.g. for [^{14}C]-glucose. But, as we measured extracellular enzymatic substrate depolymerization, we could show that there might be significant regulatory mechanism in the water, which contributes to the processing of polymeric DOM. There was on average a release of about 15.6 nmol l^{-1} h^{-1} of glucose from added synthetic MUF-glucose by glucosidase activity (Fig. 3). In comparison to average glucose uptake, this means that theoretically there is an overflow of DF-glucose into the UDOM pool. Since such glucose overflow has been never reported, this may raise the question, whether the synthetic MUF-compounds represent a good natural substrate to the enzyme systems to calculate together with glucose uptake data the flow rates of natural substrates in the DOM pool. According to results in Fig. 8 and previous studies on extracellular substrate depolymerization and substrate uptake, we expect a close relationship

and feed-back mechanism between substrate uptake and extracellular enzymatic release of glucose (Chróst *et al.*, 1989; Münster, 1991). According to our data, we may have a tenfold higher rate of inflow of DF-glucose by extracellular enzyme activity to the UDOM pool. Similar experiments by Hoppe *et al.* (1988) with the leu-MCA/leucine aminopeptidases system have shown that in the brackish water of Kiel Fjord, similar ratios for hydrolysis and uptake (6.8:1 and 13.4:1) of leucine occurred. We assume that there is an oscillating regulatory mechanism between substrate uptake systems and extracellular substrate depolymerization systems. This mechanism would have a much faster response of the uptake systems to the UDOM pool sizes, when compared to UDOM supply via enzymatic substrate depolymerization. This may be due to environmental parameters like pH, oxygen, HS (see Fig. 9), which influence more strongly the enzymatic hydrolytic processes than the uptake systems. Therefore, the actual rates of substrate uptake and enzymatic cleavage of polymers may be moderately coupled. We estimate from our studies of glycosidases and aminopeptidases that about 1–5% of DOC could be processed and utilized by the microorganisms. We consider our enzyme-substrate systems to be suitable tools to study detritus processing and UDOM pool size regulation on a biochemical level. This approach may provide deeper insight into ecophysiological processes and may improve our understanding of the dynamics and regulations of microbial food chain processes.

Microbial enzymatic substrate processing may certainly also exist for other, more recalcitrant substrates like HS, but we know less about the biochemical background of microbial HS depolymerization and utilization, which may explain the higher heterotrophy in humic compared to clear water lakes. Photochemical reactions may also be involved in the decomposition and (together with enzymatic attack by peroxidases of recalcitrant DOM) enhanced microbial utilization of HS may occur (Salonen & Tulonen, 1990; Münster, 1991).

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