BACTERIAL ENZYME ACTIVITIES IN GROUND WATER
DURING BANK FILTRATION OF LAKE WATER

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Abstract—The interactions between organic matter, bacterial biomass, enzyme activities and environmental factors were studied during bank filtration of humus-rich lake water. The exoenzymatic β-glucosidase, phosphatase and alanine-aminopeptidase activities in water were measured in vitro as release of fluorescing degradation products from methylumbelliferyl substrates. The total enzymatic decomposition activity was measured as the hydrolysis of fluorescein diacetate (FDA). Bacterial enzymatic activities decreased strongly after infiltration of lake water. The decrease in the enzyme activities correlated with decrease in bacterial counts and biomass production. However, the increase in specific FDA-hydrolysis activity (activity per bacterial cell) indicated that maintenance energy requirements increased during filtration in the ground. There was also an increase in the specific phosphatase activity, which might be associated with the decrease in the concentration of available phosphate. All enzyme activities depended on seasonal temperature changes. The highest FDA-hydrolysis, phosphatase and β-glucosidase activities occurred during the summer months, when the bacterial production activity and the demand of essential nutrients were highest. On the contrary, the alanine-aminopeptidase activity was highest during autumn and winter, probably as a result of infiltrated nitrogenous material from senescing and dying microbes and algae in lake water. The close correlations between enzymatic activities and other microbial parameters suggest that enzyme activities can be used to monitor the changes in microbiological quality of water during bank filtration of lake water. Copyright © 1996 Elsevier Science Ltd

Key words—bacteria, bank filtration, enzyme activities, ground water, lake water, organic matter, phytoplankton

NOMENCLATURE
AapA = alanine-aminopeptidase activity (pmol/ml h)
AODC = acridine orange direct count
AOC = assimilable organic carbon (µg acetate eq-C/l)
ATCC = American Type Culture Collection
BP = bacterial production (ng C/ml h)
CFU/ml = colony forming units/ml
Chl-a = chlorophyll-a (µg/l)
COD = chemical oxygen demand (mg KMnO4/l)
DO% = oxygen saturation per cent (%)
FDA = fluorescein diacetate
fl-GIcA = β-glucosidase activity (pmol/ml h)
HFrS = the sum of UV absorbing humus peak heights (mm)
PhoA = phosphatase activity (pmol/ml h)
SPSS/PC+ = trade mark of SPSS Inc. statistical program
TOC = total organic carbon (mg C/l)

INTRODUCTION
In soil, where most of the microbial biomass and enzymes are associated with soil particles (Burns, 1983), these immobilized enzymes have great importance (Tabatabai, 1994). When lake water is filtrated through soil, the nutrients of water are consumed by microbes living in water or attached to soil particles. Infiltrated water and its nutrients affect the nutrient status of the ground. The bacterial populations living in the ground adapt to the nutritional conditions caused by the infiltrated water.

In pelagic waters the growth of heterotrophic bacteria is affected by the availability of organic matter (Chrost et al., 1986). Organic residues like proteins, lipids and carbohydrates originating from dead organisms are rapidly assimilated by heterotrophic bacteria (Sundh and Bell, 1992). Easily available organic compounds make up only a small portion of the total dissolved organic carbon. However, heterotrophic bacteria are able to degrade organic compounds of high molecular weight with various enzymes (Chrost et al., 1986). In humic lake waters the major part of the organic matter consists of unlabile high molecular weight compounds (Munster, 1985), which have to be degraded by
exoenzymes to compounds of lower molecular weight before microbial assimilation.

Phosphatases, glucosidases and aminopeptidases are considered to be the major exoenzymes in humic surface waters (Münster et al., 1989). Because water ecosystems are often limited by the available phosphorus (Wetzel, 1975), bacteria need phosphatase enzyme activity for mobilizing organic phosphorus to soluble inorganic phosphate. Glucosidases are needed for the depolymerization of polysaccharides, especially after algal blooms (Chrost and Overbeck, 1990). Dissolved free amino acids may be used as energy and nitrogen sources for heterotrophic bacteria. Peptidases are needed to degrade peptides to amino acids, which together with proteins, lipids, carbohydrates and humic substances form the majority of organic compounds in natural waters (Chrost et al., 1989; Münster and Chrost, 1990). The activities of glucosidase, phosphatase and peptidase can be measured with fluorogenic methyllumbiferyl (MUF) substrates. The total microbial activity can be estimated as the hydrolysis of fluorescein diacetate (FDA), because FDA hydrolysis correlates with bacterial respiration activity (Schnurer and Roswell, 1982). FDA is hydrolysed unspecifically by several enzymes like esterases, proteases and lipases (Gullbault and Kramer, 1964; Lundgren, 1981).

We studied the changes in enzymatic activities at different filtration distances in a full-scale bank filtration water works. The water works studied bank filtrated humus-rich lake water. Bank filtrated water was used for further chemical purification before entering the water distribution network of Kuopio city. The spatial and seasonal changes in alkaline phosphatase, β-glucosidase, alanine-aminopeptidase and FDA-hydrolysis activities were measured. We also studied the interactions between enzymatic activities, bacterial abundance and transformation of organic matter during the filtration.

**MATERIALS AND METHODS**

**Sampling site**

The samples were collected from the bank filtration water plant described earlier in detail (Miettinen et al., 1994). The plant is located on a ridge-like island (62°51'N, 27°45'E) in the humus-rich (20–50 mg K MnO₄/l) Lake Kallavesi in Central Finland. The production wells were located on the north-west side of the island. The location of the production wells divides the island into two opposite infiltration sites: the north-west direction (short filtration site, retention time of water in ground, approximately 1 week) and the south-east direction (long filtration site, retention time of water in ground, 4 weeks). Observation wells W1 and W2 were located at the short filtration, and W3, W4 and W5 at the long filtration site.

**Sampling**

Fourteen samplings, covering all seasons, were carried out during the research period in 1988–1989. The observation wells were pumped for 5 min before sampling. The mixed water samples of the production wells were collected at the pumping station. The surface water samples from Lake Kallavesi and a ground water pond located in the middle of the island were taken with a Ruttner sampler at the depth of 1 m. Lake water samples were taken at the front of the filtration shore.

**Enzyme activities**

All enzyme activities were measured in triplicate using modified fluorometric methods presented by Holzapfel-Pichorn et al. (1987). The parallel samples of surface water (lake and pond) were filtrated with Millipore membranes (5 μm) to remove algae. Substrates were added as shown in Table 1. The standardizations were done using the fluorescein end-products (Table 1). The samples (3 ml) for FDA-hydrolysis and β-glucosidase measurements were incubated in a water bath at 20°C. The samples (3 ml) for the phosphatase and alanine-aminopeptidase measurements were incubated at 30°C in a water bath. Incubation time for the surface water samples (lake and pond samples) ranged from 3 to 9 h. The ground water samples were incubated for 10 to 16 h. Before the fluorescence measurements for the FDA, phosphatase and aminopeptidase activities, the samples were buffered with 500 μl of 0.05 M Hepes buffer to pH 7.5. β-glucosidase samples were buffered with 750 μl of 0.1 M glycine buffer to pH 10 before the analyses. The emission of degradation products (Table 1) was measured using a Hitachi F-4000 fluorescence spectrophotometer. The specific enzymatic activities were calculated by dividing the activities by the microbial biomass (presented below).

**Assimilable organic carbon (AOC)**

A method presented by Van der Kooij (1982) was used for the AOC analyses. The samples were stored in polyethylene flasks at −18°C. The content of AOC in water samples from the lake, observation wells (W2, W3) and production wells was analyzed. Microbial activity in samples was eliminated by heating the samples for 30 min at 60°C in a water bath. These pasteurized samples were inoculated with *Pseudomonas fluorescens* strain P17 biotype 7.2 (ATCC 19642) and *Pseudomonas fluorescens* strain MMV90 isolated from Finnish drinking water. A 100 μl sample of mineral salt solution (4.55 g (NH₄)₂SO₄, 0.2 g KH₂PO₄, 0.1 g MgSO₄·7H₂O, 0.1 g CaCl₂·2H₂O and 0.2 g NaCl in 1000 ml deionized water) was added to each sample to ensure that organic carbon was the only factor limiting the bacterial growth. The inoculated samples were incubated at 20°C in the dark. Bacterial growth was followed as viable counts (D). The AOC concentration in the samples was determined from the maximum counts of P17 and MMV90. The growth yield of the strains was determined with sodium acetate standards.

**Table 1. Substrates, standards and excitation/emission (Ex/Em) wavelengths in FDA-hydrolysis (A), phosphatase (B), β-glucosidase (C) and alanine-aminopeptidase (D) measurements**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Final concentration (μg/ml)</th>
<th>Standard</th>
<th>Ex/Em (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Fluorescein diacetate</td>
<td>13</td>
<td>Fluorescein-Na₂ salt</td>
<td>480/505</td>
</tr>
<tr>
<td>B. 4-Methylumbelliferylphosphate-Na₂ salt</td>
<td>565</td>
<td>4-Methylumbelliferyl-H₂O</td>
<td>360/440</td>
</tr>
<tr>
<td>C. 4-Methylumbelliferyl-β-glucoside</td>
<td>270</td>
<td>4-Methylumbelliferyl-H₂O</td>
<td>360/440</td>
</tr>
<tr>
<td>D. L-Alanine-4-methoxy-β-naftylamide</td>
<td>250</td>
<td>4-Methoxy-β(2)-naftylamine</td>
<td>330/420</td>
</tr>
</tbody>
</table>
Bacterial enzymes in water

Bacterial number, biomass and production

Total counts of bacteria and bacterial size were determined using acridine orange (AO) direct counting (Hobbie, 1977). Biomass carbon was calculated using a conversion factor of 350 fg C per μm³ bacterial cell material (Bjørnsen, 1986). Heterotrophic bacteria were enumerated using R2A agar plate counting (Reasoner and Geldreich, 1985). Bacterial production was determined with an ³H-thymidine incorporation method (Fuhrman and Azam, 1980). The amount of ³H-thymidine incorporated was converted to bacterial production using a factor 1.1 x 10⁹ cells per nmol thymidine (Riemann et al., 1987).

The bacterial cells were converted to bacterial carbon using the same conversion factor as above.

Chlorophyll-a and amount of phytoplankton

Phytoplankton biomass was determined with a light microscope and chlorophyll-a according to the Finnish Standard SFS 3013 (1983). Both the phytoplankton and chlorophyll-a determinations were made at the Savo-Karjala Water Protection Association and North-Savo Regional Environment Centre (Kuopio, Finland).

Amount of total organic carbon (TOC), chemical oxygen demand (COD) and UV absorbing humus fractions

TOC was determined with a Horiba PIR-2000, Ionics Model 555 carbon analyzer. COD (KMnO₄ values) was determined according to the Finnish Standard (1981). The UV absorbing humus fractions were measured using size exclusion chromatography (Vartiainen et al., 1987).

Water physico-chemical characteristics

Temperature was measured in situ with a Ysi Incorporated Model 57 oxygen meter. Colour was determined in the laboratory against platinum standards using a Hitachi 100-20 spectrophotometer (wavelength 420 nm). Content of total phosphorus was measured according to the Finnish Standard 3026 (1986) and content of total nitrogen by the method of Koroleff (1983).

Statistical calculations

SPSS/PC + version 2.0 statistical program was used in statistical calculations. Logarithmic transformation was used to obtain the normal distribution of the enzyme activities before Pearson correlation analysis and one-way analysis of variance. Principal component analysis was used to characterize the interactions between enzyme activities and physico-chemical parameters of water.

RESULTS

Temperature

The seasonal variation in temperature was highest in the observation wells near the shore line (W2). In the long filtration (W3) the changes in temperature were less than 5°C (Fig. 1). The changes in temperature were delayed by 1 week during the short filtration and 2 to 3 weeks during the long filtration, compared to the temperature changes in the lake (Fig. 1).

Amount of phosphorus, chlorophyll-a and phytoplankton

The total amount of phosphorus decreased during the filtration (Table 2). The phosphorus content in the lake was stable until water circulation in spring, which caused a short phosphorus peak. The contents of chlorophyll-a and phytoplankton had maximum values in the middle of summer (data not shown).

FDA-hydrolysis activity

The FDA-hydrolysis activity decreased strongly soon after the water was infiltrated. The esterase activity in water from production wells was 3% of the activity in unfiltered lake water (Table 3). The FDA-hydrolysis activity in the membrane filtrated samples from the lake was less than 15% of the activity in the samples without filtration (Table 3). Only a slight decrease in the FDA-hydrolysis activity occurred during the filtration in the ground (Table 3). Similarly, the specific FDA-hydrolysis activity decreased after infiltration from the lake.
Fig. 3. Seasonal variation in the activities of β-glucosidase (A) and alanine-aminopeptidase (B). Symbols: (+) lake; (△) W2; and (●) W3.

The specific β-glucosidase activity increased during filtration in ground close to the FDA-hydrolysis activity in the lake water (W2 = 0.4 ± 0.7 pmol/ng C h; W3 = 0.3 ± 0.8 pmol/ng C h). The FDA-hydrolysis activity was highest during the summer months (Fig. 2A).

**Phosphatase activity (PhA)**

The phosphatase activity decreased during filtration in the ground and was 16% of the activity in unfiltrated lake water samples (Table 3). Differences in the phosphatase activities after the long and the short filtrations were minor. Surprisingly, the specific phosphatase activity increased during filtration in ground. The specific phosphatase activity was four times higher in mixture water of production wells (8.7 ± 7.2 pmol/ng C h) than in lake water (2.2 ± 1.7 pmol/ng C h). The seasonal variation in the phosphatase activity was highest in the lake and pond waters. The phosphatase activities were highest during the summer months (Fig. 2B). The phosphatase activity in the short filtration site followed more closely the changes in lake water than the activities in the long filtration site. In the lake water samples, in contrast to the samples from the ground water, there was an increase in the phosphatase activity during the winter (Fig. 4B).

**β-glucosidase activity (β-GlcA)**

The β-GlcA decreased strongly shortly after the infiltration. Like esterase activity, the β-GlcA also was higher in the unfiltrated and membrane filtrated (5 μm) lake water (Table 3). There was a steady decrease in the β-GlcA during the filtration. The specific β-GlcA in mixture water of production wells (0.2 ± 0.1 pmol/ng C h) was also lower than in lake water (0.4 ± 0.2 pmol/ng C h). The highest β-glucosidase activities occurred early in the spring (Fig. 3A).

**Alanine-aminopeptidase activity (AapA)**

The alanine-aminopeptidase activity decreased strongly after the infiltration. Differences in the activities in the unfiltrated and membrane filtrated lake waters were minor (Table 3). Both the alanine-aminopeptidase and specific alanine-aminopeptidase activities decreased strongly during the filtration in the ground. However, the AapA was slightly higher after the long filtration than after the short filtration. The AapA in the water mixture from production wells was 5% of the AapA in lake water (Table 3). The specific alanine-aminopeptidase activity was

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### Table 2. Contents of total organic carbon (TOC), assimilable organic carbon (AOC), total nitrogen and total phosphorus at different sampling points (means ± standard deviations; number of observations in parentheses)

<table>
<thead>
<tr>
<th>Group</th>
<th>TOC (mg/l)</th>
<th>AOC* (μg C/l)</th>
<th>Nitrogen (mg/l)</th>
<th>Phosphorus (μg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake water</td>
<td>12.1 ± 1.4</td>
<td>350 ± 270</td>
<td>1.9 ± 1.7</td>
<td>80 ± 90</td>
</tr>
<tr>
<td>Short filtration</td>
<td>9.7 ± 1.5</td>
<td>70 ± 30</td>
<td>1.3 ± 1.4</td>
<td>40 ± 80</td>
</tr>
<tr>
<td>W1 and W2</td>
<td>9.7 ± 1.5</td>
<td>(29)</td>
<td>(8)</td>
<td>(14)</td>
</tr>
<tr>
<td>Long filtration</td>
<td>4.4 ± 1.4</td>
<td>60 ± 20</td>
<td>1.7 ± 1.9</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>(W3, W4 and W5)</td>
<td>(43)</td>
<td>(8)</td>
<td>(20)</td>
<td>(19)</td>
</tr>
<tr>
<td>Product wells</td>
<td>7.3 ± 1.7</td>
<td>110 ± 60</td>
<td>0.9 ± 0.8</td>
<td>7 ± 7</td>
</tr>
<tr>
<td>(78)</td>
<td>(10)</td>
<td>(27)</td>
<td>(32)</td>
<td></td>
</tr>
</tbody>
</table>

*Unit = μg acetate eq. C/l.
Bacterial enzymes in water

9.7 ± 3.8 pmol/ng C h in lake water and after the bank filtration in mixture water of production wells it was 2.2 ± 1.4 pmol/ng C h. Surprisingly, the highest AapA values were measured during autumn (Fig. 3B).

**Environmental factors, microbiology and enzyme activities**

All the enzyme activities correlated positively with the AODC counts, bacterial biomass and viable counts (Table 4). β-glucosidase had the highest correlation coefficients of all enzyme with COD ($r = 0.75$; $P < 0.005$), TOC ($r = 0.63$; $P < 0.001$), colour ($r = 0.59$; $P < 0.001$) and the sum of the UV absorbing humus peaks ($r = 0.60$; $P < 0.001$).

In principal component analysis all enzyme activities and other microbiological parameters were associated with each other (Fig. 4). TOC, temperature and the sum of UV absorbing humus peaks (HFrS) were located in a different component than the microbiological parameters. Filtration distance had strong negative interactions with other parameters (Fig. 4).

**Amount of assimilable organic carbon (AOC)**

The AOC content was highest in the lake samples and decreased during the filtration (Table 2). The AOC content had high seasonal variation in lake water, being highest in late winter and in early spring, and lowest in autumn (Fig. 5). At the short filtration site the AOC content was highest during summer. On the contrary, at the long filtration site the AOC content was lowest during summer and autumn (Fig. 5).

**DISCUSSION**

In general, the numbers of free-living bacteria in natural ground waters are low compared to the bacterial numbers associated with soil particles (Chapelle, 1992). In this research only water samples from the ground could be taken. It is obvious that both free-living microbes and microbes associated with soil particles are metabolizing nutrients in infiltrated water. It can be assumed that the bacterial populations living on soil particles are in balance with the free-living bacteria. Therefore, the relative changes in total bacterial activities during water infiltration can be estimated using water samples.

Our results showed that the enzymatic activities on ground water correlated with bacterial production activity and bacterial abundance. This idea agrees with results from surface waters, where the enzymatic activities are associated with other bacterial metabolic activities (Chrost and Overbeck, 1990). We found that the enzyme activities decreased with bacterial abundance and production activity. The decrease in microbial enzymatic activities during bank filtration of surface water supports some earlier observations (Obst, 1984; Obst et al., 1988). The enzyme activities were strongly affected by seasonal temperature changes. At the short filtration site the buffering effect of ground on temperature was weak, causing high seasonal variation in enzymatic activities.

In general, the phosphatase activity was highest in summer when also the biomass production and the
phosphorus demand were highest (unpublished results). The decrease in the phosphatase activity in autumn might be a result of decrease in temperature and increase in the content of phosphorus in the water. The increase in content of phosphorus in autumn occurred simultaneously with the decrease in microbial activity (unpublished results). This increase would be associated with the lower need for available phosphorus in autumn.

Increase in alanine-aminopeptidase activity in autumn and winter, with a simultaneous decrease in bacterial numbers, indicated that alanine-aminopeptidase activity was induced by proteinous compounds originating from the activities of lake algae and bacteria. The maximum in alanine-aminopeptidase activity during August suggested that some bacterial and algal metabolites had infiltrated to the ground, increasing the alanine-aminopeptidase activity. This agrees with the observations that decomposition of proteinous organic matter by aminopeptidases and endopeptidases is highest after phytoplankton blooms (Halemejko and Chrost, 1986). Our results showed that the majority of the proteolytic alanine-aminopeptidase activity in surface waters was associated with the <5 µm fraction (bacterial cell size), agreeing with some earlier results (Rosso and Azam, 1987; Unanue et al., 1993). In contrast, the activities of esterase, phosphatase and β-glucosidase in surface water were probably associated mainly with the algae size fraction (algae or bacteria attached to algae sized particles), because the majority of these enzyme activities were reduced by filtration with <5 µm membranes.

The specific FDA-hydrolysis activity (FDA reflects the total microbial activity; Schnurer and Roswell, 1982) increased during filtration. The ratio of FDA hydrolysis to bacterial biomass might depend on environmental conditions (Jørgensen et al., 1992). Increase in the specific FDA-hydrolysis activity would indicate unfavourable growth conditions in the ground which, like environmental stress in general, increase the maintenance energy requirements of microbes (Anderson and Domsch, 1993). Some of this energy could have been used to produce phosphatases, because the specific activity of phosphatases strongly increased along with filtration. There was probably a shortage of available phosphorus in this humus-rich environment. Available phosphorus can be used instantly for growth or stored as polyphosphates, which enables bacterial growth under low phosphate concentrations (Schlegel, 1988).

The β-glucosidase is controlled by repression-induction mechanisms regulated by concentrations of easily available substrates and polymeric polysaccharides. The highest β-glucosidase activities in lake waters have been found after breakdown of phytoplankton blooms, as a result of polysaccharides excreted (autolysis products) from algae (Chrost, 1989). Some of the autochthonous organic compounds infiltrated into the ground might have changed the bacterial biomass and the β-glucosidase activity. The decrease in the β-glucosidase activity in lake and ground waters in early summer suggested that the β-glucosidase activity might be repressed by the increase in the content of easily assimilable organic carbon (Fig. 5), which might be associated with the beginning of algal bloom, as presented by Chrost (1989). The reduction in easily assimilable organic carbon during filtration showed that these compounds were mineralized at a higher rate than they were produced from higher molecular weight organic matter by hydrolytic enzymes. The bacterial enzyme activities could strongly affect the quality of organic matter, like the molecular size of organic compounds. The decrease in the amount of assimilable organic carbon (AOC) during filtration indicated that the microbiological stability of infiltrated lake water increased during filtration.

Many xenobiotics inhibit exoenzyme activities. This inhibition has been used as a biochemical toxicity test of polluted surface waters (Obst et al., 1985). However, in unpolluted surface waters like Lake Kallavesi, occurrence of enzyme inhibitors is unlikely. Therefore, the close association of bacterial counts with enzyme activities indicates that enzyme activities can be used to monitor changes in water microbial/biochemical characteristics during processing of drinking water from surface water in the ground.

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