Microb Ecol (1995) 29:1–17

MICROBIAL ECOLOGY

A Between-River Comparison of Extracellular-Enzyme Activity

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Received: 22 February 1994; Revised: 27 May 1994

Abstract. River-water extracellular-enzyme activity in the lowland Rivers Ouse and Derwent, northeast England, had much in common. In both rivers, the mean enzyme activities over 15 months differed in the following order: leucine aminopeptidase > phosphatase > β-D-glucosidase > β-D-galactosidase and β-D-xylosidase. None of the five enzymes assayed had significant between-river difference in activity, and there was significant between-river correlation of β-D-glucosidase, phosphatase, and leucine-aminopeptidase activity. The common enzyme regimes were probably more due to between-river similarity of planktonic microbiota than to similar physico-chemical conditions. The potential for glucose uptake by bacterioplankton closely followed β-D-glucosidase activity in magnitude and periodicity. The potential for leucine uptake, however, was much less than leucine-aminopeptidase activity; hence rate of leucine release probably did not limit leucine uptake. There was an appreciable and highly variable proportion of free (<0.2 μm) enzyme activity in river water; ranges were β-D-glucosidase 10–30%, phosphatase 53% to apparently 104%, and leucine aminopeptidase 22–98%. These free enzymes did not necessarily originate from planktonic microbiota and may explain the fairly loose coupling between whole-water enzyme activity and microbial variables. Marked downstream increase in enzyme activity, along about 104 km of the River Derwent, was found on only one of three sampling days; hence the single site used for regular sampling was reasonably representative of most of the river.

Introduction

Extracellular enzymes are important in natural waters, being responsible for the hydrolysis of high-molecular-weight organic compounds to the low-molecular-weight moieties which are available for microbial uptake [10]. Research on rivers has emphasized extracellular-enzyme activity at submerged surfaces of stones [6, 7, 40], wood and abscised leaves [18, 39], and artificial (glass) substrata [30, 31,

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37], and also in sediments [32, 33] and organic detritus [38]. Less is known about activity in river water, although preliminary information suggests that it is ecologically interesting. For example, phosphatase and peptidase were related to bacterial and/or phytoplankton abundance and/or productivity in such contrasting rivers as the European Rhine [1] and the Australian Kiewa River [2]. Furthermore, pollution can influence enzyme regimes in river waters. Thus enzyme activity may be increased by effluents from sewage works [8] and fish farms [4, 13], or may potentially be inhibited by toxic discharges [42].

In the work described here we measured extracellular-enzyme activity over 15 months in water from two geographically close lowland rivers in northeast England. The aim was to establish whether their enzyme regimes (1) were unique to each river, or (2) shared a between-river similarity, perhaps in response to common underlying variation by microbial and/or physico-chemical characteristics. We also examined enzyme activity in fractions of river water partitioned by differential filtration and, in one river, downstream change in enzyme activity.

Materials and Methods

Sites and Sampling

The sites for regular sampling were the River Ouse at York (National Grid Reference SE 603 514), and the River Derwent at Kexby (SE 705 511) 10 km east of York. Both rivers flow across the Plain of York at an altitude of <15 m but have different upland catchments. The main rivers that feed the Ouse rise in the Pennines about 60–85 km northwest of York, while the principal tributaries of the River Derwent rise on the North York Moors about 50 km north of Kexby. Current velocity at both sites is reduced by weirs, both sites support coarse fisheries, and both are potentially enriched by upstream discharges of sewage-works effluent [20].

Routine sampling was at about monthly intervals from November 1990 to January 1992. Surface-water samples for enzyme and bacteriological assays were collected in sterile glass bottles and were transported on ice. Other samples were collected in polythene bottles. Water temperature and pH were measured in the field using a mercury-in-glass thermometer and a pHox System (Shefford, Bedfordshire, UK) type 42D pH meter.

Some water samples were partitioned [11]. This was by 3-μm filtration, followed by 0.2-μm filtration of a portion of the first filtrate. Track-etched, 47-mm diameter, polycarbonate membrane filters were used (Isopore, Millipore UK, Watford), with gentle (<13 kPa) suction.

To investigate downstream change in enzyme activity, and the extent to which the Kexby site was representative of the whole River Derwent, water samples were collected at intervals from about 3 km to 107 km upstream of the barrage at Barmby on the Marsh (National Grid Reference SE 681 287). The barrage prevents tidal inflow of water into the River Derwent. Kexby is about 30 km upstream of the barrage. This sampling was on three days in November–December 1992. On one of these days (9 December) the river was in spate and in places overflowing its banks.

Enzyme Assays

Enzyme assays used analogue substrates which were hydrolyzed to give fluorescent products. The substrates were 4-methylumbelliferyl (MUF) β-D-glucopyranoside, MUF β-D-galactopyranoside, MUF β-D-xylopyranoside, MUF phosphate, and L-leucine-7-amido-4-methylcoumarin, all from Sigma Chemical Co., Poole, UK. These were for assay, respectively, of the glycosidases, β-D-glucosidase (EC 3.2.1.21), β-D-galactosidase (EC 3.2.1.23), β-D-xylosidase (EC 3.2.1.37), and phosphatase (EC 3.1.3.1-2), and leucine aminopeptidases (EC 3.4.1.1.) [10, 23, 24]. These enzymes

Table 1. Comparison of extracellular-enzyme activities[a] at a single analogue-substrate concentration (50 μmol liter^{-1}) with V_{max} values from incubation over a range of substrate concentrations

Enzyme	Rate at 50 μmol liter^{-1} (nmol liter^{-1} h^{-1})	V_{max} (nmol liter^{-1} h^{-1})
β-D-glucosidase	52.5 (52.0–52.8)	53.6 (49.5–58.6)
β-D-galactosidase	19.2 (18.9–19.3)	20.2 (18.6–22.1)
β-D-xylosidase	10.8 (8.8–13.1)	11.3 (10.6–12.2)
Phosphatase	27.1 (26.8–27.8)	27.8 (26.5–29.2)
Leucine aminopeptidase	41.6 (38.5–44.9)	65.9 (61.9–70.4)

[a] Values are rates of analogue-substrate hydrolysis; those at 50 μmol liter^{-1} were from three replicate incubations (with range in brackets); values of V_{max} were from incubation of three replicates at each of six or eight substrate concentrations from 0.5 to 100 μmol liter^{-1} and were calculated using the Wright and Hobbie [44] kinetic model (95% confidence intervals are in parentheses). Water samples were from the River Derwent at Kexby, June–July 1990; incubations were for 2–5 h at river temperature (15–18°C)

were chosen because of their probable importance in fresh waters. They are involved in the degradation of β-linked polysaccharides, orthophosphoric monoesters, and proteins that are plant and/or animal polymers which originate in rivers from both autochthonous and allochthonous sources.

Incubations for assay of enzyme activity were carried out after addition of 0.1 ml of 10 mmol liter^{-1} analogue-substrate stock, made up in 40% methanol [22], to three replicate 20-ml subsamples of river water and a boiled-water blank (final concentration 50 μmol liter^{-1}). The incubations were for 1.25–6.7 h, with the longer incubations for lower enzyme activities, at river temperature (1–18°C) in darkness in an orbital incubator at 80 oscillations min^{-1}. Postincubation river water was mixed 1:0.08 v/v with pH 10 buffer (BDH Laboratory Supplies, Poole, UK) [23], and fluorescence intensity was read at 455 nm, with excitation at 366 nm, using an EEL 244 fluorimeter (Evans Electroselenium, Halstead, UK). Subtraction of the fluorescence intensity of the blank allowed for nonenzymatic hydrolysis of substrate and/or fluorescent impurities. The postincubation fluorescent product (4-methylumbelliferone or 7-amino-4-methylcoumarin) was quantified using straight-line calibration graphs prepared from buffered standard solutions made up in fresh river water.

The analogue-substrate concentration of 50 μmol liter^{-1} was chosen to represent a saturation concentration; hence the rates of substrate hydrolysis measured should approximate to V_{max}. Preliminary trials (Table 1) confirmed that activities of β-D-glucosidase, β-D-galactosidase, β-D-xylosidase, and phosphatase measured at 50 μmol liter^{-1} equalled V_{max} values determined by incubation over a range of substrate concentrations, while leucine aminopeptidase at 50 μmol liter^{-1} approached but possibly underestimated V_{max}.

Microbial and Environmental Variables

The total glucose and leucine uptake capacity of bacteria in river water was determined by measuring both assimilation and mineralization of ^{14}C-labeled substrate. These substrates were chosen because they are the products of glucosidase and leucine aminopeptidase activity. To measure assimilation into cell biomass, three replicate, 15-ml water samples, plus two blanks killed by formaldehyde at 2% w/v, were incubated with [^{14}C]glucose or [^{14}C]leucine. Immediately after incubation, subsamples (2–5 ml) of each replicate and blanks were filtered through 0.2-μm polycarbonate membrane filters (Nuclepore, Costar UK, High Wycombe) and washed with 10 ml of distilled water. To measure mineralization, five replicate, 10-ml water samples, plus two acidified blanks (0.25 mol liter^{-1} H_2SO_4), were incubated with [^{14}C]glucose or [^{14}C]leucine in gas-tight serum bottles. These incubations were stopped by acidification, and released $^{14}CO_2$ was absorbed on 2-phenylethylamine-impregnated paper wicks [16]. Incubations were for 1.5–5 h, with the longer incubations when uptake rates were lower, in darkness at

river temperature; radioactivity on filters and wicks was assayed by liquid-scintillation counting. The radioactive substrates used in these incubations (D-[U-^{14}C]glucose, specific activity 56.0 MBq mg^{-1} or L-[U-^{14}C]leucine, 81.4 MBq mg^{-1}, from Amersham International, Amersham, UK) equalled about 0.74 kBq ml^{-1} (assimilation) or 0.37 kBq ml^{-1} (mineralization). Further, nonradioactive substrate was added to give a total substrate concentration of about 210 μg liter^{-1}. This should represent a saturation concentration for natural populations of aquatic bacteria [17, 21, 34, 43]; hence the values obtained for rate of substrate uptake should approximate to V_{max}.

Direct counts of acridine orange-stained bacteria were made using epifluorescence microscopy after concentration on black 0.2-μm polycarbonate (Nuclepore) membrane filters [12]; procedure followed Carr and Goulder [5]. More than 600 free-living cells were counted from each of two replicate subsamples. Particle-bound bacteria were counted separately in the same microscope fields, and these counts were doubled to allow for hidden bacteria on the underside of particles [15].

Phytoplankton chlorophyll *a* was determined spectrophotometrically following filtration (GF/C glass-fiber filters, Whatman Labsales, Maidstone, UK) of two replicate, usually 1-liter, subsamples and overnight extraction in refrigerated 90% acetone [41]. For later work on downstream change in the River Derwent, single 0.5- or 1-liter subsamples were filtered and extraction was for 2 min in boiling 95% ethanol [29].

Suspended solids and particulate organic matter were determined gravimetrically by GF/C filtration of two replicate, usually 1-liter, subsamples followed by drying at 80°C, and subsequent ashing in a muffle furnace for 0.5 h at 400°C [28]. The concentration of presumed coliform bacteria, included as an environmental variable since it was a potential indicator of recent enrichment by sewage-works effluent, was determined by spreading 0.2 ml of undiluted river water on ten replicate agar plates (membrane enriched teepol broth, Oxoid Ltd, Basingstoke, UK [14], plus 15 g liter^{-1} agar); yellow colonies were counted after 18 h incubation at 37°C. Values of mean river flow over roughly the 24 h prior to sampling were provided by the National Rivers Authority. These were from gauges on the River Ouse at Skelton and the River Derwent at Buttercrambe, respectively, about 6 km and 10 km upstream of the sampling sites.

Results and Discussion

Between-River Comparison

The river-water extracellular-enzyme regimes in the Ouse and the Derwent had much in common (Table 2). The ranking of mean enzyme activities was essentially the same in both rivers, i.e., leucine aminopeptidase > phosphatase > β-D-glucosidase > β-D-galactosidase and β-D-xylosidase. Furthermore, none of the five enzymes assayed had a significant between-river difference in activity ($P > 0.05$, two-tailed Mann-Whitney U-test, $n = 13$–15). Also, temporal variation by β-D-glucosidase in the two rivers was significantly correlated (Table 2); phosphatase and leucine aminopeptidase (but not β-D-galactosidase or β-D-xylosidase) were similarly correlated.

The detail of temporal variation by extracellular-enzyme activity is shown in Fig. 1. The between-river correlation of β-D-glucosidase activity was highlighted by shared maxima in March. Similarly, there were shared peaks for phosphatase in January and March, and for leucine aminopeptidase in March and May. In contrast, May maxima of β-D-galactosidase and β-D-xylosidase in the River Ouse were not found in the River Derwent.

Extracellular-enzyme activity in aquatic environments has been ascribed to microorganisms, including bacteria and algae [10]. It follows that the similarity between enzyme regimes in the Rivers Ouse and Derwent might be a consequence of a high degree of resemblance between the microbial populations in the plankton

Table 2. Between-river comparison and summary of extracellular-enzyme activities and other variables[a] in river waters; Rivers Ouse at York and Derwent at Kexby, November 1990–January 1992

	River Ouse		River Derwent		
	Mean (range)	CV[b] (%)	Mean (range)	CV (%)	r_s[c]
Extracellular-enzyme activities[d] (nmol liter^{-1} h^{-1})					
β-D-glucosidase	17.5 (3.23–62.8)	83	21.3 (6.16–72.5)	77	0.71**
β-D-galactosidase	6.15 (0.93–19.1)	79	7.60 (1.61–14.1)	63	0.28^{NS}
β-D-xylosidase	6.86 (1.75–22.3)	80	6.61 (1.13–19.2)	74	0.17^{NS}
Phosphatase	37.9 (12.8–140)	97	39.8 (16.8–135)	72	0.64**
Leucine aminopeptidase	502 (99.1–2217)	114	364 (108–986)	69	0.95***
Microbial variables					
Glucose assimilation (nmol liter^{-1} h^{-1})	5.45 (0–17.8)	86	7.24 (1.63–21.6)	79	0.85***
Glucose mineralization (nmol liter^{-1} h^{-1})	3.28 (0.42–8.03)	72	5.38 (0.60–20.9)	102	0.75**
Total glucose uptake (nmol liter^{-1} h^{-1})	8.92 (1.23–25.8)	80	12.6 (2.37–42.5)	89	0.81***
Leucine assimilation (nmol liter^{-1} h^{-1})	2.53 (0–9.37)	92	2.14 (0–7.08)	94	0.70**
Leucine mineralization (nmol liter^{-1} h^{-1})	0.32 (0–0.94)	88	0.22 (0–0.74)	109	−0.14^{NS}
Total leucine uptake (nmol liter^{-1} h^{-1})	2.99 (0.14–9.97)	87	2.54 (0.06–7.82)	91	0.57*
Free-living bacteria (×10^6 ml^{-1})	5.97 (3.33–12.6)	40	4.98 (2.18–8.57)	35	0.77***
Particle-bound bacteria (×10^6 ml^{-1})	1.31 (0.34–4.23)	79	1.00 (0.25–2.80)	64	0.80***
Total bacteria (×10^6 ml^{-1})	7.28 (4.25–12.9)	31	5.98 (3.27–8.82)	23	0.34^{NS}
Chlorophyll (μg liter^{-1})	3.35 (0.39–14.0)	110	1.62 (0.39–4.43)	83	0.86***
Environmental variables					
Temperature (°C)	9.1 (1.0–18.0)	59	9.0 (1.0–17.0)	56	1.00***
pH	7.5 (6.8–8.7)	6	7.5 (7.0–8.1)	4	0.81***
Suspended solids (mg liter^{-1})	25.3 (2.85– > 150)	206	15.7 (2.20– > 150)	247	0.66**

Table 2. Continued

	River Ouse		River Derwent		
	Mean (range)	CV[b] (%)	Mean (range)	CV (%)	r_s[c]
Particulate organic matter (mg liter^{-1})	3.51 (0.50–22.5)	166	1.85 (0.45–4.90)	71	0.92***
Coliforms (ml^{-1})	106 (0–555)	125	93 (0–255)	95	0.63**
Flow (m^3s^{-1})	46.1 (4.91–258)	162	11.2 (2.92–48.1)	104	0.83***

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, $P > 0.05$; n = 13–15 for all variables

[a] Of the 21 variables summarized only flow showed significant between-river difference in magnitude ($P < 0.01$, two-tailed Mann-Whitney U-test, $n = 15$)

[b] CV = coefficient of variation

[c] r_s = Spearman's rank correlation coefficient (one-tailed probability values are indicated)

[d] Enzyme activities are given as rate of analogue-substrate hydrolysis

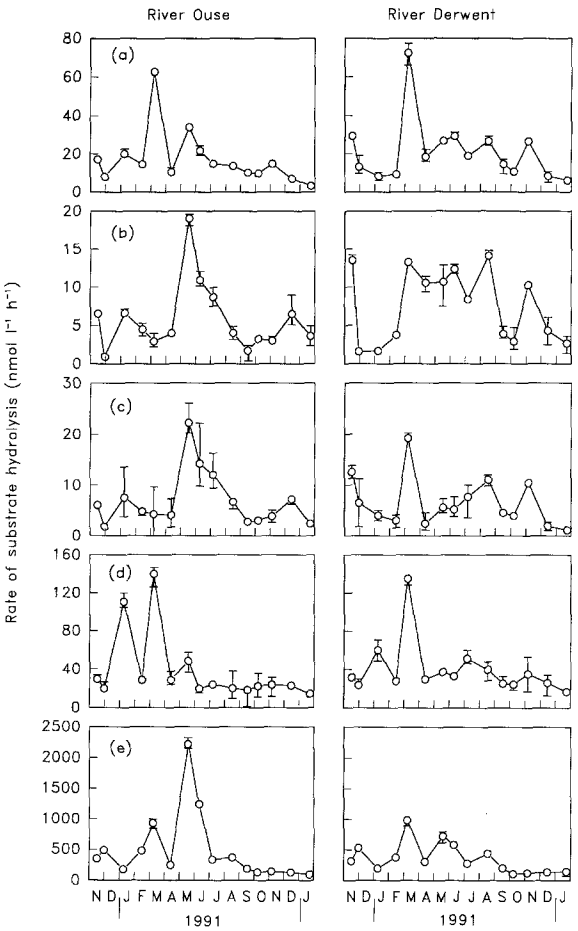

Fig. 1. Temporal variation of extracellular-enzyme activity in river waters; Rivers Ouse at York and Derwent at Kexby, November 1990–January 1992: **a,** β-D-glucosidase, **b,** β-D-galactosidase, **c,** β-D-xylosidase, **d,** phosphatase, and **e,** leucine aminopeptidase. Mean values from three incubations are given; *vertical bars* indicate range, except where the values virtually coincided.

of the two rivers. Some evidence for this resemblance was provided by earlier (1982–1983) work on the Rivers Ouse and Derwent at York and Kexby [20] in which abundance and metabolic activity of planktonic bacteria showed significant between-river correlation. In the present study there was also much between-river similarity. Thus none of the ten microbial variables which are summarized in Table 2 had significant between-river difference in their values ($P > 0.05$, two-tailed Mann-Whitney U-test, $n = 13$–15). Furthermore, temporal variation by microbial variables tended to be similar in the two rivers (Fig. 2). Indeed, of the ten microbial variables included in Table 2, all except leucine mineralization and total bacterial abundance had significant between-river correlation.

The extent to which extracellular-enzyme activity may depend on microbial variables in these rivers can, to some degree, be appraised by juxtaposition of Figs. 1 and 2, especially in the River Ouse where May maxima for β-D-galactosidase, β-D-xylosidase, and leucine-aminopeptidase activity conspicuously coincided with the maxima for glucose assimilation and mineralization, leucine assimilation, abundance of free-living bacteria, and phytoplankton chlorophyll. Since insuffi-

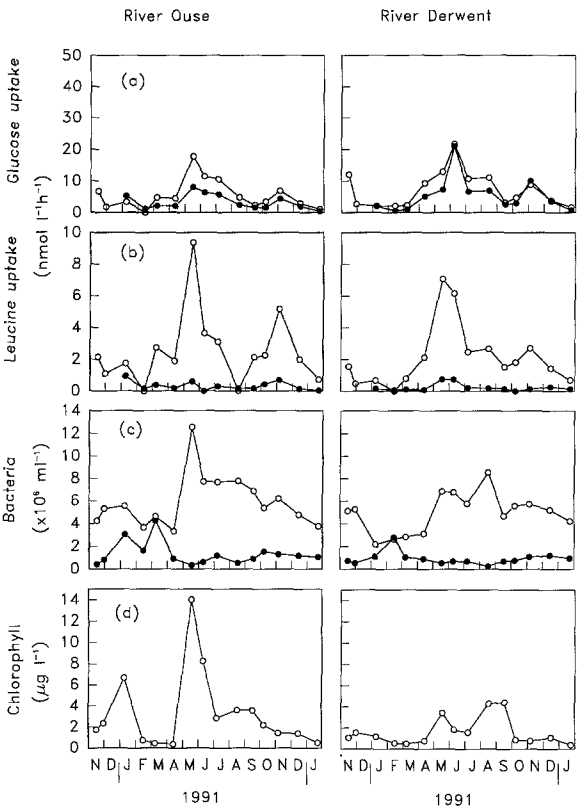

Fig. 2. Microbial variables in the Rivers Ouse at York and Derwent at Kexby, November 1990–January 1992: **a,** glucose assimilation *(open circles)* and mineralization *(closed circles),* **b,** leucine assimilation *(open circles)* and mineralization *(closed circles),* **c,** free-living bacteria *(open circles)* and particle-bound bacteria *(closed circles),* and **d,** phytoplankton chlorophyll.

cient data were available for multivariate statistical analysis, the relationships were further explored by calculation of Spearman's rank correlation coefficients. In the River Ouse there were many significant correlations between enzyme activities and microbial variables (Table 3). There were, however, fewer such correlations in the River Derwent; indeed no significant relationships were found between β-D-xylosidase, phosphatase, or leucine-aminopeptidase activity and microbial variables. This partial uncoupling of enzyme activity from microbial variables in the River Derwent may have contributed to the breakdown of between-river correlation shown by some enzymes (Table 2).

The causes of the uncoupling are unknown, but one potential cause was augmentation of native river enzyme activity by inputs of enzymes in effluents. Discharges from sewage works have high extracellular-enzyme activity which can bring about substantial increase over distances of several kilometres in the recipient rivers [8]. In the River Derwent at Kexby, however, there were no positive correlations between enzyme activities and presumptive coliforms. Indeed, unexpectedly, β-D-glucosidase, phosphatase, and leucine-aminopeptidase activities were negatively related to coliform concentration (Table 4). There was, therefore, no support here for the suggestion that a substantial proportion of the river enzyme activity originated in sewage-works effluents.

Table 3. Relationships between extracellular-enzyme activities and microbial variables[a] in river waters; Rivers Ouse at York and Derwent at Kexby, November 1990–January 1992

Enzyme	Microbial variables that showed significant correlation[b]
River Ouse	
β-D-glucosidase	Glucose assimilation (0.69**), glucose mineralization (0.76**), total glucose uptake (0.73**), leucine assimilation (0.56*), total leucine uptake (0.63*), attached bacteria (0.66**), total bacteria (0.61*)
β-D-galactosidase	Glucose assimilation (0.45†), glucose mineralization (0.53†)
β-D-xylosidase	Glucose assimilation (0.65**), glucose mineralization (0.81***), total glucose uptake (0.71**), free bacteria (0.49†), total bacteria (0.55*), chlorophyll (0.51†)
Phosphatase	Leucine mineralization (0.61*)
Leucine aminopeptidase	Glucose mineralization (0.56*), total glucose uptake (0.51*)
River Derwent	
β-D-glucosidase	Glucose assimilation (0.70**), glucose mineralization (0.60*), total glucose uptake (0.64*), leucine assimilation (0.60*), total leucine uptake (0.68**)
β-D-galactosidase	Glucose assimilation (0.72**), glucose mineralization (0.60*), total glucose uptake (0.61*), leucine assimilation (0.64**), total leucine uptake (0.69*), total bacteria (0.46†)
β-D-xylosidase	No significant correlations ($P > 0.1$)
Phosphatase	No significant correlations ($P > 0.1$)
Leucine aminopeptidase	No significant correlations ($P > 0.1$)

†, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; $n = 13$–15

[a]The full set of ten microbial variables is listed in Table 2

[b]Values in brackets are Spearman's rank correlation coefficient (two-tailed probability values are indicated)

So far in this discussion the rates of glucose and leucine uptake (assimilation and mineralization) have been considered as measures of bacterial metabolism and potentially as indicators of the ability of bacteria to produce extracellular enzymes. Uptake, however, is potentially limited by availability of the low-molecular-weight substrates that are themselves the products of extracellular enzymatic hydrolysis. Rates of glucose uptake in the Rivers Ouse and Derwent were up to 21.6 nmol liter^{-1} h^{-1} for assimilation and 20.9 nmol liter^{-1} h^{-1} for mineralization (Table 2). These were similar, for example, to rates of up to 41.7 nmol liter^{-1} h^{-1} for glucose mineralization in the River Hull, northeast England [19]. It was of special interest, however, that glucose uptake rates (approximations of V_{max}) were quantitatively of the same order as the V_{max} values of the principal β-glycosidases. Thus, Fig. 3 shows that the rate of total glucose uptake (assimilation + mineralization) in both rivers was frequently similar to β-D-glucosidase activity, and that the two variables had very similar temporal variation, apart from aberrant peaks of β-D-glucosidase activity in March (River Ouse, two-tailed Spearman's rank correlation coefficient $r_s = 0.73$, $n = 13$, $P < 0.005$; River Derwent, $r_s = 0.64$, $n = 13$, $P < 0.02$). This suggests that glucose uptake might have been limited by β-glycosidase capacity. There are, however, other potential sources of glucose for bacterial uptake, especially hydrolysis of α-linked polysaccharides which may well be important in rivers because of the role of starch as a plant product.

Table 4. Relationships between extracellular-enzyme activities and environmental variables[a] in river waters; Rivers Ouse at York and Derwent at Kexby, November 1990–January 1992

Enzyme	Environmental variables that showed significant correlation[b]
River Ouse	
β-D-glucosidase	Suspended solids (0.51†), particulate organic matter (0.73**)
β-D-galactosidase	Coliforms (−0.64*)
β-D-xylosidase	Coliforms (−0.67**)
Phosphatase	Particulate organic matter (0.53†), flow (0.55*)
Leucine aminopeptidase	pH (0.55*)
River Derwent	
β-D-glucosidase	Temperature (0.52*), coliforms (−0.52*)
β-D-galactosidase	Temperature (0.48†)
β-D-xylosidase	No significant correlations ($P > 0.1$)
Phosphatase	pH (0.48†), coliforms (−0.75***)
Leucine aminopeptidase	pH (0.53*), coliforms (−0.49†)

†, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; $n = 14$ or 15

[a]The full set of six environmental variables is listed in Table 2

[b]Values in brackets are Spearman's rank correlation coefficient (two-tailed probability values are indicated)

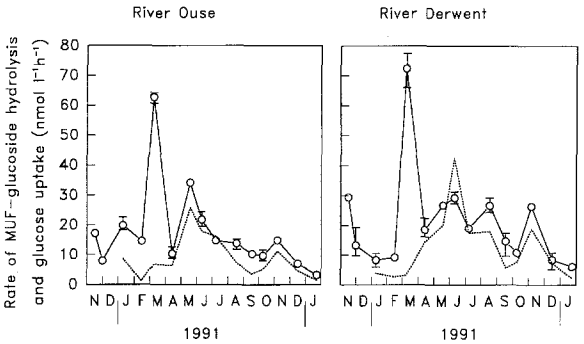

Fig. 3. β-D-glucosidase activity *(open circles)* and bacterioplankton glucose uptake rate (assimilation + mineralization) *(broken line)* in the Rivers Ouse at York and Derwent at Kexby, November 1990–January 1992. Values of β-D-glucosidase activity are means from three incubations; *vertical bars* indicate range, except where the values virtually coincided.

Rates of leucine uptake in the Rivers Ouse and Derwent were up to 9.4 nmol liter^{-1} h^{-1} for assimilation and 0.94 nmol liter^{-1} h^{-1} for mineralization (Table 2). They were similar, for example, to V_{max} values of about 2.6–47 nmol liter^{-1} h^{-1} for incorporation of leucine into the trichloroacetic-acid-insoluble fraction of planktonic bacteria in the River Seine, France [36]. However, the leucine uptake rates in the Rivers Ouse and Derwent, which were probably approximations of V_{max}, were generally much less than the values of leucine-aminopeptidase activity, which ranged from 99 to 2217 nmol liter^{-1} h^{-1} (Table 2), and which were possibly underestimates of V_{max} (Table 1). It is unlikely, therefore, that lack of leucine-aminopeptidase activity limited leucine availability and uptake in these river waters. This conclusion contrasts with (1) the observation that turnover rate for leucine

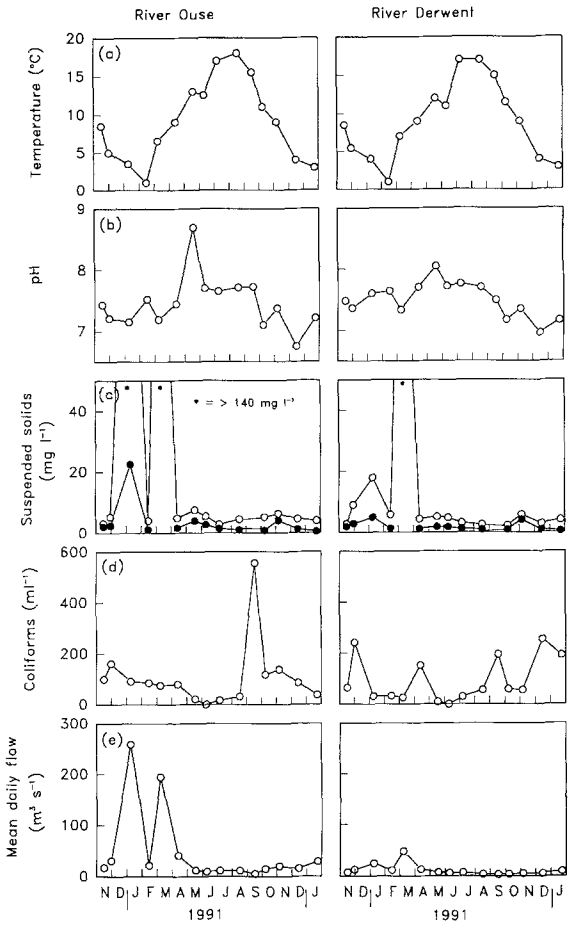

Fig. 4. Environmental variables in the Rivers Ouse at York and Derwent at Kexby, November 1990–January 1992: **a,** water temperature, **b,** pH, **c,** suspended solids *(open circles)* and particulate organic matter *(closed circles)*, **d,** coliforms, and **e,** flow.

uptake in Baltic sea water usually about equalled or exceeded that for hydrolysis of L-leucine-7-amido-4-methylcoumarin [25] and (2) the suggestion that bacterial production at depth (down to 500 m) in North Atlantic sea water was limited by slow peptidase hydrolysis of particulate-organic-nitrogen compounds [26].

Extracellular-enzyme activity in river waters is liable to be influenced by environmental variables as well as by the microbiota. Thus, for example, phosphatase and aminopeptidase activities in the Australian Kiewa River were related to river level, temperature, and suspended solids [2]. The Rivers Ouse and Derwent are geographically close to each other and had fairly similar physico-chemical environments. Of the six environmental variables summarized in Table 2, only flow had significant between-river difference, being generally greater in the River Ouse ($P < 0.01$, two-tailed Mann-Whitney U-test, $n = 15$). Environmental variables, like microbial variables, tended to follow a similar temporal pattern in both rivers (Fig. 4). Thus, for example, both rivers had maxima of temperature in August, and pH in May, and there were simultaneous peaks of suspended solids and flow in January and March 1991. All six environmental variables showed significant between-river

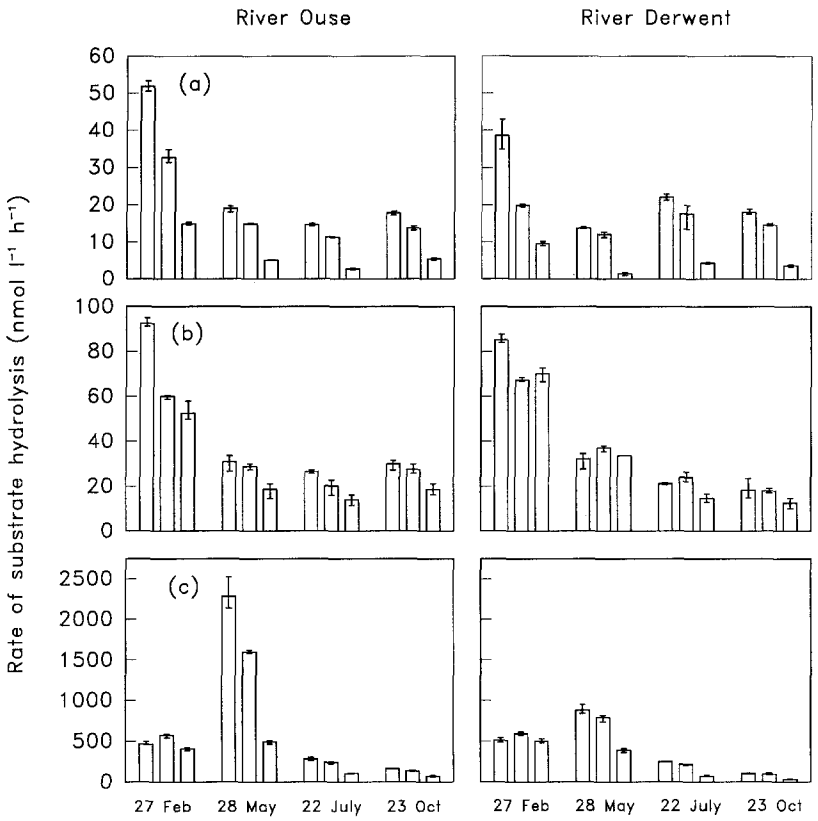

Fig. 5. Results of partitioning of extracellular-enzyme activity in water from the Rivers Ouse at York and Derwent at Kexby, February–October 1991: **a,** β-D-glucosidase, **b** phosphatase, and **c** leucine aminopeptidase. For each sampling date the left-hand column represents whole water, the center column represents 3-μm filtered water, and the right-hand column represents 0.2-μm filtered water. Mean values from three incubations are given; *vertical bars* indicate range, except where the values virtually coincided.

correlation (Table 2). There were, however, few significant relationships between extracellular-enzyme activities and environmental variables (Table 4)—and among these the puzzling negative correlations with coliform concentration were conspicuous. It follows that, although the similarity of the enzyme regimes in the two rivers might in some part have been a result of common response to similar physicochemical environments, the between-river resemblance of the microbiota was perhaps more important.

Partitioning of Enzyme Activity

Figure 5 gives results from partitioning of β-D-glucosidase, phosphatase, and leucine-aminopeptidase activity in water samples collected from the River Ouse at York and the River Derwent at Kexby in February, May, July, and October 1991. The rate of analogue-substrate hydrolysis in 0.2-μm filtered subsamples repre-

sented free extracellular-enzyme activity. That in the 3-μm-filtered fraction roughly represented free activity plus activity associated with free-living bacterial cells, probably bound to cell walls and in periplasmic space. The rate in whole water represented total extracellular-enzyme activity, i.e., also including activity associated with particle-bound bacteria, phyto- and zooplankton, and bound to nonliving particulate matter. The differences between appropriate column heights in Fig. 5 gave enzyme activity of (1) free-living bacteria, and (2) plankton and nonliving particulates.

β-D-Glucosidase activity in both rivers was mostly associated with free-living bacteria and/or other particulate components; only 18.5–30.2% of total activity in the River Ouse and 10.3–24.8% in the River Derwent was free in the river water (Fig. 5). The proportion of free phosphatase activity tended to be greater, 52.7–62.6% in the River Ouse and 69.4%–apparently 104% in the River Derwent. Such anomalous results, with the summed activity of fractions exceeding that of the unfractionated sample, can be a feature of partitioning of enzyme activity and may be related to release of enzymes during fractionation. In an extreme case the sum of phosphatase activity in individual size fractions from the Ovens River, Australia equalled 933% of the activity in unfractionated river water [3]. The proportion of free leucine-aminopeptidase activity was extremely variable, 21.5–86.3% in the River Ouse and 29.6–97.6% in the River Derwent.

Most partitioning of extracellular-enzyme activity in fresh water has been done on lakes. In eutrophic lakes in northern Germany, for example, the proportion of free enzyme activity (<0.2-μm fraction) ranged from 0 to 7% for β-D-glucosidase [9], 10 to 50% for phosphatase [11], and 10 to 30% for aminopeptidase [27]. These results agree with those from the Rivers Ouse and Derwent to the extent that the proportion of free phosphatase was notably greater than β-D-glucosidase. Less information is available about river waters. Boon [3] found that although much phosphatase activity in Australian rivers was associated with the 0.2- to 1-μm size fraction, which corresponded to many planktonic bacteria, the combined activity of size fractions less than 0.2 μm sometimes exceeded that of the 0.2- to 1-μm fraction. Furthermore, Unanue et al. [43] found a very variable proportion (0–59%) of free (<0.2 μm) aminopeptidase activity in the Butrón River, Spain. Relatively high proportions of free enzymes in river waters have also been associated with pollution-enhanced extracellular-enzyme activity, for example, (1) a mean of 87% of phosphatase downstream of a fish farm on the River Hull, northeast England [4], and (2) 30–32% of β-D-glucosidase, 55–59% of phosphatase, and 55–71% of leucine aminopeptidase in a disused canal, and 16–78% of β-D-glucosidase, 66–82% of phosphatase, and 43–75% of leucine aminopeptidase in a small calcareous stream, northeast England, both downstream of discharge of sewage-works effluent [8].

The observation that free enzymes (<0.2 μm) were responsible for an appreciable and variable proportion of whole-water extracellular-enzyme activity in the Rivers Ouse and Derwent (Fig. 5) perhaps, in part, explains the relatively loose nature of the relationship between whole-water enzyme activity and microbial variables (Table 3). The free enzymes probably had several origins; for example, release from surfaces and sediments, release from microorganisms and larger fauna in the water column, inputs in ground water, and probably inputs in sewage-works effluents.

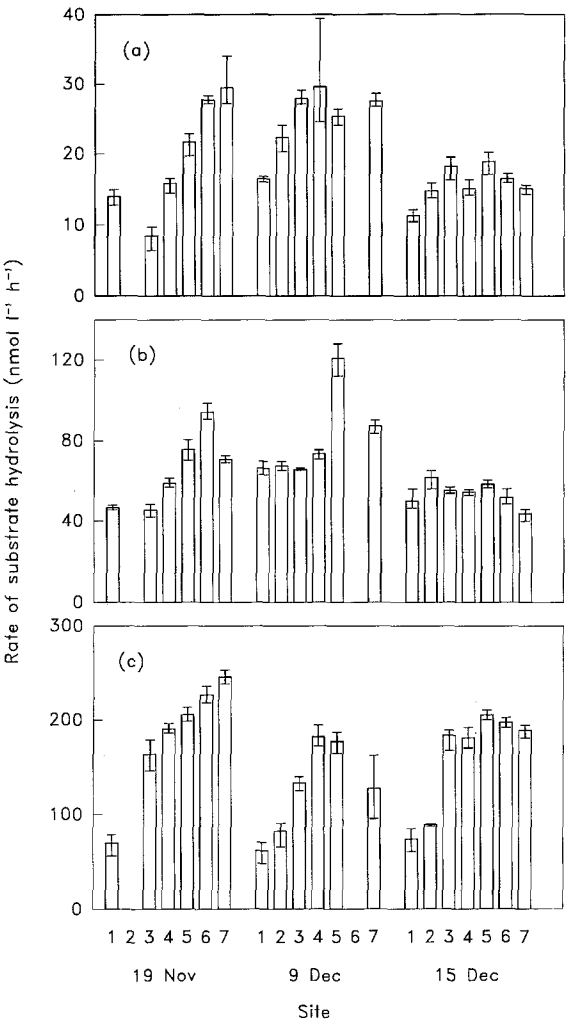

Fig. 6. Downstream change in extracellular-enzyme activity in water from the River Derwent, November–December 1992: **a,** β-D-glucosidase, **b,** phosphatase, and **c,** leucine aminopeptidase. The sites were about (1) 107 km, (2) 97 km, (3) 79 km, (4) 51 km, (5) 30 km, (6) 19 km, and (7) 3 km upstream of the tidal barrage (Kexby is site 5). Mean values from three incubations are given; *vertical bars* indicate range.

Downstream Change in Enzyme Activity

Enzyme activities along the River Derwent are given in Fig. 6. On 19 November, β-D-glucosidase, phosphatase, and leucine-aminopeptidase activities all tended to increase with distance downstream. There were, however, no such pronounced increases on 9 and 15 December, although β-D-glucosidase and aminopeptidase activities were lowest at the most upstream site.

Bacterial abundance and chlorophyll concentrations are shown in Fig. 7. The abundance of both free-living and particle-bound bacteria was lowest at the most upstream site on all three days, and the low enzyme activities at that site (Fig. 6) may be related to this. There was downstream increase in bacterial abundance on 15 December (Fig. 7) but this was not mirrored by enzyme activities (Fig. 6). Such downstream increase by bacteria is usual in headstreams [35] but the results for 19

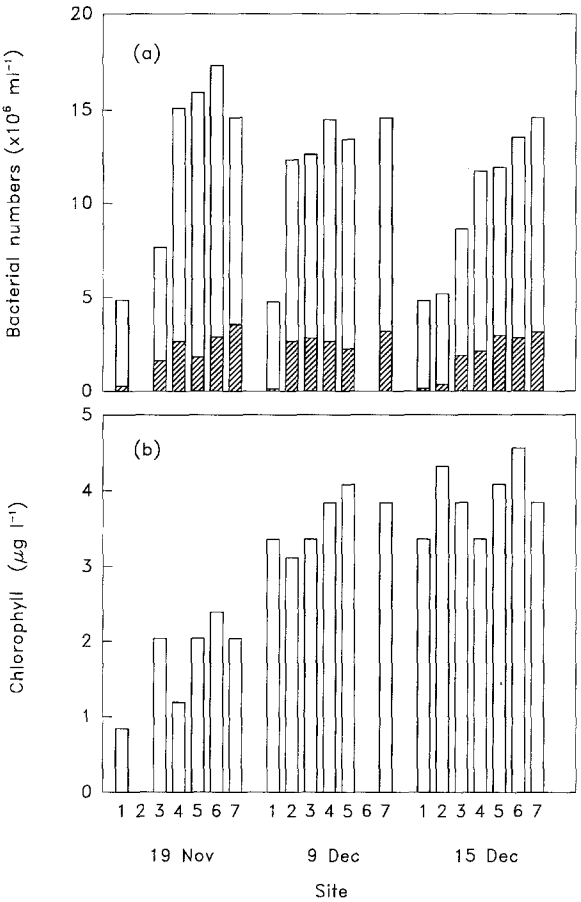

Fig. 7. Downstream change in **a,** bacterioplankton abundance (*open column* represents free-living bacteria, 95% confidence intervals were less than plus or minus 8% of the mean, *hatched column* represents particle-bound bacteria, full column height equals total bacteria), and **b,** phytoplankton chlorophyll in the River Derwent, from extraction of single subsamples, November–December 1992. See legend to Fig. 6 for site locations.

November and 9 December indicated that there was not always such an increase along the River Derwent.

Chlorophyll concentrations, up to 4.6 μg liter^{-1} (Fig. 7), measured after hot-ethanol extraction, were high compared to winter values in the River Derwent in 1991 (Fig. 2). It is possible, however, that cold-acetone extraction, used during 1991, was less effective and led to underestimation of phytoplankton chlorophyll. The chlorophyll concentration was fairly uniform along the river. There was a low value at the most upstream site, corresponding to low enzyme activity, only on 19 November. The Kexby site was evidently, at least on some winter sampling days, reasonably representative of most of the river.

Acknowledgments. We thank S. Lythe for technical assistance and the National Rivers Authority for flow data. The work was supported by a research grant from the Natural Environment Research Council.

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