

Rapid potential rates of extracellular enzymatic hydrolysis in Arctic sediments

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Abstract

The extracellular enzymatic hydrolysis rates of three fluorescently-labeled polysaccharides (pullulan, laminarin, and xylan) were measured in the upper ~11 cm of sediment cores collected near Svalbard in the Arctic Ocean. The three polysaccharides differ in molecular weight (200,000, ~6,000, and ~8,000 Da for pullulan, laminarin, and xylan, respectively), as well as in monomer composition, linkage position, and anomeric configuration, and are most probably hydrolyzed by distinctly different enzymes. Potential hydrolysis rates of pullulan and laminarin were rapid throughout the sediment cores (average rates of 52 cuts per nmol glucose $\text{cm}^{-3} \text{h}^{-1}$ for pullulan, 38 cuts per nmol glucose $\text{cm}^{-3} \text{h}^{-1}$ for laminarin) and were comparable to rates measured in sediment cores from more temperate sites. Xylan potential hydrolysis rates were considerably slower at all Svalbard stations (average of 3 cuts per nmol xylose $\text{cm}^{-3} \text{h}^{-1}$). Rapid microbial remineralization of particulate organic carbon requires high levels of extracellular enzyme activity; the high potential hydrolysis rates of fluorescently-labeled pullulan and laminarin in Svalbard sediments demonstrate that at least some types of extracellular enzymes can function rapidly in permanently cold environments.

Sedimentary microbial communities in deep-ocean sediments have the capability to rapidly metabolize an influx of fresh particulate organic matter (Lochte and Turley 1988; Gooday and Turley 1990; Poremba 1994). Investigations of extracellular enzymatic activities, however, have suggested that potential rates of enzymatic hydrolysis are markedly slower in deep-ocean sediments than in shallower and more temperate sites (Boetius 1995; Poremba and Hoppe 1995). Although enzymatic activities are not greatly affected by pressure (Helmke and Weyland 1986; Meyer-Reil and Koster 1992; Poremba 1995), temperature optima of extracellular enzymes isolated from marine bacteria from the deep and polar oceans frequently have been found to be far above ambient growth temperatures (Reichardt 1987; Helmke and Weyland 1991). These studies seem to present a paradox: rapid microbial utilization of particulate organic matter implies rapid hydrolysis of organic macromolecules, because substrates ≥ 600 Da must be hydrolyzed enzymatically outside the cell prior to transport into the periplasmic space (Weiss et al. 1991), yet there is little evidence that extracellular enzymes function rapidly in deep and cold environments.

One possible explanation lies in the fact that the specificities and activities of macromolecule-hydrolyzing enzymes of marine bacteria are still only poorly understood. Bacteria have the capability of producing a wide range of enzymes with precise substrate specificities. In marine systems, however, a limited range of low-molecular-weight substrates

have been used as proxies for organic macromolecules to measure extracellular enzyme activities in seawater and sediments. Methylumbelliferyl (MUF) substrates, for example, consist of a MUF fluorophore attached to a monosaccharide or amino acid. Hydrolysis of a MUF substrate leads to an increase in fluorescence signal from the MUF fluorophore (e.g. Hoppe 1983; Somville 1984; Meyer-Reil 1987; Boetius 1996). The MUF substrates, although convenient to use, are poor proxies for many high-molecular-weight substrates such as polysaccharides (Helmke and Weyland 1991). In addition, hydrolysis of MUF substrates measures activities of periplasmic as well as extracellular enzymes (Martinez and Azam 1993), so their relevance as measures of extracellular enzymatic activity is doubtful. A number of laboratory studies have used specific polysaccharide substrates, not proxies, to measure the hydrolytic capabilities of isolated species of bacteria (Norkrans and Stehn 1978; Reichardt 1988; Helmke and Weyland 1991). Isolation of bacteria, however, necessarily selects a sub-population from the total community originally present in the sediments; culture conditions that favor specific bacteria may not accurately reflect the microbial community responsible for macromolecule hydrolysis in sediments.

In order to investigate the hydrolytic potential of microbial communities in permanently cold sediments, a new technique, based on fluorescently-labeled (FLA) polysaccharides (Arnosti 1995, 1996), was used to measure rates of extracellular enzymatic hydrolysis in sediment cores. Because FLA polysaccharides are far too large to be transported intact across bacterial membranes, enzymatic hydrolysis of these substrates must occur outside of the microbial cell. Polysaccharides were selected as the target macromolecular substrates because they are major components of marine organic matter such as phytoplankton (Parsons et al. 1961). Three specific polysaccharide substrates (pullulan, laminarin, and xylan) were used because they occur in marine algae and/or their enzyme activities have been measured in marine bacteria. Pullulanase can function as a debranching enzyme of starch (White and Kennedy 1988), and starch is a

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common storage product of dinophytes, chlorophytes, and rhodophytes (Lee 1980). Starch-hydrolyzing activity appears to be widespread among polar bacteria (Helmke and Weyland 1991; Norkrans and Stehn 1978), and pullulanase activity has been characterized specifically in a number of thermophilic bacteria (Brown et al. 1990; Antranikian 1992) and among mesophilic bacteria in coastal sediments (Arnosti and Repeta 1994; Arnosti 1995). Laminarin is an energy-storage product of bacillariophytes and phaeophytes, and may also occur in chlorophytes and chrysophytes (Painter 1983). Laminarinase activity has been found in algae, bacteria, mollusks, a range of marine invertebrates (Kristensen 1972; Wainwright 1981), and in coastal marine sediments (Arnosti et al. 1994; Arnosti 1996). Xylans are found among the rhodophytes and chlorophytes (Painter 1983), and xylanase activity has been reported in extracts of a number of marine detritus feeders and omnivores (Kristensen 1972). Xylanase enzymes have also been purified and characterized from several species of thermophilic bacteria (Bragger et al. 1989; Shao et al. 1995; Winterhalter and Liebl 1995).

Pullulan, laminarin, and xylan also differ from one another in molecular weight, monomer composition, linkage position, and anomeric linkage, providing an opportunity to consider the effects of different aspects of chemical structure on enzymatic hydrolysis rates. Note that hydrolysis rates measured here must be considered "potential" rates, since the additional substrate competes with substrate naturally present in the sediment for enzyme active sites.

This work was carried out as part of an integrated study of carbon cycling in permanently cold environments (Sagemann et al. 1998; Thamdrup and Fleischer 1998). Sediments were collected at several sites near Svalbard in the Arctic Ocean, where both the shallow water column (depths at sampling sites ranged from 115 to 175 m) and the sediments experience permanently cold temperatures. Sediment cores could be retrieved and processed rapidly without complications due to pressure effects or warming during retrieval through the water column. Because most oceanic sediments experience permanently cold temperatures, the rates at which extracellular enzymes function at low temperatures have significant implications for studies of sedimentary organic carbon remineralization.

Methods

Preparation of substrates—Pullulan [$\alpha(1,6)$ -linked maltotriose [$\alpha(1,4)$ linkages], MW 200 kDa], laminarin [$\beta(1,3)$ -linked glucose; MW 6 kDa], and xylan [$\beta(1,4)$ -linked xylose; MW ~ 8 kDa] were obtained from Sigma. Fluorescently-labeled pullulan, laminarin, and xylan were prepared and characterized following the method of Glabe et al. (1983), as described in Arnosti (1995, 1996). To summarize briefly, 20 mg of polysaccharide was dissolved in Q-H₂O, 10 mg CNBr was dissolved in 200 μ l Q-H₂O, and the two solutions were rapidly mixed. (Note that the xylan solution was filtered through 0.45- μ m and then 0.2- μ m pore-size filters in order to remove insoluble material prior to activation with CNBr.) To keep the solution pH above 10.0 for 7 min, 25 μ l portions of 0.25 M NaOH were added as needed. The

reaction mixture was immediately desalted on a column of Sephadex G-25 gel (18 \times 1 cm), with a mobile phase of Na₂B₄O₇ (flow rate of 1.5 ml min⁻¹). The fraction corresponding to the void volume of the column was collected in a vial containing 2 mg fluoresceinamine (isomer II; Sigma), and was incubated for 18 h in the dark at room temperature. Labeled polysaccharide was separated from unreacted fluoresceinamine by injecting the reaction mixture on a 26.5 \times 1-cm column of Sephadex G-25 gel with a mobile phase of 100 mM NaCl + 50 mM NaH₂PO₄/Na₂HPO₄, pH 8.0. The bright yellow fraction that eluted in the void volume of the column was collected; reinjection of a few microliters of the FLA polysaccharides on the column demonstrated that all fluorescence eluted in the void volume of the column, and was therefore attached to the polysaccharide. The synthesis procedure results in the formation of stable covalent bonds between the fluorophores and the polysaccharide (Kohn and Wilchek 1978).

The labeling density of the polysaccharides (μ M label/ μ M polysaccharide) was determined by measuring absorbency at 490 nm against a series of fluoresceinamine standards, and measuring carbohydrate concentration using the phenol-sulfuric acid method (Chaplin and Kennedy 1986) with pullulan, laminarin, and xylose as the standards. On average, 1 in 60 glucose monomers of pullulan, 1 in 148 glucose monomers of laminarin, and 1 in 38 xylose monomers of xylan bore a fluorescent tag. (For a discussion of factors controlling labeling density, see Arnosti 1996.) The presence of fluorescent tags does not measurably affect the biological activity of labeled polysaccharides, which exhibit endothelial monolayer binding activities and inhibit lectin-mediated hemagglutination activity to the same extent as their unlabeled counterparts (Glabe et al. 1983). Tests demonstrate (data not shown) that the FLA polysaccharides are stable with time and temperature, showing slow loss of label from the polysaccharides only after months of storage at room temperature or days of incubation at temperatures $>45^{\circ}\text{C}$.

Study sites—Sediments were collected at three sites near Svalbard in the Arctic Ocean in September–October 1995. Hornsund is located at 76°58'20"N, 15°34'50"W. Water depth was 155 m, and the bottom water temperature was 2.6°C. Sediments were light brown in the upper 5 cm, and contained numerous worm burrows. Below 5 cm, the sediments were clayey in consistency and mottled dark brown and gray. Sedimentary organic carbon content was 2.0% at the surface and decreased to 1.7% at 10 cm. Van Mijenfjorden is at 77°45'67"N, 15°03'94"W, with water depth of 115 m and a bottom-water temperature of 0.2°C. Sediments were light brown in the upper 3–5 cm, and dark brown to very dark brown below a depth of ~ 5 cm. Sedimentary organic carbon content was close to 2.0%. Storfjorden is at 77°33'00"N, 19°05'00"W, with a water depth of 175 m and a bottom water temperature of -1.72°C . Sediments were light brown in the top ~ 3 cm, and graded to gray-brown at greater depths. Sedimentary organic carbon content was $\sim 2.4\%$ throughout the cores.

Core collection, preparation, and processing—All sediments were collected with a multicorer; each multicore was

Table 1. Potential rates of FLA pullulan hydrolysis in cores collected at Hornsund and Van Mijenfjorden. Depths are 0–2 cm (surface), 4–6 or 5–7 (middle), and 9–11 or 10–12 (bottom). Rates are expressed in standard enzymatic terms, cuts per nmol glucose cm⁻³ h⁻¹.

		Hornsund	Van Mijenfjorden
Averages (±SD)		60±25 57±33 70±42	28±7 47±26
Individual cores			
Time (h)			
12	Surface	24	
	Middle	36	
	Bottom	341	
18	Surface	110	
	Middle	137	
	Bottom	56	
24	Surface		
	Middle		17
	Bottom		58
30	Surface	76	
	Middle	46	32
	Bottom	67	74
30	Surface		
	Middle		32
	Bottom		25
36	Surface	67	
	Middle	78	
	Bottom	67	
36	Surface	72	
	Middle	68	
	Bottom	115	
48	Surface	49	
	Middle	55	23
	Bottom	156	73
48	Surface	52	
	Middle	23	28
	Bottom	21	11
48	Surface	68	
	Middle	55	26
	Bottom	64	68
60	Surface	41	
	Middle	43	41†
	Bottom	31	54
60	Surface	37	
	Middle	30	21†
	Bottom	55	14

* Not including 12-h deep sample.

† Fifty-eight hours.

subcored using 2.6 × 20-cm core liners with silicone-stoppered injection ports at 1-cm intervals. Several centimeters of overlying water were retained in each subcore.

For each subcore, one FLA polysaccharide solution was injected at three depths, typically 1, 5, and 9 or 11 cm below the surface. Injection volumes were 200, 300, and 500 μl for FLA xylan, FLA pullulan, and FLA laminarin, respectively, representing additions of 1.1 μmol xylose, 3.0 μmol glucose, and 6.3 μmol glucose per injection point, respectively. At Storfjorden, only FLA xylan was used, and 400

Table 2. Potential hydrolysis rates of FLA laminarin in cores collected at Hornsund and Van Mijenfjorden. Depths are 0–2 or 3 cm (surface), 5–7 or 6–8 cm (middle), and 9–11 or 10–12 (bottom) (rates are expressed in standard enzymatic terms, cuts per nmol glucose cm⁻³ h⁻¹).

		Hornsund	Van Mijenfjorden
Averages (±SD)		66±19 56±21 58±21	14±7 15±10 16±3
Individual cores			
Time (h)			
18	Surface	86	
	Middle	78	
	Bottom	81	
36	Surface	49	33
	Middle	35	26
	Bottom	41	20
48	Surface	64	12
	Middle	54	6
	Bottom	53	15
48	Surface		23
	Middle		12
	Bottom		13

μl was injected at each depth. For all stations and samples one subcore was sectioned immediately as the zero-time sample, and the remaining subcores were incubated at in situ temperature. Uninjected cores were also incubated for measurement of background fluorescence, pore-water volume and carbohydrate content. At each time point (incubation times ranging from 12 to 60 h; Tables 1–3), one or more cores was sectioned and centrifuged for 5 min at 4,000 η

Table 3. Potential hydrolysis rates of FLA xylan in cores collected at Hornsund, Van Mijenfjorden, and Storfjorden. Depths 0–2 cm (surface), 4–6 or 5–7 cm (middle), and 8–10 or 9–11 (bottom) (rates are expressed in standard enzymatic terms, cuts per nmol xylose cm⁻³ h⁻¹).

		Hornsund	Van Mijenfjorden	Storfjorden
Averages (±SD)		8±4 3±3 4±2	2±1 1±0 1±3	6±4 0±0 4±6

Individual cores

Time (h)

18	Surface	12		3
	Middle	0		0
	Bottom	4		0
36	Surface	5	3	6
	Middle	4	1	0
	Bottom	3	0	11
48	Surface	4	2	3
	Middle	6	1	0
	Bottom	6	4	0
48	Surface		3	11
	Middle		1	0
	Bottom		0	1

in a cool (2°C) room. The overlying pore water was filtered through a 0.2- μm pore-size filter. A few samples were analyzed immediately to determine the extent of hydrolysis and to decide on subsequent core harvesting times, and the rest were stored frozen until analysis. Comparison of the same samples run immediately and then after freezing and thawing demonstrated that freezing the samples did not affect elution profiles or fluorescence intensity.

Sample analysis—FLA polysaccharides and their hydrolysis products were analyzed using a low-pressure gel-permeation chromatography (GPC) system consisting of a peristaltic pump (Multistatic) connected to two columns: a Sephadex G-50 gel column connected to a Sephadex G-75 gel column, both equilibrated in a buffer of 100 mM NaCl + 50 mM $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$, pH 8.0. Shipboard analyses were made with two $19 \times 1\text{-cm}$ columns at a flow rate of 1.0 ml min^{-1} . Shore-based analyses were made with a newly poured set of columns (18.3 cm G-50, 15 cm G-75, flow rate of 0.82 ml min^{-1}). The G-75 columns had a 1-cm layer of G-25 gel poured at the base in order to prevent clogging of the column frit through compaction of the soft G-75 gel. Each set of columns was individually calibrated with FITC-dextran molecular weight standards (Sigma; 150 kDa, 40 kDa, 10 kDa, 4 kDa, FITC-glucose, and free FLA tag) to determine the elution time for a given molecular weight range. The columns were connected to a Waters model 470 fluorescence detector (shipboard) or a Hitachi 7480 detector (shore), operated at excitation and emission wavelengths of 490 and 530 nm, respectively, with data collected on an HP 3395 integrator. Total injection volume for each sample was 2,000 μl , with each injection consisting of 500–2,000 μl of pore-water sample plus any necessary makeup volume.

Fluorescence recovery—The cores were sectioned into 2-cm intervals; in a few cases, the surface section was 3 cm. In many cases, the injected fluorescence was found to spread beyond a 1-cm interval on either side of the injection point. Therefore, the fluorescence of all of the core sections for all of the cores was measured to determine the distribution of fluorescence in the cores. For uninjected sections that were found to contain fluorescence, rate calculations (*see below*) were made by assuming that the fluorescence of the uninjected sections came from the neighboring injected section(s). When two injected sections bracketed an uninjected section that contained fluorescence, half of the fluorescence was assumed to come from each injected section. For calculations of fluorescence in surface (injected) sections, an amount of fluorescence equal to the amount in the section below was assumed to have escaped into the overlying water. In some cases, the overlying water was visibly colored by escaped FLA polysaccharide. For Sta. 3 pullulan surface injections (injection depth typically 1 cm), recovery of fluorescence was too low for reliable integration, so no hydrolysis rates were calculated for these samples.

Recovery of total injected fluorescence in pore water varied significantly. Recoveries were calculated by multiplying the fluorescence concentration of pore water (fluorescence units/ μl pore water injected) by the total pore water of the uninjected core collected for each station. Average recovery

for FLA pullulan cores from Sta. 2 was 22% (range of 13–43%); the average for Sta. 3 was 25% (range of 9–46%). Average recoveries for FLA laminarin were somewhat higher, 49% for Sta. 2 (range of 42–57%) and 30% for Sta. 3 (range of 25–38%). Recoveries of FLA xylan fluorescence were lower on average—16% for Sta. 2 (range of 10–24%), 12% for Sta. 3 (range of 9–16%), and 34% for Sta. 5 (range of 14–58%). There was no correlation between fluorescence recovery in the cores and potential hydrolysis rates.

Rate calculations—All rate calculations represent potential hydrolysis rates, since the substrate added to the sediment competes with naturally occurring substrate for enzyme-active sites. Rate calculations were made using a conservative calculation that sets a lower boundary on potential hydrolysis rates, described in detail in Arnosti (1996). To summarize briefly, all of the fluorescence is initially attached to a polysaccharide of known molecular weight. As a polysaccharide is hydrolyzed, the distribution of fluorescence in a chromatogram is observed to change. The changes, corresponding to changes in molecular weight distribution, can be quantified because the GPC column is calibrated with a series of polysaccharide standards of known molecular weights. By quantifying the fluorescence observed in a given molecular weight class at a known time, the minimum number of hydrolyses required for the original polysaccharide to be reduced to that molecular weight can be calculated.

Analytical variation and precision—The variation in elution times for laminarin and xylan zero-time samples (i.e. for all of the samples run from the zero-time cores) was ~8%; the same degree of variability was seen for the poisoned controls. Neither the zero-time samples nor the poison-control samples exhibited any of the characteristic peak shape features (broadening, development of shoulders) observed for the incubated cores. Xylan samples not showing changes greater than the zero-time and poison control samples have potential hydrolysis rates of zero (Table 3). No variability was observed for pullulan samples. Owing to the high molecular weight of pullulan (200,000 Da), zero-time FLA pullulan eluted in the void volume of column, whereas FLA laminarin and FLA xylan both eluted in the included volume of the column. Analytical precision of replicate injections of the same sample was 5%.

Controls and blanks—Controls were prepared by mixing 5 ml sediment with 30 ml water collected from the bottom water overlying the multicores. Two hundred microliters of a 50 mg ml^{-1} HgCl_2 solution was added to the slurries, and after a 5–10-min delay, 200 μl of FLA polysaccharide was added. One uninjected core from each station was incubated for the duration of the experiment, then sectioned in the same manner as the injected cores in order to obtain pore water for background fluorescence measurements and total carbohydrate content. The background fluorescence from pore water was zero at excitation and emission wavelengths of 490 and 530 nm. Total pore-water carbohydrates were measured using the phenol-sulfuric acid method (Chaplin and Kennedy 1986), and total water weight was determined

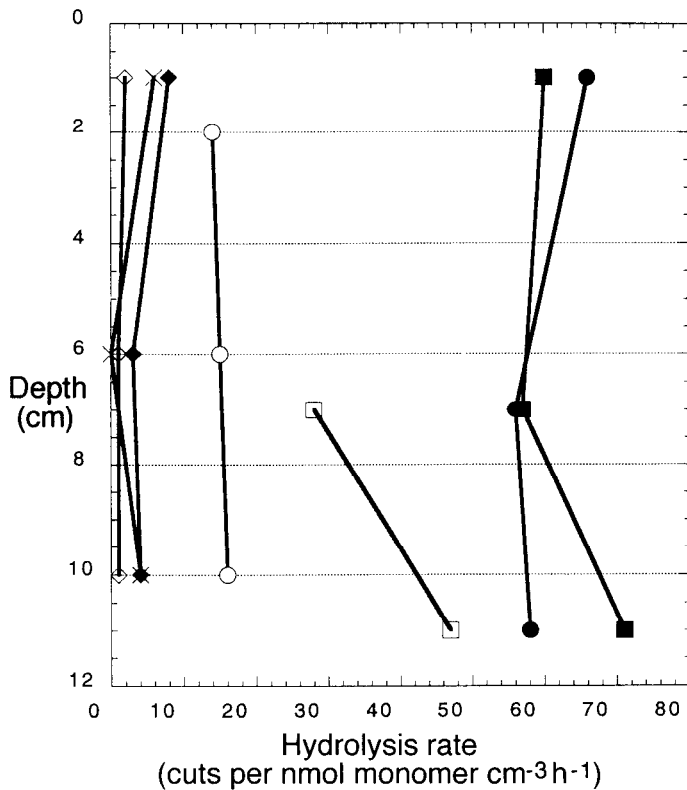


Fig. 1. Average potential hydrolysis rates of FLA polysaccharides in intact sediment cores collected at Hornsund, Van Mijenfjorden, and Storjorden (FLA xylan only). (For individual rates, see Tables 1–3.) ■, Hornsund FLA pullulan; □, Van Mijenfjorden FLA pullulan; ●, Hornsund FLA laminarin; ○, Van Mijenfjorden FLA laminarin; ◆, Hornsund FLA xylan; ◇, Van Mijenfjorden FLA xylan; ×, Storjorden FLA xylan.

by sectioning and drying a second uninjected core from each station.

Results

FLA polysaccharides were hydrolyzed at all three sites. Hydrolysis activity was higher for pullulan and laminarin, the two glucose polysaccharides, and was lower for xylan, the xylose polysaccharide. In nearly every case, hydrolysis was measurable from the top to the bottom of the subcores (Fig. 1). Potential hydrolysis rates differed considerably between stations (Tables 1–3). For both FLA pullulan and FLA laminarin, potential hydrolysis rates at Hornsund were generally higher than rates measured at Van Mijenfjorden. Potential hydrolysis of FLA xylan also was slightly faster at Hornsund than at Van Mijenfjorden, although the absolute difference between the rates was very small. The potential FLA xylan hydrolysis rates at Storjorden were also close to the potential rates measured at the other stations.

At Hornsund, average FLA pullulan and FLA laminarin hydrolysis rates were nearly identical: 60, 57, and 70 cuts per nmol glucose $\text{cm}^{-3} \text{h}^{-1}$ for surface, middle, and bottom FLA pullulan samples, and 66, 56, and 58 cuts per nmol glucose $\text{cm}^{-3} \text{h}^{-1}$ for surface, middle, and bottom FLA lam-

inarin samples (Tables 1, 2). FLA xylan potential hydrolysis rates were lower: 8, 3, and 4 cuts per nmol xylose $\text{cm}^{-3} \text{h}^{-1}$ for surface, middle, and bottom samples, respectively (Table 3). At Van Mijenfjorden, FLA pullulan hydrolysis was on average more rapid than FLA laminarin hydrolysis: 28 and 47 cuts per nmol glucose $\text{cm}^{-3} \text{h}^{-1}$ for middle and bottom FLA pullulan samples, as compared to 14, 15, and 16 cuts per nmol glucose $\text{cm}^{-3} \text{h}^{-1}$ for surface, middle, and bottom FLA laminarin samples. As at Hornsund, FLA xylan hydrolysis was slower than hydrolysis of the glucose polysaccharides, with average potential hydrolysis rates of 2, 1, and 1 cuts per nmol xylose $\text{cm}^{-3} \text{h}^{-1}$ for surface, middle, and bottom samples. At Storjorden, average rates of FLA xylan hydrolysis were similarly low: 6, 0, and 4 cuts per nmol xylose $\text{cm}^{-3} \text{h}^{-1}$ for surface, middle, and bottom samples.

The extent of horizontal variability in enzyme concentrations and(or) activities in the sediments was investigated by harvesting multiple FLA pullulan cores at three time points. Previous measurements of FLA pullulan hydrolysis in homogenized sediment samples showed up to 12% variation in potential hydrolysis rates for replicate samples (Arnosti 1995). For the deeper samples, variations in potential hydrolysis rates were >12%. For Hornsund, standard variations at 36 h were 5, 10, and 37% for surface, middle, and deep sediments. At 48 h, standard variations of replicate cores were 19, 41, and 86%, and at 60 h, standard variations were 6, 26, and 39% for surface, middle, and deep samples. Variability at Van Mijenfjorden was also significant: standard variations were 0 and 71% for middle and deep samples at 30 h, 9 and 68% at 48 h, and 45 and 81% at 58 h. The significance of these variations is discussed further below.

Discussion

Organic carbon remineralization in cold environments—

The rates of organic carbon remineralization in permanently cold environments, as well as possible temperature limitations on biological processes, have been the focus of a number of investigations (e.g. Pomeroy et al. 1991; Wiebe et al. 1992; Vetter and Deming 1994; Wheeler et al. 1996). A study of bacterial activity in the water column off Newfoundland showed that microorganisms were slow to respond to a spring phytoplankton bloom (Pomeroy et al. 1991). Pomeroy and colleagues postulated that bacterial growth at low temperatures might be limited at the cellular level by factors including “limited temperature ranges of enzyme activity.” Extracellular enzymes isolated from polar bacteria have frequently exhibited optimal temperatures of enzyme activity far higher than ambient environmental temperatures (Reichardt 1988; Helmke and Weyland 1991). The seeming lack of cold adaptation among extracellular enzymes led Reichardt (1987) to suggest that organic matter degradation in permanently cold environments may be limited in its initial step, unless increased enzyme production or accumulation could compensate for low levels of activity.

An increasing number of studies, however, have suggested that permanently cold temperatures are not intrinsically a barrier to high turnover rates of organic carbon. Bacterial activity in Antarctic and Arctic sites has been found to be

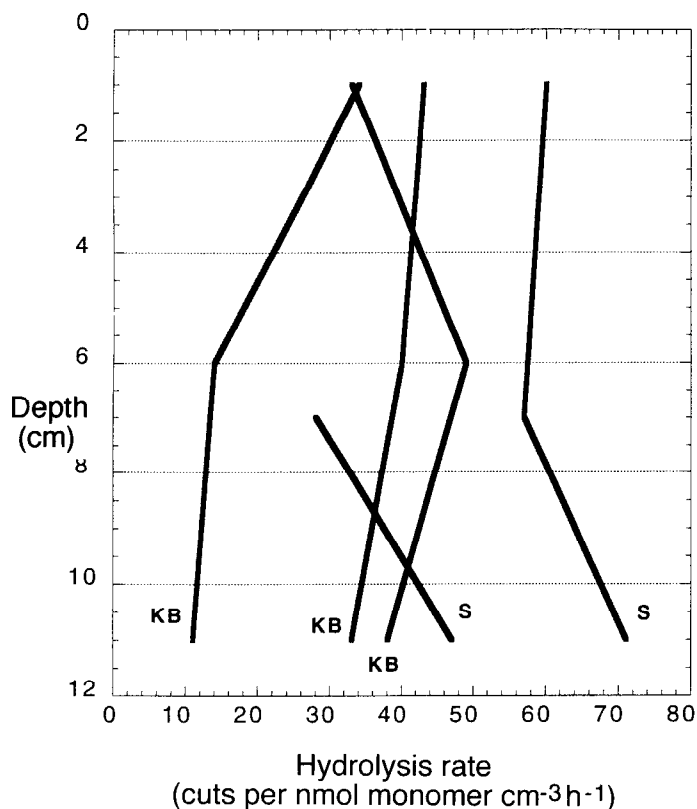


Fig. 2. Average potential hydrolysis rates of FLA pullulan in intact sediment cores at two stations at Svalbard (S) and three stations at Kiel Bight in the Baltic Sea (KB). (Kiel Bight data are from Arnosti 1995.)

comparable to activity in more temperate environments (Hodson et al. 1981; Thingstad and Martinussen 1991; Vetter and Deming 1994; Wheeler et al. 1996). Work undertaken at Svalbard showed that sulfate reduction rates in Svalbard sediments are comparable to rates measured in more temperate environments (Sagemann et al. 1998). Deming and Baross (1993) have pointed to a strong correlation between bacterial biomass and particulate organic carbon flux in deep-sea sediments as evidence that bacterial activity is regulated more by organic carbon supply than by temperature. The same conclusion was reached by Nedwell et al. (1993), who found that seasonal variations in benthic metabolism in Antarctic sediments were controlled primarily by organic matter input. At Svalbard, a comparison of the temperature responses of the initial and terminal steps of organic carbon remineralization—extracellular enzymatic hydrolysis, and sulfate reduction and oxygen consumption, respectively—also supports the suggestion that microbial respiration may be controlled more by organic matter supply than by temperature (Arnosti et al. 1998).

If particulate organic carbon is remineralized quickly in permanently cold environments, bacteria must have the means to hydrolyze rapidly the macromolecules that comprise most organic matter. The high potential hydrolysis rates of FLA pullulan and FLA laminarin measured at Svalbard demonstrate that at least some extracellular enzymes can function rapidly in permanently cold sediments. Potential

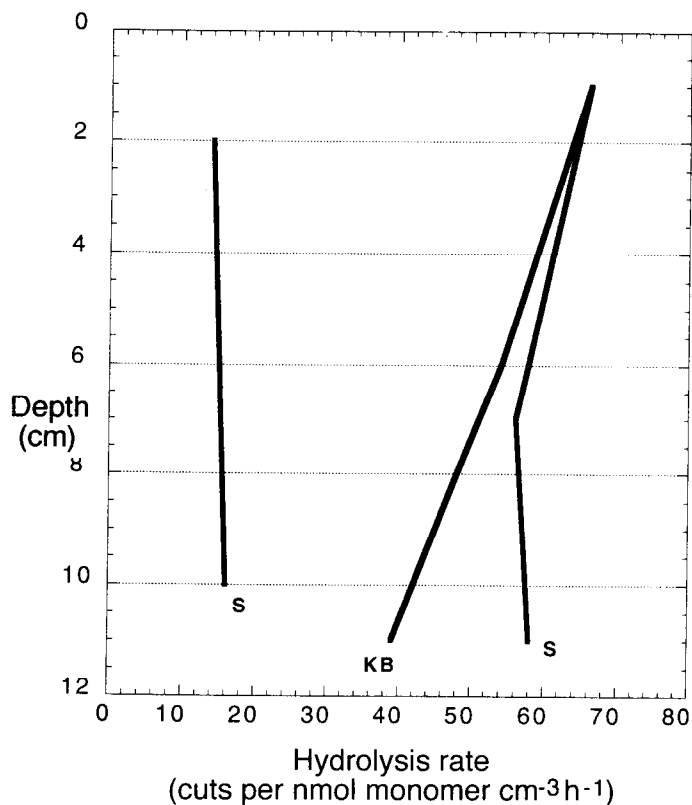


Fig. 3. Average potential hydrolysis rates of FLA laminarin in intact sediment cores at two stations in Svalbard (S) and one station at Kiel Bight in the Baltic Sea (KB). (Kiel Bight data are from Arnosti 1996.)

rates of pullulan hydrolysis at the Svalbard stations were in fact as high or higher than potential hydrolysis rates measured in the more temperate environment of Kiel Bight in the Baltic Sea (Fig. 2), whereas rates of FLA laminarin hydrolysis in Svalbard bracketed rates measured in Kiel Bight (Fig. 3). The close correspondence between potential hydrolysis rates in permanently cold Arctic sediments and in temperate sediments contrasts with results of studies using MUF substrates, which have found potential hydrolysis rates of MUF- α -glucose and MUF- β -glucose in permanently cold deep-sea sediments to be far less rapid than in shallow and temperate sediments (Boetius 1995; Poremba and Hoppe 1995). In the case of pullulanase and laminarinase, permanently cold temperatures clearly do not preclude high hydrolytic activity. Polysaccharide-hydrolyzing enzymes found in permanently cold environments may be isozymes of mesophilic enzymes, with structural adaptations providing greater flexibility and enabling them to function with higher efficiency at cold temperatures than do mesophilic enzymes. These types of adaptations (reduction in the number of salt bridges and weakly polar interactions, lower hydrophobicity) have been found in an α -amylase of a psychrophilic Antarctic bacterium (Feller et al. 1994).

Although FLA pullulan and FLA laminarin are hydrolyzed in Arctic sediments as rapidly as in temperate environments, these results should not automatically be extrapolated to other extracellular enzymes that have different

substrate specificities and activities and might not be adapted as well to cold temperatures. Hydrolysis of FLA xylan in Svalbard sediments, for example, was considerably slower than hydrolysis of FLA pullulan or FLA laminarin. Although no comparable measurements of FLA xylan hydrolysis were made in Kiel Bight sediments, hydrolysis rates of FLA pullulan, FLA laminarin, and FLA xylan in homogenized surface sediments from the North Sea were rapid and similar to one another (Arnosti unpubl. data). It is possible, therefore, that hydrolysis rates of FLA xylan in Svalbard sediments may be slower than in temperate sediments. If FLA xylan is hydrolyzed more slowly in Svalbard sediments than in temperate sediments, causes could include kinetic barriers to xylanase activity at low temperatures or a scarcity of xylanase enzymes among bacteria in Svalbard sediments. A more extensive survey of the activities of extracellular enzymes is needed in order to determine whether rapid potential hydrolysis rates are characteristic of a wider range of extracellular enzymes in Arctic sediments. The fact that deep-sea sedimentary microbial communities react rapidly to influxes of organic matter (Lochte and Turley 1988; Gooday and Turley 1990; Poremba 1994), however, requires that bacteria in permanently cold environments have sufficient enzymes to rapidly convert particulate organic substrates to molecular weights low enough to be transported across bacterial membranes.

Depth- and station-related trends—On average, FLA laminarin and FLA xylan potential hydrolysis rates were relatively constant with depth at both Hornsund and Van Mijenfjorden, whereas potential hydrolysis rates of FLA pullulan were higher in deep sediment sections than in the surface sections of both stations (Fig. 1). The potential hydrolysis rate measured at a given depth in sediments may be due to activities of enzymes produced by bacteria living at that depth, as well as to the activities of enzymes originally present in surface layers that were buried by sedimentation. Studies of soil-associated enzymes and investigations of the properties of immobilized enzymes have determined that enzymes attached to particles may be relatively more resistant to degradation and denaturation than are free enzymes in solution (Nannipieri et al. 1982; Monsan and Combes 1988). Measurements of substrate hydrolysis rates in sediments do not differentiate between enzymes attached to cell surfaces, free enzymes in solution, newly secreted enzymes associated with sedimentary organic matter, and enzymes “preserved” through association with sediment particles. All of these differently associated enzymes may in fact contribute to the hydrolysis of macromolecular substrates that fuel microbial metabolism.

Because the addition of FLA polysaccharides increased the total carbohydrate concentration of the pore waters on the order of 10- to 100-fold, competition from naturally occurring substrates for enzyme active sites is most probably negligible. The potential hydrolysis rates measured with FLA polysaccharides therefore should be zero-order (independent of substrate concentration), and are a measure of the rates at which specific enzymes could function, given non-limiting substrate concentrations. Actual rates of hydrolysis at any depth in sediments may be limited by substrate sup-

ply. The observation that relatively high levels of sulfate reduction occurred in the 3–10-cm interval at all stations (Sagemann et al. 1998), however, demonstrates that there is an active microbial community in subsurface sediments, and indicates that substrates of some type suitable to fuel this level of sulfate reduction must be produced at these depths in the sediments. Generally higher hydrolysis rates measured at Hornsund relative to Van Mijenfjorden (Fig. 1, Tables 1–3) also correspond to more rapid rates of sulfate reduction at Hornsund as compared to Van Mijenfjorden (Sagemann et al. 1998).

Average rates of pullulan and laminarin hydrolysis were similar at Hornsund, whereas at Van Mijenfjorden, average rates of pullulan hydrolysis were somewhat more rapid than average rates of laminarin hydrolysis. At both Hornsund and Van Mijenfjorden, rates of xylan hydrolysis were far slower than rates of laminarin and pullulan hydrolysis. Hydrolysis rates of xylan were also slow at Storfjorden, which suggests that low rates of xylan hydrolysis may be a general characteristic of Svalbard sediments rather than a specific feature of one station. There was considerable horizontal heterogeneity in the sediments, as shown by the variability of potential hydrolysis rates among multiple cores harvested at single time points (Tables 1–3). A maximum of 12% variation in potential hydrolysis rates had been measured in replicate homogenized surface sediments from the Baltic Sea (Arnosti 1995). Variations in potential hydrolysis rates of replicate cores at Svalbard were frequently far greater than 12%, but were within the range previously observed for intact sediment cores from the Baltic Sea (Arnosti 1995, 1996). Sulfate reduction rates measured in intact sediment cores at the same Svalbard stations demonstrated equally high variability among replicate cores (Sagemann et al. 1998).

Relationships between substrate structures and hydrolysis rates—The rates at which organic macromolecules are hydrolyzed in marine systems depends in part on the abundance and activities of enzymes that exhibit distinct substrate specificities, specificities determined by chemical structures. A comparison of the potential hydrolysis rates of the three FLA polysaccharides lends insight into some of the problems inherent in attempting to link hydrolysis rates directly with individual structural features. Most notably, the fact that both pullulan and laminarin were rapidly hydrolyzed at both stations suggests that gross molecular weight of a polymeric substrate may have little effect on extracellular enzymatic hydrolysis rates. FLA pullulan, with a molecular weight of 200,000 Da, was hydrolyzed at rates similar to (Hornsund) or somewhat faster than laminarin (Van Mijenfjorden), even though laminarin has a molecular weight of only 6,000 Da. Similarly rapid rates of pullulan hydrolysis have also been measured in the more temperate environment of Kiel Bight (Fig. 2). Although xylan and laminarin have molecular weights of ~8,000 and 6,000 Da, respectively, their hydrolysis rates were significantly different. Individual structural features such as linkage position (i.e. (1,3) or (1,4) or (1,6) linkages) or anomeric linkage (α or β) likewise cannot be associated in a simple fashion with rates of enzymatic hydrolysis. Both pullulan and xylan, for example, include (1,4) linkages, and laminarin and xylan both include β linkages,

yet rates of xylan hydrolysis were consistently lower than either pullulan or laminarin hydrolysis rates.

Monomer composition likewise is unlikely to be a simple key to hydrolysis rates, even though both of the polysaccharides that were hydrolyzed rapidly are glucose polysaccharides. Another type of glucose polysaccharide, cellulose, would not most likely have been hydrolyzed as rapidly in sediments as laminarin and pullulan. Cellulose and laminarin are both β -linked glucose polysaccharides, whose glucose monomers are connected via (1,4) and (1,3) linkages, respectively. The seemingly small differences in the primary structures of these two polysaccharides are belied by their significantly different physical and chemical properties and biological roles—cellulose is a cell wall component of rhodophytes and phaeophytes (Lee 1980), while laminarin is an energy-storage polysaccharide of a range of organisms including bacillariophytes and phaeophytes (Painter 1983). Dahlbäck et al. (1982) isolated bacteria from water, surface microlayers, and sediments of the Arctic Ocean (including stations near Svalbard) and tested their ability to hydrolyze a variety of polymeric substrates. None of the isolates was able to degrade cellulose. A similar result had been found in a survey of bacteria isolated from the deep Norwegian Sea, in which no cellulolytic activity was detected (Norkrans and Stehn 1978).

The absence of a simple and direct connection between relative rates of polysaccharide hydrolysis and single structural parameters highlights a problem inherent in generalizations derived from rate measurements made with low-molecular-weight substrate proxies. Studies utilizing MUF substrates have frequently focused on differences between rates of MUF- α -glucose and MUF- β -glucose hydrolysis, with the rationale that α -MUF substrates are proxies for α -linked polysaccharides such as starch, while β -MUF substrates represent β -linked glucose polysaccharides such as cellulose (Boetius 1995). Lower levels of MUF- α -glucose activity relative to MUF- β -glucose activity in deep-ocean and Arctic sediments have been attributed to the idea that organic matter reaching deep sediments would be low in easily degradable organic compounds such as starch, leading to a decreased requirement for amylase activity, whereas less degradable compounds such as cellulose are more likely to reach the sediments, hence higher levels of MUF- β -glucose activity (Boetius 1995; Poremba 1995). Because β -linked glucose polysaccharides such as laminarin serve as energy reserves in plankton, however, this generalization about α - and β -linked glucose polysaccharides is inaccurate. FLA polysaccharides, unlike MUF substrates, could in fact be used to distinguish between the activities of enzymes that hydrolyze structurally related polysaccharides such as laminarin and cellulose. The relationships between substrate chemical structure and hydrolysis rates are doubtless complex: different enzymes exhibit distinct substrate specificities, which are determined by the interactions of individual structural features. Hydrolysis rates are likely to be affected by enzyme abundance and activity, as well as by factors such as solution conformation, solubility, and surface interactions of specific polysaccharides.

Conclusions

Rapid extracellular enzymatic hydrolysis of organic macromolecules is a prerequisite for high turnover rates of particulate organic carbon. The investigations at Svalbard clearly show that at least some types of extracellular enzymes have the potential to function at high rates in permanently cold sediments: hydrolysis rates of FLA pullulan and FLA laminarin in Svalbard sediments were comparable to rates measured in the more temperate sediments of Kiel Bight. The consistently high potential hydrolysis rates of FLA pullulan and FLA laminarin through the upper ~ 11 cm of the sediments demonstrate that the pullulanase and laminarinase enzymes have the potential to be highly active at depth in the sediments, given sufficient substrates. The sulfate-reduction rates measured through the same depth intervals suggest that an active microbial community is present, producing substrate sufficient to fuel moderately high levels of sulfate reduction. The differences in potential hydrolysis rates observed between Hornsund and Vanmijenfjorden also paralleled differences in sulfate reduction rates at the two stations (Sagemann et al. 1998).

The observation that FLA xylan was not hydrolyzed at the same rate as FLA pullulan and FLA laminarin, however, shows that not all polysaccharides are hydrolyzed equally rapidly in Svalbard sediments. Although polysaccharides of different structures may be hydrolyzed at different rates, differences in potential hydrolysis rates among polysaccharides cannot be traced directly to single structural factors such as molecular weight, linkage position, anomeric linkage, or monomer composition. The rate at which organic macromolecules are hydrolyzed is determined in part by the abundance and activity of enzymes that exhibit distinct substrate specificities—substrate specificity is determined by a complex combination of structural features. This study extends the range of environments in which pullulanase and laminarinase activity has been measured, and represents the first measurement of xylanase activity in marine sediments.

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