Vol. 152: 27-39, 1997 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published June 26

Microbiological studies along a gradient of eutrophication in a shallow coastal inlet in the southern Baltic Sea (Nordrügensche Bodden)

Marion Köster*, Sven Dahlke, Lutz-Arend Meyer-Reil

Institut für Ökologie der Ernst-Moritz-Arndt-Universität Greifswald, D-18565 Kloster/Hiddensee, Germany

ABSTRACT: Measurements of microbial biomass and activity were carried out at 6 representative locations along a gradient of eutrophication in the Nordrügensche Bodden (southern Baltic Sea, Germany). Measurements in the water column revealed that turbidity, seston content, and concentrations of chlorophyll *a* and inorganic nutrients (ammonia, nitrite, nitrate, phosphate) increased from the outer to the inner parts of the Bodden whereas salinity decreased. Investigations of sediments confirmed this gradient of eutrophication. Whereas in the outer parts of the Bodden sandy sediments prevailed, sandy mud and muddy sediments dominated towards the inner parts. Generally, organic carbon and nitrogen, concentrations of chlorophyll *a* and phospholipids (indicator of microbial biomass), oxygen consumption and hydrolytic enzyme activities increased with increasing level of eutrophication. At the relatively unpolluted location in the outer parts of the Bodden, proteolytic enzymes dominated at the sediment surface. In subsurface horizons carbohydrate-decomposing enzymes gained more importance. At the heavily polluted locations in the inner parts of the Bodden, proteolytic enzymes were even more important. With increasing sediment depth enzyme activities were greatly reduced, however, and shifts in the spectrum of hydrolytic enzymes were less pronounced. Among the biological and chemical parameters, characteristic patterns of interrelationships became obvious, which led to the conclusion that microbial biomass and enzyme activities in sediments of the outer and central parts of the Bodden are limited by organic carbon. The organic-rich sediments of the inner parts of the Bodden, however, did not support further increases in microbial biomass and decomposition activities. Enzyme activities are discussed in relation to the composition and degradability of substrates. The enzymatic decomposition potential was measured by means of fluorogenic model substrates. Methodological investigations showed that the methylumbelliferyl substrates used reacted specifically enough to justify their use in ecological studies. Sediments can be stored refrigerated for over 2 mo without changes in the spectrum of hydrolytic enzymes.

KEY WORDS: Nordrügensche Bodden (Southern Baltic Sea) · Sediment · Eutrophication · Microbial biomass · Microbial activities · Enzymatic activities · Storage of sediments

INTRODUCTION

The Nordrügensche Bodden, part of the Baltic coast of Mecklenburg-Vorpommern (Germany), consists of a chain of sheltered shallow water basins, some of which communicate only via small channels. Whereas the outer parts of the Bodden are strongly influenced by their connection with the Baltic Sea, the inner parts are mainly influenced by terrestrial sources. The Bodden acts as drainage for natural and anthropogenic inputs resulting from agricultural sources and sewage treatment plants. The Nordrügensche Bodden comprises the following main basins (Fig. 1): Libben, Rassower Strom, Breetzer Bodden, Breeger Bodden, Großer Jasmunder Bodden and Kleiner Jasmunder Bodden, covering an area of 158.6 km^2 with a water volume of 553.5×10^6 m^3 (mean water depth 3.5 m; Lampe 1994). Due to the low ratio of water volume to sediment surface, the sediment is of great importance for the turnover of organic material. Besides the impact of eutrophication, the Bodden is

*E-mail: koesterm@rz.uni-greifswald.de

© Inter-Research 1997
Resale of full article not permitted

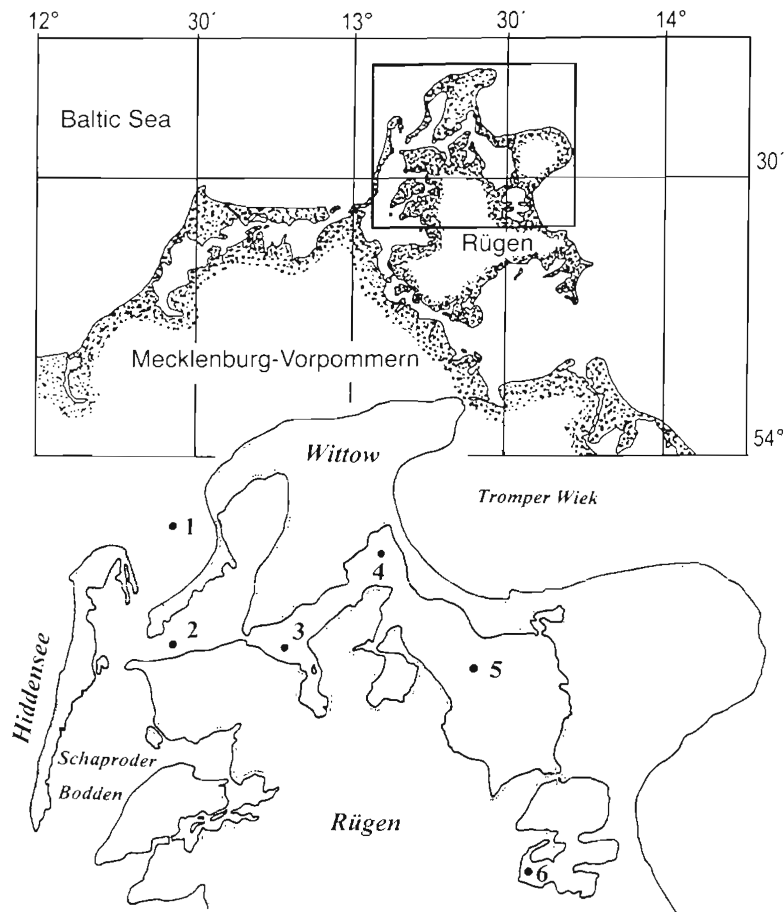

Fig. 1 Nordrügensche Bodden, showing locations sampled: 1, Libben; 2, Rassower Strom; 3, Breetzer Bodden; 4, Breeger Bodden; 5, Großer Jasmunder Bodden; 6, Kleiner Jasmunder Bodden

strongly influenced by hydrographical conditions, such as exchange with the Baltic Sea, river discharge, wind and temperature.

In the past, microbiological studies in the Nordrügensche Bodden concentrated on measurements of physico-chemical parameters, inorganic nutrients, primary production and aspects of the nitrogen cycle. Since the Bodden was a prohibited area in the former German Democratic Republic (GDR), only a selection of the data could be published. Other data were summarized in the form of unofficial or official reports with limited availability. Hübel (1969) investigated primary production as related to gradients of nutrients and salinity in the Bodden. Hübel showed that with increasing nutrient contents and decreasing salinity, phytoplankton primary production greatly increased from the outer to the inner parts of the Bodden. For more than 25 yr, salinity, temperature, primary production and concentrations of inorganic nutrients were measured at representative locations in the Nordrügensche Bodden (Hübel 1984). Intensive studies were carried out with regard to aspects of the nitrogen cycle: fixation of nitrogen by autotrophic and heterotrophic microorganisms (summary in Hübel 1984), and denitrification (Dahlke 1990). Investigations of the metabolism of the Bodden basins revealed that these are highly dynamic systems dominated by metabolic processes in the sediments and strongly influenced by hydrographical conditions (Dahlke & Hübel 1994). For the Kleiner Jasmunder Bodden, the innermost part of the Nordrügensche Bodden chain, measurements of the microbial metabolism were compared to earlier data to assess the degree of eutrophication and to discuss possibilities for the restoration of this heavily polluted area (Dahlke & Hübel 1996). This is of special importance as water exchange occurs only via a small connection with the Großer Jasmunder Bodden.

In the present study physico-chemical, chemical and microbiological parameters were determined at 6 representative locations along the gradient of salinity and eutrophication in the Nordrügensche Bodden. The aim of the study was to identify interrelationships between microbiological and chemical parameters as characteristics of the individual locations.

MATERIALS AND METHODS

Sampling. In the Nordrügensche Bodden water and sediment samples were withdrawn at 6 stations (Libben, Rassower Strom, Breetzer Bodden, Breeger Bodden, Großer and Kleiner Jasmunder Bodden) located on a west-east profile from the outer part to the inner part of the Bodden (Fig. 1). Sampling was carried out in January, March and May 1994; additional samples restricted to characteristic locations were taken in September and October 1994, and in March and June 1995. Prior to sampling, salinity, temperature (Microprocessor conductivity meter LF 196 with integrated temperature sensor, Wissenschaftliche-Technische Werkstätten GmbH), and Secchi depth were measured. During sampling, no stratification could be detected in the water column. Water samples were taken 1 m below the surface using a sterile water sampler. Undisturbed sediment samples with overlying water were withdrawn using a multiple corer (Barnett

et al. 1984) modified for application to shallow waters (H. J. Black pers. comm.). Samples were kept at *in situ* temperature and processed in the laboratory within 3 h after sampling.

Biological and chemical measurements. In the water column, seston, chlorophyll *a*, and inorganic nutrients (ammonia, nitrate, nitrite, phosphate) were analysed according to standard procedures (Grasshoff et al. 1983, HELCOM 1988). From the sediments, the 0–1 cm horizon was separated. In samples withdrawn in June 1995, 2 sediment horizons (0–1 and 9–10 cm) were analysed. The material from the corresponding horizons of 3 sediment cores was combined, carefully mixed, and various biological and chemical parameters were determined in the slurry. Water content of sediments was determined after drying at 60°C for 48 h. For determination of organic carbon and nitrogen concentrations, dried, homogenized and preweighed sediments were exposed for 48 h to concentrated HCl vapour and subsequently treated with 1 or 2 drops of concentrated HCl to remove inorganic carbon. Sediments were dried again, and concentrations of organic carbon and nitrogen were determined using a Perkin-Elmer CHN-240C-analyser (Verardo et al. 1990, Köster 1992). Phospholipids were measured spectrophotometrically according to Findlay et al. (1989). For the determination of benthic oxygen consumption, the overlying water of enclosed sediment cores (3 replicates) was sampled in time course experiments and analysed for oxygen concentration by the Winkler method (Grasshoff et al. 1983). Before sampling, the overlying water was stirred with a glass spatula to avoid concentration gradients.

Enzymatic activities. For determination of enzyme activities in sediments, fluorescence labelled model substrates were used. Fluorescein diacetate (FDA) is hydrolysed unspecifically by esterases. To determine the spectrum of hydrolytic enzymes, the following methylumbelliferyl substrates (MUF derivatives) were used: MUF-phosphate, MUF-sulfate, MUF-α-D-glucoside, MUF-β-D-glucoside, MUF-N-acetyl-β-D-glucosaminide, and MCA-leucine (L-leucine-4-methylcoumarinyl-7-amid HCl). For enzyme assays, 5 cm^3 of sediment were diluted 1:20 with phosphate buffer (1 part of 0.067 M KH_2PO_4 and 15.6 parts of 0.084 M Na_2HPO_4; pH 7.8). Sediment slurries for the phosphatase assay were prepared with sterile filtered bottom water. Aliquots (2 ml) of the slurry were pipetted into Eppendorf caps (water bath, 37°C), and the reaction was started by adding the substrates at saturation level (final concentration of FDA and MUF derivatives was 100 µM). Enzyme assays were run in duplicate; for substrates hydrolysed at low rates, triplicate assays were performed. After 10, 20, 30, 40, 50 and 60 min, 40 µl of the slurry was removed and transferred to 1 ml of buffer (pH 7.8 and 10 for samples treated with FDA and MUF derivatives, respectively) in an ice bath. Samples were centrifuged at 5870 × *g* for 8 min at 0°C and analysed spectrofluorometrically (Kontron SFM 25). FDA samples were examined at 370 nm excitation and 410 nm emission, MUF samples at 365 nm excitation and 445 nm emission wavelength against standards of fluorescein and methylumbelliferone, respectively. As the excitation and emission characteristics of the fluorochromes MUF and MCA are similar, MUF and MCA samples were treated in the same manner. Enzyme activity rates were calculated from the linear part of time-dependent activity curves. Correlation coefficients were significant at 95% confidence level or higher.

Methodological investigations. In laboratory experiments, the influence of different methods of sediment storage, the specificity of hydrolysis of the model substrates, and effects of pH and temperature on the stability of the model substrates and the corresponding fluorescent dyes were investigated.

For experiments on the influence of storage, aliquots of mixed sediments (Rassower Strom, 0–1 cm horizon) were either stored at 2°C, or frozen at –20°C, or freeze dried (LYOVAC GT2). After various times the aliquots were removed and analysed as described above.

The specificity of hydrolysis of the model substrates was investigated by adding the following commercially available enzymes to FDA and MUF derivatives: protease (Type XIV: from *Streptomyces griseus*), leucine-aminopeptidase (Type III-CP: from porcine kidney), N-acetylglucosaminidase A and B (from bovine epididymis), α- and β-glucosidase (from *Bacillus stearothermophilus* and from almonds, respectively), phosphatase (Type X: from sweet potato) and sulfatase (Type VI: from *Aerobacter aerogenes*). All enzymes were purchased from Sigma Company.

The influence of pH was examined by adding FDA and MUF derivatives and the corresponding dyes (fluorescein and methylumbelliferone, respectively) to buffer solutions of pH ranging between 1 and 14. Thermal stability of substrates was investigated at 37°C. Enzyme assays were run as described above.

RESULTS

Methodological investigations

Effect of storage

Variations in selected hydrolytic enzymes (leucine-aminopeptidase, N-acetylglucosaminidase, α- and β-glucosidase, phosphatase, and sulfatase) were especially pronounced during the initial time of storage (Fig. 2). In both freeze-dried and frozen sediment sam-

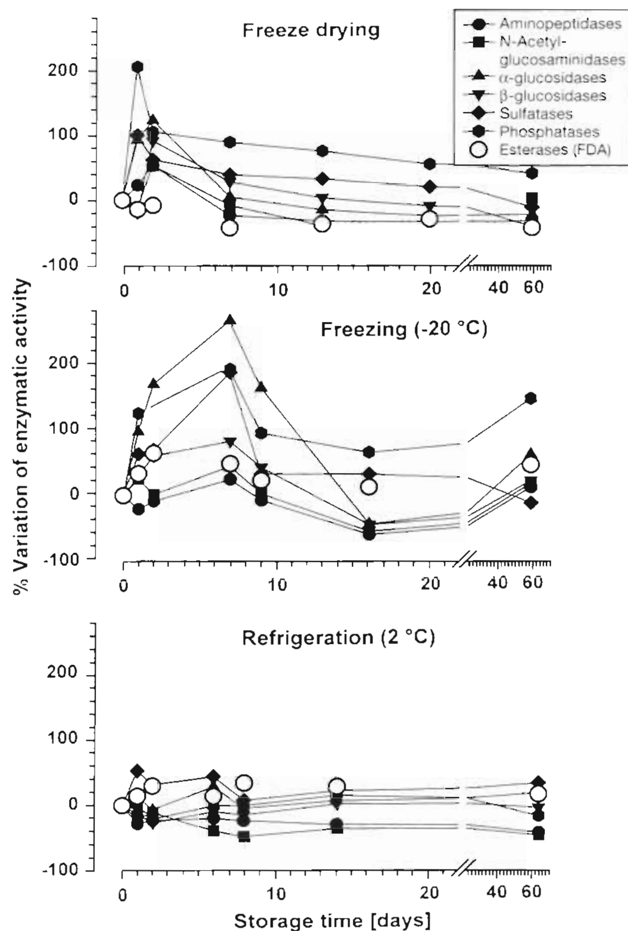

Fig. 2. Influence of storage on enzymatic activities (measured by means of hydrolysis of fluorescein diacetate and selected MUF-derivatives). Aliquots of sediments (Rassower Strom, March 1995) were kept freeze dried, frozen (−20°C) or refrigerated (2°C). Enzymatic activities determined immediately after sampling were set to 100%

ples, the most striking observation was the increase in activity during the initial phase of storage. In sediments kept at 2°C, variations in activities in selected hydrolytic enzymes were lowest. For all 3 preservation methods, enzymatic activities decreased to values comparable to the initial rates measured in the untreated samples after approximately 60 d of storage. However, this did not apply to phosphatase activity in frozen samples, which increased again after the initial increase and subsequent decrease. The contribution of selected enzymes to the pool of hydrolytic enzymes remained remarkably constant during the storage period of 2 mo.

Generally, variations in specific hydrolytic enzymes were much more pronounced in comparison to general activities of esterases (cleavage of FDA). In frozen and refrigerated sediment samples, variations in selected hydrolytic enzymes were paralleled by variations in esterase activities. However, in freeze-dried samples a short-term enhancement of activity of selected hydrolytic enzymes was not observed.

Specificity of hydrolysis of model substrates

MUF substrates were hydrolysed quite specifically by commercially available enzymes (protease, leucine-aminopeptidase, N-acetylglucosaminidase A and B, α- and β-glucosidase, phosphatase, sulfatase). Only MUF-phosphate and MUF-N-acetyl-β-D-glucosaminide showed weak unspecific reactions with sulfatase. Whereas the MUF derivatives were quite specifically cleaved by the corresponding enzymes, the model substrate FDA was unspecifically hydrolysed by all enzymes applied (Table 1). During incubation (1 h, 37°C) no spontaneous hydrolysis of the model substrates was observed.

Effect of pH

Enzymatic reactions and fluorescence measurements require distinct pH values for optimal reaction of

Table 1. Hydrolysis of model substrates by commercially available enzymes. +, positive; –, negative reactions; (+) refers to weak positive reactions. MCA-leu: L-leucine-4-methylcoumarinyl-7-amid HCl; MUF-N-Ac-glu: MUF-N-acetyl-β-D-glucosaminide; MUF-α-D-glu: MUF-α-D-glucoside; MUF-β-D-glu: MUF-β-D-glucoside; MUF-pho: MUF-phosphate; MUF-sul: MUF-sulfate; FDA: fluorescein diacetate

Enzymes	Substrates						
	MCA-leu	MUF-N-ac-glu	MUF-α-D-glu	MUF-β-D-glu	MUF-pho	MUF-sul	FDA
Protease	+	–	–	–	–	–	+
Leu-aminopeptidase	+	–	–	–	–	–	+
N-acetyl-glucosaminidase A	–	+	–	–	–	–	+
N-acetyl-glucosaminidase B	–	+	–	–	–	–	+
α-D-glucosidase	–	–	+	–	–	–	+
β-D-glucosidase	–	–	–	+	–	–	+
Phosphatase	–	–	–	–	+	–	+
Sulfatase	–	(+)	–	–	(+)	+	+

enzymes and stability of model substrates. FDA was stable in the pH range from 1 to 7.8. Higher pH values led to a drastic increase in relative fluorescence due to chemical hydrolysis. Compared to FDA, MUF derivatives were stable over a broader pH range (from 1 to 12). At pH values above 12, the fluorescence decreased rapidly. The autofluorescence of the substrate solutions was less than 1% as compared to the fluorescence measured in the samples. Stock solutions of FDA and MUF derivatives (5 mM) which were frozen at −20°C for 6 mo showed a slightly higher autofluorescence (data not shown).

Maximum fluorescence intensity of the dyes fluorescein and methylumbelliferone was reached at pH values above 7.8 and 9.0, respectively. A shift to lower pH values led to a drastic reduction of relative fluorescence of the dyes. Therefore, measurements are optimal in buffer solutions of pH 7.8 and 10, respectively (see 'Materials and methods').

Ecological observations

Physico-chemical and chemical parameters in the water above the sediments revealed pronounced gradients throughout the Bodden. In general, temperature, concentrations of seston, chlorophyll *a* and inorganic nutrients (ammonia, nitrite, nitrate, phosphate) increased, whereas salinity and Secchi depth decreased from the outer part (Libben) to the inner part (Kleiner Jasmunder Bodden) of the Bodden. The data are summarized in Table 2.

Salinity ranged between 8.4 and 12.2‰ in the outer part (Libben) and between 4.4 and 5.4‰ in the inner part (Kleiner Jasmunder Bodden) of the Bodden. Changes in temperature followed the seasonal cycle. Secchi depth was between 1.4 and 3.8 m in the outer part, and less than 0.7 m in the inner part of the Bodden. The seston content varied between 7 and 20 mg l^{-1} in the outer part, and between 32 and

Table 2. Temperature (T), salinity (S), Secchi depth (SD), seston, chlorophyll *a* (chl *a*) and nutrient concentrations at different stations along the Nordrügensche Bodden (LI: Libben; RS: Rassower Strom; BRZ: Breetzer Bodden; BRG: Breeger Bodden; GJB: Großer Jasmunder Bodden; KJB: Kleiner Jasmunder Bodden). nd: not determined

	T (°C)	S (PSU)	SD (m)	Seston (mg l^{-1})	Chl *a* (µg l^{-1})	NH_4 (µM)	NO_3 (µM)	NO_2 (µM)	PO_4 (µM)
25 Jan 1994									
LI	2.5	12.1	1.4	20	2	1.9	11.2	1.0	0.7
RS	2.3	11.4	1.4	19	3	2.5	11.5	1.0	0.7
BRZ	2.3	10.9	2.0	34	3	2.6	9.0	1.4	0.8
BRG	1.9	8.6	0.7	38	19	7.2	35.8	1.8	1.0
GJB	1.9	8.6	0.8	40	27	8.6	26.7	7.8	1.0
KJB[a]	2.3	4.7	0.5	55	132	27.0	53.1	1.2	0.6
16 Mar 1994									
LI	2.8	12.2	3.8	9	1	1.5	9.6	0.1	0.9
RS	2.9	11.1	2.1	14	2	1.5	11.5	27.2	0.8
BRZ	3.2	9.8	1.9	22	5	1.3	38.7	1.0	0.9
BRG	3.7	8.1	1.2	59	22	4.8	33.7	0.8	5.9
GJB	3.4	8.0	1.2	47	21	5.6	51.5	1.1	1.4
KJB[b]	3.6	4.4	0.6	61	113	23.8	49.9	1.6	0.8
25 May 1994									
LI	10.8	8.4	3.2	7	3	0.6	<0.1	0.3	<0.1
RS	13.6	8.6	1.7	14	10	<0.1	<0.1	0.1	<0.1
BRZ	13.9	8.5	1.5	16	11	<0.1	<0.1	0.1	<0.1
BRG	13.8	8.2	1.0	34	30	<0.1	<0.1	0.1	<0.1
GJB	13.9	8.0	0.9	33	46	<0.1	0.2	0.1	0.1
KJB[c]	14.9	4.4	0.4	84	149	2.4	0.1	<0.1	1.8
13 Sep 1994									
RS	14.8	9.0	2.5	8	14	0.2	0.2	0.1	0.7
GJB	15.3	8.3	1.1	28	44	0.9	0.4	0.1	5.9
KJB[d]	16.7	5.4	0.6	32	73	0.1	nd	nd	nd
10 Oct 1994									
RS	10.0	nd	2.7	10	6	nd	0.2	<0.1	6.3
GJB	9.6	8.3	1.7	14	29	nd	0.7	<0.1	7.0
KJB[e]	9.6	5.2	0.6	34	86	0.3	1.6	<0.1	0.3

Sampling dates in the Kleiner Jasmunder Bodden: [a]3 Feb 1994; [b]22 Mar 1994; [c]7 Jun 1994; [d]7 Sep 1994; [e]6 Oct 1994

84 mg l^{-1} in the inner part of the Bodden. Concentrations of chlorophyll *a* were lowest at the Libben station (between 1 and 3 µg l^{-1}) and highest at the Kleiner Jasmunder Bodden (between 73 and 149 µg l^{-1}). Concentrations of inorganic nutrients (ammonia, nitrite, nitrate, phosphate) were highest in winter and early spring and decreased towards summer and autumn (Table 2). As an example of increasing eutrophication along the Bodden, concentration gradients measured in the water column in March 1994 are illustrated in Fig. 3.

The gradient of eutrophication from the outer to the inner parts of the Bodden was especially pronounced in sediments (Table 3). Concentrations measured in March 1994 are illustrated in Fig. 4. In the outer part of the Bodden, sandy sediments prevailed (Libben, water content 17 to 24%), whereas muddy sand and sandy mud sediments dominated towards the inner part of the Bodden (water content: Rassower Strom 28 to 68%, Breetzer Bodden 26 to 43%, Breeger Bodden 52 to 71%). The innermost parts were characterized by muddy sediments (water content: Großer Jasmunder Bodden 85 to 94%, Kleiner Jasmunder Bodden 87 to 95%; Fig. 4). Oxygen consumption rates varied between 14 and 65 mmol m^{-2} d^{-1} along the transect (Table 3).

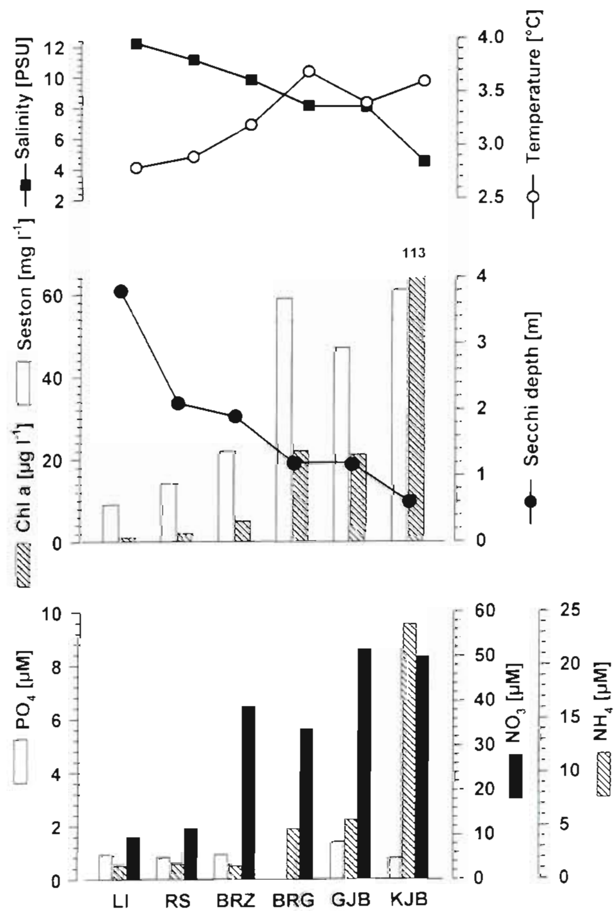

Fig. 3. Temperature, salinity, Secchi depth and concentrations of seston, chlorophyll *a*, and inorganic nutrients in the water column along a gradient of eutrophication in the Nordrügensche Bodden (March 1994). Locations sampled: LI, Libben; RS, Rassower Strom; BRZ, Breetzer Bodden; BRG, Breeger Bodden; GJB, Großer Jasmunder Bodden; KJB, Kleiner Jasmunder Bodden. For concentration measurements the average values of 2 parallel measurements are shown

Concentrations of organic carbon in surface sediments (0–1 cm) were lowest at Libben (0.1%) and increased towards the inner parts of the Bodden (Rassower Strom, Breetzer and Breeger Bodden: 0.4 to 2.5%). Maximum concentrations of organic carbon were reached at the Großer and Kleiner Jasmunder Bodden (7.4 to 12.7% and 8.8 to 15.1%, respectively). Generally, a slight increase in the ratio of carbon to nitrogen was observed along the transect (Table 3).

In general, chlorophyll *a* concentrations were highest in surface sediments (0–1 cm) of the Rassower Strom and Breetzer Bodden (6 to 25 µg cm^{-3}) and decreased drastically towards the light-limited sediments of the Großer and Kleiner Jasmunder Bodden (2 to 13 µg cm^{-3}). Relatively high concentrations of chlorophyll *a* (26 µg cm^{-3}) measured in sediments from the Großer Jasmunder Bodden in October 1994 might have been caused by inhomogeneity of the sample (note high standard deviation; Table 3). Concentrations of phospholipids as a biomarker for microbial biomass ranged between 19 and 219 nmol cm^{-3} and revealed peaks at the Rassower Strom and Kleiner Jasmunder Bodden. Occasionally, relatively high values also appeared at the Breetzer Bodden station (Table 3). Based on the conversion factor given by Findlay et al. (1989) this means that living biomass represents only a few percent (between 1 and 8%) of the total organic pool. These data were comparable to findings of Meyer-Reil (1993).

Parallel with increasing organic matter content and microbial biomass along the transect, enzymatic decomposition activities (FDA hydrolysis) in surface sediments increased from 49–92 nmol cm^{-3} h^{-1} at the Libben station to 340–710 nmol cm^{-3} h^{-1} at the Großer Jasmunder Bodden. Generally, highest degradation rates were measured at the Kleiner Jasmunder Bodden (419 to 710 nmol cm^{-3} h^{-1}; Fig. 4). An exception were the samples measured in October 1994, when enzymatic activities were lower (by a factor of 2) than activities of sediments from the Großer Jasmunder Bodden. Relatively high enzymatic degradation rates were measured in January and September 1994 in sediments from the Rassower Strom station which were muddy sand sediments with a water content of up to 68% (Table 3).

At 2 characteristic locations in the outer and inner part of the Bodden (Rassower Strom and Kleiner Jasmunder Bodden, respectively), the pool of esterases

Table 3. Water content (WC), organic carbon (C), carbon to nitrogen ratio (C:N), chlorophyll *a* (chl *a*) and phospholipid (PL) concentrations, enzymatic activity (EA, measured as FDA hydrolysis) and oxygen consumption (O_2) in surface sediments (0–1 cm) of different locations in the Nordrügensche Bodden (LI: Libben; RS: Rassover Strom; BRZ: Breetzer Bodden; BRG: Breeger Bodden; GJB: Großer Jasmunder Bodden; KJB: Kleiner Jasmunder Bodden). Standard deviations are given in brackets (ss: analysis of a single sample). nd: not determined

	WC (%)	C (%)	C:N	Chl *a* (µg cm^{-3})	PL (nmol cm^{-3})	EA (nmol cm^{-3} h^{-1})	O_2 (mm m^{-2} d^{-1})
25 Jan 1994							
LI	23 (ss)	0.1 (0.01)	9.2 (1.4)	2 (<0.5)	48 (5)	49	nd
RS	66 (ss)	1.6 (0.05)	9.4 (1.4)	6 (<1)	120 (8)	556	nd
BRZ	41 (ss)	0.7 (0.02)	9.2 (0.1)	14 (2)	174 (9)	259	nd
BRG	56 (ss)	1.5 (0.04)	9.7 (0.2)	6 (1)	53 (12)	318	nd
GJB	92 (ss)	nd	nd	4 (1)	95 (7)	561	nd
KJB[a]	93 (ss)	10.6 (ss)	9.2 (ss)	5 (1)	119 (ss)	567	nd
16 Mar 1994							
LI	17 (ss)	0.1 (0.00)	8.6 (1.7)	3 (<0.5)	40 (6)	92	17
RS	28 (ss)	0.4 (0.01)	8.9 (0.5)	13 (2)	118 (19)	223	27
BRZ	26 (ss)	0.5 (0.02)	8.5 (0.5)	13 (3)	85 (25)	395	26
BRG	52 (ss)	1.4 (0.04)	9.2 (0.1)	10 (<0.5)	71 (21)	504	nd
GJB	85 (ss)	7.4 (ss)	nd	2 (<0.5)	90 (12)	468	nd
KJB[b]	95 (ss)	15.1 (0.2)	9.1 (0.7)	7 (3)	219 (22)	710	24
25 May 1994							
LI	24 (<1)	0.1 (0.002)	7.9 (0.7)	4 (1)	19 (1)	68	14
RS	38 (<1)	0.6 (0.03)	8.8 (0.1)	19 (3)	145 (20)	384	19
BRZ	43 (<1)	0.7 (0.01)	8.3 (0.5)	15 (3)	133 (19)	324	31
BRG	71 (3)	2.3 (0.2)	9.1 (0.3)	10 (1)	86 (2)	291	33
GJB	93 (<1)	9.7 (0.04)	10.0 (0.3)	9 (<1)	216 (22)	340	28
KJB[c]	94 (<1)	13.6 (0.02)	9.1 (0.5)	13 (1)	122 (15)	473	65
13 Sep 1994							
RS	68 (<1)	2.5 (0.07)	9.1 (0.6)	25 (2)	170 (30)	664	26
GJB	94 (<1)	11.1 (0.6)	nd	11 (1)	116 (13)	352	55
KJB[d]	90 (<1)	10.4 (0.4)	9.9 (0.5)	7 (1)	188 (21)	419	nd
10 Oct 1994							
RS	35 (<1)	0.7 (0.05)	8.8 (0.5)	22 (1)	211 (38)	495	28
GJB	93 (<1)	12.7 (0.2)	8.8 (0.1)	26 (9)	150 (5)	710	33
KJB[e]	87 (<1)	8.8 (0.5)	10.3 (0.8)	2 (<1)	nd	290	nd

Sampling dates in the Kleiner Jasmunder Bodden: [a]3 Feb 1994; [b]22 Mar 1994; [c]7 Jun 1994; [d]7 Sep 1994; [e]6 Oct 1994

(FDA hydrolysis) and the spectrum of selected enzymes were analysed in surface (0–1 cm) and subsurface (9–10 cm) horizons. In the muddy sand sediments at the Rassower Strom station, concentrations of organic carbon in both horizons were about 1% with a C:N ratio of 9.5. Muddy sediments from the Kleiner Jasmunder Bodden had concentrations of 12 and 5% C in surface and subsurface horizons, respectively, with a C:N ratio of 9.5 and 12.0, respectively (data not shown).

As shown in Fig. 5, proteolytic decomposition activities prevailed in surface sediments (0–1 cm) from the Rassower Strom station, followed by activities of β- and α-glucosidases. Sulfatase, phosphatase and N-acetyl-glucosaminidase contributed far less to the pool of hydrolytic enzymes. In deeper sediment horizons (9–10 cm), a pronounced shift in the spectrum of hydrolytic enzymes activities occurred: aminopeptidase activities decreased by a factor of 4, α-glucosidase by a factor of 17, sulfatase and phosphatase by a factor of 8. Whereas the activities of β-glucosidase remained almost constant, activities of N-acetyl-glucosaminidase drastically increased by a factor of 9. In muddy surface sediments of the Großer and Kleiner Jasmunder Bodden activities of aminopeptidases dominated; carbohydrate-decomposing enzymes, sulfatase and phosphatase were far less important compared to surface sediments of the Rassower Strom station. In the 9–10 cm sediment horizons, the enzyme activities were generally reduced. However, the extent of reduction differed at both locations (Großer Jasmunder Bodden: factor 11 to 30; Kleiner Jasmunder Bodden: factor 2 to 5; Fig. 5).

DISCUSSION

The methodological investigations revealed that measurements of enzymatic activities by means of fluorescent-labelled model substrates require distinct pH ranges. This applies to the stability of the model substrates as well as to the optimum sensitivity of the

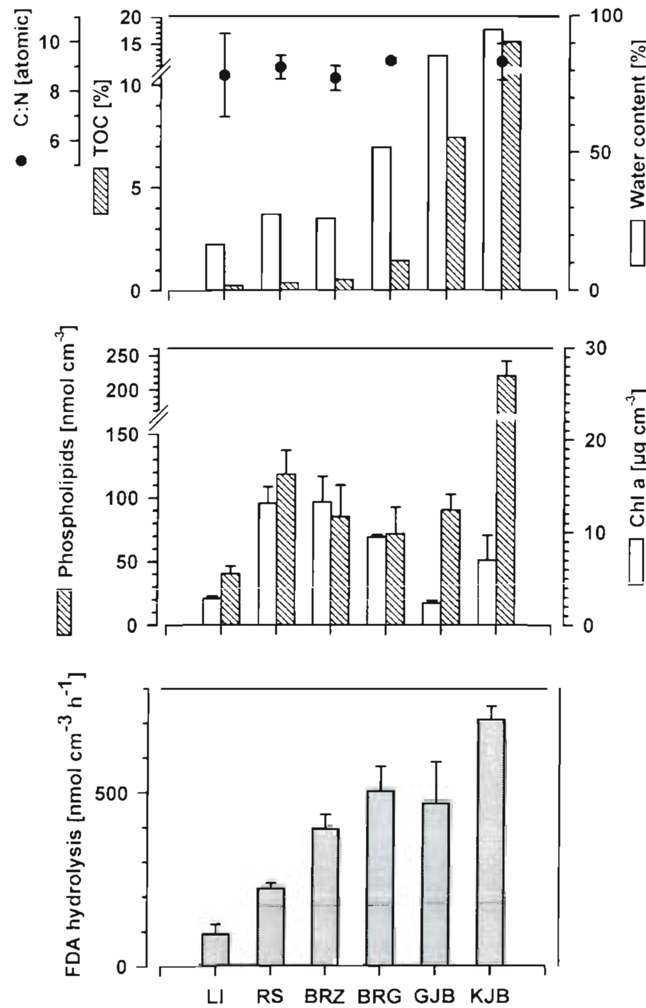

Fig. 4. Concentration of organic carbon (TOC), C:N ratios, water content, concentrations of phospholipids and chlorophyll *a*, and enzymatic activity (FDA hydrolysis rates) in surface sediments (0–0.5 cm) of the Nordrügensche Bodden (March 1994). Locations sampled: LI, Libben; RS, Rassower Strom; BRZ, Breetzer Bodden; BRG, Breeger Bodden; GJB, Großer Jasmunder Bodden; KJB, Kleiner Jasmunder Bodden. Error bars are standard deviations of 3 to 6 parallels

analysis of the fluorescent dyes released during cleavage. Autofluorescence during storage of stock solutions of the substrates and during performance of the enzyme assays can be neglected. Furthermore, it could be shown that the methylumbelliferyl derivatives used reacted specifically enough to justify their use in ecological research. As previous investigations have shown, enzyme assays should be carried out at substrate saturation (Meyer-Reil 1990).

Generally, information concerning chemical and biological alterations of stored samples is scarce. This applies especially to variations of enzyme activities. A variety of different preservation procedures (freezing at different temperatures, freeze drying, refrigeration) have been applied in previous studies without assessing the effectiveness of storage in relation to chemical and biological parameters (Chrost & Velimirow 1991, Christian & Karl 1995). Effective storage will only be achieved if the original properties of the sample are preserved. Damage to organisms and enzymatic degradation processes in stored samples should be minimized. Furthermore, the preservation procedure should be practicable immediately after sampling. Most of the information

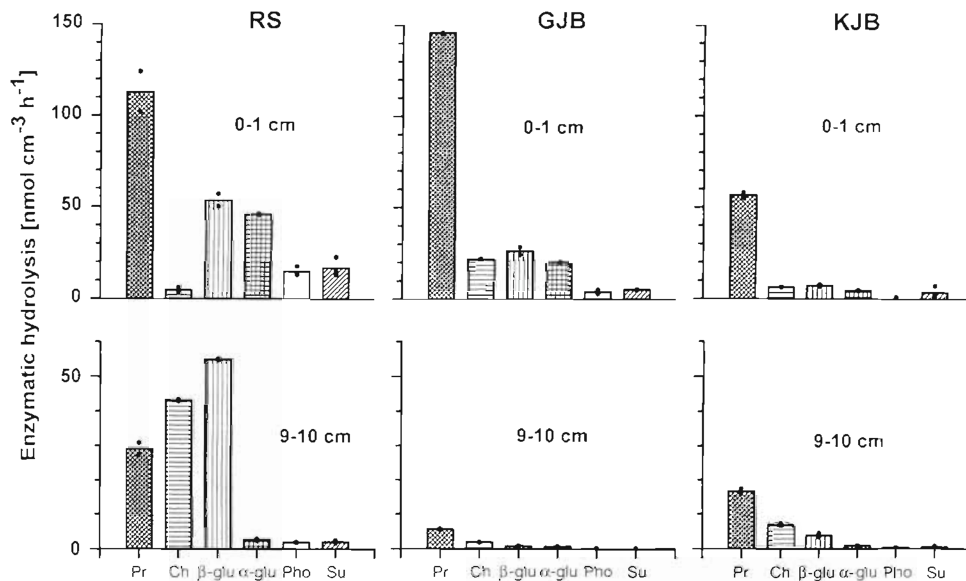

Fig. 5. Spectrum of selected enzymatic activities in the sediment horizons 0–1 cm and 9–10 cm in Rassower Strom, Großer Jasmunder Bodden and Kleiner Jasmunder Bodden sampled in June 1995. Pr: proteolytic activity, Ch: chitinolytic activity, β-glu: β-glucosidase activity, α-glu: α-glucosidase activity, Pho: phosphatase activity, Su: sulfatase activity

about storage effects refers to water samples or individual organisms (e.g. Chrost & Velimirow 1991, Ohman 1996); however, almost nothing is known about methods appropriate to the preservation of sediments for later enzymatic analyses.

As shown, enzyme activities are strongly affected by mode and duration of storage. Highest variations occurred during the initial period of storage. This applies especially to samples frozen at −20°C and freeze-dried sediments. It is well known that freezing and thawing processes alter the physical and chemical structure of sediments as well as the composition of pore water. The formation of ice crystals leads to mechanical damage to organisms causing the release of cell constituents including intracellular enzymes. The critical step of freezing is also required if samples are stored by freeze-drying. Chrost & Velimirov (1991) concluded from their investigations that water samples frozen at −20°C cannot be used for later estimation of enzyme activity. However, Christian & Karl (1995) stated that preservation of sea water by freezing is feasible for later enzyme analyses. Several authors found that enzymes are still active at −20°C (e.g. Scopes 1994 cited in Ohman 1996), thus continuously altering quality and quantity of organic matter.

Best results in respect to sample storage were obtained by refrigeration of sediments at 2°C. Generally, the pool of hydrolytic enzymes and the spectrum of selected enzymes investigated revealed lowest variations as compared to preservation by freezing and freeze-drying. Comparable results were reported by Federle & White (1982), who found a decrease in lipid phosphate but no change in fatty acids in refrigerated sediments. In frozen sediments, however, a drastic decline in lipid phosphate and a significant decrease in many fatty acids was measured. If samples cannot be analysed immediately after sampling, which is preferable, as an acceptable compromise, it is suggested that the sediments should be stored refrigerated for a few days prior to analysis of enzyme activities. It has to be considered, however, that different types of sediments with different benthic colonization may react differently to preservation.

The study showed that the Bodden is characterized by pronounced gradients of chemical and biological parameters. Salinity and Secchi depth decreased, whereas seston and organic matter content, nutrient concentrations, microbial biomass and enzyme activities generally increased from the outer to the inner parts following increasing eutrophication. Corresponding observations were reported by Dahlke (1990) and Dahlke & Hübel (1994) for the Nordrügensche Bodden, and by Nausch & Schlungbaum (1991) for the Darß-Zingster Bodden (western part of the Baltic coast of Mecklenburg-Vorpommern). The Bodden may be regarded as a model system for the study of interactions between chemical and biological parameters along gradients of salinity and eutrophication.

By analysing various chemical and biological parameters, characteristic patterns of interrelationships in sediments with different levels of eutrophication became obvious (Fig. 6). Sandy sediments from the outer part of the Nordrügensche Bodden (Libben), where water exchange with the Baltic Sea is strong, were characterized by low organic matter content together with low microbial biomass and decomposition potential. In muddy sand sediments of the central parts of the Bodden (Rassower Strom, Breetzer and Breeger Bodden), microbial biomass and degradation activities increased in parallel with the organic carbon content. Muddy sediments from the Großer and Kleiner Jasmunder Bodden revealed a further increase in microbial biomass, decomposition potential and organic carbon. However, the pronounced increase in organic carbon was not paralleled by corresponding increases in microbial biomass and decomposition activities.

These observations lead to the conclusion that the development of microbial biomass and decomposition activity in sediments of the outer and central parts of the Nordrügensche Bodden is limited by organic carbon concentrations (range 0.1 to 2.5% C). The organic-rich sediments of the inner parts of the Bodden (range 7.4 to 15.1% C), however, did not support a corresponding increase in microbial biomass and activity. As possible limiting factors, the availability of organic carbon and/or oxygen for microbial metabolism as well as physicochemical characteristics of the muddy sediments (grain size, availability of surfaces for microbial colonization) have to be considered. Corresponding observations were reported by Meyer-Reil (1993), who found that higher microbial biomass was supported in sandy mud than in muddy sediments in the Kiel Bight.

Comparable relationships were found in previous investigations of sediments from the Rassower Strom station. Black et al. (1995) showed for 20 stations with different sediment properties that microbial biomass and activity were directly related to carbon concentrations up to 2%. At the higher concentrations found in muddy sediments, relationships became less significant. In sediments from an intertidal mudflut, Mayer (1989) found no close relationship between proteolytic activities and either microbial number or biochemical compounds (protein, chlorophyll, pheophytin). In the water column of marine and freshwater environments, however, interrelationships between bacterial biomass, secondary production and enzymatic activity have been shown in several studies (e.g. Karner et al. 1992, Rath et al. 1993). In investigations by Nedoma et al. (1994) carried out in different freshwater environments, activities of N-acetyl-glucosaminidases

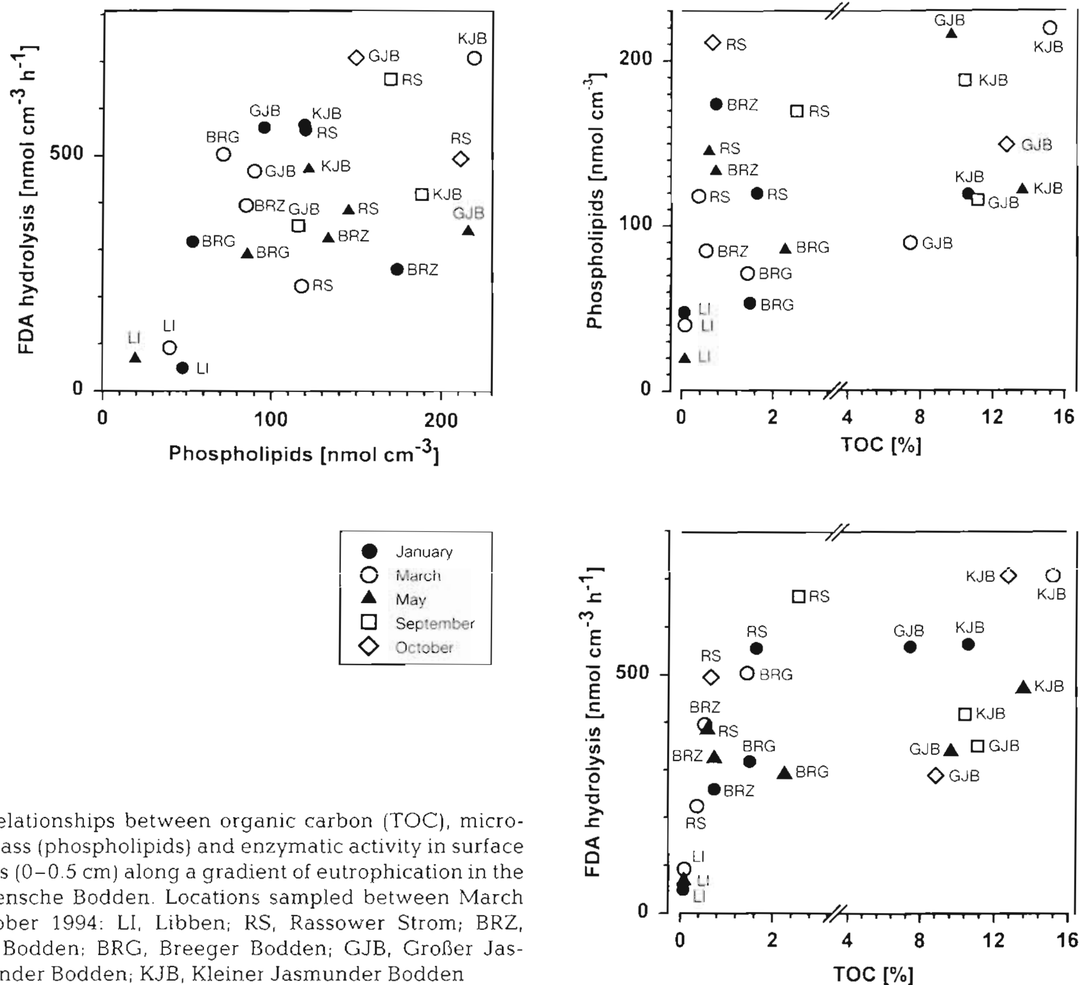

Fig. 6. Relationships between organic carbon (TOC), microbial biomass (phospholipids) and enzymatic activity in surface sediments (0–0.5 cm) along a gradient of eutrophication in the Nordrügensche Bodden. Locations sampled between March and October 1994: LI, Libben; RS, Rassower Strom; BRZ, Breetzer Bodden; BRG, Breeger Bodden; GJB, Großer Jasmunder Bodden; KJB, Kleiner Jasmunder Bodden

were closely correlated with microbial parameters (number of active bacteria, N-acetylglucosamine incorporation). Differences in relationships between enzymatic activities and microbial parameters reported in the literature may be explained by the specific properties of the individual environments investigated.

Studies relating measurements of enzymatic activities to eutrophication are scarce. In the supralittoral zone of the coast of Japan, Kondrat'eva & Pavlyushina (1991) reported highest activities in areas with high anthropogenic loads near ports, fish and wood industry. Kuhbier et al. (1995) characterized different eutrophicated zones (industrial inputs) of a stream by determining activity of esterases and number of saprophytes, indicating the degree of organic pollution caused by diffusive and distinct inputs of organic material. Esterase activity and number of saprophytes were significantly correlated. Along a trophic gradient in the northern Adriatic Sea, Karner et al. (1992) observed positive trends in bacterial secondary production and leucine-aminopeptidase activity in the water column while α-glucosidase did not exhibit such a clear trend. In water samples taken at different trophic sites along the Atlantic Barrier Reef off Belize (Central America) Rath et al. (1993) also measured distinct differences in bacterial biomass and production as well as in the activity of certain enzymes: α- and β-glucosidase increased from the oligotrophic to the eutrophic sites while leucine-aminopeptidase was inversely correlated with the trophic status of the environment. Relationships between microbial parameters and the degree of eutrophication correspond to findings of the present study, where organic carbon, microbial biomass and enzymatic activities in surface sediments generally increased along the gradient of eutrophication from the outer to the inner parts of the Nordrügensche Bodden.

In very few studies have the activities of specific hydrolytic enzymes been followed as a reflection of the composition and degradability of inorganic and organic material altered by biological processes. Sin-

sabaugh & Linkins (1990) noted that the activities of carbohydrate and lignin-degrading enzymes measured on benthic particulate organic matter from a boreal river were significantly correlated with concentrations of carbohydrates and lignin. Frølund et al. (1995) measured esterase activity and chemical composition of activated sludge-flocs in treatment plants. The authors found that esterase activity in the sludge increased with an increased content of humic compounds. Esterase activity and protein concentration were not correlated. Variations of specific enzymes present in sludge-flocs at different waste-water treatment plants were explained by differences in the substrate composition (Nybroe et al. 1992). From a limited number of investigations in marine and limnic environments it can be concluded that spatial and temporal variations of enzymatic activities may reflect changes in nutrient supply (Chrost & Rai 1993, Boetius & Lochte 1994, Vetter & Deming 1994) and/or changes in the composition of the microbial population (Martinez et al. 1996).

The ratio of β-glucosidase to α-glucosidase was used as an indicator of the quality of organic material (Herndl 1992); a low ratio points to easily degradable material, a high ratio to refractory material. Nedoma et al. (1994) measured the activity of N-acetylglucosaminidase and β-glucosidase in various freshwater environments. The authors related differences in the ratios of both enzymes to the availability of substrates. Boetius (1995) assumed that differences in the spectrum of hydrolytic enzymes measured in different environments (deep-sea sediments, intertidal sediments and fjord water) were caused by different availabilities of organic substrates.

The present study reveals that at the relatively unpolluted location of the outer part of the Nordrügensche Bodden, protein decomposing enzymes prevailed at the sediment surface; in deeper sediments the activities of carbohydrate decomposing enzymes (β-glucosidases, N-acetylglucosaminidases) dominated. This certainly points to a shift in the spectrum of organic compounds available for microbial metabolism. It may be expected that the largest variety of decomposable organic compounds exists at the sediment surface. This situation is reflected by the broad spectrum of hydrolytic enzymes. With increasing sediment depth, quantity and quality of decomposable organic material is reduced; the material becomes more and more resistant to microbial attack. As shown, the decomposition of structural polysaccharides dominated in subsurface horizons. In parallel, the ratio of β-glucosidases to α-glucosidases drastically increased as an indication of reduction in the quality of decomposable organic material (Herndl 1992). At the polluted locations in the inner parts of the Bodden, proteolytic enzymes were even more important as compared to the less-polluted location in the outer part of the Bodden. With increasing sediment depth, enzyme activities were generally greatly reduced. However, shifts in the spectrum of enzyme activities were far less pronounced. Thus, although the decomposable material was reduced in quantity, it did not change much in quality with increasing sediment depth. It should be considered that, in contrast to the sandy sediments, anaerobic conditions prevailed in these muddy sediments.

Variations in the activities of hydrolytic enzymes reflect variations in the composition and degradability of inorganic and organic material. However, the relevant processes are only poorly understood. Experiments have shown that enzymatic activities are stimulated as a response to nutrient enrichment in sediments (Köster et al. 1991, Boetius & Lochte 1994). The immediate response implies that microorganisms play a dominant role in the enzymatic decomposition of substrates (Meyer-Reil & Köster 1992). This may be by stimulation of enzymes already present or by production of new enzymes.

Not very much is known about the origin, location and fate of enzymes in sediments. The pool of enzymes in sediments comprises enzymes of different origin: those secreted by cells and those liberated by the lysis of cells. What is the contribution of each enzyme 'group' to the hydrolysis of substrates? It is well known that the bulk of enzymatic activity in sediments is bound to particles or cell surfaces; the activity of free dissolved enzymes in the pore water is usually very low (Boon 1989, Meyer-Reil 1991). As far as cell-associated enzymes are concerned, one precondition for organic matter decomposition is the colonization of the particles by microbes. For the hydrolysis processes, a close connection between microbes and substrates is necessary. However, not very much is known about the fate of the enzymes, especially those which are bound to particles or entrapped in the organic matrix of sediment particles. As shown by Burns (1978), enzymes liberated from cells may retain their activities by adsorption to clay particles and humic acids. However, the question arises: how long do these enzymes retain their activities?

Only if enzyme activities decrease with decreasing substrate concentrations can a direct relationship between concentration and decomposition of substrates be expected. Measurements of enzyme activities by means of fluorogenic model substrates are relatively easy to perform. However, the information gained is difficult to interpret. It has to be considered that the spectrum of hydrolytic enzymes measured represents an arbitrary choice of the pool of enzymes naturally present. Further progress can only be

expected if along with enzyme analysis their natural substrates can be identified. It might also be useful to characterize the prevailing physiological groups of microorganisms responsible for the substrate decomposition. The difficulties in determining both natural substrate concentrations and active microorganisms are well known.

Acknowledgements. Part of the investigations were financed by the German Environmental Protection Agency (Umweltbundesamt, UBA; 'Ökosystemanalyse Kleiner Jasmunder Bodden'; grant 102 04 397). The authors are especially grateful to Tina Brüggmann, Mechthild Gau, Sandra Kläber and Ingrid Kreuzer for their excellent technical assistance.

LITERATURE CITED

Barnett PRO, Watson J, Conelly D (1984) A multiple corer for taking virtually undisturbed samples from shelf, bathyal and abyssal sediments. Oceanol Acta 7:339–409

Black HJ, Köster M, Meyer-Reil LA, Andres D (1995) Dokumentation von Benthosgemeinschaften in Flachwasserbereichen der Nordrügenschen Boddengewässer. Abschlußbericht, Deutsche Bundesstiftung Umwelt, Osnabrück

Boetius A (1995) Microbial hydrolytic enzyme activities in deep-sea sediments. Helgol Meeresunters 49:177–187

Boetius A, Lochte K (1994) Regulation of microbial enzymatic degradation of organic matter in deep-sea sediments. Mar Ecol Prog Ser 104:299–307

Boon PJ (1989) Organic matter degradation and nutrient regeneration in Australian freshwaters: I. Methods for exoenzyme assays in turbid aquatic environments. Arch Hydrobiol 115:339–359

Burns RG (1978) Enzyme activity in soil: some theoretical and practical considerations. In: Burns RG (ed) Soil enzymes. Academic Press, London, p 295–340

Christian JR, Karl DM (1995) Measuring bacterial ectoenzyme activities in marine waters using mercuric chloride as a preservative and control. Mar Ecol Prog Ser 123: 217–224

Chrost RJ, Rai H (1993) Ectoenzyme activity and bacterial secondary production in nutrient-impoverished and nutrient-enriched freshwater mesocosms. Microb Ecol 25: 131–150

Chrost RJ, Velimirov B (1991) Measurement of enzyme kinetics in water samples: effect of freezing and soluble stabilizer. Mar Ecol Prog Ser 70:93–100

Dahlke S (1990) Denitrification in sediments of the North Rugian estuaries, preliminary communication. Limnologica 20:145–148

Dahlke S, Hübel H (1994) Stoff- und Energietransfer in den flachen Küstengewässern der deutschen Ost- und Nordsee und Entwicklung von dazugehörenden Meßtechnologien und Systemen (Rügensche Boddengewässer/SWH-Wattenmeer). Abschlußbericht BMBF, Warnemünde

Dahlke S, Hübel H (1996) Der Kleine Jasmunder Bodden: Entwicklung eines hypertrophen Gewässers in Vergangenheit, Gegenwart und Zukunft. BODDEN 3:83–96

Federle TW, White DC (1982) Preservation of estuarine sediments for lipid analysis of biomass and community structure of microbiota. Appl Environ Microbiol 44:1166–1169

Findlay RH, King GM, Watling L (1989) Efficacy of phospholipid analysis in determining microbial biomass in sediments. Appl Environ Microbiol 55:2888–2893

Frølund B, Griebe T, Nielsen PH (1995) Enzymatic activity in the activated-sludge floc matrix. Appl Microbiol Biotechnol 43:755–761

Grasshoff K, Ehrhardt M, Kremling K (1983) Methods of seawater analysis. Verlag Chemie, Weinheim

HELCOM (1988) Guidelines for the Baltic Sea Monitoring Programme for the third stage, Part 27D. Baltic Marine Environmental Protection Commission, Helsinki

Herndl GJ (1992) Marine snow in the Northern Adriatic Sea: possible causes and consequences for a shallow ecosystem. Mar Microb Food Webs 6:149–172

Hübel H (1969) Die Primärproduktion des Phytoplanktons in Brackgewässern unterschiedlichen Salz- und Nährstoffgehaltes. Limnologica 7:185–190

Hübel H (1984) Die Bindung molekularen Stickstoffs durch autotrophe und heterotrophe Mikroorganismen in Küstengewässern der DDR und einigen Stationen der Ostsee. Habilitationsschrift, University of Greifswald

Karner M, Fuks D, Herndl G (1992) Bacterial activity along a trophic gradient. Microb Ecol 24:243–257

Kondrat'eva LM, Pavlyushina OV (1991) Diversity of littoral microbial community under conditions of anthropogenic pollution. Biol Morya/Mar Biol 3:38–45

Köster M (1992) Mikrobieller Abbau von organischem Material an Grenzzonen—erläutert an Beispielen von Sedimenten der Nordsee und des Europäischen Nordmeeres. Ber Sonderforschungsbereich 313, 35, Kiel University

Köster M, Charfreitag O, Meyer-Reil LA (1991) Availability of nutrients to a deep-sea benthic microbial community: results from a ship-board experiment. Kiel Meeresforsch Sonderh 8:127–133

Kuhbier S, Lorch HJ, Ottow JCG (1995) Charakterisierung unterschiedlicher belasteter Zonen eines Fließgewässers mit Hilfe der Enzymaktivität der Esterasen. Anwendung enzymatischer Tests im aquatischen Bereich. Bundesanstalt für Gewässerkunde, Berlin

Lampe R (1994) Die vorpommerschen Boddengewässer—Hydrographie, Bodenablagerungen und Küstendynamik. Die Küste 56:25–49

Martinez J, Smith DC, Steward GF, Azam F (1996) Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. Aquat Microb Ecol 10:223–230

Mayer LM (1989) Extracellular proteolytic enzyme activity in sediments of an intertidal mudflat. Limnol Oceanogr 34: 973–981

Meyer-Reil LA (1990) Microorganisms in marine sediments: considerations concerning activity measurements. Arch Hydrobiol Beih Ergeb Limnol 34:1–6

Meyer-Reil LA (1991) Ecological aspects of enzymatic activity in marine sediments. In: Chróst RJ (ed) Microbial enzymes in aquatic environments. Springer-Verlag, New York, p 84–95

Meyer-Reil LA (1993) Mikrobielle Besiedlung und Produktion. In: Meyer-Reil LA, Köster M (eds) Mikrobiologie des Meeresbodens. Gustav Fischer Verlag, Jena, p 38–81

Meyer-Reil LA, Köster M (1992) Microbial life in pelagic sediments: the impact of environmental parameters on the enzymatic degradation of organic material. Mar Ecol Prog Ser 81:65–72

Nausch G, Schlungbaum G (1991) Eutrophication and restoration measures in the Darß-Zingst Bodden chain. Int Rev Ges Hydrobiol 76:451–463

Nedoma J, Vrba J, Hejzlar J, Simek K, Straskrabova V (1994) N-acetylglucosamine dynamics in freshwater environ-

ments: concentration of amino sugars, extracellular enzyme activities, and microbial uptake. Limnol Oceanogr 39: 1088–1100

Nybroe O, Jørgensen PE, Hence M (1992) Enzyme activities in wastewater and activated sludge. Water Res 26: 579–584

Ohman MD (1996) Freezing and storage of copepod samples for the analysis of lipids. Mar Ecol Prog Ser 130:295–298

Rath R, Schiller C, Herndl GJ (1993) Ectoenzymatic activity and bacterial dynamics along a trophic gradient in the Caribbean Sea. Mar Ecol Prog Ser 102:89–96

Sinsabaugh RL, Linkins AE (1990) Enzymic and chemical analysis of particulate organic matter from a boreal river. Freshwat Biol 23:301–309

Verardo DJ, Froelich PN, McIntyre A (1990) Determination of organic carbon and nitrogen in marine sediments using the Carlo-Erba NA-1500 analyzer. Deep Sea Res 37:157–165

Vetter YA, Deming JW (1994) Extracellular enzyme activity in the Arctic Northeast Water polyna. Mar Ecol Prog Ser 114:23–34

This article was submitted to the editor

Manuscript first received: November 27, 1996
Revised version accepted: April 24, 1997