Vol. 108: 185–192, 1994 | MARINE ECOLOGY PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published May 12

Storm-stimulated enzymatic decomposition of organic matter in benthic/pelagic coastal mesocosms

Ryszard J. Chróst[1], Bo Riemann[2]

[1]Microbial Ecology Department, University of Warsaw, Ul. Karowa 18, PL-00-927 Warsaw, Poland
[2]International Agency for ^{14}C Determination, Water Quality Institute, Agern Allé 11, DK-2970 Hørsholm, Denmark

ABSTRACT: Sediment resuspension caused by simulated storms led to a dramatic short-term increase of aminopeptidase (AMPase) and β-glucosidase (GLCase) activities and bacterial secondary production in coastal waters during a mesocosm experiment in Knebel Vig, Denmark. Perturbation of sediments strongly affected the kinetic parameters of AMPase and GLCase in the water column of the enclosures. V_{max} values of AMPase and GLCase determined 4 h after a simulated storm event were 24‰ and 43% higher, respectively, than those assayed during the calm period. Sediment resuspension in enclosures ('storm enclosures') had the most pronounced effect on GLCase apparent K_m (Michaelis constant) values, which increased 2.6 times (163%) after sediment resuspension. AMPase K_m values were approximately 1.6 times higher after sediment resuspension than before sediment resuspension. Changes in of the V_{max} and K_m values of GLCase and AMPase in storm enclosures were accompanied by longer turnover times for enzymatic hydrolysis of substrates. GLCase and AMPase after sediment resuspension had noticeably higher specific activities than before the storm event. GLCase and AMPase specific activities produced by an 'average' single bacterium were 2.4 and 2.1 times higher, respectively, after the storm event. Sediment in the water caused a situation in which most GLCase activity (68%) was associated with the particle size fraction >10 µm. AMPase activity after a storm event also shifted towards the >10 µm size fractions. Activities of free AMPase and GLCase dissolved in the water were low and contributed 8% and 14–16%, respectively, to the total activity of these enzymes. Results of simultaneously radiolabeling size-fractionated samples with [^3H]thymidine and [^{14}C]protein hydrolysate showed that the water column of storm enclosures was inhabited by metabolically different bacterial populations before and after sediment resuspension. The response of the suspended microbial assemblages to the storm event was very rapid but short-term; enzymatic activity levels returned to pre-storm levels within 24 h. Our mesocosm experiments demonstrated that sediment resuspension caused by simulated storms had a pronounced stimulatory effect on the rates of microbial enzymatic degradation of organic matter in a coastal ecosystem.

KEY WORDS: Organic matter · Enzymes · Decomposition

INTRODUCTION

It is generally accepted that heterotrophic bacteria are the major component of the aquatic biota which steers the processes of organic matter decomposition and nutrient remineralization in aquatic environments. Bacteria are also primarily users of the dissolved organic matter (DOM) in the water column and sediments. However, most organic matter (both dissolved and particulate) is composed of polymeric compounds which are not directly transportable through bacterial membranes because of their high molecular weight and the large size of the molecules. To be readily utilizable by bacteria, the polymers and high-molecular-weight constituents of organic matter must undergo enzymatic depolymerization and transformation to smaller transportable subunits (Chróst 1990). Depending on the chemical structure of the compound and on the enzymatic action, the final products of enzymatic hydrolysis of organic matter are released into the water as organic

substrates or as ions of inorganic nutrients (N, P, S) that are accessible to bacteria and other aquatic microorganisms (Chróst 1991a, b, 1992). Among aquatic microorganisms, the heterotrophic bacteria are superior producers of a variety of hydrolytic enzymes which associate with the cell surface (ectoenzymes) and perform degradation of organic polymers in close proximity to the bacteria (Ammerman 1991, Billen 1991, Chróst 1991c, Martinez & Azam 1993). Bacterial ectoenzymes not only act on dissolved components but also solubilize the particulate fraction of organic matter (Hoppe et al. 1993). It is now clear that heterotrophic bacteria serve as a primary pathway by which DOM and bound inorganic nutrients are made available to higher trophic levels (Chróst 1993a). As a consequence of their pivotal position in aquatic food webs, bacterial activity, production and processing of organic matter, and their role in inorganic nutrient cycling have been extensively studied in the last decade (Joint & Morris 1982, Azam & Cho 1987, Ducklow & Carlson 1992, Chróst 1993b, Chróst & Rai 1993a, b).

The impact of storms on bacterial activity in marine coastal waters has not yet been intensively studied owing to the unpredictable timing and severity of any given storm as well as the hazards of being on the open water during stormy weather. During periods of high wind and rainfall, rapid mixing of a given body of water coupled with increased resuspension of sediments could alter the rates of bacterial activity and the nutrient flow through benthic/planktonic microbial communities. Previous studies have shown that sediment resuspension in natural coastal systems is an important factor controlling activity of benthic microbial communities (Demers et al. 1987, Riemann & Hoffman 1991, Troussellier et al. 1993). A major goal of the present study was to examine the impact of resuspension of coastal sediments due to storm events on bacterial enzymatic decomposition of organic matter in benthic/pelagic microbial communities. Using coastal mesocosm enclosures, we simulated storm-driven resuspension of sediments but without the lateral transport which normally occurs during a natural storm event. We investigated 2 model ectoenzyme systems (Chróst 1989, 1991c), which are responsible for hydrolysis of predominant organic constituents in the DOM and POM pool, i.e. β-linked polysaccharides and proteins; these were: β-D-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21; GLCase) and leucine aminopeptidase (EC 3.4.1.1; AMPase).

MATERIALS AND METHODS

Mesocosm experiment. Enclosure experiments were carried out in Knebel Vig, Denmark, in July 1992. Knebel Vig is a small coastal embayment on the east side of Kalø Vig, which is part of the Bay of Århus. Knebel Vig has a maximum depth of 16 m and a surface area of 37 km. Four large plastic enclosures (1.5 m in diameter, approximate volume 6500 l, open at the bottom), filled with coastal water, were set up on top of the sediment at 3.6 to 3.8 m depth (Riemann et al. 1988, Nybroe et al. 1992, Bratbak et al. 1992). The enclosures were fixed to a pontoon bridge situated about 100 m from shore. The water in the enclosures was kept circulating by wind-driven mills. The sediment at the site was silty, with no macrophytes or macrofauna. The benthic phototrophic community was dominated by pennate diatoms and cyanobacteria which created a coherent mat. The phytoplankton community was mainly composed of *Prorocentrum minimum* (N. P. Sloth et al. unpubl.).

Two enclosures with resuspended sediment ('storm enclosures') and 2 associated control enclosures were set up. Four days later, the sediment (1 to 2 cm surface layer) in each storm enclosure was resuspended using a propeller (150 × 800 mm) driven by an electric motor at a speed of 25 rpm for 2 h. The propeller was 10 cm from the sediment surface. To estimate the depth of sediment resuspension, 250 g of colored glass beads (2 mm in diameter) were distributed on the sediment surface before resuspension. After the experiment, 10 cores from each storm enclosure were sampled and the depth of sediment resuspension was estimated by retrieving the colored beads. Beads were also distributed in control enclosures, from which 5 sediment cores were analyzed. Water samples were taken daily (10:00 h) with a Ruttner sampler from 1 m depth in the storm and control enclosures and transported to the laboratory within 15 min.

Enzymatic activity. GLCase and AMPase activity were measured fluorometrically (Chróst & Rai 1993a). GLCase activity was measured as an increase in fluorescence as the nonfluorescent substrate 4-methylumbelliferyl-β-D-glucopyranoside (MUF-GLC; Sigma) was hydrolyzed to the fluorescent product, 4-methylumbelliferone (MUF). The amount of MUF-GLC cleaved was equivalent to the amount of highly fluorescent MUF anion. L-leucine-4-methyl-coumarinylamide hydrochloride (Leu-MCA; Fluka) was used for determination of AMPase activity, which was equal to the amount of fluorescent 7-amino-4-methylcoumarin (AMC) produced after Leu-MCA hydrolysis. The fluorometer (Aminco, American Instruments) was calibrated with standard solutions (100 to 1000 nM) of MUF (Sigma) or AMC (Fluka) prepared in particle-free (Whatman GF/F) water samples.

Stock solutions of MUF-GLC and Leu-MCA were prepared to a concentration of 5 mmol l^{-1} in deionized 0.2 µm filtered water and stored at a tempera-

ture of −25 °C. The stock substrate solutions were thawed at room temperature immediately before assay. For all GLCase and AMPase kinetic assays, varying amounts (10 to 500 µl) of substrate solutions were added to triplicate 5 ml water samples, yielding final substrate concentrations in assays of 19.9, 49.5, 98, 238 and 454 µmol l^{-1}. Potential activity of AMPase and GLCase and enzyme activities associated with the different size fractions of seston (0.2–1.0, 1–3, 3–10 and >10 µm), as well as activity of dissolved AMPase and GLCase (<0.2 µm fraction), were measured according to Chróst (1989) at substrate concentration of 454 µmol l^{-1}. Samples at time zero (in which fluorescence was measured immediately after addition of substrate) were treated as blanks. Samples were incubated at *in situ* temperature (18 to 21 °C) for 3 to 4 h (AMPase) or 5 to 6 h (GLCase). GLCase and AMPase activities after incubation of samples were terminated using the deep-freezing method of Chróst & Velimirov (1991).

Different concentrations of substrates were added to the samples to establish enzyme-substrate saturation levels and to allow calculation of the Michaelis-Menten kinetic parameters (V_{max} and K_m) of GLCase and AMPase (Chróst 1990). The kinetic parameters were calculated by non-linear regression analysis of a direct plot of reaction velocity versus substrate concentration using the IBM PC computer program 'Enzfitter' (Elsevier-Biosoft, Cambridge, UK) to determine the best fit of the rectangular hyperbola (Leatherbarrow 1987). Turnover time (T_t; hours) for GLCase and AMPase hydrolysis was calculated from K_m and V_{max} as $T_t = K_m/V_{max}$. GLCase and AMPase specific activities were calculated by dividing V_{max} values by the number of bacteria, determined using an epifluorescence microscope in samples stained with acridine orange.

Size-fractionated [^{3}H]thymidine incorporation and uptake of [^{14}C]protein hydrolysate. Bacterial incorporation of [^{3}H]thymidine (TdR, specific activity 20 Ci $mmol^{-1}$; New England Nuclear) and the uptake of [U-^{14}C]protein hydrolysate (Amersham, spec. act. 56 mCi mg-at.$^{-1}$ C) were determined in 10 ml water samples supplemented with 25 nmol l^{-1} or 0.5 µg-at. C l^{-1} (final concentrations) of radiolabeled TdR or protein hydrolysate, respectively. Samples were incubated for 30 min and 1 h for TdR and protein hydrolysate, respectively, and fixed with 3% formalin. TdR-labeled material was precipitated (20 to 30 min) with ice-cold TCA (5% final conc.). Radiolabeled water samples were fractionated by filtration through 0.2, 1.0, 3.0 and 10.0 µm pore size Nuclepore filters. Radioactivity of filters was determined using the liquid scintillation counting method in a Rack-Beta counter (LKB Instruments, Inc., Piscataway, NJ, USA).

RESULTS

Enzymatic activity

Excluding the 24 h interval after resuspension of sediments in storm mesocosms (July 18), GLCase and AMPase activities of both control and manipulated storm enclosures were similar (Fig. 1). GLCase and AMPase activity patterns coincided. AMPase activities of storm and control mesocosms were almost identical (average 453 ± 57 and 452 ± 55 nmol l^{-1} h^{-1} in storm and control enclosures, respectively) and varied from 335 to 516 nmol l^{-1} h^{-1}. Activity of GLCase in the enclosures fluctuated between 26 and 39 nmol l^{-1} h^{-1} (average 32 ± 6 and 31 ± 7 nmol l^{-1} h^{-1} in the storm and control enclosures, respectively).

Sediment resuspension in the storm mesocosms caused a dramatic short-term increase in AMPase and GLCase activities (Fig. 1). Sediment resuspension strongly affected the kinetic parameters of both enzymes studied (Table 1). V_{max} of AMPase and GLCase determined 4 h after simulation of the storm event increased significantly and were 24% and 43% higher, respectively, than those assayed 1 h before sediment disturbance. Sediment resuspension in storm mesocosms had the most pronounced effect on the GLCase apparent K_m values, which increased 2.6 times (163%), i.e. from 22 ± 0.4 µmol l^{-1} before to 58 ± 3 µmol l^{-1} after sediment resuspension. AMPase apparent K_m values increased from 32 ± 1 µmol l^{-1} before to 52 ± 2 µmol l^{-1} after the artificial storm event, i.e. 1.6 times. Changes in the V_{max} and K_m values of

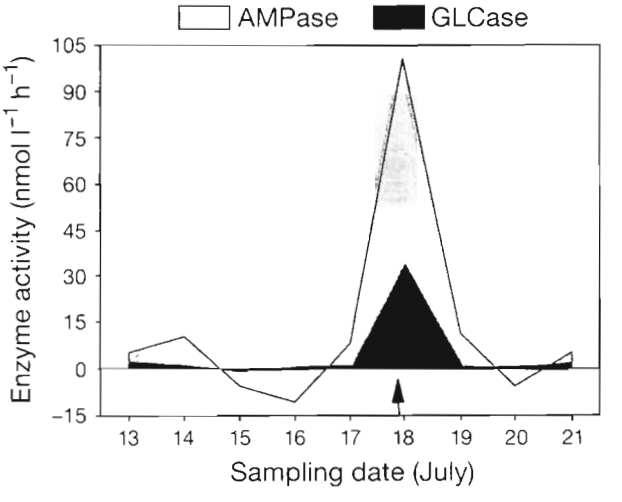

Fig. 1 Differences of aminopeptidase (AMPase) and β-glucosidase (GLCase) activities between storm and control enclosures during the mesocosm experiment in Knebel Vig, Denmark. Enzyme activity = (storm) − (control). Arrow indicates time of sediment resuspension in storm enclosures

Table 1. β-Glucosidase and aminopeptidase kinetics in the storm and control enclosures. SD in parentheses

Parameter	β-Glucosidase			Aminopeptidase		
	Storm		Control[b]	Storm		Control[b]
	Calm[a]	Storm[b]		Calm[a]	Storm[b]	
V_{max} (nmol l^{-1} h^{-1})	28 (± 4)	117 (± 8)	41 (± 3)	278 (± 9)	990 (± 27)	463 (± 13)
Apparent K_m (μmol l^{-1})	22 (± 0.4)	58 (± 2.8)	21 (± 1.3)	33 (± 1)	52 (± 2)	31 (± 3)
Turnover time (h)	458 (± 13)	840 (± 18)	488 (± 22)	70 (± 4)	89 (± 7)	67 (± 7)
Number of bacteria (10^9 cells l^{-1})	0.616	1.043	0.788	0.616	1.043	0.788
Specific activity[c] (amol $cell^{-1}$ h^{-1})	46 (± 5)	112 (± 11)	52 (± 8)	451 (± 23)	950 (± 34)	587 (± 43)

[a]Samples were taken during the calm period, 1 h before sediment resuspension
[b]Samples were taken 4 h after sediment resuspension
[c]Specific enzyme activity calculated per bacterium

GLCase and AMPase in the storm mesocosms were accompanied by longer turnover times for enzymatic hydrolysis of substrates. The turnover times for polysaccharide hydrolysis by GLCase and protein degradation by AMPase were, respectively, 1.8 and 1.3 times longer after resuspension of the sediment (Table 1). GLCase and AMPase produced in the storm mesocosms 4 h after sediment resuspension also had noticeably higher specific activities than the same enzymes synthesized by bacteria before the storm event (Table 1). GLCase and AMPase specific activities produced by an 'average' single bacterium were 2.4 and 2.1 times higher 4 h after sediment resuspension than 1 h before it.

Changes in enzyme kinetics studied after sediment resuspension in the storm mesocosms were connected with a shift in the association of GLCase and AMPase with different partocle sizes (Fig. 2). Before resuspension of the sediment, the bulk of GLCase (62 to 73%) and AMPase (85 to 91%) activity in the storm and control mesocosms was associated with particulates <10 μm. Resuspension of the sediment in the water caused the majority of GLCase activity (68%) to be associated with the fraction >10 μm. AMPase activity after the storm event also moved towards larger particle sizes; however, this change was not so evident as for GLCase. There was some variation in the GLCase and AMPase association with particulate size fractions <10 μm, but both enzymes displayed a similarly large change (79 to 80%) in the extent to which their activities were associated with the size fraction >10 μm (Fig. 2). Activities of free enzymes dissolved in the water, i.e. associated with the sample size fraction <0.2 μm, were insignificant and contributed 8% and 14–16% to the total activity of GLCase and AMPase, respectively. Changes in the activity of free enzymes after resuspension of the sediments were also small (GLCase 3%, AMPase 13%).

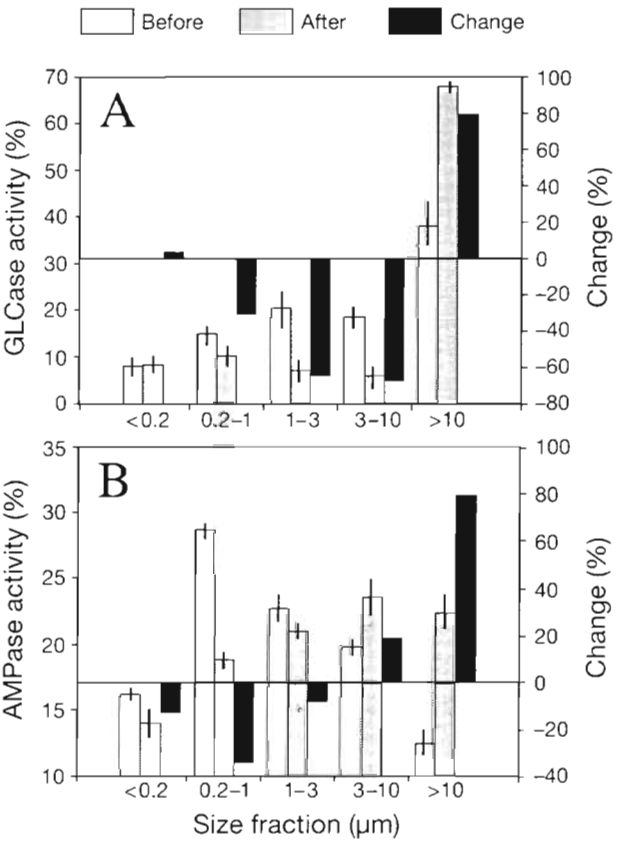

Fig. 2. Percentage contribution, and change, of (A) β-glucosidase (GLCase) and (B) aminopeptidase (AMPase) activities associated with different size fractions in water samples from storm enclosures sampled 1 h before and 4 h after sediment resuspension. Thin black vertical bars indicate standard deviation of the mean of triplicate samples

TdR incorporation and uptake of protein hydrolysate

Results of simultaneously radiolabeling size fractionated seston with [^{3}H]thymidine and [^{14}C]protein hydrolysate showed that the water column of the storm

mesocosms was inhabited by metabolically different bacterial populations before and after sediment resuspension. Resuspension of the sediment led to 79% and 57% higher uptake of protein hydrolysate by bacteria associated with the particle size fractions 3–10 µm and >10 µm respectively (Fig. 3B). In contrast to the uptake of protein hydrolysate, after resuspension of sediments in storm enclosures TdR incorporation by bacteria was higher (19%) in the smallest particle size fraction (0.2–1.0 µm) (Fig. 3A). In these enclosures the incorporation of TdR by microorganisms of size fractions 3–10 µm and >10 µm was 16% and 41% lower, respectively, than before the storm event.

DISCUSSION

Several reports note that resuspended sediments are an intermittent source of inorganic nutrients and organic matter which may affect autotrophic and heterotrophic production of the marine ecosystem (Rowe et al. 1975, Aller 1980, Callender & Hammond 1982, Hopkinson 1987, Riemann & Hoffmann 1991). In 2 l laboratory mesocosms, Wainright (1987) showed that resuspension of coastal sediments stimulated bacterial production and increased their biovolume within 32 h. He suggested that dissolved substances are released from the sediments into the water column, where they will presumably benefit both free and attached microbes.

Our large-volume mesocosm field experiments, which simulated storm conditions leading to resuspension of the sediment, clearly demonstrated rapid stimulation of microbial enzymatic degradation of polysaccharides (by GLCase) and proteins (by AMPase) in the water column. The response of the suspended microbial assemblages to the storm event was very rapid but short-term. Enzymatic activity returned to pre-storm levels within 24 h (Fig. 1). Resuspension of sediments in storm mesocosms stimulated activity of GLCase more strongly than AMPase (Fig. 1, Table 1). Very similar results have been described for *in situ* studies of Lake Arlington (Texas, USA), where bacterial heterotrophic activity increased immediately after storms. Hubbard & Chrzanowski (1986) reported that storm conditions resulted in increased uptake rates and decreased turnover times of glutamate and acetate in the lake.

Sediment resuspension led to high flux of polysaccharides and proteins (i.e. enzyme substrates) into the water column of storm mesocosms. This was indicated by the significant increase in the apparent K_m values of GLCase and AMPase (Table 1). Resuspension of sediments in the storm mesocosms resulted in higher enzymatic activities of seston size fractions larger than 3 µm (Fig. 2). Moreover, GLCase and AMPase after the storm event had higher specific activities (i.e. enzyme activity calculated per bacterium) than under calm conditions (Table 1). We suggest that the higher V_{max} values and specific activity of the enzymes produced after sediment resuspension was mainly due to bacteria which were attached to the sediment particles or free-living bacteria larger than 3 µm. In accordance with this hypothesis, Chróst (1990, 1993a) reported higher specific activities of GLCase and AMPase produced by attached bacteria than by free-living bacteria.

The elevated V_{max} and higher specific activity of AMPase and GLCase in storm mesocosms, however, did not counterbalance the turnover times of the GLCase and AMPase substrates. Presumably, a high flux in polymeric substrates enlarged the pool of polysaccharides and proteins in the entire water column after resuspension of sediments in storm mesocosms, resulting in their longer turnover times (Table 1). Comparison of the changes in GLCase and AMPase kinetic parameters after simulated storm event shows that GLCase was more drastically affected by the sediment resuspension than AMPase. Significant changes in GLCase potential activity (Fig. 1) and its kinetics (V_{max} and apparent K_m) suggest that sediments of Knebel Vig were composed primarily of polysaccharides rather than proteins.

Radiolabeling of various seston size fractions indicated that sediment resuspension in the water column of the storm mesocosms also resulted in metabolic change of the microbial assemblage. This change could have resulted from rapid attachment of free-living bacteria to suspended particles and/or resuspension of sediment-attached bacteria (Fig. 3). The most pronounced changes were in thymidine incorporation and the uptake of protein hydrolysate occurred in the size fraction >3 µm. Our results from the storm mesocosms suggest that bacteria associated with particles 3–10 µm and >10 µm divided less frequently than free-living microorganisms. It would appear that sediment resuspension benefited the production of the free-living bacteria rather than that of the attached cells (Fig. 3). Protein hydrolysate uptake data, however, show that attachment of bacteria to the sediment particles resulted in higher uptake rates of amino acids and short peptides. This would mean that the free-living bacteria, when attached to the particles, had lower division rates (as indicated by lower thymidine incorporation) but were metabolically very active and grew, increasing their cell-size. This assumption is consistent with the findings of several authors (Goulder 1977, Harvey & Young 1980, Kirchman & Mitchell 1982, Palumbo et al. 1984).

Our mesocosm experiments demonstrated that resuspension of sediments caused by simulated storms

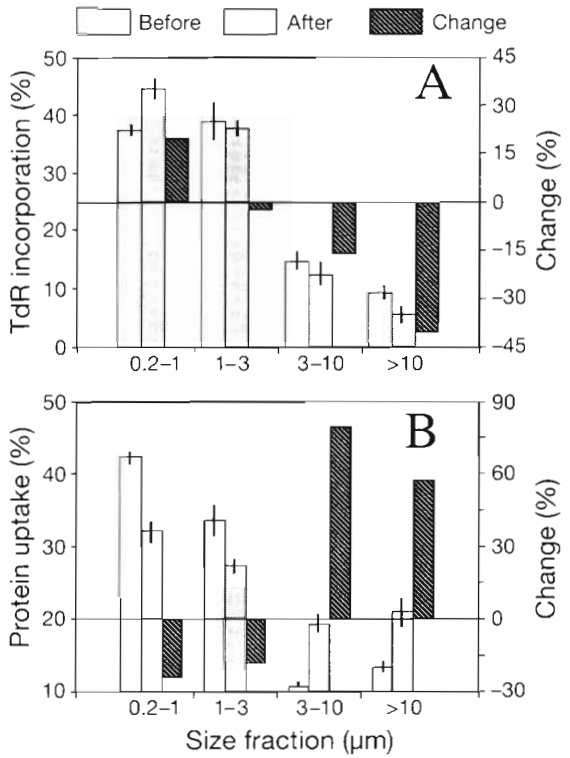

Fig. 3. Percentage contribution, and change, of (A) [^{3}H]thymidine (TdR) incorporation and (B) uptake of [^{14}C]protein hydrolysate by bacteria associated with different size fractions of water samples from storm enclosures sampled 1 h before and 4 h after sediment resuspension. Thin black vertical bars indicate standard deviation of the mean of triplicate samples

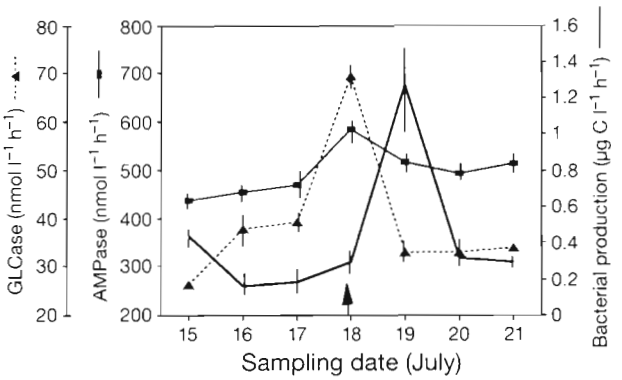

Fig. 4. Aminopeptidase (AMPase) and β-glucosidase (GLCase) activities and rates of bacterial secondary production in storm enclosures during the mesocosm experiment in Knebel Vig, Denmark. Arrow indicates time of sediment resuspension in enclosures. Vertical bars indicate standard deviation of the mean

had a pronounced short-term stimulatory effect on microbial enzymatic degradation of organic matter and subsequently enhanced the rates of bacterial secondary production in the coastal ecosystem (Fig. 4). Perturbation of the surface layer of coastal sediments by rapid water movement due to stormy weather, strong wind and waves (Troussellier et al. 1993) is usually linked directly or indirectly to an increase in concentrations of dissolved organic and inorganic nutrients and to the resuspension of particulate organic matter (POM) (Demers et al. 1987). These processes influence environmental and nutritional conditions for metabolism and production of heterotrophic bacteria in coastal ecosystems (Kirchman et al. 1989, Ducklow & Carlson 1992). Results of our experiments indicated that resuspension of sediments also induced rapid colonisation of POM by attached bacteria, which produced enzymes (AMPase and GLCase; Fig. 2) that degraded proteins and polysaccharides. Combined processes of enzymatic depolymerisation of organic matter and subsequent release and utilization of directly transportable substrates (Hoppe et al. 1988) can result in higher rates of bacterial secondary production (Chróst 1989, 1993a, Chróst & Rai 1993a, b, Hoppe et al. 1993). It was interesting that bacterial assemblages reacted very rapidly to the influx of organic matter. Almost immediately, they produced enzymes with high specific activity which were detected within 4 h after resuspension of sediments in storm enclosures (Table 1). Their response in secondary production, however, was slower, showing a delay of about 24 h (Fig. 4). N. P. Sloth et al. (unpubl.) report that before sediment resuspension (July 15 to 17) in the storm enclosures, bacterial production (BP) did not vary noticeably between the different mesocosms. Resuspension of sediments on July 18, however, induced a spectacular increase in rates of bacterial secondary production 24 h later. On July 19, BP rates in storm enclosures approached 1.26 ± 0.45 µg C l^{-1} h^{-1} and were 2.5 times higher than those found in control enclosures (0.50 ± 0.05 µg C l^{-1} h^{-1}). Two days after sediment resuspension (July 20) BP in storm mesocosms rapidly decreased and did not differ substantially from BP in control enclosures (Fig. 4).

We postulate that rapid changes in microbial enzymatic processing of organic matter and bacterial secondary production may be very important factors influencing the overall rates of regeneration of inorganic nutrients and carbon dynamics in storm-perturbated coastal ecosystems. If aquatic bacterial community structure and abundance are regulated by a balance between carrying capacity of the habitat (bottom-up control) and predation (top-down control), then storm events may pulse the system with forms of substrates that will allow a brief surge in microbial activity. Thus the balance of factors limiting bacterial populations may be temporarily disturbed, resulting in an increase of bacterial activity and production.

Acknowledgements. This study was supported by the EC MAST I program, Contract No. 0020, and by the Danish Natural Science Research Council, Danish Technical Research Council (J. No. 11-8630 and 16-48-6), and National Agency of Environmental Protection (HAV90 contract 1.23). We thank 3 anonymous reviewers for their critical comments which helped us to improve the final version of the manuscript.

LITERATURE CITED

Aller, R. C. (1980). Quantifying solute distributions in the bioturbated zone of marine sediments by defining an average microenvironment. Geochim. Cosmochim. Acta 44: 1955–1965

Ammerman, J. W. (1991). Role of ecto-phosphohydrolases in phosphorus regeneration in estuarine and coastal ecosystems. In: Chróst, R. J. (ed.) Microbial enzymes in aquatic environments. Springer-Verlag, New York, p. 165–186

Azam, F., Cho, B. C. (1987). Bacterial utilization of organic matter in the sea. In: Fletcher, M., Gray, T. R. G., Jones, J. G. (eds.) Ecology of microbial communities. Cambridge University Press, Cambridge, p. 261–281

Billen, G. (1991). Protein degradation in aquatic environments. In: Chróst, R. J. (ed.) Microbial enzymes in aquatic environments. Springer-Verlag, New York, p. 123–143

Bratbak, G., Heldal, M., Thingstad, T F., Riemann, B., Haslund, O. H. (1992). Incorporation of viruses into the budget of microbial C-transfer. A first approach. Mar. Ecol. Prog. Ser. 83: 273–280

Callender, E., Hammond, D. (1982). Nutrient exchange across the sediment-water interface in the Potomac River estuary. Estuar. coast. Shelf Sci. 15: 395–413

Chróst, R. J. (1989). Characterization and significance of β-glucosidase activity in lake water. Limnol. Oceanogr 34: 660–672

Chróst, R. J. (1990). Microbial ectoenzymes in aquatic environments. In: Overbeck, J., Chróst, R. J. (eds.) Aquatic microbial ecology: biochemical and molecular approaches. Springer-Verlag, New York, p. 47–78

Chróst, R. J. (ed.) (1991a). Microbial enzymes in aquatic environments. Springer-Verlag, New York

Chróst, R. J. (1991b). Ectoenzymes in aquatic environments: microbial strategy for substrate supply. Verh. int. Ver. theor. angew. Limnol. 24: 2597–2600

Chróst, R. J. (1991c) Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. In: Chróst, R. J. (ed.) Microbial enzymes in aquatic environments. Springer-Verlag, New York, p. 29–59

Chróst, R. J. (1992). Significance of bacterial ectoenzymes in aquatic environments. Hydrobiologia 243/244: 61–70

Chróst, R. J. (1993a). Microbial enzymatic degradation and utilization of organic matter. In: Overbeck, J., Chróst, R. J. (eds.) Microbial ecology of Lake Plußsee. Springer-Verlag, New York, p. 118–174

Chróst, R. J. (1993b). Enzymatic transformation of organic matter in aquatic environmnets. In: Guerrero, R., Pedrós-Alió, C. (eds.) Trends in microbial ecology. Spanish Society for Microbiology, Barcelona, p. 401–406

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

Chróst, R. J., Rai, H. (1993b). Bacterial secondary production. In: Overbeck, J., Chróst, R. J (eds.) Microbial ecology of Lake Plußsee. Springer-Verlag, New York, p. 92–117

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

Demers, S., Theriault, J.-C., Bourget, E., Bah, A. (1987). Resuspension in the shallow sublittoral zone of a macrotidal estuarine environment: wind influence. Limnol. Oceanogr. 32: 327–339

Ducklow, H. W., Carlson, C. A. (1992). Oceanic bacterial production. Adv. microb. Ecol. 12: 113–181

Goulder, R. (1977). Attached and free bacteria in an estuary with abundant suspended solids. J. appl. Bacteriol. 43: 399–405

Harvey, R. W., Young, L. Y. (1980). Enumeration of particle-bound and unattached respiring bacteria in the salt marsh environment. Appl. environ. Microbiol. 40: 156–160

Hopkinson, C. S. (1987). Nutrient regeneration in shallow-water sediments of the estuarine plume region of the nearshore Georgia, USA. Mar. Biol. 94: 127–142

Hoppe, H.-G., Ducklow, H., Karrasch, B. (1993). Evidence for dependency of bacterial growth on enzymatic hydrolysis of particulate organic matter in the mesopelagic ocean. Mar. Ecol. Prog. Ser. 93: 277–283

Hoppe, H.-G., Kim, S.-J., Gocke, K. (1988). Microbial decomposition in aquatic environments: a combined process of extracellular enzyme activity and substrate uptake. Appl. environ. Microbiol. 54: 784–790

Hubbard, J. G., Chrzanowski, T. H. (1986). Impact of storms on heterotrophic activity of epilimnetic bacteria in a southwestern reservoir. Appl. environ. Microbiol. 51. 1259–1263

Joint, I. R., Morris, R. J. (1982). The role of bacteria in the turnover of organic matter in the sea. Oceanogr. mar. Biol. A. Rev. 20: 65–118

Kirchman, D., Mitchell, R. (1982). Contribution of particle-bound bacteria to total microheterotrophic activity in five ponds and two marshes. Appl. environ. Microbiol. 43: 200–209

Kirchman, D., Soto, Y., Van Wambeke, F., Bianchi, M. (1989). Bacterial production in the Rhône River plume: effect of mixing on relationships among microbial assemblages. Mar. Ecol. Prog. Ser. 56: 267–275

Leatherbarrow, R. J. (1987). Enzfitter. A non-linear regression data analysis program for the IBM PC. Elsevier-Biosoft, Cambridge

Martinez, J., Azam, F. (1993). Periplasmic aminopeptidase and alkaline phosphatase activities in a marine bacterium: implications for substrate processing in the sea. Mar. Ecol. Prog. Ser. 92: 89–97

Nybroe, O., Christoffersen, K., Riemann, B. (1992). Survival of *Bacillus licheniformis* in seawater model ecosystems. Appl. environ. Microbiol. 58: 252–259

Palumbo, A. V., Fergusson, R. L., Rublee, P. A. (1984). Size of suspended bacterial cells and association of heterotrophic activity with size fractions of particles in estuarine and coastal waters. Appl. environ. Microbiol. 48: 157–164

Riemann, B., Hoffmann, E. (1991). Ecological consequences of dredging and bottom trawling in the Limfjord, Denmark. Mar. Ecol. Prog. Ser. 69: 171–178

Riemann, B., Nielsen, T G., Horsted, S. J., Bjørnsen, P. K., Pock-Steen, J. (1988). Regulation of phytoplankton biomass in estuarine enclosures. Mar Ecol. Prog. Ser. 48: 205–215

Rowe, G. T., Clifford, C. H., Smith, K. L., Hamilton, P. L. (1975). Benthic nutrient regeneration and its coupling to primary productivity in coastal waters. Nature 255: 215–217

Troussellier, M., Cahet, G., Lebaron, P., Baleux, B. (1993). Dis-

tribution and dynamics of bacterial production in relation to wind perturbations in a Mediterranean lagoon. Limnol. Oceanogr. 38: 193-201

Wainright, S. C. (1987). Stimulation of heterotrophic microplankton production by resuspended marine sediments. Science 238: 1710-1712

This article was presented by G. Rheinheimer, Kiel, Germany

Manuscript first received: October 5, 1993
Revised version accepted: February 16, 1994