Seasonal and Spatial Distribution of Extracellular Enzymatic Activities and Microbial Incorporation of Dissolved Organic Substrates in Marine Sediments

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Seasonal and spatial distributions of extracellular enzymatic activities and microbial incorporations of dissolved organic substrates were followed in sediments of the brackish water Kiel Bight (Baltic Sea, Federal Republic of Germany). Enzymatic hydrolysis of polymeric organic compounds was determined by means of fluorogenic substrates (4-methylumbelliferyl-β-D-glucoside, L-leucine-4-methylcoumarinyl-7-amide hydrochloride); incorporation of dissolved organic substrates into microbial biomass was measured by using tritiated substances (acetate, leucine, and thymidine). Based on a recently developed core injection technique, substrates were injected in microliter portions into undisturbed sediment cores. Enzymatic and incorporation activities underwent strong seasonal variations related to the enrichment of organic material in the sediment surface following sedimentation events. The input of the phytoplankton bloom during autumn caused stimulation of both enzymatic hydrolysis of polymeric organic compounds and microbial incorporation of dissolved organic substrates. Following input by spring phytoplankton bloom, mainly incorporation activities were stimulated. In late spring the development of the benthic fauna obviously greatly influenced microbial activities. During summer individual periods of high microbial activities were observed which might be traced back to short-term sedimentation events. The high microbial incorporation of leucine and thymidine during winter demonstrated that the nutrient supply rather than temperature is the dominating factor determining microbial production. Stimulation of microbial activities arose from the sediment surface and spread out relatively quickly into deeper horizons. Generally, the sediments were characterized by distinct patterns of interrelationships between the individual parameters of microbial activities measured.

Benthic ecosystems are greatly dependent upon the supply of organic material, most of which enters the sediment as polymeric organic compounds. Prior to incorporation into microbial cells, the polymeric material has to be decomposed by extracellular enzymes which are secreted from living cells or liberated through the lysis of cells. In soil some of these enzymes may even retain their activity by the formation of humus-enzyme complexes bound to clay particles (3). The enzymatic hydrolysis of polymers is generally considered the rate-limiting step in sediment carbon flow. However, detailed information on the dynamics and controls of these initial processes is limited and rather scattered throughout the literature (2, 9, 15, 18, 22, 26, 31). In recent investigations, interest was focused again on the measurement of enzymatic activities in sediments (14, 19), certainly a reflection on the availability of suitable methods. Through the application of artificial substrates which are hydrolyzed by natural enzymes, thus releasing highly fluorescent compounds, sensitive methods for the study of enzymatic processes are available (12, 29).

By enzymatic hydrolysis, polymeric organic compounds are degraded to oligomers or monomers which can be taken up by microbial cells to meet their energy requirements and to build up biomass. These subsequent events in carbon flow in marine sediments have been studied in more detail, using radioactive labeled substrates (for example, see references 4, 5, 7, 10, 11, 16, 19–21).

To obtain a better insight into the dynamics and the controls of sediment carbon flow, detailed studies of temporal and spatial distributions of extracellular enzymatic activities and incorporation of dissolved organic substrates into microbial biomass were carried out in undisturbed sediments of the brackish water Kiel Bight (Baltic Sea, Federal Republic of Germany).

MATERIALS AND METHODS

Sampling. A total of 26 sediment samples were collected between March 1985 and April 1986, using a Reineck grab from a muddy sand station (“Gabelsflach”; water depth, 17 m) located in the central brackish water Kiel Bight. On board ship, subsamples were withdrawn from the grab in Plexiglas tubes (Rohm & Haas Co.) (see below).

Redox potential; organic matter. Redox potential was measured with an E9 electrode (Ingold Pt-4800-M5). At least three profiles (1-cm intervals down to a sediment depth of 8 cm) were analyzed. Sediments with a redox potential above 300 mV were regarded as oxic; sediments between 300 and 100 mV, as suboxic; and sediments below 100 mV, as anoxic (see reference 8). For the surface horizons (0 to 1 and 1 to 2 cm), the total organic matter content was determined as the difference between the dry weight of the ground sediment and the residue left after combustion.

Microbiological parameters. Extracellular enzymatic activities and incorporation of dissolved organic substrates into microbial biomass were analyzed in undisturbed sediment cores, using a core injection technique as described in detail in a recent publication (19). Brieﬂy, undisturbed sediment cores were taken from the grab in Plexiglas tubes (1.2 cm in diameter). Prior to sampling, the tubes were perforated at 0.5-cm intervals with 1-mm-wide injection ports and sealed with silicone rubber. With a gas-tight Hamilton syringe, 10
μl of the substrates tested for enzymatic hydrolysis or incorporation was injected into the center of the sediment horizons. After incubation at in situ temperature for 1 h, microbial activities were terminated by quick-freezing in dry ice. For the analysis the cores (0 to 8 cm in depth) were sectioned at 1-cm intervals, the center of the horizons was cut out with a cork drill (4 mm in diameter), and the sediments were treated as described below. All manipulations were carried out under ice.

Extracellular enzymatic activities were followed with 4-methylumbelliferyl-β-D-glucoside (MUF-glu) and L-leucine-4-methylcoumarinyl-7-amide hydrochloride (MCA-leu) as substrates. Solutions (5 mM) of the substrates were prepared in brackish water (salinity, approximately 10%), filter sterilized, flushed with nitrogen, temperature equilibrated, and injected in 10-μl portions into three parallel cores. After the general treatment of the cores (see above), the sediment was centrifuged, and the fluorescence of the supernatant was read in a spectrofluorometer (Jasco FP-550) at 455 nm under 365-nm excitation against a standard solution.

Incorporation of dissolved organic substances into microbial biomass was measured with the following substrates: [1-3H]acetate (42.7 mCi/mg), L-[4,5-3H]leucine (880 mCi/mg), and [methyl-3H]thymidine (355 mCi/mg). The substrates were diluted in brackish water to an activity of 0.25 μCi per 10 μl, flushed with nitrogen, temperature equilibrated, and injected in 10-μl portions into three parallel sediment cores. After the general treatment of the cores (see above), the sediments were washed, dried, oxidized in a Tri-Carb oxidizer (Packard Instrument Co., Inc.), and analyzed in a liquid scintillation counter (Betazint 5000).

Controls were run with sediment from the individual horizons sterilized by autoclaving. The extracellular enzymatic hydrolysis and incorporation of dissolved organic substrates into microbial biomass reported represent potential and relative activities, respectively, because the concentrations of natural substrates were not known (see Discussion).

**RESULTS**

For a graphic presentation of the seasonal and depth-dependent variations of redox potential, organic matter, and microbiological parameters, isopleth diagrams were chosen. Since between December and January and between February and mid-March no samples could be obtained because of the ice cover of the Kiel Bight, the diagrams remained incomplete during these periods (Fig. 1 to 6).

Based upon the specific gravity (graph not shown), it can be concluded that the sediments investigated were comparable, since great changes in the physical composition were not observed. The redox potential of the sediments investigated revealed strong seasonal variations which influenced the whole sediment profile (Fig. 1). During spring 1985 individual periods of introduction of oxygen into the sediment were observed: the discontinuity layer (0 mV) varied between 1 and 5 cm. In summer the sediments were horizontally stratified with suboxic to anoxic conditions at the surface and a gradual decrease of redox potential with increasing sediment depth. Starting in November, a breakup of summer stagnation was observed. Due to the introduction of oxygen, first (towards mid-November) the sediment surface and later (in January) also deeper horizons became oxic. In spring of the following year, sediments were horizontally stratified again withoxic conditions at the surface, suboxic conditions in medium horizons, and anoxic conditions in deeper sediments.

Interpretations of the total organic matter content (ignition loss) seemed to be meaningful only at the sediment surface (Fig. 2). It must be assumed that most of the organic matter in medium and deeper sediment horizons is refractory material and that inorganic material (carbonate) interfered with
the analysis. During summer variations of the total organic matter content in the sediment surface were low. Enrichments of organic material, however, were recorded in autumn (beginning and end of October, end of November) and spring (end of March, mid-May to mid-June 1985).

Extracellular enzymatic activities were strongly influenced by the season. Since the hydrolysis of MUF-glu and MCA-leu revealed a similar variation pattern, only the later is discussed (Fig. 3). During spring 1985 a continuous increase of enzymatic activities was observed. Stimulation arose from the sediment surface and affected deeper sediment horizons. Since two samples were lacking during summer, the summer aspect remained incomplete. Pronounced stimulation of enzymatic activities, however, was recorded in autumn (beginning and end of October, end of November). Winter and spring 1986 were characterized by relatively low hydrolysis rates of MCA-leu at the sediment surface and a slow continuous decrease with depth. In some sediment profiles the enzymatic activities were homogeneously distributed.

Strong seasonal variations were also noticed for the incorporation of dissolved organic substrates into microbial biomass (Fig. 4 to 6). Highest incorporations of acetate were measured during spring (mid-April, mid-June). For leucine and thymidine, however, highest incorporation rates were recorded in spring, autumn, and, surprisingly enough, also during winter at a time when the incorporation of acetate showed the lowest values. Again, stimulation arose from the surface and affected deeper sediment horizons. Generally, incorporation of leucine and thymidine revealed similar variation patterns differing from that of acetate.

**DISCUSSION**

Extracellular enzymatic hydrolysis of polymeric organic compounds and incorporation of dissolved organic substrates into microbial biomass were examined in undisturbed sediment profiles of the brackish water Kiel Bight to characterize general features of seasonal and depth-dependent variations in sediment carbon flow. To follow the enzymatic hydrolysis of natural β-D-glucosidase and L-leucine-amino-
$^3$H-Acetate incorporation \(
\left[ 10^{-4} \text{ng cm}^3 \text{ h}^{-1} \right]\)

FIG. 4. Isopleth diagram of seasonal and spatial distribution of microbial incorporation of tritiated acetate in sediments of the Kiel Bight (station Gabelsflach).

$^3$H-Leucine incorporation \(
\left[ 10^{-2} \text{ng cm}^3 \text{ h}^{-1} \right]\)

FIG. 5. Isopleth diagram of seasonal and spatial distribution of microbial incorporation of tritiated leucine in sediments of the Kiel Bight (station Gabelsflach).
peptidase, fluorogenic substrate analogs (12, 29) were used, the hydrolysis of which describes potential activities (14, 19). Incorporation of dissolved organic substrates into microbial biomass was determined with tritiated acetate and leucine applied in trace concentrations. Since their natural concentrations (see references 1 and 13) and microbial availability (4, 30) could not be evaluated, the activities reported here describe relative incorporation. Parallel measurements of the incorporation of tritiated thymidine were used as a relative indicator for microbial growth without the intention to calculate growth rates (24).

From the graphs (Fig. 1 to 6) it becomes obvious that extracellular enzymatic hydrolysis of polymeric organic compounds and incorporation of dissolved organic substrates into microbial biomass were strongly influenced by the season. In summer the sediment was suboxic to anoxic with relatively low variations in organic matter. Only in mid-August was a slight increase in organic matter observed which was paralleled by a stimulation of the hydrolysis of MCA-leu. However, microbial incorporation of acetate, leucine, and thymidine revealed periods of high activities (end of July, September).

From the main sedimentation events in autumn and spring in the Kiel Bight (23, 27), the response of the benthic community in autumn could be well documented based upon a high-time resolution in sampling. In the beginning and at the end of October and November, enrichments of organic material were observed in the sediment surface obviously caused by the sedimentation of the autumn phytoplankton bloom. Not until November did the redox potential increase significantly. Parallel to the accumulation of organic material, enzymatic activities culminated, yielding the highest annual values. It is interesting to note that incorporation of dissolved organic substrates varied with the substrate. The highest incorporation of acetate and leucine was measured after the first period of input of organic material into the sediments (beginning of October). Subsequent enrichment of organic material resulted in lower incorporation. For thymidine, however, a pronounced stimulation of incorporation was not observed before the second and third input of organic material. It may be concluded that microorganisms reacted on the availability of organic material primarily with biomass production (incorporation of acetate and leucine) and secondarily with reproduction (incorporation of thymidine). This is consistent with earlier observations of seasonal variations in microbial numbers and biomass in sediments of the Kiel Bight (18).

Because of the ice cover of the Kiel Bight, sampling in winter was restricted to January. Surface sediments were oxic with relatively low organic matter content except for the sample taken at the end of January. Hydrolysis of MCA-leu and incorporation of acetate were low. Surprisingly enough, high values were measured for the incorporation of leucine and thymidine, which were comparable to incorporation recorded in autumn and spring. The nutrient basis for the high microbial production during winter is difficult to evaluate. There is some indication from earlier investigations that macrophyte material eroded by winter storms represented the major nutrient source for the high microbial biomass production observed during winter (8).

Evidence for the sedimentation of the spring phytoplankton bloom could be obtained only in spring 1985, in which organic material accumulated in the sediment at the end of March. Although the enzymatic hydrolysis of MCA-leu remained low, incorporation of acetate, leucine, and thymidine was stimulated. In spring 1986 the relatively short period of input of the phytoplankton bloom was obviously missed, since between mid-March and the beginning of April no samples could be withdrawn. It is interesting to note, however, that incorporation of acetate, leucine, and thymidine already increased in mid-March prior to the assumed period of sedimentation.
The input of the phytoplankton bloom must be regarded as the trigger for initiating benthic activities (8, 18). After decomposition of the sedimented organic material, microbial activities revealed individual periods of stimulation which have to be characterized as the most productive ones during the year. In mid-April and in the beginning of May, incorporation of acetate and leucine culminated; between the end of March and mid-May, incorporation of thymidine was almost unchanged on a high level. The considerable accumulation of organic material observed in the sediment surface between mid-May and mid-June was obviously due to a mass development of benthic fauna (polychetes). During this period microbial activities were relatively low, not to say depressed. Not before mid-June did enzymatic hydrolysis of MCA-leu and incorporation of dissolved organic substrates into microbial biomass increase again, obviously as a result of the breakdown of the fauna population. Based on microbial numbers and biomass, corresponding observations were made in previous years (18).

Interrelationships between the individual parameters of microbial activities measured were very complex and could not be generally described by simple, linear interdependencies. From the plots (Fig. 7 to 9), it became obvious that the data points for most of the samples (with the exception of the uppermost sediment horizon) were arranged in distinct patterns “characteristic” for the correspondent sediment profile. This means that most of the sediments were characterized by specific relationships of microbial activities which had been developed as a result of both history of the sediment and impact of environmental conditions. It must be assumed that variations in environmental parameters (e.g., nutrients) affected the whole sediment core (at least down to a sediment depth of 8 cm) relatively quickly. As possible mechanisms, bioturbation and physical exchange processes driven by density gradients (25, 28) have to be considered.

The plot of the interrelationships between microbial incorporation of acetate and leucine for samples taken in autumn (period of a high-time resolution in sampling) demonstrated the existence of complex, sediment-specific interdependences (Fig. 7). In the sediment profile of 4 November, leucine incorporation varied by a factor of at least 2; acetate incorporation, however, showed only very little variations. In the sediment of 2 December, acetate incorporation differed by a factor of at least 2; the incorporation of leucine, however, varied only insignificantly. From the sediment profile of 28 October and 25 November, one might derive a direct, linear relationship between the incorporation rates of both substrates. The same applied for the lower horizons of the sediment sample of 22 October. Finally, from the sediment profile of 2 and 16 October, no distinct patterns of relationships could be detected. Similar observations could be derived for interrelationships between the enzymatic hydrolysis of MCA-leu and the incorporation of leucine (Fig. 8). For some profiles a direct, linear relationship might be assumed; in other sediments the parameters varied independently of each other.

Microbial incorporations of thymidine plotted against incorporation of leucine gave the best impression of a general linear relationship (Fig. 9). If leucine incorporation represents a measurement of protein synthesis (biomass production; 17) and thymidine incorporation characterizes nucleic acid synthesis (reproduction; 24), both activities should be synchronized at least during balanced growth. However, as shown by the sediment profile of 2 December, situations of unbalanced growth have to be taken into account.

In conclusion, general features of the carbon flow in boreal coastal sediments could be described. The activity of the benthic microbial community is limited by the supply of organic material, most of which enters the sediment via sedimentation from the water column. Correspondent to pelagic primary production, the main sedimentation events occur in autumn and spring. Generally, enrichments of

FIG. 7. Interrelationship between microbial incorporation of acetate and that of leucine in sediments of the Kiel Bight (station Gabelsflech). The graph summarizes the data from seven samples withdrawn in autumn 1985. Numbers with the symbols characterize the individual sediment horizons.
FIG. 8. Interrelationship between microbial incorporation of leucine and extracellular enzymatic hydrolysis of MCA-leu in sediments of the Kiel Bight (station Gabelsfalch). For further explanations, see legend to Fig. 7.

FIG. 9. Interrelationship between microbial incorporation of thymidine and that of leucine in sediments of the Kiel Bight (station Gabelsfalch). For further explanations, see legend to Fig. 7.
organic material in the sediment surface led to stimulation of extracellular enzymatic hydrolysis of the polymeric organic compounds. Subsequently, incorporation of dissolved organic substrates into microbial biomass increased. Besides the two main events, short-term sedimentations might have occurred, responsible for individual periods of stimulation of microbial activities (mainly in summer), although correspondent enrichment of organic material in the sediment surface could not be detected. Stimulation arose from the sediment surface and spread out relatively quickly into deeper horizons obviously caused by biological and physical processes. The high microbial production even at low temperature during winter makes clear that the nutrient supply rather than temperature is the dominating factor determining microbial activities. From most of the previous studies in marine sediments, it has been concluded that microbial numbers and activities followed the temperature cycle (e.g., see references 6 and 10). The existence of distinct patterns of interrelationships between individual parameters of microbial activities demonstrates that in natural sediments relationships between microbial activities may not generally be described by simple linear interdependencies. Although the patterns cannot be interpreted at present, the ratios of different parameters of microbial activities might be useful indicators of changes in sediment metabolism.

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LITERATURE CITED