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ZAHL, BIOMASSE UND ABBAU VON ORGANISCHEM MATERIAL

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Lutz-Arend Meyer-Reil

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Benthic response to sedimentation events during autumn to spring at a shallow-water station in the Western Kiel Bight

I. Analysis of processes on a community level *

G. Graf, R. Schulz, R. Peinert and L.-A. Meyer-Reil

Institut für Meereskunde an der Universität Kiel; Düsternbrooker Weg 20, D-2300 Kiel 1, Germany (FRG)

Abstract

The response of the benthos to the break up of anoxia in the Kiel Bight (Western Baltic Sea), and to three succeeding events of "external" food supply, consisting of a settled autumn plankton bloom, resuspended matter and macrophyte input during winter, and of a sedimented spring phytoplankton bloom, is described on a community level. The first input of oxygen broke up anoxic conditions and made stored food resources available to decomposition. This "internal" food supply, mainly consisting of protein (folin positive matter), was followed by a drastic increase in heat production and ATP-biomass and caused a period of low redox potential, which lasted for several weeks. During this phase, a plankton bloom (dinoflagellates and diatoms) settled to the sea floor. Although there was an immediate response of benthic activity, this food input was not completely consumed by the strongly disturbed benthic community. During winter resuspended matter and the input of macrophyte debris caused another maximum in benthic activity and biomass despite the low temperature. The response to sedimentation of cells from a diatom bloom during mid March was also without any time lag and was consumed within 5–6 wk. A comparison of the amount of particles collected in a sediment trap with the increase of organic matter in the sediment demonstrated that the sediment collected four times (autumn) and seven to eight times (spring) more than measured by the sediment trap. Strong indications of food limitation of benthic activity were found. During autumn and winter these indications were caused more by physical than by biological processes. The three events of "external" food supply caused a temporary shift in the type of metabolism towards fermentation processes and reduced the redox

potential. In spring the development of the benthic community was still being strongly influenced by the events of the preceding summer and autumn.

Introduction

In recent years pelagic-benthic coupling has been found to be a highly dynamic process occurring over very short time scales. Especially the investigations of plankton blooms which, via sedimentation, represent a large food supply for the benthic system, have demonstrated the necessity of investigating sedimentation and the subsequent benthic response over time scales of days to a few weeks (Smetacek et al., 1978; Smetacek, 1980; Graf et al., 1982). This is particularly important in shallow water ecosystems, such as the Kiel Bight (Western Baltic Sea), where studies on benthic-pelagic coupling are additionally complicated by the close interaction of short-term weather conditions with the ecosystem, e.g. irradiance, wind, and horizontal advection.

The input of particulate organic matter to the benthic system is generally investigated by near-bottom sediment traps. However, large discrepancies have been found between the amount of matter collected in the trap and the benthic consumption immediately below (Wiebe et al., 1976; Graf et al., 1982). These authors explain the discrepancy with lateral advection and near-bottom transport of organic matter and show that a significant link in the chain of processes extending from production in the surface and incorporation of particles into the sediment is still to be identified. If, however, sedimentation of particles is strongly concentrated in certain periods of the year, it should be possible to measure the pelagic input directly in the sediment itself, even in the presence of a high background level of organic matter.

This report gives the first results of an interdisciplinary project carried out by the SFB 95. It will be followed by a paper dealing with the special role of benthic bacteria

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(Meyer-Reil, 1983). A report on the role of macrofauna, meiofauna, ciliates and benthic diatoms will be published later. This first part analyses pelagic-benthic coupling on a community level.

The response of the benthos to a settling phytoplankton bloom in spring 1980 was reported by Graf *et al.* (1982) and first results on the role of bacteria within this process have already been described by Meyer-Reil (1981). Consequently, our original intention was to investigate the response of the benthos to the settling autumn phytoplankton bloom of 1981. The project was extended, however, to the following spring because in 1981 there was unusually extensive anoxia in the Kiel Bight during the summer. Thus, the investigation of pelagic-benthic coupling ran into additional complexities.

Material and methods

From September 9, 1981 to April 26, 1982, 20 cruises with RV 'Littorina' to the 'Hausgarten', an area restricted for research in the Western Kiel Bight (Western Baltic Sea), were carried out. The sediment station was situated at a water depth of 18 m on the slope of the Kiel Bight channel system. The sediment is sandy mud. The dry weight of 1 cm³ is 1.300 ± 0.064 g.

Temperature was measured with a TS probe (Electronic Switchgear, London). Redox potential of the sediment was measured with an Eh-electrode (Ingold, Pt-4800-M5, FRG) and a mV-meter (Knick, FRG). Water samples were taken from seven depths using 5-l Niskin bottles. The sediment trap (Zeitzschel *et al.*, 1978) collected particles at two-day intervals. The handling and the analysis of the water samples and that of trapped matter is described by Peinert *et al.* (1982). Chlorophyll *a* analyses were carried out as recommended by Edler (1979). For the trapped matter, chlorophyll *a* equivalents are given according to Peinert *et al.* (1982).

Sediment was collected using a Reineck grab (20×28 cm surface area). Subsamples were taken with Plexiglas corers with 5-cm diameter and, for direct calorimetry, with a plastic syringe (top cut off) with 2.3-cm diameter. The different sediment horizons were separated on board.

For the chemical analyses, sediments from three different grabs were combined, whereas for calorimetry three slides of the parallel grabs were carefully placed in one calorimeter chamber.

ATP-concentration, electron-transport (ETS-)-activity and heat production were measured according to Pamatmat *et al.* (1981). ETS-activity is given in μl O_2. For the recently suggested unit electrochemical microequivalents (μeq) (Packard *et al.*, 1983) the results have to be converted into $\mu \cdot$ mol and multiplied by four. Unlike in earlier investigations, direct calorimetry was run at *in-situ* temperature ($\pm 1\,C°$) and ETS-activity at 10 °C. Thermograms of anoxic and suboxic sediments were not evaluated before a constant heat flow was observed (6–7 h) (c.f. Pamatmat, 1982). Protein and carbohydrate were assayed as described by Meyer-Reil (1983).

Results and discussion

Physical environment

During summer, the oxygen tension in the bottom water of the Western Kiel Bight is generally low. Hydrogen sulfide, however, can only be observed in the deeper parts of the Kiel Bight channel system. The station investigated had originally been chosen to avoid anoxic conditions. During 1981, however, large areas of Kiel Bight became anoxic (Ehrhardt and Wenck, in press).

To distinguish oxic from anoxic conditions, the redox potential in the sediment was measured (Fig. 1). The first autumn storms in early October broke up the anoxic conditions and increased the redox potential from $Eh = -160$ mV on September 23 to $Eh = +420$ mV on October 8. This break, however, was only observed at the sediment surface; the deeper horizons of the sediment were less affected. From October 28 to December 3 the redox potential of the top centimeter of the sediment was within the range of $Eh = +100$ mV to $Eh = +300$ mV. In Fig. 1, this range is marked by dashed lines and is termed suboxic. In the sediments of the study site, $Eh = +100$ mV is the upper limit for the occurrence of free hydrogen sulfide (Reimers, 1976). Jørgensen and Fenchel (1974) also indicated an $Eh = +100$ mV as the lower limit for free oxygen. Accordingly $Eh = +300$ mV is typical for oxic conditions.

The final termination of the suboxic conditions occurred during early December and produced oxic conditions down to sediment depth 5 cm. Although the hydrographical processes transporting oxygen rich water to the sediment surface are not yet completely understood, it is
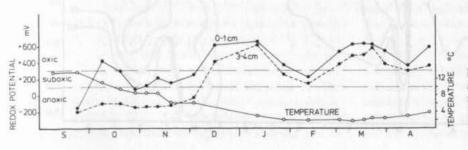

Fig. 1. Redox potential in the sediment and temperature of the sea floor. The range between $Eh = +100$ mV and $Eh = +300$ mV is marked with dashed lines and termed suboxic

probable that the final break was induced by heavy north-west storms during the last few days of November, which after some delay in time were followed by an inflow of saline rich bottom water from the Great Belt, causing a lateral exchange of water masses (cf. Wittstock, 1982). A comparable displacement of bottom water, however, was not found when the first hydrographical change in early October occurred.

The process of terminating the summer anoxic period as described above, i.e. a first autumnal break followed by a suboxic period lasting several weeks and a final break in early winter, seems to be typical for the area. The same pattern was observed in the years 1971 to 1973 by oxygen measurements in the near bottom water of the Western Kiel Bight (v. Bodungen, 1975).

The decrease of the redox potential following the first break may have been caused by both a chemical oxygen demand for the oxidation of hydrogen sulfide and also, as will be demonstrated, by increased biological activity. The latter would also explain the temporary reduction of the redox potential from late January to mid February and during early April.

The temperature development in the near-bottom water is typical for a boreal region. Temperature decreased from 13.9 °C on September 23 to 2 °C on January 27 and increased slowly at the end of the investigation period. For the Western Kiel Bight the temperature curve is well within the range given for the statistical mean recorded over many years (Krey et al., 1978).

Food supply to the benthos

During recent years the importance of the phytoplankton spring bloom for benthic nutrition has been postulated (Hobro et al., as cited by Jansson, 1978; Skjoldal and Lännergren, 1978; Walsh, 1981). Studies in the pelagic system of the Kiel Bight have demonstrated that large amounts of freshly produced matter during both spring and autumn bloom do not enter the pelagic food web but rather settle to the sea floor, representing up to 2/3 of the yearly input from the pelagic to the benthic system (Smetacek, 1980). The consequence of such a food input

on the benthic system during spring was described by Graf et al. (1982).

During autumn 1981, the plankton population maintained a high ATP-biomass level from September to early November with peak values above 1.4 μg ATP l^{-1}. Phytoplankton growth was terminated by heavy storms followed by sedimentation of cells in late November (Figs. 2 and 3). At the beginning of the investigation period, the mixed phytoplankton population was dominated by the armoured dinoflagellates *Ceratium tripos* and *C. fusus*, whereas at the end diatoms became more important in terms of biomass. There was, however, no distinct separation of these populations, as recorded in 1973 (Smetacek, 1980).

From the integrated POC values of the water column, we presume that the autumn bloom started to settle at the end of October, and in fact, there was a significant increase of the protein content of the top centimeter of the sediment (Fig. 4). Unfortunately, no sediment trap data are available for this period as the trap was lost. During November there is a steady vertical flow of freshly produced cells to the sediment, as indicated by the high amount of chlorophyll *a* equivalents collected by the sediment trap and the low C:chlorophyll *a* ratios of the collected matter (< 100:1).

During mid November, high chlorophyll *a* concentrations in the water column concomitant with high primary production rates (Peinert, unpublished data) might be related to a nutrient release from the sediment following the first break (v. Bodungen, 1975). The storms at the end of November were followed by sedimentation of the entire phytoplankton population within 5 to 6 d (Fig. 3).

During the period of December 16 to March 2 sediment traps were not employed. The plankton standing stock was followed in terms of ATP-biomass. As depicted in Fig. 2, it was very low during this period.

The spring bloom in 1982 started in early March and was mainly composed of the diatom *Detonula confervacea* as in other years (Smetacek, 1975; Peinert et al., 1982). During mid March it reached its maximum ATP-biomass (4 μg l^{-1}) and settled out of the water column within a few days shortly thereafter (Fig. 3). The amount of chlorophyll
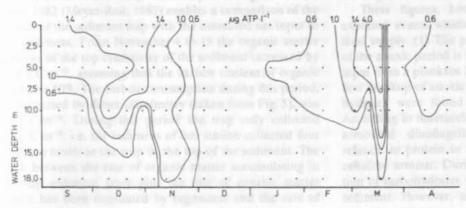

Fig. 2. Isopleth diagram of ATP-biomass in the water column, describing the biomass development of the autumn and spring plankton bloom
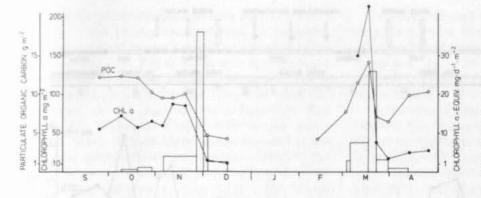
Fig. 3. Particulate organic carbon and chlorophy'l *a* in the water column (integrated for 18 m) compared to sedimentation rate of particles collected by a bottom near sediment trap as analysed in terms of chlorophyll *a* equivalents. For the period Dec. 17 to Feb. 10 no values are available
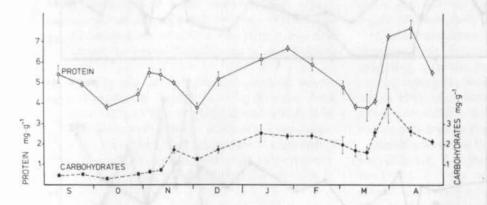

Fig. 4. Protein and carbohydrate concentration in the top centimeter of the sediment (according to Meyer-Reil, 1983, modified)

a equivalents collected by the trap suggest that there was a considerable loss of cells already during early to mid March, when the bloom was still growing vigorously. However, for technical reasons the trap covered the period from March 5 to 17. Compared to the increase of protein in the sediment (Fig. 4), it is most likely that the bulk of the trapped matter settled during the last days of this period. C:chlorophyll *a* ratios in the water column were also < 100:1. The carbon input of both blooms to the sediment, as determined by sediment traps, was in the range of 10–15 g C m^{-2}.

Earlier calculations for an annual budget of the year 1980 at the same station suggest that the vertical input of phytoplankton cells via sedimentation as determined by sediment traps only explains 15 to 25% of the observed activity (Graf *et al.*, 1982). The analysis of organic matter 1981/1982 (Meyer-Reil, 1983) enables a comparison of the results of the sediment trap with the measured net input to the sediment. From November 4 to 19 the organic matter content of the top centimeter of the sediment increased by 15.9 g C m^{-2}, assuming that the carbon content of organic matter is 50%. The carbon consumption during this period, as estimated by direct calorimetry (taken from Fig. 5), was 2.6 g C m^{-2}. During this period the trap only collected 4.8 g C m^{-2}, i.e. the sediment of our station collected four times as much as the trap in the top of the sediment. The ratio between the rate of organic matter accumulating in the top sediment layer plus the rate of organic matter which has been consumed by organisms and the rate of

organic matter which sedimented in the sediment trap will be called the "advection factor".

The type of sediment trap used gives rather realistic values when used for budgetary calculations of the pelagic system (Peinert *et al.*, 1982). Thus, the observed discrepancy cannot, at least not exclusively, be explained by the sediment trap methodology. According to the topography of the investigation area, it is more likely that the organic matter was accumulated at our station by horizontal near-bottom transport down the slope. A corresponding calculation for the period March 10–30 revealed an advection factor of 7 to 8. The increase of freshly produced organic matter from the autumn bloom as well as from the spring bloom in the top centimeter of the sediment is also documented by increased protein and carbohydrate contents (Fig.4).

These figures, however, also document some unexpected events which partly represent other sources of food supply. (1) The protein level still present at the end of the anoxic period is in the same range as found after the input from a plankton bloom; it decreases rapidly after the first breakup of anoxic conditions. Contrary to this, carbohydrates were found to be at a very low level. (2) According to Smetacek and Henrikson (1979), a bloom of armoured dinoflagellates increases carbohydrates in relation to protein in the water column because of their cellulose armour. During October, in terms of concentration of carbohydrates, no such record is to be seen in the sediment. However, as described by Meyer-Reil (1983),
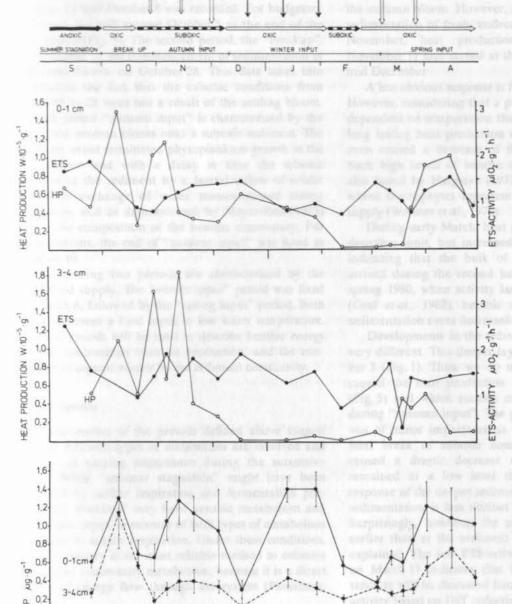
Fig. 5. The scheme at the top of the figure demonstrates the periods as defined according to physical conditions and the sedimentation events. The curves below give activity of the sediment in terms of heat production (HP) and the activity of the electron transport system (ETS) and ATP-biomass in two different strata of the sediment

an increase of α-amylase activity could be observed. (3) The most surprising finding, however, is the continuous increase of protein and carbohydrates during December and January. The processes leading to this accumulation have been summarized by Graf *et al.* (1983): the resuspension of organic particles in the deeper parts of the channel system and their redeposition in the shallower areas, and the near bottom transport of macrophytes that are torn off and macerated during these months (Schomann, 1977), documented by the high chlorophyll *a* concentrations in the sediment during late January (up to $4.7 \, \mu g \, cm^{-3}$), a range that is only found after an input of freshly produced phytoplankton (Schulz, 1983). The input of macrophyte debris would also explain the high concen-

tration of carbohydrates that is present in spring. This is most likely due to resistant, structural carbohydrates. The protein level, however, during early March is as low as during early October and early December.

Defining the relevant periods

For a further presentation of the results and discussion it seems useful to define certain periods within the entire 8-month period observed, based on the changing physical environment and the food supply of the benthos. We do not know for how long the anoxic conditions had prevailed before the program was started. Thus, for the first

period, called "summer stagnation" only the end between September 23 and October 8 was recorded. For budgetary calculations, we will assume October 1 as the end of the anoxic period (Fig. 5). The second period, the "breakup", was terminated by the commencement of sedimentation of the autumn bloom on October 28. This date takes into consideration the fact that the suboxic conditions from October 19 to 28 were not a result of the settling bloom. The third period "autumn input" is characterized by the input of the autumn bloom onto a suboxic sediment. The same storm event terminated phytoplankton growth in the water column and with a delay in time the suboxic conditions in the sediment by a lateral inflow of colder water. This exchange of water masses caused strong resuspension and as demonstrated by Meyer-Reil (1983) changed the composition of the benthic community. For the calculations, the end of "autumn input" was fixed as December 10.

The following two periods are characterized by the type of food supply. The "winter input" period was fixed until March 6, followed by the "spring input" period. Both periods represent a food input at low water temperature. These five periods will be used to describe benthic energy flow, the concomitant biomass production, and the consumption of organic matter by the sediment community.

Benthic response

The characteristics of the periods defined above suggest that very different types of metabolism are involved and they are of varying importance during the successive periods. While "summer stagnation" might have been dominated by sulfate respiration and fermentation processes, the "breakup" may have aerobic metabolism and the "autumn input" a mixture of both types of metabolism in addition to nitrate respiration. Under these conditions, direct calorimetry is the most reliable method to estimate total benthic community metabolism, because it is a direct measure of energy flow through the system (Pamatmat, 1982).

The benthic response in terms of heat production in the top sediment layer is given in Fig. 5. In the top centimeter, there is a strong response to the "breakup", increasing heat production by a factor of 3. During this period there is a significant loss of protein (folin positive matter) in the sediment (Fig. 4), suggesting that it was consumed. From a microbiological point of view, there is no reason why bacteria should not be able to decompose protein under anoxic conditions (Fenchel and Blackburn, 1979). The high concentrations found, however, suggest that it was not available to them. Meyer-Reil (1983) reported a strong decrease of the activity of proteolytic enzymes in anoxic sediments. Thus, the stored protein food became available during the "breakup" when the anoxic situation terminated. The fast decomposition of large amounts of protein probably significantly contributed to the following buildup of suboxic conditions by oxygen consumption.

A second response is caused by the sedimentation of the autumn bloom. However, although there was a steady sedimentation of fresh, undecomposed matter throughout November, heat production already decreased on November 11 and stayed at this low level thereafter until mid December.

A less obvious response is found during "winter input". However, considering that a part of benthic metabolism is dependent on temperature, there is still a considerable and long lasting heat production during this period, and this even caused a decrease of the redox potential (Fig. 1). Such high levels of benthic activity during winter were also found by Hargrave (1973) in Saint Margaretes Bay, where macrophytes represent a major part of the food supply (Webster *et al.*, 1975).

During early March, heat production was at the lower detection limit, but increased drastically after March 17, indicating that the bulk of the settling spring bloom arrived during the second half of March. In contrast to spring 1980, when activity lasted at least until mid May (Graf *et al.*, 1982), benthic activity following the 1982 sedimentation event decreased as early as late April.

Developments in the sediment horizon 3 to 4 cm were very different. This deeper layer was anoxic until December 3 (Fig. 1). Thus, we do not know which mechanism caused the heat production response to the "breakup" (Fig. 5) and which reasons may explain the fluctuations during "autumn input". The possible role of bioturbation was of minor importance as will be discussed later. The final break of suboxic conditions in early December caused a drastic decrease of heat production, which remained at a low level throughout the winter. The response of the deeper sediment layer to the spring bloom sedimentation is less distinct than in the top centimeter. Surprisingly, however, the response seems to start 5 d earlier than at the sediment surface and this cannot be explained. The low ETS-activity simultaneously observed on March 17 indicates that fermentation was of importance as will be discussed later. The measurement of ETS-activity based on INT reduction comprises several types of respiration chains (cf. Tabor and Neihof, 1982). Besides O_2-respiration, the method positively responds to nitrate respiration (Packard *et al.*, 1983) and most likely to sulphate respiration (Bengtsson, 1982). During this program, however, the method turned out not to be very informative. Two factors strongly influencing *in-situ* activity, namely food limitation and low temperature, are optimised or kept constant in the enzyme reaction. Thus, only potential activity is given. The high ETS-activity found during "summer stagnation" obviously indicates the importance of sulfate respiration (Fig. 5). In the top centimeter of the sediment (Fig. 5), there is only a slight response in ETS-activity to "autumn input" and to the preceding changes in the physical environment and to "spring input". No response is to be seen of "winter input" and the low activity during the end of this period.

Benthic biomass excluding macrofauna was determined by ATP-measurements. The ATP-concentration

comprises the biomass of bacteria, protozoa (especially ciliates, flagellates, and foraminifera), meiofauna, and temporary meiofauna. As shown in Fig. 5, there is a good agreement between the development of the ATP-biomass with the defined periods given. The consumption of stored food during the "breakup", as well as the three following external supplies of organic matter, elicit immediate response by an increase in ATP-biomass.

Changes in the physical environment as indicated by redox potential was one factor explaining the decrease of ATP-biomass at the end of the periods "breakup", "autumn input", and "winter input". The concomitant minima of protein concentration in the sediment, however, also suggest that food limitation might have been of importance and that organic matter, including attached organisms, were resuspended.
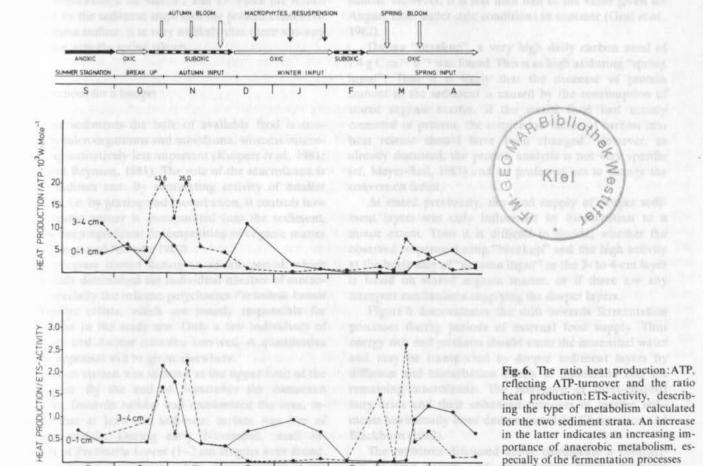
The deeper sediment horizon (3 to 4 cm) showed a corresponding biomass pattern (Fig. 5). The effects, however, are found to be less pronounced, especially during "autumn input" and "winter input".

The ratio of heat production:ATP reflects ATP-turnover. Pamatmat *et al.* (1981) demonstrated that the heat loss and ATP-turnover of a bacteria culture is most pronounced during exponential growth, but can be significantly reduced during steady state conditions. A corresponding explanation may be given for the shape of the

curve in Fig. 6. In the top centimeter of the sediment, every increase in ATP-biomass (Fig. 5) is accompanied or preceded by an increase in ATP-turnover. Thus, it is likely that the high ratio of 11.0×10^3 W mol^{-1} found on December 16 indicates that the population responsible for the high ATP-biomass during January had already started to grow, although biomass itself is still rather low. For this type of process, periods between sampling were too long.

The high ratios of heat production:ATP found in the deeper sediment horizon during the anoxic period indicate a faster turnover of ATP under these conditions. Especially such extreme ratios of 43.6×10^3 and 26.0×10^3 W mol^{-1} as found in October and November have otherwise only been reported from the exponential growth phase of an obligate anaerobic bacteria culture (Pamatmat *et al.*, 1981).

Type of metabolism

As already discussed, direct calorimetry measures all types of metabolism, but it also includes the activity of exo-enzymes and the heat release of chemical oxidation. The latter two components, however, are thought to be of minor importance for the heat loss of the sediments (Pamatmat, 1982; Bengtsson, 1982).

Fig. 6. The ratio heat production:ATP, reflecting ATP-turnover and the ratio heat production:ETS-activity, describing the type of metabolism calculated for the two sediment strata. An increase in the latter indicates an increasing importance of anaerobic metabolism, especially of the fermentation processes

The difference between the activity as determined by direct calorimetry and ETS-activity, which describes the activity of respiration chains, should thus mainly indicate substrate phosphorilation. i.e. fermentation processes. ETS-activity, however, is only a potential measure and it is not possible to calculate an absolute amount of fermentation. Therefore, it was preferred to establish the quotient heat production:ETS-activity as a qualitative indicator for changes in the type of metabolism (Pamatmat *et al.*, 1981). This calculation employs a conversion of ETS-activity into heat using the Ivlev coefficient (Ivlev, 1934).

In the top centimeter of the sediment, the three "external" food supplies during "autumn input", "winter input", and "spring input" were accompanied by a shift in the type of metabolism towards fermentation (Fig. 6). This change is especially pronounced during the suboxic period of the "autumn input". Unfortunately, ETS-activity was not measured on October 8 and it is unknown whether such a shift also occurred when the "internal" food store was used during "breakup".

For the deeper sediment horizon (3 to 4 cm), no values for the organic content are available. Therefore, it is not known whether the food supply influenced changes in the type of metabolism, as evident from Fig. 6, for the periods "autumn input" and "spring input". During "winter input" there was no response to the food supply of the sediment surface and it is not clear which process influenced the shift to fermentation on March 2 and 17. From the results obtained by the sediment traps and the protein content of the sediment surface, it is very unlikely that there was any connection with the spring bloom.

Considerations for a budget

In marine sediments the bulk of available food is consumed by microorganisms and meiofauna, whereas macrofauna is quantitatively less important (Kuipers *et al.*, 1981; Kemp and Boynton, 1981). The role of the macrofauna is a more indirect one. By stimulating activity of smaller organisms, i.e. by grazing and bioturbation, it controls how much organic matter is incorporated into the sediment, and how deep significant decomposition of organic matter occurs (Yingst and Rhoads, 1980).

Our program started during an anoxic period, which considerably diminished the individual number of macrofauna, especially the infauna polychaetes *Pectinaria koreni* and *Nephtys ciliata*, which are mainly responsible for bioturbation in the study site. Only a few individuals of *Astarte* sp. and *Arctica islandica* survived. A quantitative faunistic appraisal will be given elsewhere.

The 18-m station was situated at the upper limit of the anoxic area. By the end of September the cumacean crustacean *Diastylis rathkei* had recolonized the area, indicating that at least the sediment surface was free of hydrogen sulfide. During early November, small individuals of *Pectinaria koreni* (1–2 cm length) were found. In the course of the winter, other species settled at the station, most likely from shallower areas, e.g. *Nephtys ciliata*. During spring, the composition of macrofauna was still very different from that in other years (cf. Arntz, 1978). The low individual numbers, comprised largely of small molluscs, and the smaller size of the individuals present, support the finding of A. ıtz (1981), who described the development of macrofauna after anoxic periods.

From the results presented thus far, it appears that the low response of the deeper sediment layers during "autumn input" and "winter input" is due to the reduced effect of bioturbation. During "spring input", bioturbation already reached the 3- to 4-cm horizon. Differences in the type of macrofauna also represents one reason for the higher advection factor of particulate organic matter in the sediment from the spring bloom (advection factor 7–8), when compared to the atumn bloom (4).

The strong response of the deeper sediment layer during "breakup" is very surprising and can hardly be explained by bioturbation. One possible explanation is that this horizon had become temporarily oxic during the period September 23 to October 8. As a consequence, for budget calculations, different sediment depths have to be considered for the different period (Table 1). During the anoxic period, the consumption of organic matter is $0.8 \text{ g C m}^{-2} \text{ d}^{-1}$, demonstrating that under these conditions a considerable amount of organic matter can also be consumed. However, it is less than half of the value given for August 1980 under oxic conditions in summer (Graf *et al.*, 1982).

During "breakup", a very high daily carbon need of $1.4 \text{ g C m}^{-2} \text{ d}^{-1}$ was found. This is as high as during "spring input". Thus it is likely that the decrease of protein content in the sediment is caused by the consumption of stored organic matter. If the stored food had mainly consisted of protein, the conversion factor of carbon into heat release should have been changed. However, as already discussed, the protein analysis is not very specific (cf. Meyer-Reil, 1983) and we preferred not to change the conversion factor.

As stated previously, the food supply of deeper sediment layers was only influenced by bioturbation to a minor extent. Thus it is difficult to decide, whether the observed response during "breakup" and the high activity at the beginning of "autumn input" in the 3- to 4-cm layer is based on stored organic matter, or if there are any transport mechanisms supplying the deeper layers.

Figure 6 demonstrates the shift towards fermentation processes during periods of external food supply. Thus energy rich end products should enter the interstitial water and may be transported to deeper sediment layers by diffusion and bioturbation effects of meiofauna and the remaining macrofauna. The relevance of short-chained fatty acids and their enhanced turnover in marine sediments has recently been demonstrated by Christensen and Blackburn (1982).

The problems discussed above complicate a comparison of food supply and consumption during "autumn

Table 1. Budget for the periods defined in Fig. 5 based on direct calorimetry

	Sediment depth cm	Energy flow KJ m^{-2}	Carbon flow gC m^{-2}	Daily energy flow KJ m^{-2} d^{-1}	Daily carbon flow gC m^{-2} d^{-1}
Summer stagnation 9. 9.–1. 10. 81	5	7	16.8	32	0.8
Breakup 2.10.–27. 10. 81	5	1 411	35.3	54	1.4
Autumn input 28.10.–10. 12. 81	2	589	14.7	13	0.3
	5	1 700	42.5	38	0.9
Winter input 11.12.–6. 3. 82	2	588	14.7	7	0.2
Spring input 7.3.–26. 4. 82	4	2 817	70.4	55	1.4

The top two centimeters were calculated with the upper sediment layer (0–1 cm) and the deeper sediment layers with the 3- to 4-cm layer. For the conversion of Joule into C it was assumed that 1 g C is equivalent to 40 KJ

input". Therefore, two alternatives were calculated (Table 1). Although the consumption of 42.5 g C m^{-2} estimated for 5-cm sediment depth fits very well with the trapped input of 10–15 g C m^{-2} and an advection factor of 4, it is more likely that this fit occurred merely by chance. The sudden decrease of protein from November 19 to December 3, which is not accompanied by comparable levels of heat production, indicates that the sediment lost a considerable amount of organic matter, possibly by horizontal transport due to resuspension during the stormy period in late November.

Thus, it can be stated that the input of the autumn bloom is not completely consumed rapidly. This was also concluded by Smetacek and Hendrikson (1979) from the analysis of particulate organic matter from the water column during autumn and winter, which, in terms of chemical composition, remained similar to freshly produced phytoplankton.

The increase in protein (folin positive matter) and carbohydrates during December and January can thus also be an effect of resuspended matter still derived from the autumn bloom. However, it is not possible to separate this part of accumulation from the input of macrophytes.

Although there is a small maximum in heat production during winter, total consumption of organic matter is only 14.7 g C m^{-2}. This amount is not sufficient to explain the decrease of organic matter from January 27 to March 10, which was in the range of 54 g C m^{-2} (Meyer-Reil, 1983). Again physical processes such as resuspension and erosion must have been involved. A corresponding loss of organic matter was described by Graf et al. (1983) for a 10-m station at the same slope.

During spring, the latter processes are less important and thus the budget for "spring input" fits very well. 70.4 g C m^{-2} corresponds to an input of 10 to 15 g C m^{-2} and an advection factor of 7–8. The spring bloom was consumed within 5–6 wk and the decreasing activity at the end of the period was caused by declining food supply.

General conclusions

The extraordinary position of the 18- to 20-m zone within the channel system of the Kiel Bight was already pinpointed by Arntz (1978), who demonstrated that highest diversity and biomass production of macrofauna is to be found in this zone and who thought that this zone is of utmost importance for the food supply of demersal fish. On artificial substrate raised above the sediment level, macrofauna was able to produce as much as 600 g fresh weight m^{-2} yr^{-1} (Arntz and Rumohr, 1982). Graf et al. (1982) estimated the consumption of organic matter at a 20-m station to be 405 g C m^{-2} yr^{-1} under the assumption that no anoxic period occurs and that the macrofauna population is dominated by Pectinaria koreni. The peculiarities of the physical environment and the reduced role of macrofauna during the study period explain the lower carbon consumption during the period from September 1981 to April 1982. However, the advection factors observed sufficiently underline the favourable situation of the study site compared to other areas in the Western Kiel Bight. This preference might even be more improved, when an intact macrofauna population, which is able to diminish the loss of sedimentary organic matter by erosion, and resuspension, and by vertical transport into deeper sediment strata, is present.

In spite of the abundant food supply, there are still strong indications that benthic activity is food limited. This is demonstrated by the consistent pattern of food

input and activity as determined by direct calorimetry and by the budget for the spring bloom. The similar minima of protein on October 8, December 3, and March 17 suggest that they represent the lower limit of available protein. Because of the unspecific analysis, the bulk of the remaining protein (folin positive matter) is represented by humic acids. Although there is a high level of carbohydrates present during the last protein minimum, benthic activity in terms of heat production is nearly zero. The latter finding would strongly support the hypothesis of Hargrave (1980) and Hanson (1982) that benthic activity and microbial decomposition of detritus is limited by nitrogen supply. However, as already discussed, the remaining carbohydrates are difficult to decompose because of their macrophyte origin. Thus, we can only postulate the limitation of available food as a controlling factor of benthos.

Physical and biological processes in the shallow water ecosystem of the Kiel Bight turned out to proceed on the same time scales. At certain periods of the year, the processes are so quick that they have to be followed within days. Directly coupled to the weather conditions, processes such as sedimentation, accumulation, and inflow of oxygen rich water happen within few days and are immediately responded to by the biological component of the system without any time delay. Especially during autumn and winter, physical processes dominate the system. The food limitation during early December and early March discussed above is due much more to resuspension than to biological activity. One might speculate that there are areas, most likely in the deeper parts of the channel system, where no food limitation exists and where no zero activity occurs during winter.

The benthic response also includes a significant increase of ATP-biomass. This biomass is a storage for essential elements and in combination with fermentation end products it will lead to a delayed nutrient release from the sediment to the water column as was also suggested by Pollehne (1981).

As late as spring 1982, the benthic community was still being influenced by processes that took place in autumn 1981, indicating that the system has a historical aspect. Considering the coupling to weather and regional hydrography, it is easy to imagine how variable these processes may be. The input of the autumn bloom and of the macrophytes during winter will vary within 6 to 8 wk from year to year, whereas the input of the spring bloom is a more regular event and settles during mid March to mid April. Benthic response to the autumn and winter input might also be very different from the one described above. Whether a settling autumn bloom can be completely consumed by a well established benthic community remains to be seen. Under oxic conditions it is most likely. The minimum activity found during the period before the spring bloom input was 0.03×10^{-5} W g^{-1} in 1982, but 0.3×10^{-5} W g^{-1} in 1980 (Graf et al., 1982). In the spring periods analysed thus far, the input of freshly produced phytoplankton cells turned out to be a trigger for benthic

activity, however, the starting level each year was very different. This variability will represent one reason for the completely opposite shift in the type of metabolism, from anaerobic to aerobic, found in spring 1980 (Graf et al., 1982). Another reason is obviously given by the different type of macrofauna in 1980, which will have caused a much more extensive bioturbation especially caused by the very abundant polychaete *Pectinaria koreni*. However, as we do not know the history of the benthic community of spring 1980, this is only speculation. The problem encountered is that the benthic community is influenced by several years' cycles and that it is very difficult to set a zero point for an annual cycle in the Western Kiel Bight. This was already stated by Arntz (1981) for macrofauna.

The present investigation started during an anoxic period, which, during 1981, was obviously longer than in other years. It remains a matter of controversy whether this is due to the cumulative effect of increasing eutrophication or has a natural cause in unusual hydrography. This is not the place to enter this discussion. The processes described in this paper, however, are of relevance for further eutrophication studies.

The food supplies during winter, spring and most likely during autumn led in 1981/1982 to a temporary decrease of redox potential, indicating an oxygen debt. This has already been postulated by Pamatmat (1977) and Hargrave (1980). In an oxidized sediment, this debt is resolved rapidly and even an advection factor of 7–8, as found in spring 1982, decreased the redox potential only temporarily.

The critical point of such a system is therefore the time interval between the major sedimentation events. The variability of the processes during autumn and winter discussed here can lead to the following hypothetical pattern of processes. The oxygen debt caused by the winter input of macrophyte debris is not yet compensated when the spring bloom comes in. Thus, the spring bloom is burned less efficiently and when the water column becomes stratified during summer there will still be available food and an uncompensated oxygen debt. Such a pattern would strongly increase the probability of an anoxic period during summer.

An anoxic period that results in an incomplete consumption of the following autumn bloom will increase the probability of an anoxic period in the following year, because it increases the starting level of benthic activity and the amount of available food during the next spring. This effect, however, is partly compensated for by the disturbed macrofauna and the concomitantly reduced activity of the community. In summary it can be stated that it is possible to analyse relevant ecological processes and to estimate energy flow in benthos on a community level. This holistic approach to ecosystem analysis can, however, be significantly strengthened if combined with analyses at more specific levels. This has been demonstrated by the work of Meyer-Reil (1983), who analysed the bacterial component of the community simultaneously with this investigation.

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Benthic response to sedimentation events during autumn to spring at a shallow water station in the Western Kiel Bight

II. Analysis of benthic bacterial populations *

L.-A. Meyer-Reil

Institut für Meereskunde an der Universität Kiel; Düsternbrooker Weg 20, D-2300 Kiel 1, Germany (FRG)

Abstract

Seasonal variations in bacterial populations (total number, biomass, biomass-spectrum, number of dividing cells) as well as in concentrations and decomposition rates of particulate organic material were followed in a sandy mud sediment of the Western Kiel Bight (Baltic Sea; FRG). The strong seasonal variations observed could be traced back to the effect of certain ecological situations and events in the sediment from which the input of the phytoplankton blooms in autumn and spring, respectively, the accumulation of organic material during winter, and the spring development of the benthic fauna turned out to be the most important. Bacterial carbon net production following the breakdown of the phytoplankton blooms ranged between $9 \mu g$ (autumn) and $16 \mu g$ (spring) per g of dry weight sediment per day. The consequences of shifts in the size composition of the bacterial populations as well as the importance of the measurement of enzymatic decomposition rates of particulate organic material in sediments are demonstrated and discussed in relation to the events mentioned above.

Introduction

The role of bacteria in marine coastal sediments is only poorly understood, although sediments play an important function in nutrient regeneration for marine ecosystems. Most of the microbiological work was concentrated on nutrient cycles in mostly anoxic sediments. Indirectly, the activity of the benthic bacteria was concluded from changes in concentrations or turnover rates of inorganic

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and organic chemical parameters. The bacterial populations themselves, however, were regarded as a "black box", mediating any kind of substrate turnover, which was traced back to bacterial metabolism since other organisms could not be responsible.

Direct observations of number, biomass and activity of bacterial populations in marine sediments are rare. Meadows and Anderson (1966) and Weise and Rheinheimer (1978) analysed marine sandy sediments using scanning electron and epifluorescence microscopy. The authors could demonstrate by impressive photos that bacteria colonize the crevices and depressions of sand grains in high numbers and large diversity. They are protected there against mechanical demages. From the microscopic analysis, the complexity of the particle surface as a microenvironment became obvious. It consists of an organic matrix of polysaccharides and detritus to which the bacteria are attached or embedded in.

Through the investigations of Dale (1974), the high number of bacteria in marine sediments was quantitatively documented. Subsequent studies (Griffiths et al., 1978; Meyer-Reil et al., 1978; Kepkay et al. 1979; Weise and Rheinheimer, 1979) confirmed these observations, analysing different types of sediment. Information on bacterial biomass is limited. However, the analysis of Meyer-Reil et al. (1980) and Moriarty (1980) have demonstrated that, correspondent to their high number, bacteria contribute significantly to the living carbon standing stock in sediments.

In agreement with the fragmentary knowledge about benthic bacteria, investigations of the seasonal variations of number, biomass and composition of the bacterial populations are practically lacking. The question, however, arises how bacterial populations react to ecological situations and events, which occur seasonally-dependent in boreal coastal ecosystems. Among these events, the input of the phytoplankton blooms into the sediment in autumn and spring, respectively, (Graf et al., 1983 a), the accumulation of organic material during winter, and the

development of the benthic fauna in late spring are probably the most important.

During an interdisciplinary joint research program at Kiel University (Sonderforschungsbereich 95), the pelagic-benthic coupling of processes in the Western Kiel Bight (Baltic Sea; FRG) was followed. The first paper of this series deals with the analysis of pelagic and benthic processes on a community level (Graf et al., 1983 b). Closely related to this first report, this paper describes the response of the benthic bacterial populations to seasonal variations in the availability of organic material. Special emphasis was laid on the analysis of variations in the composition of the bacterial populations. Since particulate organic material represents the primary carbon source in sediments, the measurement of enzymatic decomposition rates of carbohydrate and protein seemed to be the most promising to be included in the investigation.

Materials and methods

Sampling

A series of sediment samples was withdrawn between September 9, 1981 and June 7, 1982, using a Reineck grab (surface area 20×28 cm) from an 18-m station located on the slope of the Kiel Bight channel system. This area, known as "Hausgarten", is restricted to research and has been subject to several investigations during recent years. At the station investigated, sandy mud prevails. For the analysis of parameters determined in this study and in Graf et al., 1983 b, the 0 to 1-cm horizons from three grabs were combined on board ship and transported to the laboratory in insulated containers.

Redox potential, temperature

Redox potential was measured immediately after sampling with an Eh-electrode (Ingold, Pt-4800-M5) connected to a MV-meter (Knick). Temperature was determined with a portable TS-probe (Electronic Switchgear).

Organic matter

Organic material was analysed from dried (60 °C, 24 h) and ground sediment samples (mortar Pulverisette 1; Fritsch GmbH). Total organic matter content was reported as the difference between the dry weight of the sediment and the residue left after combustion at 550 °C. Protein analysis was based on the method of Lowry et al. (1951) using bovine serum albumin (Boehringer GmbH) as a standard. As discussed in detail by Hendrikson (1975), various organic material (for example humic acids) interfere with the protein determination, so that the expression "Folin-positive" material would be more appropriate. Carbohydrates were assayed according to Handa (1967) as

modified by Hendrikson (1975) using soluble starch as a standard. Since the acid hydrolysis of the sediment had already resulted in a brown colour (the intensity of which was seasonal-dependent), these values (designed as acid-soluble material) were regarded as controls and subtracted from the sulfuric acid-phenol positive material (carbohydrates).

Decomposition of particulate organic material

The extracellular enzymatic decomposition rates of carbohydrates (α-amylase activity) and protein (proteolytic enzymes) were followed using Amylopectin Azure (Kim and ZoBell, 1974) and Hide Powder Azure (Little et al., 1979; Meyer-Reil, 1981), respectively, as substrates (Calbiochem). These covalent-bound dye derivates are stable, water insoluble and sensitive against enzymatic reaction. Sediment samples were suspended in the six-fold amount of ice-cold homogenization buffer and homogenized in a mortar (cf. above). The homogenization buffer consisted of KH_2PO_4 (0.07 M) and Na_2HPO_4 (0.07 M), pH 6.0, with the addition of 2 ml of Triton X-100 and 1.5 g of polyvinylpyrrolidon (per 1 000 ml of buffer; cf. Bengtsson, 1982). Later experiments have shown that the homogenization procedure can be omitted. The experiments described, however, were based on homogenized samples. This means that the analysis of the decomposition rates of particulate organic material relates to the potential of the total sediment sample, including all organisms present. The enzymatic reaction was started by adding substrate at concentrations high enough to saturate the enzyme systems (200 mg of Amylopectin Azure and Hide Powder Azure, respectively, per 30 ml of homogenized sediment). The samples were incubated under shaking (200 rpm) at room temperature. At 0.5-h (α-amylase) and 3-h (proteolytic enzymes) intervals, 1.5 ml of the sample was removed, transferred into centrifuge tubes, and the enzymatic reaction was terminated by the addition of 0.5 ml of a stopper solution, consisting of one part formalin (4%) and one part H_3PO_4 (1 M). After centrifugation (5 000 rpm, 15 min), the release of the dye was measured in the supernatant (spectrophotometer Zeiss PM 2K) at 595 nm. Controls received the stopper solution prior to substrate addition. At least five time-dependent readings were made comprising three parallels. Preliminary experiments have shown that the enzymatic reaction was linear for at least 24 h.

Enzymatic decomposition rates (changes in absorbance per h per g of sediment) were calculated from the slope of the time-dependent activity curves by linear regression (r^2 was usually above 0.96 and always above 0.90). Using conversion factors of 100 (Amylopectin Azure) and 118 (Hide Powder Azure), respectively, activity rates were converted into mg of substrate decomposed per h per g of sediment. The conversion factors were obtained from decomposing a known amount of substrate with enzymes commercially available (α-amylase, type III-A; protease,

type V; Sigma Chemical Co.). and measuring the resulting absorbance. The measurement of the decomposition rates of particulate organic material used in this study represents a further development of the method described by Meyer-Reil (1981).

Bacterial parameters

One cm³ sediment (3 parallels) was added to 10 ml of freshly prepared double distilled water supplemented with formalin (2%). Samples were sonicated (Sonifier B12; Branson Sonic Power) at 50 W for 1 min (ice bath) following a 30-s brake. This procedure was repeated three times. Scanning electron photographs demonstrated that this treatment liberates the overwhelming portion of the bacteria from the particles. After allowing the coarse particles to settle (30 s). subsamples were withdrawn from the supernatant and diluted 100 times with double distilled water supplemented with formalin (2%). Portions of the subsamples were added into the funnel of a filtration unit (Schleicher and Schüll) equipped with a Nuclepore filter (0.2-µm pore size, 25-mm diameter) prestained with Sudan Black (Zimmermann *et al.*, 1978). For a better distribution of the bacteria, a silver filter (Selas Flotronics) was positioned between the Nuclepore filter and the filter support (Zimmermann, 1977). The diluted subsamples were stained for 3 min with acridine orange (final concentration 1:10 000), and wedges of the filter were analysed with a drop of Cargille's immersion oil (type A) by epifluorescence microscopy (Zeiss Universal microscope, magnification × 1 600) using blue light excitation and an Osram HBO 200 burner (Zimmermann, 1977). Generally, bacteria were counted by means of a microscopic grid (40 × 40 µm) in a total of 40 microscopic fields distributed on three filters prepared from parallel samples. The microscopic fields were chosen at approximately even intervals between the periphery and the center of the filter to account for an uneven distribution of bacteria on the filter surface. The cell density was between 10 and 25 bacteria per grid. Only bodies with clear outline, bacterial shape and distinct fluorescence (orange or green) were counted as bacterial cells (Meyer-Reil, 1977). Conversions from bacterial number per cm³ of wet sediment to number per g of dry weight sediment were carried out after determining the dry weight content of the individual samples counted.

On the same epifluorescence microscopy preparations, the number of dividing bacteria defined as cells with a clearly visible invagination, were analysed. At least 100 dividing bacteria were counted on filters prepared from three parallel samples (cf. above). Although the counting procedure was very time consuming, and the values obtained certainly represent an underestimation of the actual number of dividing cells, this parameter turned out to be valuable for the interpretation of the seasonal variations of the bacterial communities.

For biomass determinations, colour slides were prepared from characteristic microscopic fields (Kodak

Ektachrome 400 film; exposure time 20–30 s) using a Zeiss CS-matic camera. The slides were projected onto the screen of a semi-automatic image analyser (MOP AM-02; Kontron). With a pencil, the inner outline of the sharply defined bacteria was copied and traced with the detection pen of the analyser. The data printed by the analyser comprise: maximum diameter, outline, area, and form factor. Assuming an ellipsoid, the volume of the individual bacteria can be calculated from area and maximum diameter as known variables. Biovolume (µm³) was converted into biomass (mg) by presuming a bacterial specific gravity of 1. For conversion into bacterial carbon, a factor of 0.1 was used. At least 60 bacteria were analysed, and the mean biomass per cell (mg of carbon) was calculated. The biomass of the total population (mg of bacterial carbon per g of dry weight sediment) followed from multiplying the mean biomass per cell by the total number of bacteria in the corresponding sample.

For the analysis of the biomass spectrum of the individual populations, bacteria were grouped into three size classes according to their volume: < 0–$0.3 \mu m^3$, 0.3–$0.6 \mu m^3$, and $> 0.6 \mu m^3$. Although as many size classes could have been established as bacteria were measured, these three size classes seemed to be the most suitable to demonstrate easy to survey seasonal variations in the composition of bacterial biomass. Subsequently, the total bacterial biomass associated with each size class was determined for the individual samples.

Bacteria ATP was calculated from carbon assuming a conversion factor of 1/250. For extrapolation, it has to be presumed furthermore that all bacteria contribute equally to the total bacterial ATP-pool, i.e. all bacteria counted were active. This assumption is certainly not justified. However, since the bacterial ATP data gained fit into the overall picture, the results were accepted, although the absolute ATP values remain questionable. Bacterial ATP was expressed as percentage of the total ATP content of the sediments as determined in Graf *et al.*, 1983 b.

Unless otherwise stated, chemicals were of p.a. grade, purchased from Merck Chemical Co.

Results

Redox potential, temperature

At the end of September, anoxic conditions (Eh −140 mV) were observed in the 0- to 1-cm horizon of the sediment. A storm at the beginning of October temporarily led to the introduction of oxygen into the sediment surface (Eh + 300 to + 420 mV). During November, suboxic conditions (Eh + 70 to + 200 mV) prevailed. Stable oxic conditions, however, were achieved in December and maintained throughout the winter with the exception of a decrease in redox potential in February and April. Temperature dropped gradually towards the winter (September 13.9 °C; January 2.0 °C) and increased slowly in spring (April 3.9 °C). An early drastic decrease in temperature
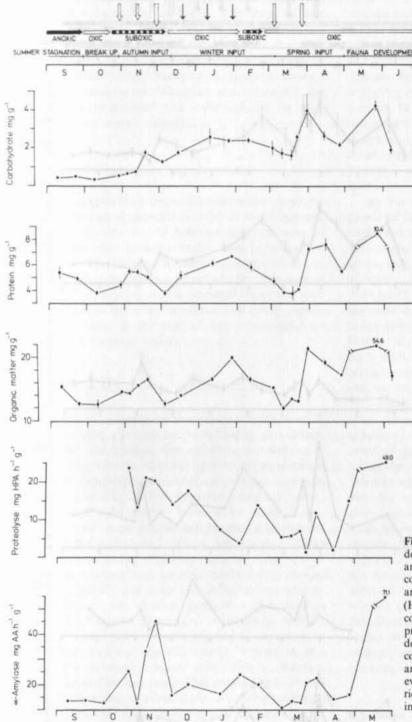
Fig. 1. Seasonal variations in concentrations and decomposition rates of organic material. Illustrated are: carbohydrates, protein, total organic matter content, activity of proteolytic enzymes and α-amylase expressed as mg of Hide Powder Azure (HPA) and Amylopectin Azure (AA), respectively, decomposed per h per g of dry weight sediment. Bars represent standard deviation of the mean (95% confidence level). For details of the measurement of the decomposition rates of organic material cf. "Materials and methods". The headline on top characterizes the events affecting the sediments. Arrows indicate the periods and the intensity of the input of organic material into the sediment

(from 6.2° to 3.2 °C) was observed in the first part of December. Details of the variations of redox potential and temperature are documented in Graf *et al.*, 1983 b. During the observation period, the water content (27.3% ± 1.5%) remained fairly constant. One cm³ of wet sediment corresponded to 1,300 ± 0.064 g of dry weight sediment (± values represent standard deviation of the mean, 95% confidence level).

Organic matter

The variations of organic matter in the sediment surface were followed by total organic matter content (ignition lost after combustion), protein (serum albumin equivalents) and carbohydrate (starch equivalents). In general, variations of these parameters were comparable (Fig. 1), although the ratios between the individual parameters
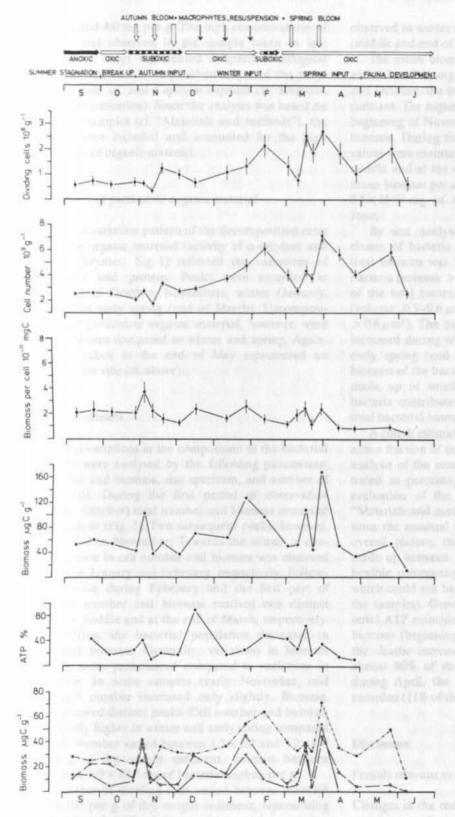
AUTUMN BLOOM • MACROPHYTES, RESUSPENSION • SPRING BLOOM

ANOXIC OXIC SUBOXIC OXIC SUBOXIC OXIC

SUMMER STAGNATION BREAK UP, AUTUMN INPUT WINTER INPUT SPRING INPUT FAUNA DEVELOPMENT

S O N D J F M A M J

Fig. 2. Seasonal variations in microbiological parameters. Illustrated are: number of dividing cells, total number of cells, mean biomass per cell, total biomass, bacterial ATP (% of the total ATP), and biomass-spectrum. The biomass-spectrum comprises: small-size bacteria of a volume $> 0-0.3\ \mu m^3$ (closed circles), medium-size bacteria of a volume $0.3-0.6\ \mu m^3$ (open circles), and large-size bacteria of a volume $> 0.6\ \mu m^3$ (crosses). Bars represent standard deviation of the mean (95% confidence level). For explanations of the headline on top see Fig. 1

varied strongly, reflecting quite different nutritional conditions for the benthic community. The variations of organic matter were characterized by the following pattern: peaks in autumn (mid November), high concentrations during winter (peaks in January), and maximum concentrations in early spring (end of March). Generally, concentrations of organic material were higher in winter and spring compared to autumn. Total organic matter varied between 12.1 and 21.5 mg per g of dry weight sediment, protein between 3.8 and 7.7 mg per g, and carbohydrates

between 0.4 and 4.0 mg per g. The high concentrations of organic material observed in the sample taken in late spring (end of May) represented a specific ecological situation characterized by high abundances of the polychaetes *Polydora* sp. and *Capitella capitata* (H. Rumohr, personal communication). Since the analysis was based on homogenized samples (cf. "Materials and methods"), the polychaetes were included and accounted for the high concentrations of organic material.

Decomposition of particulate organic material

Generally, the variation pattern of the decomposition rates of particulate organic material (activity of α-amylase and proteolytic enzymes; Fig. 1) reflected the variations of carbohydrates and protein. Peaks were recorded in autumn (end of October, November), winter (January, February) and early spring (end of March). Decomposition rates of particulate organic material, however, were higher in autumn compared to winter and spring. Again, the sample taken at the end of May represented an exception of the rule (cf. above).

Bacterial parameters

The seasonal variations in the composition of the bacterial populations were analysed by the following parameters: total number and biomass, size spectrum, and number of dividing cells. During the first period of observation (September, October) total number and biomass remained almost constant (Fig. 2). Two subsequent peaks, however, were recorded in November. Towards the winter, a continuous increase in cell number and biomass was observed with peaks in January and February, respectively. Following a decrease during February and the first part of March, cell number and biomass reached two distinct peaks in the middle and at the end of March, respectively. Towards spring, the bacterial population decreased in number and biomass. Generally, variations in biomass were much more pronounced compared to variations in cell number. In some samples (early November, mid March), cell number increased only slightly. Biomass, however, showed distinct peaks. Cell number and biomass were generally higher in winter and early spring compared to autumn. Number varied between 1.7×10^9 and 7.2×10^9 cells per g of dry weight sediment, biomass between 12×10^{-3} and 169×10^{-3} mg of bacterial carbon per g.

The number of dividing cells varied between 0.3×10^8 and 2.7×10^8 per g of dry weight sediment, representing between 2.0 and 5.9% of the total bacterial population (Fig. 2). Generally, the number of dividing cells was higher in winter and spring compared to autumn. During the first period of observation, the number of dividing cells was almost constant. The peaks recorded at the end of November corresponded to peaks in total bacterial number. The same applied to the high number of dividing cells

observed in winter (February) and late winter/early spring (middle and end of March, respectively).

The mean biomass per cell varied between 0.5×10^{-11} and 3.7×10^{-11} mg of carbon (Fig. 2). During September and October, the mean biomass per cell remained almost constant. The highest biomass values were recorded at the beginning of November, leading to the first peak in total biomass. During the winter, relatively high mean biomass values were maintained. Subsequent peaks occurred in the middle and at the end of March. Towards late spring, the mean biomass per cell strongly decreased to a minimum of 0.5×10^{-11} mg of bacterial carbon at the beginning of June.

By size analysis, the contribution of different size classes of bacteria (cf. "Materials and methods") to the total biomass was followed (Fig. 2). Generally, small-size bacteria (volume $> 0-0.3 \mu m^3$) represented the major part of the total bacterial biomass, followed by medium-size (volume $0.3-0.6 \mu m^3$) and large-size bacteria (volume $> 0.6 \mu m^3$). The biomass of all three size classes strongly increased during winter (January, February) and again in early spring (end of March). Towards late spring, the biomass of the bacterial population was almost exclusively made up of small-size cells. Individual size classes of bacteria contributed differently to the peaks observed in total bacterial biomass (cf. "Discussion").

A rough estimate on the contribution of bacteria to the active fraction of the benthic community is possible by the analysis of the seasonal variations of bacterial ATP illustrated as percentage of the total ATP (Fig. 2). For the evaluation of the data, the reservations mentioned in "Materials and methods" have to be considered. However, since the seasonal variations of bacterial ATP fit into the overall picture, the data were included. Bacterial ATP made up between 11 and 88% of the ATP content of the benthic community (with the exception of macrofauna, which could not be considered because of the small size of the samples). Generally, peaks in the percentage of bacterial ATP coincided with peaks in bacterial number and biomass (beginning of November, mid March) except for the drastic increase observed in mid December (up to almost 90% of the total ATP). In late November and during April, the lowest values in bacterial ATP were recorded (11% of the total ATP).

Discussion

Periods relevant to the benthic community

Changes in the redox-potential and the different stages of enrichment of organic material in the sediment surface gave rise to the description of certain periods relevant to the benthic community (cf. Graf *et al.*, 1983 b). The first period of observation coincided with the termination of the "summer stagnation", a period in which anoxic conditions prevailed in the sediment as a consequence of stratification in the water column overlying the sediment.

This "break up" of summer stagnation was characterized by oxic conditions at the beginning and by suboxic conditions at the end of October. The enrichment of organic material during November could be traced back to the input of the autumn phytoplankton bloom ("autumn input") dominated by armoured dinoflagellates. During winter, a continuous slow increase of organic material was observed in the sediment surface ("winter input"). It may be speculated that part of this material was derived from macrophytes eroded by winter storms (cf. Webster et al., 1975; Graf et al., 1983b). Material from terrestrial origin as well as resuspended sediment could have represented another part. The breakdown of the spring phytoplankton bloom (mainly diatoms) led to an enrichment of organic material in the sediment surface during late March to mid April ("spring input"). In late spring, the benthic fauna started to develop with high abundances of polychaetes. This period is called "fauna development". Seasonal variations in the enzymatic decomposition of particulate organic material and in benthic bacterial populations have to be interpreted as a reflection upon these different ecological situations and events in the sediment (cf. headline to Figs. 1, 2).

Enzymatic decomposition of particulate organic material

The overwhelming portion of the input of organic material into the sediment is particulate organic carbon, which has to be enzymatically decomposed, at least partly, prior to incorporation into cells. The pool of extracellular enzymes in sediments comprises those actively secreted by living bacteria (Corpe and Winters, 1972) as well as those liberated during the lysis of dead and decaying cells. Some of these enzymes may retain their activity by the formation of humic-enzyme complexes bound to clay particles (cf. model by Burns, 1980). If this hypothesis is valid, the continuous and wasteful production of bacterial enzymes is avoided. Since homogenized samples were assayed, the enzymatic activity rates determined in this study relate to the decomposition potential of the total benthic community with the exception of macrofauna.

Seasonal variations in the enzymatic decomposition of particulate organic material in relation to sedimentation events are only poorly understood. As could be shown in this study, the enrichment of organic material (carbohydrate, protein) in the sediment surface led to a corresponding increase in the enzymatic decomposition rates (activity of α-amylase, proteolytic enzymes). Generally, relationships between α-amylase activity and concentrations of carbohydrates were much more pronounced than relationships between proteolytic enzymes and protein. This may be explained by the specificity of carbohydrates and protein measurements (cf. "Materials and methods"). Enzymatic responses were higher in autumn compared to winter and spring. This is obviously a reflection of both the higher temperature and the higher benthic biomass in autumn. During the autumn and spring input, respec-

tively, high enzymatic decomposition rates already occurred when concentrations of carbohydrates and protein started to accumulate in the sediment surface, indicating an induction of enzymatic activity by increasing concentrations of suitable substrate.

There is evidence from the data that during the anoxic period of summer stagnation, protein accumulated. However, parallel to the break up of summer stagnation (temporary introduction of oxygen into the sediment), concentrations of protein decreased, whereas concentrations and decomposition rates of carbohydrate remained almost unchanged on a low level. A tentative explanation for these observations is provided by laboratory experiments which have shown that the decomposition of protein is strongly reduced under anoxic conditions. Under the same circumstances, the decomposition of carbohydrate is much less affected (Meyer-Reil, unpublished data). Unfortunately, during the period of decreasing protein concentrations, data on the decomposition rates are lacking.

Peaks in enzymatic activity rates coincided or were related to peaks in bacterial parameters (mainly cell number; cf. below). This stresses the important role of bacteria in the decomposition of particulate organic material, a process by which high molecular weight material from the primary production becomes available for higher trophical levels.

Seasonal development of the benthic bacterial community

The total number of bacteria as determined by epifluorescence microscopy (between 17.3×10^8 and 71.7×10^8 cells per g of dry weight sediment) agree well with data reported in the literature for different types of sediment (Dale, 1974; Griffiths et al., 1978; Meyer-Reil et al., 1978, 1980; Weise and Rheinheimer, 1979). Bacterial carbon (between 12 and 169 μg per g of sediment) contributed significantly to the biomass standing stock in the sediments investigated (cf. data on ATP). Again, the range of the bacterial carbon data is in accordance with the literature, although the information is limited (cf. Meyer-Reil et al., 1980; Moriarty, 1980).

Information on number, biomass and size spectrum of bacteria in the literature is almost exclusively based on single observations from which no general trend for seasonal variations could be detected (Cammen, 1982; Montagna, 1982). However, as shown in this study by a high time resolution in sampling, the development of the bacterial community is strongly influenced by seasonal variations in the nutrient supply closely connected to the specific ecological situations and sedimentation events mentioned above.

Changes from anoxic to oxic conditions (break up of summer stagnation) led to an internal shift in the composition of the bacterial biomass: large-size bacteria ($> 0.6 \, \mu m^3$) tended to decrease. Parallel to this, bacterial ATP, as percentage of the total ATP, strongly decreased

from 43% (anoxic conditions) to 18% (oxic conditions). With decreasing Eh-values towards the beginning of November, however, bacterial ATP again increased (up to 40% of the total ATP), indicating an increasing importance of bacteria in benthic metabolism under suboxic conditions. Corresponding observations could be made following drastic changes in temperature. In mid December, an early strong decrease in temperature was observed (from 6.2° to 3.2°C within less than two weeks). Bacterial ATP as percentage of the total ATP increased drastically (from 17 to 88%), indicating a strong reduction in the active biomass of the remaining benthic community and a dominance of bacterial biomass.

The accumulation of the more refractory organic material during the winter input was accompanied by a slow continuous increase in total bacterial number, number of dividing cells and biomass. In January/February bacterial parameters reached values that were even higher than those observed in autumn following the input of the phytoplankton bloom. This is surprising when the low temperature is taken into account. However, the limited number of grazers during winter and the relatively long time the bacterial population had available for its "undisturbed" development may explain the high bacterial standing stock in winter. In this respect, the development of the bacterial population differed basically from its development during autumn and spring, respectively (cf. below). Generally, it has to be considered that, with the sedimentation of particles onto the sediment (material from terrestrial origin, phytoplankton blooms), bacteria attached to particles enter the sediment. From the low density of bacteria on particles in the water column, a significant contribution of these bacteria to the autochthonous benthic bacterial population must be doubted.

Compared to the development of the bacterial population following changes in redox-potential, temperature and the input of organic material during winter, the response of the bacteria to the input of the phytoplankton blooms in autumn and spring, respectively, is much more complex. Generally, two separate, distinct peaks in bacterial parameters have to be distinguished. The first peak occurred when concentrations of organic material from the phytoplankton blooms started to accumulate in the sediment surface. Parallel to this, bacterial ATP significantly increased (up to 65% of the total ATP). This demonstrates that the bacterial population reacted almost immediately to the availability of decomposable organic material. The second peak in bacterial parameters was observed to coincide with the main input of organic material into the sediment following the final breakdown of the phytoplankton blooms.

Differences in the response of the bacterial populations to the input of organic material in autumn and spring, respectively, were certainly caused by differences in both the nutrient supply and the history of the bacterial populations. The autumn phytoplankton bloom was a mixed population dominated by armoured dinoflagellates, whereas at the end of the bloom, diatoms became more

important. Part of the settled organic material may indeed be worked up by organisms in the water column. The bacteria faced with the input of this material were derived from an anoxic population (mainly fermentative bacteria, sulfate reducers) prevailing during the period of summer stagnation. Within this population, the input of freshly produced organic material caused a drastic shift. Bacteria primarily reacted with a strong increase in cell volume (biomass production). Not till the main input of organic material did the bacteria subsequently respond with cell division (increase in cell number). The spring phytoplankton bloom was mainly composed of diatoms, which at that time of year almost totally sink to the bottom due to the absence of zooplankton (v. Bodungen et al., 1975). This material represents one third of the total yearly input into the sediment (Smetacek, 1980). Compared to autumn, the history of the bacterial population was quite different. Oxic conditions prevailed in the sediment during winter. Bacterial number, biomass and organic material were obviously declining due to the erosion of the sediment caused by winter storms. The input of freshly produced, almost unmodified organic material hit an impoverished bacterial community, which immediately reacted with both biomass production and an increase in cell number. Following a temporary decrease in bacterial parameters (cf. below), the main input of organic material into the sediment again stimulated biomass production and cell division: bacterial number and biomass reached their maximum values.

The further fate of the bacterial community in late spring was greatly dependent upon the development of the remaining benthic community. At the end of May, high abundances of the polychaetes Polydora sp. and Capitella capitata were observed in the sediment (H. Rumohr, personal communication). By their action, the sediment surface was firmly glued together. This specific ecological situation was reflected by an individual bacterial population consisting of almost exclusively small-size cells. Two reasons may be responsible for the pauperization of the benthic bacterial community: nutrient deficiency because of the limited transport through the consolidated sediment surface and, secondly, preferential grazing of medium- and small-size bacteria by the polychaetes. Since the bacteria actively grew (high number of dividing cells), the latter hypothesis is favoured. There is indeed evidence from the literature that grazing stimulates the metabolic activity of bacteria (Gerlach, 1978; Morrison and White, 1980). When the polychaetes had disappeared, bacterial parameters reached their lowest values. The population seemed to be indeed nutrient-limited.

Size spectrum of bacteria

As mentioned above, small-size cells ($< 0.3 \mu m^3$) dominated the bacterial biomass. Generally, with increasing cell size, the associated biomass decreased. This is in

agreement with the observation of Schwinghamer (1981), who found a characteristic distribution of benthic biomass ("Sheldon" spectrum) with two main peaks in the largest (> 2 mm: corresponding to macrofauna) and in the smallest size classes (< 2 μm: corresponding to bacteria), respectively. The author has already pointed out that variations from the typical pattern might be interpreted as the effects of exogenous disturbance. This can be related to the distribution of bacterial biomass as well. The input of the phytoplankton blooms in autumn and spring, respectively, into the sediment caused a drastic shift in the composition of bacterial biomass. Deviating from its "normal" distribution, the biomass was dominated by medium ($0.3–0.6 \, \mu m^3$) and large-size (> $0.6 \, \mu m^3$) bacteria. However, shortly after the exogenous "disturbance" (approximately one week), the normal pattern of distribution of the bacterial biomass was re-established: medium and large-size bacteria decreased, and small-size bacteria again dominated the biomass. Increasing predation pressure, especially on medium and large-size bacteria, may be the most important factor for the restitution of the normal distribution in connection with a stimulation of the cell-division of small-size bacteria. Analysing systems that differ from a pattern regarded as "typical" may offer a promising approach in understanding the dynamics of benthic bacterial populations.

Bacterial production

The high time resolution in sampling during autumn and spring permits an estimation of the bacterial net production in the sediment surface. As response to the input of the autumn phytoplankton bloom, bacterial production amounted to 9 μg of carbon per g of dry weight sediment per day. The corresponding values in spring were 8 and 16 μg of carbon per g per day, respectively, based upon the two peaks in bacterial biomass. However, for reasons discussed above, almost the same amount of bacterial carbon disappeared from the system within less than one week. It is interesting to note that these bacterial net production estimates correspond to the lower range of production measurements carried out in nearshore western Atlantic Ocean sediments using the thymidine uptake method (Fallon et al., 1983). With the same technique, Moriarty and Pollard (1982) found approximately one order of magnitude lower bacterial production values for surface sediments associated with seagrass beds in More-ton Bay, Queensland, Australia. Sandy beaches of the Kiel Fjord and the Kiel Bight revealed, under summer conditions, a bacterial net production of 5 μg of carbon per g per day based on the uptake of glucose (Meyer-Reil et al., 1980). This two to three times higher production corresponds to the higher bacterial standing stock carbon in shallow water sediments of the Kiel Bight (this study), indicating a similar bacterial production to biomass ratio in beaches and shallow water sediments, respectively, of the Kiel Bight.

Conclusion

This study presents a first insight into the dynamics of the decomposition of particulate organic material and the development of the bacterial populations in the shallow water marine sediments of the Kiel Bight. Based on a high time resolution in sampling, the strong seasonal variations in enzymatic decomposition rates of particulate organic material and bacterial parameters could be traced back to certain ecological situations and events in the sediment. Changes from oxic to anoxic conditions as well as drastic decreases in temperature strongly favoured the dominance of bacteria in benthic metabolism. Only during winter, in the absence of grazers, did bacteria exhibit an "undisturbed" development despite the low temperature. The bacteria almost immediately reacted to the input of freshly produced organic material into the sediment following the breakdown of the phytoplankton blooms in autumn and spring, respectively. The input of the autumn phytoplankton bloom primarily stimulated biomass production and the subsequent increase in cell number. In early spring, both bacterial biomass production and cell division were stimulated simultaneously. In late spring, the fate of the bacterial population turned out to be greatly dependent upon the development of the benthic fauna. Increasing grazing pressure led to an impoverished bacterial community consisting of almost exclusively small-size cells. Deviations from the "normal" size distribution pattern of bacteria have to be interpreted as indications of effects of exogenous disturbance factors. Generally, concentrations of particulate organic material (carbohydrate, protein) were closely related to enzymatic decomposition rates (activity of α-amylase, proteolytic enzymes). Peaks in bacterial parameters (mainly cell number) coincided with peaks in enzymatic activity rates, stressing the importance of bacteria in the decomposition of particulate organic material. Future research will be concentrated on investigations of shifts in the metabolic activity of the benthic bacterial populations as well as on interactions between bacteria and the faunal components of the benthic communities.

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A. FAUBEL and L.-A. MEYER-REIL

Measurement of enzymatic activity

of meiobenthic organisms :

methodology and ecological application.

MEASUREMENT OF ENZYMATIC ACTIVITY
OF MEIOBENTHIC ORGANISMS:
METHODOLOGY AND ECOLOGICAL APPLICATION (1)

by

A. Faubel

Zoologisches Institut und Museum, Universität Hamburg, Martin-Luther-King-Pl. 3, 2 Hamburg 13, Federal Republic of Germany.

and

L.-A. Meyer-Reil

Institut für Meereskunde, Universität Kiel, Düsternbrooker Weg 20, 23 Kiel, Federal Republic of Germany.

Résumé

Pour leur alimentation, les organismes méiobenthiques dépendent directement du stock de matière organique particulaire (POM) dans le sédiment. Abstraction faite de quelques rares espèces, on n'a guère réalisé jusqu'à ce jour d'études sur la digestion et le taux de dégradation chez des Annélides, des Turbellariés et chez d'autres taxons voisins. Pour la présente étude, les mesures d'activité protéolytique et hydrolytique (α-amylase, β-D-glucosidase) ont été choisies comme indices principaux pour estimer le taux de dégradation de la matière organique dans la méiofaune. Basés sur des expériences antérieures sur l'influence des procédés d'homogénéisation, de température et de salinité sur l'activité enzymatique, les résultats ont été assez nets en ce qui concerne la stabilité et l'activité des enzymes, pourvu que l'estimation de ces dernières se produise dans des conditions adéquates. Des expériences de dégradation de la matière organique ont été effectuées dans des systèmes sédimentaires marins pour évaluer l'importance de la méio- et de la microfaune dans la dégradation de la matière organique. Par rapport à l'activité de la β-D-glucosidase, ces expériences ont mis en évidence la contribution des organismes méiobenthiques pour le tiers de l'activité au taux de la dégradation. Dans un programme du Kieler Bucht ("Hausgarten", juin 1980), l'activité digestive des Oligochètes (Lumbricillus lineatus, Grania postclitellochaeta, Peloscolex benedeni) et des Némertiens (Cephalothrix sp.) a été étudiée pendant une journée. Les résultats obtenus ont mis en évidence que la dégradation des glucides (α-amylase-activité) par les Oligochètes et par Cephalothrix sp. suit un rythme journalier pendant lequel le maximum de l'activité a été observé le matin et vers midi.

Introduction

The aim of the investigation presented was to follow short-term activities of meiobenthic organisms from natural sediments. Enzymatic measurements promised to be adequately sensitive to achieve direct results.

Very few ecological studies have been carried out on the enzymatic decomposition of biological polymers by marine meiobenthic

organisms (Nielsen, 1966; Faubel and Meyer-Reil, 1981), especially compared with macrofaunal groups such as mollusks or arthropods (Kristensen, 1972). The most common polymers are protein and carbohydrates which undergo degradation by diverse decomposers in the benthic environment. To a certain extent, meiofauna organisms are involved in these degradation on particulate organic matter as a food source (Fenchel, 1970; Tenore, 1977; Gerlach, 1978; Riemann and Schrage, 1978; Meyer-Reil and Faubel, 1980).

Various methods have been used in the past to assess biological activity in aquatic environments and soils (references *in* Verstraete *et al.*, 1976; Wieser and Zech, 1976). Corpe and Winters (1972) and Kim and ZoBell (1972) demonstrated that α-amylase, β-D-glucosidase and proteolytic enzymes are cretion products of marine bacteria. The authors suggested that the degradation and mineralization of particulate organic matter result from the activities of these enzymes secreted from living cells as well as liberated during the lysis of cells, originating from micro-organisms either in the bottom sediments or throughout the water column or in the intestines of animals.

Generally, it is known that in lower metazoa the mode of digestion is an intra- and/or extracellular process. This takes place in the digestive tract in which larger food particles have to be introduced prior to being subjected to hydrolysis. Qualitative tests in different benthic animals revealed that the secretion of α-amylase and proteolytic enzymes is located in the digestive system (Nielsen, 1966; Michel, 1977; Marsden, 1963), known from higher metazoa. The synthesis of β-D-glucosidase by intestinal walls has been pointed out, for example, in *Mesenchytraeus glandulosus* (Nielsen, 1962) and other wrack invertebrates (Nielsen, 1966). Very little information exists about enzymes of Turbellaria (Jennings, 1974).

Based on the knowledge of determining enzymatic decomposition of organic matter by micro-organisms, pioneered by micro-biologists (Corpe and Winters, 1972; Kim and ZoBell, 1972 : Little *et al.*, 1979; Meyer-Reil, 1981), the authors intended to adopt the methods to meiobenthic organisms. Up to recently, this is the first approach to describe activities of natural meiobenthic individuals on the basis of feeding dependent enzymatic activity. Therefore, the main purpose of this ecophysiological approach is to standardize reliable methods of determining proteolytic and hydrolytic activity of meiobenthic organisms which allows to acquire information on short-term variations or rythms and to evaluate the contribution of meiobenthic animals to the decomposition of particulate organic matter in natural sediments.

Area of investigation

The organisms used in these studies were collected from the midlittoral sandy beach near "Falkenstein" in the brackish water Kiel Fjord and from a station in the investigation area called "Hausgarten", located at a depth of 10m in the brackish water Kiel Bight (Baltic Sea), during periods in June and August 1980. The

collection site at the beach near "Falkenstein" was the "Otoplanenzone", a habitat where the beach sediments were subjected to brief periods of exposure to brackish water and air. Influenced by wave action, there is a high contact of Oligochaeta and Otoplanidae (Turbellaria) with accumulated marine plant debris from bottom runoff. In this region the dominant meiofauna components are Oligochaeta (*Lumbricillus lineatus* (O.F. Müller)), the rich supply of decaying algae making this habitat favourable for this species (Giere and Hausschildt, 1979). Other important meiobenthic species are the Turbellaria Otoplanidae: *Bothriomolus balticus* Meixner, 1938; *Itaspiella helgolandica helgolandica* Sopott, 1972; *Otoplanella schulzi* (Ax, 1951).

The meiofauna found in the "Hausgarten" area represents a wide spectrum of species. Nematodes were by far the most important group, making up from 59 to 82 percent of the total. Harpacticoids were next in abundance. Other meiofaunal taxa were less numerous, but they were found in all cores every time (Scheibel, 1976).

Material and methods

Sediment sampling.

The sampling for meiofauna was carried out by pushing a short (0-15 cm) plastic core liner (4.9 cm inner diameter) into the sediment. For abundance analysis, the sediment in the core was immediately extruded and cut into segments at 2 cm levels. In the laboratory the samples were fixed with 8 percent formalin and sorted later on. Other samples were sorted within a day, while the animals remained alive. Separating the fauna from the sediment was performed by the shaking supernatant technique of Wieser (1960). Thorough shaking removed nearly all of the meiofauna organisms from the sediment. Weighing for biomass calculations was performed with a Mettler microbalance (range 10^{-2} to 10^{-7} g) and carbon was analyzed with a CHN-analyzer (Hewlett Packard 185 B). The organisms were washed well with filtered seawater (0.2μm), then pipetted onto a prepared GF/C Whatman filter and dried at 60° C. Mean grain size and organic matter content were analyzed as described by Meyer-Reil *et al.* (1978).

Enzyme preparations and assay techniques.

The enzymes (α-amylase, β-D-glucosidase, proteolytic enzymes) investigated were prepared from freshly sampled animals or homogenized sediment samples and essayed spectrophotometrically (PM 2K, Zeiss). These preparations were carried out a short time before assaying and the crude enzyme mixtures were always stored at 4°C. According to Curl and Sandberg (1961), at 25°C enzymatic activity diminishes by about 50 percent over a period of 6h and the activity is lost after 20h. The crude enzyme solutions were prepared by homogenization technique such as grinding with a glass rod, sonication (3 min, 50 watt; Sonifier B 12, Branson Sonic Power) or by Ultra-Turrax.

Animals separated from the sediment were washed well in 0.2 μm filter sterilized seawater and a distinct number depending on body length, was homogenized in 1.5 ml sterilized seawater (pH= 8.2). For determination of enzymatic activity of total fresh sediment samples (10 cm³), 10 ml sterilized seawater was added and the mixture sonified. The general assay procedure outlined below was followed in the enzymatic activity determinations of α-amylase, β-D-glucosidase, and proteolytic enzymes. For the determination of the activity of α-amylase and proteolytic enzymes, the reaction mixture contained as substrate amylopectin azure (AA) (Kim and ZoBell, 1972) and hide poder azure (HPA) (Little et al., 1979), respectively. The assay mixture of β-D-glucosidase contained p-nitrophenyl-β-D-glucosid dissolved in NaHCO₃ buffer, pH 9, (Morrison et al., 1977) as the substrate. Variables, as indicated in the text and figures, were homogenization techniques, temperature, and salinity. All experiments were performed in triplicate test tubes, accompanied by controls and blanks for each assay. Enzyme assays were carried out in glass centrifuge tubes containing: 1.5 ml sterilized seawater, 0.1 ml homogenized sample (crude enzymes) and the respective substrate. Incubation experiments using homogenized sediment samples were carried out in Erlenmeyer flasks (100 ml). These were charged with 10 cm³ sediment sample, sterilized seawater and the respective substrate. Measuring β-D-glucosidase activity the Erlenmeyer flasks were charged with only 10 cm³ sediment and p-nitrophenyl-β-D-glucosid. Before incubation, controls were heated at 100°C for 30 min. Blanks were charged with substrate only. The tubes were stoppered and, according to the test, placed in an incubator while gently shaking for 4 incubation times. As the end of each incubation time, the content of the tubes was centrifuged (5000 rpm for 15 min at 4°C). The supernatant was decanted and assayed spectrophotometrically at 595 nm for dye released from AA and HPA and at 410 nm for p-nitrophenol. Control values were substracted and activity rates were determined from the slope of the activity curves calculated by linear regression. The proteolytic rates were determined from the slope of the proteolysis curve of absorbances between 0.020 and 0.400. The straight lines were fitted to the data for each time course by the least squares method. It was always above 0.950 thus justifying the 95 percent confidence level.

RESULTS AND DISCUSSION

Efficiency of homogenization techniques

Different homogenization techniques were used for the preparation of crude enzyme extracts. As illustrated in Fig. 1A-C, the highest activity of α-amylase in *Lumbricillus lineatus* was obtained after grinding the tissue of whole organisms with a glass rod as well as by applying homogenization by Ultra-Turrax (Fig. 1B), and

sonication (Fig. 1C). Initially, after sonication more activity could be detected compared to treatment with Ultra-Turrax. However, after 2h of incubation, the readings of the samples homogenized by Ultra-Turrax are somewhat higher.

In order to estimate the activity of α-amylase after homogenization of the tissue which remains bound to particles, an experiment with untreated and with filtered homogenized samples

α-Amylase

Lumbricillus lineatus

FIG. 1
Lumbricillus lineatus.

Effect of different homogenization techniques on the activity development of α-amylase. Animal tissues were homogenized by sonication (A), Ultra-Turrax (B) and grinding with a glass rod (C). Removal of the particles in the homogenized tissue by filtration through 0.2μm cellulose ester membranes resulted in a drastic decrease in the activity of α-amylase (D). Activity (ΔE/58,2μg dwt/h) is expressed as changes in absorbance at 595nm (dwt = dry weight).

riment with untreated and with filtered homogenized samples (0.2 μm cellulose ester membranes) was carried out. Since in the filtered sample (Fig. 1D) the activity decreases approximately by one half, it must be concluded that most of the α-amylase activity remains bound to particles.

For instance, Verstraete et al. (1976) demonstrate in an identical experiment with saccharase that these enzymes are either intracellulary bound or are complexed to the suspended solids in the water.

Activity of individual enzymes

Proteolytic enzymes, α-amylase, and β-D-glucosidase activities ($\Delta E/\mu g$ dwt/h) (dwt=dry weight) were assayed using crude homogenates derived from *Lumbricillus lineatus* (Oligochaeta) and Proseriata (Turbellaria). By plotting incubation time versus activity, typical curves (straight lines) were obtained, as shown in Fig. 1-2. The convenient incubation temperature in these experiments was found to be 25°C for proteolytic enzymes and β-D-glucosidase and
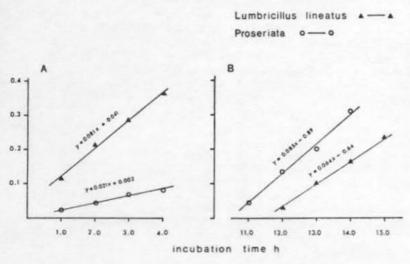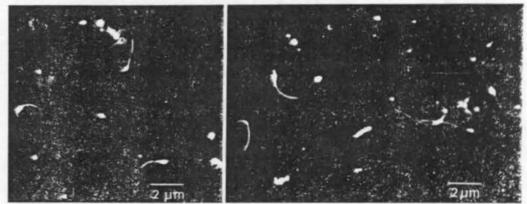
Lumbricillus lineatus ▲ —— ▲

Proseriata ○ —— ○

incubation time h

FIG. 2

Lumbricillus lineatus and Proseriata.

Time course of the activity of β-D-glucosidase (A) and proteolytic enzymes (B) in homogenized tissue from the oligochaete *L. lineatus* and Proseriata. Activity ($\Delta E/ 0.1mg$ dwt/h) is expressed as changes in absorbance at 410nm (β-D-glucosidase) and 595nm (proteolytic enzymes), respectively, (dwt = dry weight).

37°C for α-amylase. The results attained by assaying β-D-glucosidase activity ($\Delta E/100\,\mu g$ dwt/h) of Proseriata were less pronounced compared with the activity ($\Delta E/100\,\mu g$ dwt/h) of *L. lineatus* from digestive cells of animal's intestine (Fig. 2). The comparison of proteolytic activity ($\Delta E/100\,\mu g$ dwt/h) of Proseriata and *L. lineatus* (Fig. 2), however, proves that the activity of Proseriata is somewhat higher than that of *L. lineatus*. Indeed, apart from some studies on β-D-glucosidase of marine macrofauna organisms (Yokoe and Yasumasu, 1964; Okada *et al.*, 1966; Kristensen, 1972; Lewis, 1980) very little information exists on this enzyme of meiobenthic organisms (see Nielsen, 1962; 1966). As mentioned above, some animals possess cellulases, but in many cases the degradation and mineralization of these polymers is believed to result from the activities of micro-organisms (ZoBell, 1968).

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PLANCHE I

Epifluorescence photograph of micro-organisms inhabiting *Lumbricillus lineatus* (Oligochaeta). Homogenized animals (cf. Material and methods) were diluted and filtered onto Nuclepore polycarbonate membranes prior to staining with acridine orange. Bar represents 2μm.

Generally, as suggested in some reports (ZoBell and Feltham, 1938; Jannasch, 1954; Odum, 1968; Corpe and Winters, 1972; Meyer-Reil and Faubel, 1980; and other authors cited in the last paper), a considerable amount of digestive enzymes could be derived from micro-organisms inhabiting the digestive tracts for taken up with nutrient supply. As illustrated in Plate I, from homogenized *Lumbricillus lineatus*, micro-organisms colonizing the digestive tract were analyzed by epifluorescence microscopy (Meyer-Reil *et al.*, 1978). From Plate I, the occurrence of a great number of filamentous bacteria, which are very seldom found in water or sediment samples of the corresponding station, becomes obvious.

Effect of pH, temperature and salinity

It has been well established (Okada *et al.*, 1968; Kim and ZoBell, 1972) that the pH value influences enzymatic activity rates. Enzymes are catalytically active over only a restricted pH range and usually have a quite pronounced optimum pH. This optimum is generally near pH 7. The activity of α-amylase on amylopectin azure is almost linear near its optimum pH 6.0 or in seawater (Kim and ZoBell, 1972).

ᴈ-Amylase
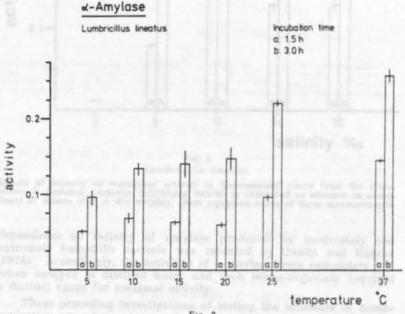
Fɪɢ. 3
Lumbricillus lineatus.

Effects of temperature on α-amylase activity in homogenized tissue from the oligochaete *L. lineatus*. Activity ($\Delta E/60.5\mu g$ dwt/h) is expressed as changes in absorbance at 595nm. Bars represent range of three measurements. (dwt = dry weight).

As illustrated in Figs. 3 and 4, increased activity of α-amylase was obtained with increasing temperature when incubated at temperatures of 5°, 10°, 15°, 20°, 25°, 37°C (pH 8.2). However, the pattern of the activity curves of α-amylase for *Lumbricillus lineatus* and Proseriata are quite different. The activity rates of crude α-amylase prepared from Proseriata as relatively low incubated at temperatures of 5° and 10°C, but relatively high activity rates of α-amylase
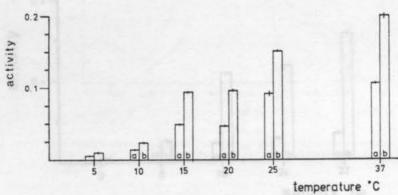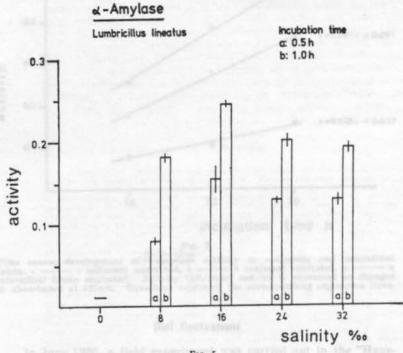

α-Amylase

Proseriata (Turbellaria)

Incubation time
a: 2.0 h
b: 4.0 h

temperature °C

FIG. 4

Effects of temperature on α-amylase activity in homogenized tissue from Proseriata (Turbellaria). Activity (ΔE/45,2μg dwt/h) is expressed as changes in absorbance at 595nm (dwt = dry weight). Bars represent range of three measurements.

were obtained from *L. lineatus* even at 5°C (approximately 32 percent of the activity measured at 37°C). In both, a very strong increase in enzyme activity was observed when the temperature was raised from 20° to 25°C. At the transition from 10° to 15°C, only α-amylase from Proseriata shows a great increase, approximately the four-fold of the activity assayed at 10°C. Comparable results were obtained from enzyme activity measurements of β-D-glucosidase in sediment cores investigated by Meyer-Reil (1981).

Within the salinity range studied (0, 8, 16, 24 and 32 permil S), different results were obtained for *Lumbricillus lineatus* and Proseriata (Fig. 5, 6). In the enzyme mixture of *L. lineatus*, the enzymatic activity ranges from 8 to 32 permil S with a peak around 16 permil S. In the extract from Proseriata, high enzymatic activity already arose at 16 permil S with a peak around 32 permil S. In both experiments, the activity of α-amylase in distilled water was completely lost. The peaks may reflect the optimal salinity range of

that habitat for these species. As reported (Giere, 1971; Sopott, 1972), both *L. lineatus* and the Proseriata (*Bothriomolus balticus, Itaspiella helgolandica* and *Otoplanella schulzi*) are also found in beaches of the North Sea with salinity values up to 32 permil S. Almost all species investigated show an eury-haline range in their enzymatic activity pattern displaying a peak proportional to the salinity of that area obviously being the proper habitat. The

α-Amylase

Lumbricillus lineatus

Incubation time
a: 0.5 h
b: 1.0 h

salinity ‰

FIG. 5

Lumbricillus lineatus.

Effects of salinity on α-amylase activity in homogenized tissue from the oligochaete *L. lineatus*. Activity (ΔE/60,5μg dwt/h) is expressed as changes in absorbance at 595nm (dwt = dry weight). Bars represent range of three measurements.
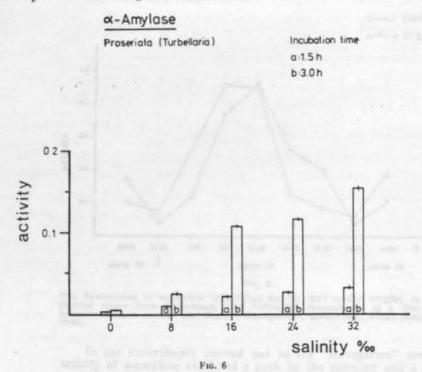
dependence on salinity of amylase produced by moderately and extremely halophilic bacteria was reported by Onishi and Hidaka (1978). Accordingly, the activities of α-amylase were completely lost when assayed in distilled water and each micro-organism required a distinct range for maximal activity.

These preceding investigations of testing the influence of homogenization procedures, temperature and salinity of enzymatic activity are believed to be of importance prior to study enzymatic degradation and activity rates in a benthic system. The results obtained reveal that there is a fairly high reliability for enzymatic stabilities and activities, if the enzymatic assays are subjected to adequate

conditions. Based on this, experiments were performed to determine the contribution of meiofauna organisms to the decomposition of POM. Furthermore, it was investigated wether the enzymatic activity of meiofauna organisms follows diurnal cycles.
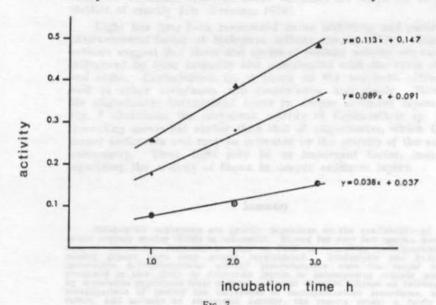
α-Amylase

FIG. 6

Effects of salinity on α-amylase activity in homogenized tissue from Proseriata (Turbellaria). Activity ($\Delta E/45.2\mu g$ dwt/h) is expressed as changes in absorbance at 595nm (dwt = dry weight). Bars represent range of three measurements.

Sediment activity

Aliquots of sediment (10cm³; 1cm depth) collected from the sandy beach of Falkenstein were used for studying the activity of β-D-glucosidase originated through the activity of micro-organisms and of meiofauna individuals. The following components of the samples were incubated for assaying β-glucosidase activity: (a) sediment samples untreated, (b) sediment samples homogenized by sonication, and (c) the interstitial fauna of the sample extracted by shaking and decanting the supernatant. Category (c) was homogenized as well as (b). As the results illustrate in Fig. 7, in each category (a, b, c) activity of β-D-glucosidase was recorded but, as expected, the enzymatic activity of each assay exhibits different activity values. In (b), the curve represents the highest activity, and comparatively in (a) and (c) lower activities are recorded.

Adding up these activity values of the components (a) and (c), the activity values of curve (b) will be obtained. The share of the degradation rate of meiobenthic individuals according to β-D-glucosidase activity amounts to about a third of the total activity.

$y = 0.113x + 0.147$

$y = 0.089x + 0.091$

$y = 0.038x + 0.037$

incubation time h

FIG. 7

Time course development of β-D-gluc. activity in sediments and interstitial fauna. - ——— - sediment untreated, Δ ——— Δ sediment sonicated, o ——— o interstitial fauna sonicated. Activity ($\Delta E/10cm^3$ sed./h) is expressed as changes in absorbance at 595nm. Equations represent the corresponding regression lines.

Diel fluctuations

In June 1980, a field experiment was carried out in the "Hausgarten" area of the Kiel Bight to study diel variations of biological and chemical parameters. The sediment sampling area was marked by a grid of squares in order to obtain statistically valid samples. Salinity, temperature and wind remained relatively constant during the study period. Samples were withdrawn from the sediment at 4h intervals during a 36h cycle by divers. Oligochaetes (*Lumbricillus lineatus*, *Grania postclitellochaeta*, *Peloscolex benedeni*) and Nemertini (*Cephalothrix* sp.) were separated by decantation while alive. For each assay, equal amounts in number and weight of both organisms groups (Fig. 8) were taken to prepare crude enzyme extracts of animal tissues. The measurements of α-amylase activity were performed after an incubation period of three hours. Plotting incubation time versus activity (Fig. 8) reveals that the decomposition of carbohydrates (α-amylase activity) in Oligochaeta and *Cephalothrix* sp. followed a diel cycle.

Comparable results of diel fluctuations could be demonstrated

for biomass and uptake of dissolved organic substances by bacteria, as well as for living biomass in the sediment cores (ATP) (G. Graf and L.-A. Meyer-Reil, unpublished data). These fluctuations may also depend on vertical movements of *Peloscolex benedeni* belonging to the Tubificidae (Oligochaeta).
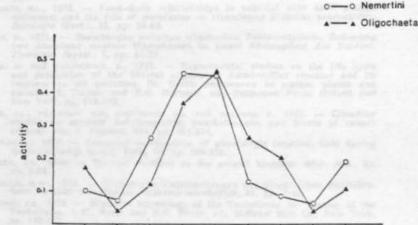

Fig. 8

Diel fluctuations of α-amylase (ΔE/26,5μg dwt/h) (dwt = dry weight) in homogenized tissue from Oligochaeta and Nemertini, respectively, at a 10m sandy sediment station in the Kiel Bight ("Hausgarten", Baltic Sea; FRG; June 12-14, 1980).

In the experiments carried out in the "Hausgarten" area the activity of α-amylase exhibited a peak in the morning and a continous decrease during afternoon and evening. The lowest amounts of relative activity were measured around midnight. The increase in α-amylase activity started again around 04.00h.

In all eucaryonts biological fluctuations or rhythms have been observed, but only recently have rhythms of enzyme activities also been detected (Harker, 1958; Halberg, 1960; Pittendrigh, 1960; Bünning, 1973; Rensing, 1973). Measurements of circadian fluctuations of enzyme activities have also been evaluated directly in crude extracts of homogenized animal tissues or indirectly through changes in metabolic activities (Glick *et al.*, 1961; van Pilsum and Halberg, 1964; Hardeland, 1969). The literature is very limited with respect to enzymatically dependent rhythms in invertebrates or even in meiobenthic organims. However, most recently diel fluctuations in microbiological and cheminal parameters could be demonstrated for bodies of water (Meyer-Reil *et al.*, 1979).

From the results presented the question arises wether these rhythms or fluctuations are of exogenous or endogenous nature. Generally, it is believed that circadian rhythms are endogenous

fluctuations, the properties of which are fixed genetically. However, this does not imply that endogenous rhythms are not directly influenced by environmental, i.e. exogenous factors such as temperature, lunarity, light intensity, atmospheric pressure, radiation and magnetic variation, since all these variables are subjected to periodicities of exactly 24h (Rensing, 1973).

Light has long been recognized as an initiating and controlling environmental factor of biological activity (Segal, 1970). Thus, the authors suggest that these diel cycles of feeding activity are strongly influenced by light intensity and coordinated with the cycle of day and night. *Cephalothrix* sp. is living on the sediment surface as well as other meiofauna and macrofauna individuals. However, the oligochaetes investigated occur in deeper sediment layers. As Fig. 8 illustrates, the enzymatic activity of *Cephalothrix* sp. starts increasing somewhat earlier than that of oligochaetes, which live in deeper sediments and may be activated by the activity of the surface community. Thus, light may be an important factor, indirectly regulating the activity of fauna in deeper sediment layers.

Summary

Meiobenthic organisms are greatly dependent on the availability of particulate organic matter (POM) in sediments. Except for very few species, however, natural enzymatic degradation rates of POM in annelids, turbellarians and related groups have been poorly investigated. Proteolytic and hydrolytic (α-amylase, β-D-glucosidase) activity measurements were the major indices examined in this study to determine trends in decomposing organic material by meiofauna organisms from natural sediment samples. Based on introductory investigations of testing the influences of homogenization procedures, temperature, and salinity on enzymatic activity, the results reveal a fairly high reliability for enzymatic stabilities and activities, if the enzymatic assays are subjected to adequate conditions. Experiments on degradation of organic matter in natural marine sediment systems were made to determine that part of decomposition which is originated by meiofauna and micro-organisms, respectively. The share of degradation rate of meiobenthic organisms according to β-D-glucosidase activity amounts to about a third of the total activity. In a field experiment in the Kiel Bight ("Hausgarten", June 1980), Oligochaeta (*Lumbricillus lineatus, Grania postelitellochaeta, Peloscolex benedeni*) and Nemertini (*Cephalothrix* sp.) were investigated for their activity of digestion over a diurnal cycle. The results reveal that the decomposition of carbohydrates (α-amylase activity) in Oligochaeta and *Cephalothrix* sp. follow a diurnal cycle showing maximum activity during morning and noon.

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Seasonal variations in bacterial biomass and decomposition of particulate organic material in marine sediments

By Lutz-Arend Meyer-Reil, Kiel

With 2 figures in the text

Abstract

Seasonal variations in bacterial populations as well as in concentrations and enzymatic decomposition rates of carbohydrate and protein were investigated in a sandy mud sediment of the Kiel Bight (Baltic Sea; FRG). Extracellular enzymatic activity rates were measured in time-course incubations using the covalent-bound dye derivates, Amylopectin Azure and Hide Powder Azure, respectively. These substrates are stable, water insoluble and sensitive against enzymatic reaction. The development of the benthic bacterial community was followed by epifluorescence microscopy. The strong seasonal variations observed in enzymatic decomposition rates of particulate organic material and in bacterial populations could be traced back to the effect of certain ecological situations and events in the sediment.

Methods

Sampling

A series of sediment samples was withdrawn between September 9, 1981 and June 7, 1982, using a Reineck grab from a 18 m station (sandy mud) located in the "Hausgarten" area on the slope of the Kiel Bight channel system.

Organic matter

Organic material (total organic matter, carbohydrate, protein) was analysed from dried and ground sediment samples according to standard procedures (for literature cf. Meyer-Reil 1983).

Decomposition of particulate organic matter

The extracellular enzymatic decomposition rates of carbohydrate (α-amylase activity) and protein (proteolytic enzymes) were followed using Amylopectin Azure (Kim & ZoBell 1974) and Hide Powder Azure (Little et al. 1979; Meyer-Reil 1981), respectively, as substrates (Calbiochem). These covalent-bound dye derivates are stable, water-insoluble and sensitive against enzymatic reaction. Sediment samples were suspended in the six-fold amount of ice-cold homogenization buffer and homogenized in a motar (Pulverisette 1; Fritsch GmbH). The homogenization buffer consisted of KH_2PO_4 (0.07 M) and Na_2HPO_4 (0.07 M), pH 6.0, with the

addition of 2 ml of Triton X-100 and 1.5 g of poly-vinylpyrrolidon (per 1,000 ml of buffer). Latter experiments have shown that the mechanical homogenization can be omitted. The enzymatic reaction was started by adding substrate (200 mg of Amylopectin Azure and Hide Powder Azure, respectively, per 30 ml of sediment). The samples were incubated under shaking at room temperature. At 0.5 h (α-amylase) and 3 h (proteolytic enzymes) intervals, 1.5 ml of the sample was removed, transferred into centrifuge tubes, and the enzymatic reaction was terminated by the addition of 0.5 ml of a stopper solution, consisting of 1 part of formalin (4 %) and 1 part of H_3PO_4 (1 M). After centrifugation (5,000 rpm, 15 min), the release of the dye was measured in the supernatant (spectrophotometer Zeiss PM 2K) at 595 nm. Controls received the stopper solution prior to substrate addition. At least 5 time-dependent readings were made comprising 3 replicates. Preliminary experiments have shown that the enzymatic reaction was linear at least for 12 h (Fig. 1). Enzymatic decomposition rates were calculated from the slope of the time-dependent activity curves by linear regression (r^2 was usually above 0.96 and always above 0.90). Using conversion factors of 100 (Amylopectin Azure) and 118 (Hide Powder Azure), respectively, activity rates could be converted into mg of substrate decomposed per h per g of sediment. The conversion factors were obtained from decomposing known amounts of substrate with enzymes commercially available (α-amylase, type III-A; protease, type V; Sigma Chemical Co.) and measuring the resulting absorbance.
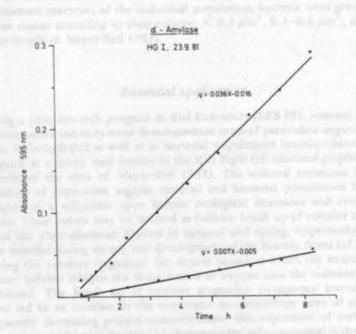

Fig. 1. Time-course development of α-amylase activity measured in a homogenized (curve above) and in a non-homogenized (curve below) natural sediment sample from the Kiel Bight (Baltic Sea; FRG). For details compare text.

Bacterial parameters

Sediment samples (3 replicates, fixed in double distilled water with 2 % formalin) were sonicated (Sonifier B 12; Branson Sonic Power) in an ice bath at 50 Watt three times in 1 min intervals following 30 sec brake. After allowing the coarse particles to settle (30 sec), subsamples were diluted in double distilled water, stained with acridine orange (1:10,000; Merck No. 1333) for 3 min, and filtered through Nucle-pore filters (0.2 μm pore size; 25 mm diameter— prestained in Sudan Black (Merck No. 1387). Wedges of the filters were analysed with a drop of Cargille's immersion oil (Type A; Cargille Laboratories, Inc.) by epifluorescence microscopy (Zeiss Universal microscope, magnification x 1,600) using blue light excitation and Osram HBO 200 burner. Generally, 400—600 bacteria were counted by means of a microscopic grid in a total of 40 microscopic fields distributed on 3 filters prepared from replicate samples. Parallel, the number of dividing cells (at least 100) defined as bacteria with a clearly visible invagination was recorded. For biomass determinations, colour slides from characteristic fields (Zeiss CS-matic camera; Kodak Ektachrome 400 film, exposure time 20—30 sec) were projected onto the screen of a half-automatic image analyser (MOP-02, Kontron). The inner outline of the bacteria sharply defined was copied and traced with the detection pen of the analyser which prints off diameter and area of the individual bacteria. Assuming an ellipsoid, the volume of the bacteria could be calculated. For conversions into biomass and carbon, a specific gravity of 1, and a factor of 0.1, respectively, was used. The biomass of the total population followed from multiplying the mean biomass per cell by the total number of bacteria in the corresponding sample. For the analysis of the biomass spectrum of the individual population, bacteria were grouped into three size classes according to their volume: $< 0.3 \ \mu m^3$, $0.3-0.6 \ \mu m^3$, and $> 0.6 \ \mu m^3$ (for details cf. Meyer-Reil 1983).

Potential application

During a joint research program at Kiel University (SFB 95), seasonal variations in concentrations and enzymatic decomposition rates of particulate organic material (protein, carbohydrate) as well as in bacterial populations (number, biomass) were investigated at a sandy mud station in the Kiel Bight (cf. idealized graphs in Fig. 2; for details of the data cf. Meyer-Reil 1983). The seasonal variations in the decomposition of particulate organic material and bacterial populations have to be understood as a reflection upon certain ecological situations and events in the sediment. These events may be defined as follows: break up of summer stagnation, input of the phytoplankton blooms in autumn and spring, respectively, input of organic material during winter, and development of the benthic fauna (cf. Fig. 2).

During the summer stagnation (an anoxic period in which the stratification in the water column inhibits the introduction of oxygen into the sediment), protein accumulated. The break up of summer stagnation (temporary introduction of oxygen) led to an increase in the enzymatic decomposition rates of protein and subsequently decreasing protein concentrations. Decomposition of carbohydrate, however, was much less influenced by changes in the redox potential in the sediment. During the break up of summer stagnation, total number and biomass of bacteria remained almost constant. Variations, however, were recorded in the composition

of the bacterial population: the biomass associated with large-size bacteria (volume $> 0.6 \ \mu m^3$) decreased.

The input of the autumn phytoplankton bloom can be characterized by primarily stimulating biomass production (increase in cell volume) and subsequently cell division of the benthic bacterial population. Obviously due to the induction of enzymatic activities, increasing decomposition rates of particulate organic material already occurred at the end of October, when concentrations of carbohydrate and protein started to accumulate in the sediment surface. Maxima in the enzymatic decomposition rates, however, coincided with peaks in bacterial number.
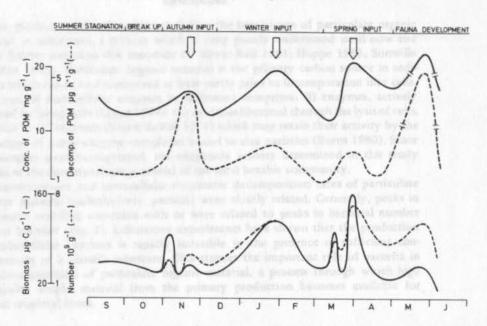
Fig. 2. Idealized graphs of seasonal variations in concentrations and decomposition rates of particulate organic material (carbohydrate, α-amylase) as well as in bacterial number and biomass recorded between September 1981 and June 1982 at a 18 m sandy-mud station in the Kiel Bight. The headline on top characterizes the events affecting the sediment. Arrows indicate the input of organic material into the sediment.

The accumulation of particulate organic material during winter (macrophytes eroded by winter storms, material from terrestrial origin, resuspended sediment) was reflected by a continuous increase in enzymatic decomposition rates of particulate organic material and bacterial parameters with peaks in January. The limited number of grazers and the log time the bacterial population had for its "undisturbed" development, may be responsible for the high bacterial number and biomass observed in winter.

Bacteria almost immediately reacted on the input of the phytoplankton bloom in spring with an increase in number and biomass. Parallel to the development of the bacterial population in autumn, two peaks were recorded. Again, the second peak coincided with a peak in the enzymatic decomposition rates of particulate organic material.

The further fate of the benthic bacterial populations in spring was greatly dependent upon the development of the remaining benthic fauna. At the end of May, high abundances of polychaetes were observed in the sediment. The bacterial population consisted of a high number of actively growing, almost exclusively small-size bacteria with a relatively low biomass. Preferentially grazing upon medium and large-size bacteria by the worms could be responsible for the pauperization of the bacterial community.

Discussion

The method described enables to follow the breakdown of particulate organic material in sediments, a process which is only poorly understood until now and needs further attention (for literature cf. Meyer-Reil 1981; Hoppe 1983; Somville & Billen 1983). Particulate organic material is the primary carbon source in sediments which has to be decomposed at least partly prior to incoroporation into cells. The pool of extracellular enzymes in sediments comprises: (i) enzymes, actively secreted by living cells (Corpe 1974), (ii) enzymes liberated through the lysis of cells, and (iii) "free" enzymes (Kim & ZoBell 1974) which may retain their activity by the formation of humic-enzyme complexes bound to clay particles (Burns 1980). Since the samples were homogenized, the enzymatic activity determined in this study relates to the decomposition potential of the total benthic community.

Concentrations and extracellular enzymatic decomposition rates of particulate organic material (carbohydrate, protein) were closely related. Generally, peaks in enzymatic activities coincided with or were related to peaks in bacterial number and/or biomass (Fig. 2). Laboratory experiments have shown that the production of extracellular enzymes is rapidly inducible in the presence of sufficient concentrations of a suitable substrate. This stresses the important role of bacteria in the decomposition of particulate organic material, a process through which high molecular weight material from the primary production becomes available for higher trophical levels.

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Authors' address:

L.-A. Meyer-Reil, Institut für Meereskunde an der Universität Kiel, Abt. Marine Mikrobiologie, Düsternbrooker Weg 20, 2300 Kiel 1, Fed. Rep. Germany

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SEASONAL DEVELOPMENT OF BACTERIAL COMMUNITIES IN A COASTAL MARINE SEDIMENT AS RELATED TO THE INPUT OF ORGANIC MATERIAL

L.-A. MEYER REIL and G. GRAF

Institut für Meereskunde an der Universität Kiel, Düsternbrooker Weg 20, 2300 KIEL (FRG)

ABSTRACT - The seasonal development of benthic communities was followed in a coastal marine sediment of the Kiel Bight (Baltic Sea ; FRG). Total benthic biomass (ATP), bacterial biomass, overall benthic activity (heat production), and enzymatic decomposition rates of carbohydrate (d-amylase activity) followed a seasonal cycle strongly related to certain events in the sediment. Among these, the input of the phytoplankton blooms in autumn and spring, the accumulation of organic material during winter, and changes in the physical characteristics of the sediment turned out to be most important for the development of benthic biomass and activity. Processes within the benthic community occurred in very short time scales (within days).

Key words : bacterial biomass, heat production, enzymatic decomposition, seasonal cycle, sediment, sedimentation.

RÉSUMÉ - Le développement saisonnier des communautés benthiques a été suivi dans les sédiments marins côtiers de la baie de Kiel (Mer Baltique, RFA). La biomasse totale benthique (ATP) la biomasse bactérienne, l'activité benthique globale (production de chaleur), et taux de décomposition enzymatique des carbohydrates (activité de l'amylase) suivent un cycle saisonnier fortement lié à certains événements dans le sédiment. Parmi ceux-ci, l'apport des blooms phytoplanctoniques en automne et au printemps, l'accumulation de matière organique en hiver et les changements dans les caractéristiques physiques du sédiment sont considérés comme les plus importants pour le développement de la biomasse et de l'activité benthique. Ces processus dans les communautés benthiques ont lieu à une échelle de temps très courte (de l'ordre de quelques jours).

Mots clés : biomasse bactérienne, production de chaleur, décomposition enzymatique, cycle saisonnier, sédiment, sédimentation.

INTRODUCTION

The supply of organic material is the dominating factor determining structure and activity of benthic communities. Whereas the benthos in shallow waters may be directly supplied with benthic primary production, the benthos in deeper waters is greatly dependent upon the sedimentation of organic material from the water column.

Based upon data published in recent publications (Graf *et al.* 1983, Meyer-Reil 1983), this paper summarizes information on the seasonal development of benthic communities in coastal sediments of the brackish water Kiel Bight (Baltic Sea ; FRG) as related to the input of organic material.

THE EXPERIMENTAL APPROACH

Various parameters related to benthic biomass as well as to metabolic activities were applied and examined for their ability to characterize the seasonal development of

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benthic communities in a sandy-mud sediment of the brackish water Kiel Bight (Baltic Sea; FRG) between September 1981 and June 1982. From these parameters, the following will be discussed in this paper. ATP-measurements were used to describe overall benthic biomass. Bacterial number and biomass were extrapolated from epifluorescence microscopy preparations. Direct calorimetry (heat production) served as an indicator for overall benthic metabolism. Decomposition of particulate organic material was derived from exoenzymatic activity measurements. Details of the methods used as well as a characterization of the sediments sampled are described in two recent papers and literature cited therein (Graf et al. 1983, Meyer-Reil 1983).
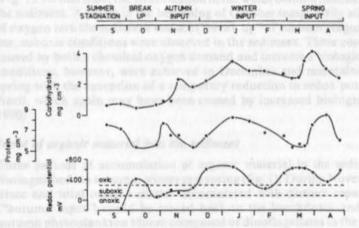
Figure 1 : Seasonal variations in redox potential, protein, and carbohydrate in the top centimeter of a sandy-mud sediment of the Kiel Bight (Baltic Sea; FRG) sampled between September 1981 and April 1982.
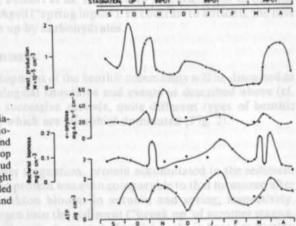
Figure 2 : Seasonal variations in ATP, bacterial biomass, α-amylase activity, and heat production in the top centimeter of a sandy-mud sediment of the Kiel Bight (Baltic Sea, FRG) sampled between September 1981 and April 1982.

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Seasonal variations in the food supply for the benthos as well as changes in the physical characteristics of the sediment gave rise to the definition of certain periods which turned out to be of high relevance for the development of the benthic communities (legends in fig. 1 and 2).

Physical characteristics of the sediment

Typical for a boreal region, temperature dropped gradually in the near bottom water from autumn to winter (14°C to 2°C) and increased slowly towards spring (4°C in April). The first period of observation coincided with the termination of "summer stagnation", a period in which anoxic conditions (hydrogen sulfide) were prevailing in the sediment (Fig. 1). At that time, the stratification in the water column limited the access of oxygen to the sediment. A storm in the beginning of October temporarily allowed the introduction of oxygen into the sediment surface ("break up" of summer stagnation). During November, suboxic conditions were observed in the sediment. These conditions may have been caused by both a chemical oxygen demand and increased biological activity. Stable oxic conditions, however, were achieved in December and maintained through winter and spring with the exception of a temporary reduction in redox potential in February and April, which again may have been caused by increased biological activity (Hargrave 1980)

Input of organic material into the sediment

Three periods of accumulation of organic material in the sediment surface could be distinguished : in autumn, winter and spring (fig. 1). During November protein, carbohydrate and total organic matter accumulated with distinct separate peaks. This input ("autumn input") could be traced back to the breakdown and sedimentation of the autumn phytoplankton bloom composed of dinoflagellates in the beginning and diatoms at the end of the bloom (Graf *et al.* 1983). During winter, we observed a continuous slow increase of organic material in the sediment surface ("winter input"). Resuspended sediment, material from terrestrial origin as well as macrophyte debris eroded by winter storms represented sources of the organic material. The breakdown of the spring phytoplankton bloom (mainly diatoms; Peinert *et al.* 1982) led to an enrichment of organic material during late March to mid April ("spring input"). In contrast to autumn, the first peak in organic material was made up by carbohydrates.

RESPONSE OF THE BENTHIC COMMUNITY

In the following , the seasonal development of the benthic community will be discussed as a reflection upon the different ecological situations and events as described above (cf. headlines in fig. 1,2). During the successive periods, quite different types of benthic metabolisms are involved, some of which are microbial-dominated (Fig. 2).

Break up of summer stagnation

During the anoxic period of summer stagnation, protein accumulated in the sediment surface. The concentration of stored protein was even comparable to that measured after the sedimentation of the phytoplankton blooms in autumn and spring, respectively. Following the introduction of oxygen into the sediment ("break up"of summer stagnation), protein concentrations significantly decreased. Parallel peaks in heat production and ATP imply that the stored protein was rapidly consumed and incorporated into

benthic biomass (Fig. 2). However, the question still remains open, which of the benthic organisms were responsible for the increase in biomass, since bacterial number and biomass remained almost constant during this period. Consequently, bacterial ATP as percentage of the total ATP strongly decreased from 43 % (anoxic conditions) to 18 % (oxic conditions). With decreasing redox potential towards the beginning of November, however, bacterial ATP again increased (up to 40 % of the total ATP) indicating an increasing importance of bacteria in benthic metabolism under anoxic and suboxic conditions (Meyer-Reil 1983). The inhibition of protein decomposition under anoxic conditions is not easy understandable. Under the same conditions, the decomposition of carbohydrates is much less affected as it could be shown by laboratory experiments (Meyer-Reil, unpublished data).

Autumn and spring input

The input of the phytoplankton blooms in autumn and spring, respectively, represent external food supplies for the benthos which generally reacted with an outburst in activity and subsequent biomass production (Fig. 2). However, the specificity of the benthic response is caused by differences in the food supply, the physical properties of the sediment, and the composition of the benthic community.

During the "autumn input" a peak in protein succeeded a peak in carbohydrate. In spring, however, the first peak in organic material was an enrichment of carbohydrate which was followed by protein. As an immediate response to the availability of organic material, heat production culminated. It is interesting to note that the peaks in heat production coincided with peaks in protein, but not with peaks in carbohydrate (Fig. 1, 2). Decomposition rates of carbohydrate (activity of α-amylase) were closely related to the enrichment of carbohydrate in the sediment. Enzymatic responses turned out to be much higher in autumn as compared to spring. This is obviously a reflection of both the higher temperature and the higher benthic biomass in autumn. Due to an induction of enzymatic activity with increasing substrate concentrations, a stimulation of enzymatic decomposition rates already occurred when concentrations of carbohydrate started to increase in the sediment surface. Since exoenzymatic activities are thought to be a minor component of the overall heat loss in sediments (Pamatmat 1982) both activity parameters showed no correlation (Fig. 2).

Whereas heat production comprises all types of benthic metabolism, electron transport activity (ETS; for data cf. Graf *et al.* 1983) relates to the activity of respiratory chains (oxygen-, nitrate, and most likely sulfate-respiration). The quotient between heat production and ETS-activity should therefore serve as an indicator for changes in the type of metabolism (Pamatmat 1982). A strong increase of this quotient following the "autumn-" and "spring-input", respectively, demonstrated a shift in the type of benthic metabolism towards fermentation. This coincided with suboxic conditions mainly caused by biological oxygen consumption (Graf *et al.* 1983).

The stimulation of benthic activity resulted in subsequent biomass production. Prior to peaks in total benthic biomass (ATP measurements) however, bacterial biomass accumulated (Fig. 2). Bacteria primarily reacted to the availability of organic material with an immediate and strong increase in cell volume (biomass production). Deviating from the "normal" distribution of bacterial biomass, medium and large size cells dominated. Subsequently, bacteria responded with cell division (increase in cell number), re-establishing the normal biomass distribution : small-size cells (volume $<0,3\,\mu m^3$) again dominated the bacterial biomass followed by medium and large-size cells (volume $0,3-0,6\mu m^3$ and $>0,6$ μm^3, respectively ; Meyer-Reil 1983).

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Winter input

Resuspended sediment, terrestrial material and eroded macrophytes represent an additional food supply for the benthic community during winter (Fig. 1). Although heat production continuously decreased, a high level of benthic biomass accumulated. This applies for bacterial biomass as well (Fig. 2). The high bacterial biomass sustained during winter is surprising taking into account the low temperature and the reduced metabolic activity rates. However, limited number of grazers and the relatively long time the bacteria had available for their "undisturbed" development may explain the biomass accumulation. Additionally, the more refractory kind of food source could have been responsible for the slow, continuous increase in bacterial biomass in winter. In this respect, the development of the bacterial population in winter differed basically from its spontaneous development in autumn and spring, respectively.

Fauna development

Additional samples taken in late spring revealed that the further fate of the bacterial community was greatly influenced by the development of the benthic fauna (data not shown; Meyer-Reil 1983). A mass occurrence of polychaetes in the sediment was accompanied by a high number of almost exclusively small-size bacteria (volume $<0,3\mu m^3$) which actively grew (high number of dividing cells). Most likely preferentially grazing of medium and large-size cells by the polychaetes was the reason for the impoverishment of the bacterial population. There is indeed evidence from the literature that grazing stimulates bacterial activity (Morrison and White, 1980).

CONCLUSION

From the foregoing discussion it becomes obvious that processes like sedimentation and input of organic material into the sediment may occur in very short time scales (within days), causing an immediate response of benthic activities. Whereas the succession of certain processes in the benthic community could be explained by the interactions between individual parameters, other processes still need explanation. This applies especially to the decline of benthic activities and biomass after certain levels were reached. Control mechanisms, such as the growth limiting effect of certain population densities and interactions between the individual components of the benthic communities, have to be considered.

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SPATIAL AND TEMPORAL DISTRIBUTION OF BACTERIAL POPULATIONS IN MARINE SHALLOW WATER SURFACE SEDIMENTS

L.-A. MEYER-REIL

Institut für Meereskunde an der Universität Kiel, Marine Mikrobiologie, Düsternbrooker Weg 20, 2300 Kiel 1 (Federal Republic of Germany)

ABSTRACT.

Meyer-Reil, L.A., 1986. Spatial and temporal distribution of bacterial populations in marine shallow water surface sediments. In: P. Lasserre and J.M. Martin (eds), Biogeochemical Processes at the Land-Sea Boundary. Elsevier, Amsterdam.

In the paper presented, spatial and temporal aspects of the distribution of bacterial populations in marine shallow water surface sediments are discussed. Bacterial number and biomass as determined by epifluorescence microscopy turned out to be closely related to sediment properties such as grain size and organic matter content. For an "average" sediment, one may expect 10^9 cells per g of dry weight sediment with a corresponding biomass of 10 ug bacterial C per cell. Benthic bacterial populations underwent strong seasonal variations which could be traced back to the accumulation of organic material in the sediment following sedimentation events. In shallow water surface sediments bacterial communities were governed by diurnal rhythms obviously closely related to microphytobenthos primary production. Estimates of bacterial production using quite different approaches demonstrated that between 10 and 800 mg C m^{-2} d^{-1} were fixed by bacterial secondary production.

INTRODUCTION

Coastal sediments play an important role in nutrient regeneration for marine ecosystems. These processes are governed by the activity of benthic bacteria. Microbiological work in sediments has been concentrated on the investigation of nutrient cycles as well as on the spatial and temporal distribution of bacteria. In the studies of nutrient cycles, the activity of benthic bacteria was indirectly concluded from changes in the concentrations or turnover rates of inorganic and organic chemical parameters. Generally, corresponding information on the development of the bacterial populations themselves could not be included.

This paper summarizes information on the spatial and temporal distribution of benthic bacterial populations. Many examples discussed originate from microbiological investigations of sediments from the brackish water Kiel Bight (Baltic Sea, Federal Republic of Germany). Existing data and concepts illustrating the spatial and temporal distribution of bacteria in shallow water surface sediments are organized under the following topics :

1) Sediments as habitats for bacteria

2) Bacterial number and biomass

3) Seasonal development of bacterial communities

4) Diurnal fluctuations of bacterial populations

5) Estimates of bacterial production.

SEDIMENTS AS HABITATS FOR BACTERIA

Sediments represent a complex en..ronment consisting of particles which are more or less densely packed and surrounded by interstitial water. In beach sediments which are extremely exposed to wave action and tidal activity more than 95 % of the bacteria are attached to particle surfaces (Meyer-Reil et al., 1978). Deeper sediments carry a much higher percentage of bacteria "free-floating" in the interstitial water (up to 50 % of the total number ; Weise and Rheinheimer, 1978 ; Weise and Rheinheimer, 1979).

Bacteria colonize only a small proportion of the available particle surface area (0.01 to 5 % ; range of the data obviously dependent upon the method used for the calculation of surface area ; cf. Hargrave, 1972 ; Rublee and Dornseif, 1978 ; Weise and Rheinheimer, 1978 ; DeFlaun and Mayer, 1983). Deep pores of wheathered feldspar and clay grains do not appear to be inhabited by bacteria (DeFlaun and Mayer, 1983). Bacteria preferentially
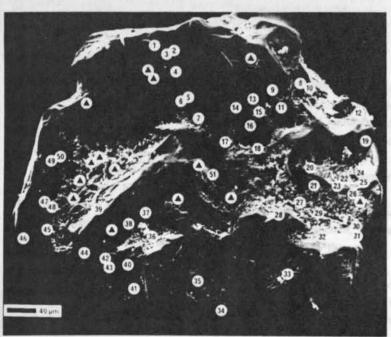
Fig. 1. Scanning electron micrograph of a 0.5 mm quartz grain. Note that organic material is concentrated in protected areas. The numbers on the quartz grain mark sites of specific investigation (for details cf. Weise and Rheinheimer, 1978).

colonize areas of low relief such as depressions and crevices of particles where the cells are protected against grazing and mechanical demages (Nickels et al., 1981 ; cf. Fig. 1). The habitation in these areas is an expression of preferential survival rather than preferential colonization as it could be demonstrated by DeFlaun and Mayer (1983).

The benthic bacterial flora is of a high diversity : rods, cocci and curved cells of different sizes. Single cells or small colonies are prevailing. Most of the bacteria are found in extracellular slime layers consisting of bacterial fibrous webs and mucus produced by diatoms (Weise and Rheinheimer, 1978 ; Moriarty and Hayward, 1982). Through the formation of this organic material of a considerable structural complexity, bacteria may influence the texture of sediments like it was demonstrated for fungi mycelium in sand dune soils (Clough and Sutton, 1978).

BACTERIAL NUMBER AND BIOMASS

As basic members of the food chain, benthic bacteria represent an important nutrient source for meio- and macrofauna. The distribution of bacterial number and biomass was shown to be closely related to sediment properties, from which organic material and grain size are probably most important.

Number and biomass as determined by epifluorescence microscopy

Beside scanning electron microscopy, epifluorescence microscopy permits a reliable estimate of number and biomass of bacteria in sediments. However, prior to counting bacteria have to be liberated from the particle surfaces. Different extraction techniques have been applied : treatment of sediments with surface active agents, homogenization (Meyer-Reil et al., 1978), and sonication (Weise and Rheinheimer, 1978 ; Ellery and Schleyer, 1984). From these techniques, sonication gave the most reliable results with the highest percentage of bacteria (approximately 95 % of the total number) liberated from the particle surfaces (Meyer-Reil, 1983). However, most recently this technique has been discussed with special regard to a possible destruction of bacterial cells (Ellery and Schleyer, 1984).

After sonication sediment samples are diluted, filtered onto prestained Nucleopore polycarbonate filters (pore size 0.2 µm) and stained with fluorescence dyes (e.g. acridine orange). Bodies with clear outline, bacterial shape and distinct fluorescence (orange or green) are counted as bacterial cells. For biomass determinations slides of characteristic microscopic fields can be prepared. The slides may be analysed by means of an image analyser which reduces the uncertainty to group the bacteria into arbitrary size classes by eye (Krambeck et al.,1981 ; Meyer-Reil, 1983). Using conversion factors, data can be extrapolated to biomass in terms of carbon (cf. Ferguson and Rublee, 1976 ; Cammen, 1982 ; Bakken and Olson, 1983).

Uncertainties arising from inadequacies of the sonication technique and the conversion factors are obviously minor as compared to the subjectivity involved in the counting

procedure. However, up to now, beside scanning electron microscopy, epifluorescence microscopy is the only direct approach to gain information on bacterial number and biom. ss (cf. Fig. 2).
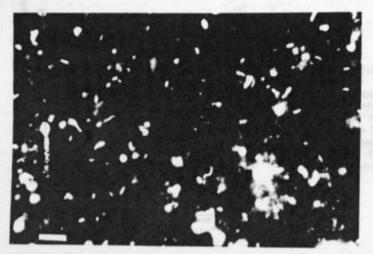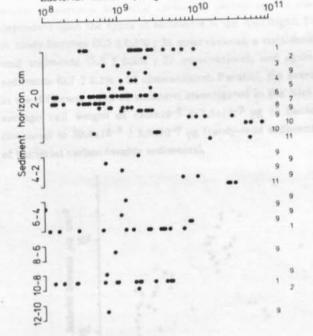

Fig. 2. Epifluorescence photograph of bacteria from a sandy sediment of the Kiel Bight (Baltic Sea ; FRG). The bacteria were liberated from the sediments by sonication. The sample was diluted and stained with acridine orange. Bar represents 3 um (from Meyer-Reil, 1984a).

Local variations of number and biomass

Data on local variations in bacterial number and biomass summarized from the literature are presented in Fig. 3 and 4. The data set comprises sediments from arctic, antarctic, boreal and tropical regions. Generally, bacterial numbers are in the range of 10^8 to 0^{11} cells per gram of dry weight sediment with a concentration of the data around 10^9 cells (cf. Fig. 3). Numbers turned out to be much more related to the type of sediment than to the region where the samples were taken. The lowest cell numbers were found in sandy sediments, the highest numbers in muddy sediments (cf. below). With increasing sediment depths, a slight decrease in bacterial numbers becomes obvious. However, even in the 11 m horizon of a sediment profile from the Antarctica, 10^9 cells per gram of sediment could be detected, only insignificantly less than in the surface horizon (Meyer-Reil, 1984 a).

Because of the time consuming procedure of size fractionation, much less data are availabl· on bacterial biomass (Fig. 4). Generally, bacterial carbon is in the range of 1 to 10^3 µg per gram of dry weight sediment thus contributing significantly to the total benthic biomass as determined by ATP measurements (for data cf. Graf et al., 1982 ; Graf et al., 1983). Again, the lowest bacterial biomass values were found in sandy sediments, the highest values in muddy sediments.

Fig. 3. Summary of data concerning bacterial numbers in sediments of different areas. Each of the dots represent one data point taken from the literature listed on the right panel : 1-Dale, 1974 ; 2-Kepkay et al., 1979 ; 3-Griffiths et al., 1978 ; 4-Meyer-Reil et al., 1981 ; 5-Meyer-Reil et al., 1978 ; 6-Meyer-Reil et al., 1980 ; 7-Meyer-Reil, 1981 ; 8-Weise and Rheinheimer, 1978, 1979 ; 9-Meyer-Reil unpublished data 10-Rheinheimer, unpublished data ; 11-Jones, 1980 ; 12-Moriarty, 1980.
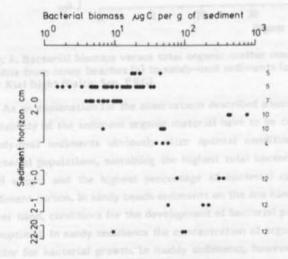

Fig. 4. Summary of data concerning bacterial biomass in sediments of different areas. For explanations see Fig. 3.
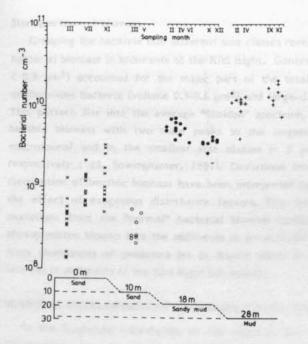
Fig. 5. Bacterial numbers per cm³ of sediment on a hypothetical horizontal profile from sandy sediments (water depth 0, 10 m) to sandy-mud sediments (water depth 18 m) and to muddy sediments (water depth 20 m) of the Kiel Bight (Baltic Sea, FRG).

Relationship between number and grain size

A horizontal profil from beaches (water depth 0 m) into deeper waters (18, 28 m) of the Kiel Bight revealed an inverse relationship between benthic bacterial number and grain size of the sediment (Fig. 5). Cell numbers increased significantly from sandy to sandy-mud and to mud sediments. This general inverse relationship certainly reflects both the greater surface area and the higher organic matter content in fine sediments as compared to coarse ones (Dale, 1974 ; Tanoue and Handa, 1979). However, the question still remains open whether the bacteria simply respond to higher organic matter level originally present in fine sediments or whether higher bacterial numbers in fine sediments results in higher organic matter accumulations (DeFlaun and Mayer, 1983).

Relationship between biomass and organic material

Relationships between bacterial biomass and organic matter content of the sediments seem to be very complex. In sediments of the Kiel Bight, bacterial carbon increased significantly from sandy beach sediments to sandy-mud sediments. However, in muddy sediments bacterial biomass did not further increase despite of the more than twofold increase in organic matter as compared to sandy-mud sediments (Fig. 6).

On an average, bacterial carbon in sediments of the Kiel Bight accounted for 0.7 ± 0.2% (standard deviation of the mean ; 76 observations) of the sediment organic carbon. In the literature, higher values have been reported (1.2 %, Dale, 1974 ; less than 2%,

Cammen, 1982) which may be partly due to methodological differences in calculating bacterial biomass. Bacterial carbon as percentage of the total sediment carbon varied dependent upon the types of sediments in the Kiel Bight. The lowest percentage was found in sandy beaches (0.5 ± 0.1% ; 36 observations), a considerable higher percentage in sandy-mud sediments (0.9 ± 0.2% ; 21 observations), and again a lower percentage in muddy sediments (0.7 ± 0.2% ; 19 observations). Parallel, the average bacterial cell weight varied in the different types of sediment investigated in the Kiel Bight. For sandy sediments an average cell weight of $15.8 \times 10^{-9} \pm 2.5 \times 10^{-9}$ µg of bacterial carbon was calculated as compared to $20.4 \times 10^{-9} \pm 3.9 \times 10^{-9}$ µg (sandy-mud sediments) and $11.3 \times 10^{-9} \pm 1.7 \times 10^{-9}$ µg of bacterial carbon (muddy sediments).
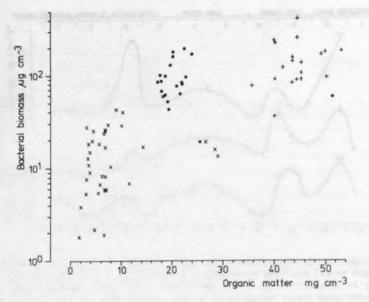

Fig. 6. Bacterial biomass versus total organic matter content on a hypothetical horizontal profile from sandy beaches (x) to sandy-mud sediments (o) and to muddy sediments (+) of the Kiel Bight (Baltic Sea, FRG).

As an explanation for the observations described above, both the concentration and the suitability of the sediment organic material have to be considered. In the Kiel Bight, the sandy-mud sediments obviously offer optimal conditions for the development of the bacterial populations, sustaining the highest total bacterial biomass, the highest average cell weight and the highest percentage of bacterial carbon as compared to the total sediment carbon. In sandy beach sediments on the one hand and in muddy sediments on the other hand, conditions for the development of bacterial populations have to be regarded as suboptimal. In sandy sediments the concentration of organic material may be the limiting factor for bacterial growth. In muddy sediments, however, much of the organic material may consist of highly refractory compounds thus restricting bacterial biomass development.

Size spectrum of biomass

Grouping the bacteria into different size classes revealed a characteristic spectrum of bacterial biomass in sediments of the Kiel Bight. Generally, small-size bacteria (volume < 0.3 μm^3) accounted for the major part of the total bacterial biomass, followed by medium-size bacteria (volume 0.3-0.6 μm^3) and large-size bacteria (volume > 0.6 μm^3). This pattern fits into the average "Sheldon" spectrum, a characteristic distribution of benthic biomass with two main peaks in the largest (> 2 mm ; corresponding to macrofauna) and in the smallest size classes (< 2 μm ; corresponding to bacteria, respectively ; cf. Shwinghamer, 1981). Deviations from this typical pattern in the distribution of benthic biomass have been interpreted by the author as an expression of the effect of exogenous disturbance factors. This interpretation can be related to deviations from the "normal" bacterial biomass spectrum as well. The input of the phytoplankton blooms into the sediments in autumn and spring, respectively, as well as mass abundances of predators let to drastic shifts in the size spectrum of bacterial biomass in sediments of the Kiel Bight (cf. below).

SEASONAL DEVELOPMENT OF BACTERIAL COMMUNITIES

In the literature information on the seasonal development of benthic bacteria is limited. From the investigations of Montagna (1982) and Cammen (1982) no general trend for seasonal variations of benthic bacteria could be detected. The studies of Rublee (1982) and DeFlaun and Mayer (1983) revealed a positive correlation between benthic bacteria and temperature. Detailed investigations in a sandy-mud sediment of the Kiel Bight using a high time resolution in sampling demonstrated strong seasonal variations in the development of bacterial populations closely related to specific ecological situations and sedimentation events (Meyer-Reil, 1983, 1984b).

Input of organic material into the sediment

In recent years it could be shown that in boreal marine systems the main sedimentation events and therefore the main food supply for the benthos occur in autumn and spring, respectively. Studies in the Kiel Bight have demonstrated that large amounts of the primary produced material in autumn and spring do not enter the pelagic food web but rather settle onto the sediment. This material already represents 2/3 of the total yearly input from the pelagic into the benthic system (Smetacek, 1980). Investigations of Peinert et al. (1982) and Gral et al. (1983) followed the biomass development of the autumn and spring phytoplankton bloom in the water column and the sedimentation of the primary produced organic material by sediment traps.

In sediments of the Kiel Bight, three periods of accumulation of organic material could be distinguished : in autumn, winter and spring (Fig. 7 ; Meyer-Reil, 1983). The enrichment of organic material during November could be traced back to the breakdown and sedimentation of the autumn phytoplankton bloom composed of dinoflagellates and

diatoms. Total organic matter, protein and carbohydrate accumulated in distinct, separate peaks. During winter a slow continuous increase of organic material was observed in the sediment surface. The organic matter consisted of resuspended sediment, material from terrestrial origin as well as of macrophyte debries eroded by winter storms. The breakdown of the spring phytoplankton bloom (mainly diatoms) let to an enrichment of organic material in the sediment surface during late March to mid April. Again separate peaks were recorded for total organic matter, protein and carbohydrae. These accumulation periods termed "autumn", "winter"- and "spring-input" turned out to be of high relevance for the development of the benthic communities in sediments of the Kiel Bight (Gral et al., 1983 ; Meyer-Reil, 1983, 1984b).
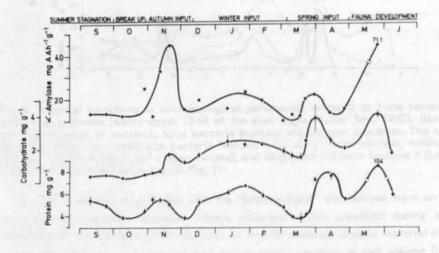

Fig. 7. Seasonal variations in concentrations and exoenzymatic decomposition rates of organic material in the O to 1 cm horizon of a sandy-mud sediment (water depth 18 m) of the Kiel Bight (Baltic Sea, FRG). Illustrated are : concentrations of protein and carbohydrate, and activity of α-amylase (mg of amylopectin azure decomposed per g of dry weight sediment per hour). The headline on top characterizes specific ecological situations and events affecting the sediments.

Heat production as measurement of community metabolism.

Direct calorimetry (heat production) represents a reliable method for the estimation of total benthic community metabolism, because it is a direct measurement of the energy flow through the system. Although the heat release from the activity of extracellular enzymes and from chemical oxidations are also included, the latter two components are thought to be of minor importance for total heat loss from sediments (Pamatmat, 1982).

As an immediate response to the "autumn"- and "spring-input" in sediments of the Kiel Bight, heat production culminated. A less obvious response was found during "winter-input". However, considering the temperature dependence of benthic metabolism, there was still a considerable and long lasting heat production during this period (Graf et al., 1983). Whereas heat production comprises all types of benthic metabolism, electron-

transport-activity (ETS) relates to the activity of respiratory chains. As pointed out by Pamatinat et al. (1981), the quotient between heat production and ETS-activity should serve as a qualitative indicator for changes in the type of metabolism. Following the "autumn-" and "spring-input", a strong increase in this quotient was observed, demonstrating a shift in the type of benthic metabolism towards fermentation. This coincided with suboxic conditions in the sediment surface mainly caused by biological oxygen consumption (Graf et al., 1983).

Decomposition of particulate organic material.

The main portion of the input of organic material into the sediment is particulate organic carbon which has to be enzymatically decomposed, at least partly, prior to incorporation into cells. In this process, extracellular enzymes are involved which are secreted by living cells (Corpe and Winters, 1972) or liberated during the lysis of dead and decaying cells. As shown by Burns (1980) some of these enzymes may retain their activity outside the cells by the formation of humic-enzyme complexes bound to clay particles.

The accumulation of organic material in sediments of the Kiel Bight during "autumn-", "winter-" and "spring-input" let to corresponding stimulations in the enzymatic decomposition rates of carbohydrate and protein (activity of $\propto$ -amylase, proteolytic enzymes, Fig. 7). Exoenzymatic responses were highest in autumn as compared to winter and spring. This is obviously a reflection of both the higher temperature and the higher benthic biomass in autumn. During "autumn-" and "spring-input" a stimulation of enzymatic decomposition rates already occurred when concentrations of particulate organic material started to accumulate in the sediment surface indicating an induction of enzymatic activities by increasing concentrations of suitable substrates (Meyer-Reil, 1983). There is strong evidence from laboratory and field data that under anoxic conditions the enzymatic decomposition of protein is retarded. During summer stagnation, an anoxic period in which hydrogen sulfide is prevailing in sediments of the Kiel Bight, protein accumulated. The concentration of the stored protein was even comparable to that measured during "autumn"- and "spring-input". Following the introduction of oxygen into the sediment (break up of summer stagnation), protein concentrations significantly decreased. Parallel peaks in heat production and ATP imply that the stored protein was rapidly consumed and incorporated into benthic biomass (cf. Graf et al., 1983 ; Meyer-Reil, 1983).

Bacterial development in autumn and spring.

As shown for sediments of the Kiel Bight, bacteria reacted to the "autumn-" and "spring-input", respectively, with two separate peaks. The first peak already occurred when concentrations of organic material started to accumulate in the sediment surface. This demonstrates that bacteria almost immediately responded to the availability of decomposable organic material. The second peak in bacterial parameters coincided with the main input of organic material following the final breakdown and sedimentation of the phytoplankton blooms (Fig. 8).
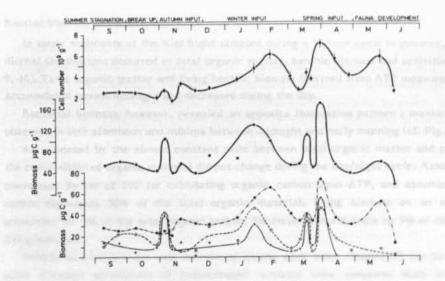
Fig. 8. Seasonal variations in microbiological parameters in the 0 to 1 cm horizon of a sandy-mud sediment (water depth 18m) of the Kiel Bight (Baltic Sea, FRG). Illustrated are: total number of bacteria, total bacteria biomass and biomass spectrum. The biomass spectrum comprises : small-size bacteria (volume < 0.3 m³ ; closed circles), medium-size bacteria (volume 0.3-0.6 µm³ ; open circles), and large-size bacteria (volume > 0.6 µm³ ; crosses). For the headline on top cf. Fig. 7.

The bacterial population faced with the "autumn-input" was derived from an anoxic population (fermentative bacteria, sulfate reducers) which prevailed during summer stagnation. Within this population the input of freshly produced organic material caused a drastic shift. Bacteria primarily reacted with a strong increase in cell volume (biomass production). Deviating from its "normal" distribution (cf. above), the size spectrum was dominated by medium and large-size cells. Following the final breakdown and sedimentation of the autumn phytoplankton bloom, the bacteria subsequently responded with cell division (increase in cell number).

Compared to autumn, the history of the bacterial population faced with the "spring-input" was quite different. During winter oxic conditions prevailed in the sediment. Due to erosion of the sediment caused by winter storms, the bacterial population was declining. The "spring-input" hit an impoverished bacterial community which immediately reacted with a strong increase in cell volume, but only a small increase in cell number. Again, deviations from the normal size spectrum were observed : small, medium and large-size cells almost equally contributed to the total biomass. Following the final breakdown and sedimentation of the spring phytoplankton bloom, bacterial number and biomass responded with a second stimulation.

Bacterial development in winter.

During winter resuspended sediment, terrestrial material and eroded macrophytes represent an additional food supply for benthic bacterial populations of the Kiel Bight. Bacterial number, biomass and cell-division activity showed a slow continuous increase up to values that were even higher as compared to those obtained following the "autumn-input" (Fig. 8). Taking into account the more refractory nature of the organic material and the low temperature with reduced matabolic activity rates, the accumulation of bacterial biomass during winter is surprising (cf. temperature-dependent development of benthic bacteria reported by DeFlaun and Mayer, 1983). However, the relatively long time the bacterial population had available for its "undisturbed development" and the limited number of grazers have to be considered. With regard to the nature of the organic material and the slow continuous development, the response of the bacterial community to the "winter-input" differed basically from the spontaneous bacterial development following the input of the phytoplankton blooms in autumn and spring, respectively.

Bacteria and fauna development.

The development of the benthic fauna in spring greatly influenced the composition of the bacterial community in sediments of the Kiel Bight. Through the activity of polychaetes the sediment surface was firmly glued together. This ecological situation was reflected by a bacterial population which consisted of a high number of almost exclusively small-size cells with a corresponding low biomass (Fig. 8). Since the bacteria actively grew as demonstrated by a high number of dividing cells, nutrient deficiency could not be the reason for the impoverishment of the bacterial population. More likely, preferential grazing on medium and large-size bacteria let to the restriction in the size spectrum of the bacteria. Although literature regarding interrelationships between bacteria and fauna components is sparse, there is some evidence for the stimulation of bacterial activity by grazing (Gerlach, 1978 ; Morrison and White, 1980).

DIURNAL FLUCTUATIONS OF BACTERIAL POPULATIONS

The strong diurnal rhythms of benthic primary production (Jorgensen et al., 1979 ; Karg, 1979 ; Revsbech et al., 1981) imply a coupling between autotrophic and heterotrophic processes in shallow water sediments. Evidence for the existence of diurnal rhythms in benthic bacterial activities obviously closely related to primary production was obtained from investigations of sediments below sea-grass beds in Moreton Bay, Queensland, Australia (Moriarty and Pollard, 1982) and from studies in sandy sediments of the Kiel Bight, Baltic Sea, FRG (Meyer-Reil and Graf, unpublished data). Since the latter study comprises various parameters related to benthic biomass and activity, the discussion of diurnal fluctuations of bacterial population will be based upon these results.

Benthic biomass and activities.

In sandy sediments of the Kiel Bight sampled during a 36 hour cycle in summer, strong diurnal fluctuations occurred in total organic matter, benthic biomass and activities (Fig. 9, 10). Total organic matter and living benthic biomass (derived from ATP measurements) accumulated around midnight and decreased during the day.

Bacterial biomass, however, revealed an opposite fluctuation pattern : maxima were observed in late afternoon and minima between midnight and early morning (cf. Fig. 9).

As indicated by the almost constant ratio between total organic matter and protein, the composition of organic material did not change during the day/night cycle. Assuming a conversion factor of 200 for calculating organic carbon from ATP, and assuming that carbon represents 50% of the total organic material, living biomass on an average accounted for 5% of the total organic carbon. Bacterial biomass made up 5% of the total living biomass.

Benthic activities were well correlated among each other despite of the fact that quite different parameters of heterotrophic activity were measured such as total metabolic activity (heat production), exoenzymatic decomposition of particulate organic material (activity of α-amylase), and bacterial uptake of dissolved organic substrates (^{14}C-labelled glucose). Benthic activities increased during the morning, culminated around noon and decreased during afternoon and night (cf. Fig. 10). A corresponding diurnal pattern in bacterial activity was reported by Moriarty and Pollard (1982) for sediments below seagrass beds based upon the incorporation of thymidine into DNA.

Trophic interrelationships.

At least in periods with sufficient light supply, shallow water sediments may represent self-supporting systems governed by benthic primary production. It may be speculated that heterotrophic benthic activities are stimulated by the excretion of substances from the benthic primary production (microphytobenthos) which is initiated by light in the early morning. Bacteria take up the excretion products and respond with a subsequent increase in biomass (note the time lag between bacterial uptake of substances and increase in biomass, Fig. 9, 10). Bacteria as basic members of the food chain are grazed by meio- and macrofauna. As a consequence, total benthic activity (heat production) and enzymatic decomposition of particulate organic material in the sediment as well as in selected meiofauna organisms (cf. Faubel and Meyer-Reil, 1983) increase. After maximum values are reached around noon, benthic activities decline towards the afternoon and night obviously due to a decreasing supply with primary produced material.
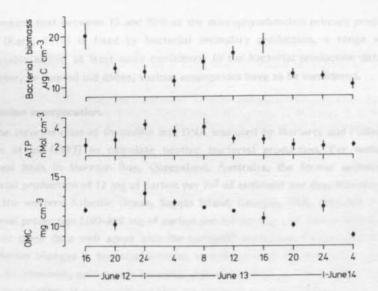
Fig. 9. Diurnal fluctuations of total organic matter, ATP, and bacterial biomass in the 0 to 1 cm horizon of a sandy sediment (water depth 10 m) of the Kiel Bight (Baltic Sea, FRG) sampled every 4 hours during a 36 hour cycle (June 12 to June 14, 1980).
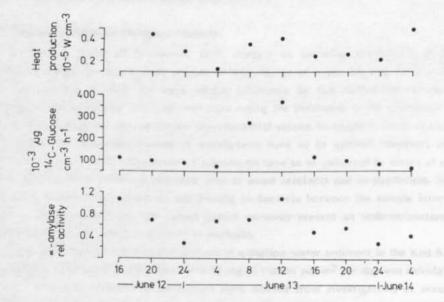

Fig. 10. Diurnal fluctuations of exoenzymatic decomposition rates of carbohydrate (activity of α-amylase), bacterial uptake of ^{14}C-glucose, and heat production in the 0 to 1 cm horizon of a sandy sediment (water depth 10 m) of the Kiel Bight (Baltic Sea, FRG) sampled every 4 hours during a 36 hour cycle (June 12 to June 14, 1980).

ESTIMATES OF BACTERIAL PRODUCTION

Since bacteria play an important role as basic members of the food chain in sediments (Yingst and Rhoads, 1980 ; Gerlach, 1978), information on bactrial production is urgently needed. In the literature, however, reliable data on bacterial production are sparse. Certainly this is a reflection of the methodological problems involved. Different approaches have been applied to gain information on bacterial production in sediments (cf. Table 1).

Glucose uptake.

In sandy beaches of the Kiel Bight, actual uptake (flux) of glucose by bacteria amounted to 0.1 µg of carbon (glucose) per gram of dry weight sediment per h (summer conditions, average of 12 observations ; Meyer-Reil, 1978 ; Meyer-Reil et al. 1980). In these sediments approximately 2% of the total dissolved organic carbon (DOC) existed in the form of labile carbon (1% amino acids, 1% monosaccharides) from which glucose roughly represents one quarter (Meyer-Reil et al., 1978). Under the assumption that bacteria take up the remaining three quarters of the DOC with the same velocity, total bacterial carbon uptake would amount to 0.4 µg per g per h. Taking into account respiration (40% as an average of the respiration of different organic substrates) and excretion (estimate of 10%, no data available) bacteria would produced 0.2 µg of carbon per g per h, which is equivalent to 28 mg of carbon per m^2 of sediment per day (Table 1).

TABLE 1.

Summary of data concerning bacterial production in sediments of different areas.

Sediment	Method	Production (mg C m^{-2} d^{-1})
(1) Sandy beaches, Kiel Bight, Baltic Sea, FRG	Glucose uptake	28
(2) Sediment associated with seagrass beds Moreton Bay, Queensland, Australia	Thymidine incorporation	12
(3) Sand, Nearshore western Atlantic Ocean Sapelo Island, Georgia, USA	Thymidine incorporation	100-800
(4) Sandy-mud/mud Kiel Bight, Baltic Sea, FRG Autumn Winter Spring	Seasonal changes in biomass	140/370 20/10 300/120
(5) Sand, Kiel Bight, Baltic Sea, FRG	Diurnal changes in biomass	80

This means that between 10 and 30% of the microphytobenthos primary production in this area (Karg, 1979) is fixed by bacterial secondary production, a range which sounds reasonable adding at least some confidence to the bacterial production data calculated. However, as pointed out above, various assumptions have to be considered.

Thymidine incorporation.

The incorporation of thymidine into DNA was used by Moriarty and Pollard (1982) and Fallon et al. (1983) to calculate benthic bacterial production. For sediments below seagrass beds in Moreton Bay, Queensland, Australia, the former authors reported a bacterial production of 12 mg of carbon per m^2 of sediment per day. Nearshore sediments from the western Atlantic Ocean, Sapelo Island, Georgia, USA, revealed a much higher bacterial production (100-800 mg of carbon per m^2 per day ; cf. latter authors). The lower limit of these data well agree with the bacterial production extrapolated from seasonal and diurnal changes in bacterial biomass in sediments of the Kiel Bight (cf. below, cf. Table 1). However, calculating bacterial production from the incorporation of thymidine requires a number of assumptions which are difficult to verify. Among these, the question of isotope dilution and the validity of conversion factors to calculate production from bacterial uptake of thymidine seem to be most important (for a detailed discussion cf. papers mentioned above and literature cited therein).

Diurnal and seasonal changes in biomass.

Calculating bacterial production from changes in bacterial biomass in samples collected frequently during short periods of time (range of hours, cf. Fig. 9) at a fixed location primises to offer the most reliable estimates. By this method the addition of exogenous substrates and artificial conditions during the incubation in the laboratory can be avoided. Except for the conversion from bacterial volume to weight in terms of carbon (cf. above), no conversion factors or assumptions have to be applied. However, other problems arise. A sufficient number of subsamples have to be collected by divers at each time interval from a defined sediment area to avoid artefacts due to patchiness. Since bacterial excretion of substrates and grazing on bacteria between the sample intervals can not be accounted for, the values gained certainly present an underestimation of bacterial production which is difficult to evaluate.

Base upon studies of diurnal fluctuations at a shallow water sediment in the Kiel Bight (cf. above), a bacterial net production of 80 mg of carbon per m^2 per day was calculated. Further estimates of bacterial production were derived from investigations of seasonal varaitions in bacterial biomass at two sediment stations in deeper waters of the Kiel Bight. As response to the input of the phytoplankton bloom in autumn, bacterial production amounted to 140 and 370 mg of carbon per m^2 per day (sandy-mud and muddy sediment, respectively). The corresponding values in spring were 300 and 120 mg of carbon per m^2 per day, respectively (cf. Table 1). As it was pointed out above, these values well agree with the lower range of bacterial production data derived from the incorporation of thymidine into DNA (Fallon et al., 1983).

CONCLUSION

From the information available it can be accepted that bacteria colonize sediments in high number and biomass. Although they represent less than 1% of the total sediment organic carbon, bacteria contribute significantly to the benthic biomass thus stressing their important role as nutrient source for the benthic fauna. Between the distribution of bacteria and sediment properties such as grain size and organic matter, close correlations exist. However, as shown for the relationship between organic material and bacterial biomass, interpretations are difficult to derive.

Processes within the benthic bacterial community may occur in very short time scales. The strong diurnal rhythms observed indicate a close coupling between autotrophic and heterotrophic processes in shallow water surface sediments. In boreal marine ecosystems the seasonal development of the benthic bacterial populations turned out to be strongly influenced by certain ecological situations and events in the sediment, from which the input of the phytoplankton blooms in autumn and spring, respectively, the accumulation of organic material during winter, and the development of the benthic fauna in spring were most important. The enrichment of organic material in the sediment surface let to corresponding stimulations in the enzymatic decomposition rates of particulate organic material. Bacteria immediately reacted on the availability of organic material with a drastic shift in the size distribution of biomass.

Reliable data on benthic bacterial production are urgently needed. From the very few data based upon quite different approaches, production ranges between 10 and some 100 mg of bacterial carbon per m^2 of sediment per day. These estimates already illustrate the importance of bacteria in the turnover of organic material in sediments.

Although basic information on the spatial and temporal distribution of benthic bacterial biomass and activity is available, a number of questions still remain open. Among these, the measurement of bacterial production, seasonal variations in the metabolic activity of bacterial populations, and interactions between bacteria and the benthic fauna need further attention.

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HEAT PRODUCTION OF MICROORGANISMS
IN EUTROPHIED ESTUARINE SYSTEMS - AN EXPERIMENTAL STUDY.

P. LASSERRE[1], T. TOURNIE[1], M. BIANCHI[2] and S. CHAMROUX[1].

[1]Station Biologique de Roscoff, Université de Paris VI & CNRS, 29211 Roscof

[2]Laboratoire de Microbiologie Marine, CNRS, Univ. de Provence, 13331 Marseille (France

ABSTRACT

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The microbially-mediated degradation of organic materials in estuaries and lagoon exerts important influences on the chemical composition of sediments and waters. I these shallow water systems, the biogeochemical processes occuring at the sediment water interface are relatively well-known, but rates and mechanisms are ill defined. The temporal behaviour of microorganisms colonising the water-sediment interface fron different estuarine and lagoonal environments was studied, in microcosms, to compare their relative ability to recover from experimental eutrophication.
The microcalorimetric data and simultaneous studies of the microbial physiologica potentialities indicate that the power-time curves describe dissipative structure, with reproducible patterns in the form of temporal successions of microorganisms. The evolution of the specific rate of heat dissipation ($\dot{q}$) showed differences in relation to seasonal thermal regimes. A characteristic feature is the increase in the specific hea production rate, shortly after nitrogen enrichment of the microcoms, followed by ; decrease indicating subsequent adaptational changes in populations. These results offer ar example of the value of direct microcalorimetry in studying homeostatic capabilities o ecosystems. Here, heat dissipation is an adequate parameter of the ability of the systen to return, after transitory oscillations, to a new steady state (a parameter of "resilience").

INTRODUCTION

Biogeochemical processes in organic-rich estuaries and lagoons are dominated by the influence of microbially mediated degradation of recently deposited organic matter These processes are relatively well defined and they take place notably at the sediment water interface (Martens, 1982 ; Nixon, 1984). Nevertheless, the difficulty in providing ; characterization of the dynamics of microbiological processes induced by eutrophicatior still remains. The kinetics of biogeochemical transformations, brought about during the degradation of complex mixtures of material of natural and anthropogenic origin, by il defined mixed populations of microbes such as occurs in the marine coastal systems, are not easily studied by the reductionist techniques applicable to homogeneous systems.

Measurement of hydrolytic activity and incorporation of dissolved organic substrates by microorganisms in marine sediments

Lutz-Arend Meyer-Reil

Institut für Meereskunde an der Universität Kiel, Abteilung Marine Mikrobiologie, Düsternbrooker Weg 20, D-2300 Kiel 1, Federal Republic of Germany

ABSTRACT: A core-injection technique is described which enables the assessment of microbial activities (extracellular enzymatic cleavage of carbohydrates and proteins, incorporation of dissolved organic substrates into microbial biomass) in natural sediment cores. Enzymatic activities were determined by means of fluorogenic substances (4-methylumbelliferyl-β-D-glucoside, L-leucine-4-methylcoumarinyl-7-amide HCl); incorporation of dissolved organic substances were measured using tritiated substrates (thymidine, leucine, acetate, glucose). Microliter portions of substrates were injected at 0.5 cm intervals into natural sediment cores, which were incubated, quick frozen, dissected and analysed. For a proper application of the core-injection technique different parameters were investigated such as volume of substrate injected, significance of diffusion of substrate, concentration of substrate and incubation time, and treatment of samples to account for the non-biological 'turnover' of substrates. In depth profiles of sediments from the Kiel Bight (Baltic Sea; FRG), microbial extracellular enzymatic activities were highest in the surface horizons and decreased steadily with sediment depth. Incorporation rates of dissolved organic substrates, however, revealed a more complex depth-dependent variation pattern indicating a pro:.unced stratification of microbial metabolism in the sediment cores.

INTRODUCTION

The microbiology of marine sediments has received increasing interest during recent years since coastal sediments have been recognized as important locations of nutrient regeneration in marine ecosystems. Most of the organic material entering the sediments by sedimentation is particulate organic carbon which is partly consumed directly by benthic organisms and partly incorporated into the pool of particulate carbon in the sediment. Through the activity of extracellular enzymes derived from living cells or liberated through the lysis of cells, particulate organic carbon is stepwise converted into low-molecular-weight dissolved organic carbon. By excreting extracellular enzymes and incorporating the products of the enzymatic hydrolysis, microorganisms play the key role in the decomposition processes of organic material. Through microbial activities, biomass is formed which serves as an important nutrient source for benthic organisms.

Recently, techniques have been reported for the measurement of extracellular enzymatic activities in natural waters using artificial substrates whose hydrolysis by natural occurring enzymes is followed by the liberation of highly fluorescent compounds (Hoppe 1983, Somville 1984). For the measurement of the microbial incorporation of dissolved organic substances, tritiated substrates can be applied in tracer concentrations thus changing natural substrate concentrations only minimally (King & Klug 1982).

Sediments exhibit a high structural complexity. Activity measurements that are hoped to reflect natural conditions require the incubation of intact sediment cores. Disruption of the system (sediment dissected and suspended in seawater) leads to a considerable stimulation of microbial metabolism (Hall et al. 1972, Meyer-Reil 1978, Novitsky 1983).

In the paper presented here, a core-injection technique is described which enables the measurement of microbial activities in intact sediment cores. For a proper application of the technique, different parameters were investigated, such as volume of substrate

injected, significance of diffusion of substrate, concentration of substrate and incubation time, and treatment of samples to account for the non-biological 'turnover' of substrate. The core-injection technique was applied to sediments from the Kiel Bight (Baltic Sea; FRG), in which depth-dependent variation of extracellular enzymatic activities and incorporation of dissolved organic substrates by microorganisms were investigated.

MATERIALS AND METHODS

Sampling. Sediment samples were collected using a Reineck grab from a muddy sand station (water depth 18 m) located on the slope of the Kiel Bight channel system. The sampling area, known as 'Hausgarten', has been subject to several investigations during recent years (see Graf et al. 1983, Meyer-Reil 1983, Balzer 1984). For the aim of this paper, 3 sediment profiles will be presented which were sampled on June 19, September 20, and November 8, 1984. Surface temperatures of these sediments were 6, 14, and 11 °C, respectively. Whereas the discontinuity layer (0 mV) of the sediments in June and September was close to the surface, the 0 mV layer in November dropped down to 1 cm sediment depth.

Core-injection technique. Activity of hydrolytic enzymes and incorporation of dissolved organic substrates by microorganisms were determined in undisturbed sediment cores (0 to 8 cm depth) which were taken on board ship 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 carefully sealed with a thin layer of a one-component silicon rubber (Elchsiegel). Immediately after sampling, 10 µl of the substrate tested for enzymatic hydrolysis or incorporation (see below) was injected at 0.5 cm intervals through the ports into the water overlying the sediment and into the sediment using a gastight syringe (Hamilton, 50 µl volume). During injection the tubes were mounted on a rack which allows accurate, horizontal injection of the substrates into the centre of the sediment horizons.

Activity of hydrolytic enzymes. As substrates for the measurement of the extracellular enzymatic activities (cleavage of carbohydrates and proteins), 4-methylumbelliferyl-β-D-glucoside (MUF-glu; Sigma) and L-leucine-4-methylcoumarinyl-7-amide HCl (MCA-leu; Serva) were used. Five mM solutions of the substrates were prepared in brackish water (salinity app. 10 ‰), filter sterilized, flushed with nitrogen, temperature equilibrated and injected in 10 µl portions into the sediment horizons as described above. Sediment cores (3 parallels) were incubated at *in situ* temperature for

1 h. For the standardization experiments, injection volume, substrate concentration and incubation time were varied. After incubation the activity was terminated by freezing the sediment core in dry ice. For the analysis, the sediment cores (0 to 8 cm depth) were sectioned at 1 cm intervals, and the centres of the horizons cut out with a cork drill (4 mm in diameter). The material from 3 parallel sediment horizons was combined in a centrifuge tube, and 3 ml of brackish water were added. All manipulations were carried out at 0 °C. After centrifugation (15 min, 5000 rpm, 0 °C), 2.5 ml of the supernatant was mixed in a cuvette with 0.25 ml of buffer (sodium tetraborate/sodium hydroxide; pH 10.8), and the fluorescence intensity was read in a spectrofluorometer (Jasco FP-550) at 455 nm under 365 nm excitation. A 1 µM solution of methylumbelliferon was used as a standard for each measurement. Since the enzymatic hydrolyses of MUF-glu and MCA-leu, respectively, yield equimolar concentrations of the fluorescent dye and the substrate (glucose and leucine, respectively), the latter could be calculated from the amount of dye released. For each section, the dry weight of the sediment was determined; and extrapolations from dry weight to volume were made by considering the specific gravity of the individual sediment horizons.

Incorporation of dissolved organic substrates. For the measurement of the incorporation of dissolved organic carbon by microorganisms, the following substrates were used: (methyl-^{3}H)thymidine (355 mCi mg^{-1}), D-(2-^{3}H)glucose (94 mCi mg^{-1}), L-(4,5-^{3}H)leucine (880 mCi mg^{-1}), (^{3}H)acetate (42.7 mCi mg^{-1}). The substrates were purchased from Amersham Buchler and diluted in particle-free brackish water to an activity of 0.25 µCi in 10 µl equivalent to the following amounts: thymidine 0.70 ng (3.66 ng), glucose 2.66 ng (13.82 ng), leucine 0.28 ng (1.48 ng), and acetate 5.85 ng (30.39 ng). The values in brackets reflect the final concentration of substrates per cm^3 of sediment. For this calculation, the volume of the sediment segment analysed was extrapolated to 1 cm^3. After injection of 10 µl portions of substrate into the sediment horizons (3 parallels), samples were incubated at *in situ* temperature for 3 h, and fixed in dry ice. For the analysis the sediment cores were sectioned at 1 cm intervals (0 to 8 cm depth), the centres of the horizons cut out with a cork drill (7 mm in diameter), the material from 3 parallel horizons combined in a centrifuge tube, and 3 ml of brackish water added. The sediment was washed at least 3 times with brackish water by centrifugation (15 min; 5000 rpm) until the radioactivity in the supernatant was removed. All manipulations were carried out at 0 °C. After drying the weight of the sediment was determined, an equal volume of cellulose powder was added, and tablets were pressed

which were then oxidized in a Tri-Carb Oxidizer (Packard), and analysed with 15 ml of Monophase 40 in a liquid scintillation counter (Betazint 5000).

Controls for the assessment of the non-biological 'activities' (enzymatic hydrolysis, incorporation of dissolved organic substrates) were run with mixed sediment from the individual horizons which were sterilized by autoclaving (30 min, 121 °C). In parallel, other fixation treatments were examined (see below).

RESULTS AND DISCUSSION

Core-injection technique

The core-injection technique described enables the measurement of microbial activities (enzymatic hydrolysis, incorporation of dissolved organic substrates) in sediment cores under conditions close to those *in situ*. Since the sediments remain intact, natural gradients and diffusion processes are only minimally altered. Similar techniques have been successfully applied by Jørgensen (1978) and Ansbaek & Blackburn (1980).

For the proper application of the core-injection technique, different parameters were examined: volume of substrate injected, significance of diffusion of substrate within the sediment volume analysed, concentration of substrate injected and incubation time, activity in 'parallel' sediment cores, treatment of sediments to account for the non-biological 'turnover' of substrates (controls), activity in undisturbed versus disturbed sediments. Most of the standardization experiments were carried out using 4-methylumbelliferyl-β-D-glucoside (MUF-glu) as substrate.

Injection volume

In natural sediment cores, 2, 5, 10 and 15 μl of MUF-glu (5 mM solutions) were injected, and enzymatic hydrolysis rates were determined. Up to 10 μl, the activities increased linearly. With 15 μl injected, there was no significant increase in the hydrolysis rates. Similar results were obtained for the incorporation of (³H)glucose. For subsequent field studies, a volume of 10 μl of substrate was chosen. The choice of this volume represented a compromise. On the one hand, substrate limitation should be avoided in the sediment horizons, and on the other hand, dilution of the pore water by the volume of substrate injected should be kept low. Considering a water content of 35 % (typical for the 0 to 1 cm horizon of the sediments investigated), the volume of substrate injected led to a dilution of the pore water by 15 %; based on a water content of 25 % (typical for deeper sediment horizons), however, the

dilution was 21 %. The calculation of these values was based on the assumption that the substrate injected was initially homogeneously distributed through the sediment segments analysed. In reality, however, the dilution rates changed with time.

Substrate transport

Through the injection of substrate, a concentration gradient was created within the sediment. Initially, the substrate was concentrated in the centre at each depth. During incubation, the substrate spread out due to active and passive transport. Two sets of experiments were carried out to assess the significance of diffusion on the activity measurements. By using cork drills of different diameters, the enzymatic hydrolysis of MUF-glu was analysed at different distances from the centre. It could be demonstrated that even in the peripheral part of the sediment segments analysed, 80 % of the activity of the centre could still be measured. Similar results were obtained in experiments in which MUF-glu was injected in every second horizon of the sediment core. On average, sediment horizons that were initially not supplied with substrate revealed a reduction in activity by 10 % as compared to the horizons that received the substrate. Parallel sediment cores in which every horizon was supplied with substrate on average showed a 20 % higher activity. This can be explained by the fact that both the substrate (MUF-glu) and the product of the enzymatic reaction (MUF) were subject to transport in the pore water. This led to a 'loss' of activity in the injected horizons of those sediment cores in which only every second horizon was supplied with substrate. Corresponding experiments with tritiated substrates were not carried out. However, the solubility of the substrates used and the incubation time of 3 h (see 'Materials and Methods') imply sufficient transport within the sediment volume analysed.

Concentration of substrate and incubation time

The proper measurement of activity rates requires: (a) linearity of activity over time (absence of substrate exhaustion or induction), and (b) minimal changes of substrate concentrations during incubation (Harrison et al. 1971). To confirm these requirements, a range of substrate concentrations was applied whose turnover was measured in time-course experiments. To reduce the heterogeneity of natural sediments, the plexiglas tubes were supplied with mixed sediment that was allowed to sit for 12 h prior to injection of substrate. Concentrations of 1, 2.5, and 5 mM MUF-glu and MCA-leu, respectively, revealed linear hydrolysis

curves over an incubation time of at least 2 h. How-
ever, even the highest concentration (5 mM) did not
saturate the enzymes. Since MUF-glu as well as MUF-
leu are soluble in brackish water only up to a concen-
tration of 5 mM, this concentration in connection with
an incubation time of 1 h was applied in subsequent
field studies. Experiments with tritiated substrates
using different concentrations and incubation times
were not always satisfactory. Parallel measurements
differed considerably which may be explained by
small-scale differences in the activity of 'parallel'
cores. With relatively low activities of ^{3}H-glucose
(0.025, 0.05, 0.1 µCi, equivalent to 0.27, 0.53, and
1.06 ng per 10 µl injected), a rapid exhaustion of sub-
strate was observed. Higher activities (0.25, 0.5 µCi,
equivalent to 2.66 and 5.32 ng, respectively, per 10 µl
injected), allowed a linear increase of incorporation
rates for at least 5 h. Corresponding straight lines were
obtained for the microbial incorporation of ^{3}H-acetate
(K. Lochte pers. comm.). Subsequent field studies were
based on an activity of 0.25 µCi (per 10 µl of substrate
injected) which guaranteed a linear activity over time
without changing the natural concentration to a great
extent (see below).

Activity in parallel sediment cores

Due to the heterogeneity of natural samples, enzy-
matic hydrolysis and incorporation of dissolved
organic substrates measured in sediment cores taken
from one grab could vary by a factor of up to 3. The
variation of 'parallel' cores was highest in the top cm
and decreased with sediment depth. 'Conservative'
parameters such as thymidine incorporation revealed
significantly less variations as compared to incorpora-
tion rates of glucose, acetate or leucine. However, if
the sediment was thoroughly mixed and subsamples
were taken immediately after mixing, the variations in
activities were greatly reduced. Experimental error
itself was generally less than 10 % (standard deviation
of the mean value). The analysis of the sediment cores
as outlined in 'Materials and Methods' represented a
compromise: 3 parallel cores per substrate were incu-
bated; for the analysis, however, the material from the
3 parallel horizons was combined.

Controls

Introductory experiments for the assessment of the
non-biological 'turnover' of substrates were carried out
with natural sediment cores that were autoclaved or in
which formalin was injected prior to the injection of
substrate. Both treatments turned out to be not very
promising. Autoclaving caused a disruption of the sed-
iment cores. Enzymatic hydrolysis and incorporation
rates of dissolved organic substrates in formalin treat-
ed cores were disproportionately high (up to 50 % of
the activity in untreated cores) even after formalin was
injected prior to the injection of substrate. For subse-
quent field studies, mixed sediment obtained from
dissecting sediment cores according to the correspond-
ing horizons served as controls. Control samples varied
significantly dependent on the sample, the substrate
and the kind of fixation. Generally, autoclaved sedi-
ments showed the lowest control values. Samples fixed
with concentrated formalin or glutaraldehyde were
higher by a factor of 2 to 4. From the dissolved organic
substrates, the lowest controls were observed with glu-
cose or thymidine as substrates; considerably higher
controls were found with charged substrates such as
acetate or leucine.

The treatment of sediment samples to account for the
non-biological 'turnover' of substrates (controls) is only
poorly addressed in the literature. Each kind of fixation
(heating, chemical treatments) causes alterations in
the structure and the adsorption properties of the sedi-
ment, so that 'absolute' control values are difficult to
obtain. The problem of proper controls arises espe-
cially in those sediment horizons where low microbial
activities may be altered considerably by questionable
controls.

Activity in undisturbed versus disturbed sediments

Exoenzymatic hydrolysis and incorporation of dis-
solved organic substrates in sediment cores that were
dissected and suspended in brackish water were on
average 1 order of magnitude higher as compared to
undisturbed sediments. The relative increase in activ-
ity in disturbed sediments varied with substrate and
sediment depth. Corresponding observations have
been reported by other authors (Hall et al. 1972,
Meyer-Reil 1978, Novitsky 1983). In seeking an expla-
nation, the complex structure of sediments and the
bacterial colonization of particles have to be con-
sidered. Through the disruption of the sediment fine
structure, microenvironments are destroyed, bacterial
aggregates are split up, and cells or groups of cells are
separated from each other. The cells are optimally
supplied with substrate; phenomena such as competi-
tion or inhibition of substrate uptake are diminished.
Based on the increase of microbial activities in dis-
turbed samples, sediments have to be characterized as
biotopes of a considerable potential activity; and one
may image the importance of natural mixing processes
such as bioturbation or mechanical disturbance by
waves for the stimulation of microbial activities.

Microbial activities in natural sediments

Based on the core-injection technique described, hydrolytic activities and incorporation rates of dissolved organic substrates by microorganisms were followed in sediment profiles sampled on June 19, September 20, and November 8, 1984, from a muddy sand station of the Kiel Bight. The activity of hydrolytic enzymes was measured using 4-methylumbelliferyl-β-D-glucoside (MUF-glu) and L-leucine-4-methylcoumarinyl-7-amide HCl (MCA-leu) as substrates (for literature see Hoppe 1983, Somville 1984). This fluorimetric assay is much more sensitive than the photometric determination based on covalent-bound dye derivates (amylopectin azure, hide powder azure) used in former sediment investigations (Meyer-Reil 1983). To my knowledge, this is the first time that the fluorimetric assay was applied to sediments. MUF-glu and MCA-leu represent model substrates which are analogs for a variety of natural occurring substrates of unknown concentration and composition. Because of this, the microbial hydrolysis rates measured describe potential activities. However, the rates measured reflect the pool of natural occurring enzymes which is the result of variations in concentration and composition of natural substrates.

For the measurement of the microbial incorporation of dissolved organic substances, glucose, leucine, and acetate were chosen which partly represent products of the hydrolytic activities and partly intermediary products of the anaerobic microbial metabolism. Parallel determinations of the incorporation of thymidine were used as a relative indicator for microbial growth (without any intention to calculate growth rates; see Pollard & Moriarty 1984).

The activity rates measured characterize the incorporation of dissolved organic substrates into microbial biomass. Respiration as well as excretion of substances could not be considered. From the analysis it could not finally be distinguished between the incorporation of the added substrate and (or) its metabolic products excreted during cell metabolism into the pore water. This applies especially to the anaerobic decomposition of glucose which yields low molecular weight fatty acids, mainly acetate (King & Klug 1982). Through the application of the tritiated substances, tracer amounts could be injected into the sediment horizons. However, the natural concentrations of the substrates are unknown. Based on the very few reliable data available for the natural concentrations of glucose (Meyer-Reil et al. 1980, King & Klug 1982) and acetate (Ansbaek & Blackburn 1980, Christensen & Blackburn 1982) in sediments, it can be derived that the concentrations added amounted to less than 10 % of the natural concentrations. This means that the incorpora-
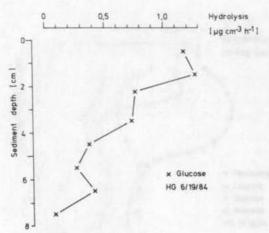

Fig. 1. Hydrolysis of 4-methylumbelliferyl-β-D-glucoside (µg glucose released). Sediment profile from Kiel Bight, Station 'Hausgarten' (HG), 19 Jun 1984
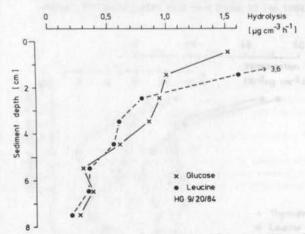

Fig. 2. Hydrolysis of 4-methylumbelliferyl-β-D-glucoside and L-leucine-4-methylcoumarinyl-7-amide HCl (µg glucose and leucine, respectively, released). Sediment profile from Kiel Bight, 20 Sep 1984
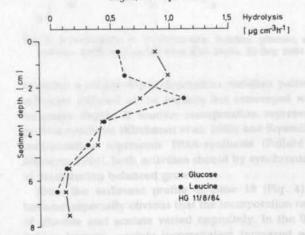

Fig. 3. Hydrolysis of 4-methylumbelliferyl-β-D-glucoside and L-leucine-4-methylcoumarinyl-7-amide HCl (µg glucose and leucine, respectively, released). Sediment profile from Kiel Bight, 8 Nov 1984

tion rates reported have to be multiplied at least by a factor of 10 in order to derive actual incorporation rates. However, even if natural concentrations are known, the question arises what percentage of the substrate is available for microbial metabolism (Christensen & Blackburn 1982, Thompson & Nedwell 1985).

In sediments of the Kiel Bight, extracellular enzymatic activities were generally highest in the surface layer (0 to 2 cm) and decreased steadily with depth (Fig. 1 to 3). The hydrolysis of MUF-glu and MCA-leu could differ considerably at the surface, but converged with depth. In the 7 to 8 cm horizon less than 15 % of the activity at the surface was measured. These observations indicate that the enzymatic decomposable material accumulated mainly at the sediment surface, obviously due to the sedimentation of organic material from the water column (see Graf et al. 1983, Meyer-Reil 1983). Although the total organic matter content (determined as ignition lost) often increased in medium sediment horizons (4 to 5 cm; data not shown), this increase was not reflected by a corresponding stimulation of extracellular enzymatic activities. Since this material obviously withstands an easy microbial hydrolytic attack, it has to be characterized as more resistant. The fate of this material is uncertain. Part of it may be buried in deeper sediment horizons (Balzer 1984); part of it may be slowly decomposed. As could be shown by Shimp & Pfaender (1985), the microbial decomposition of the more resistant organic material will be considerably stimulated by the availability of easily decomposable organic substances.

Incorporation rates of dissolved organic substrates in sediments of the Kiel Bight revealed a complex variation pattern which differs from the conventional picture of steadily decreasing microbial parameters with increasing sediment depth (Fig. 4 to 6). Generally, the highest incorporation rates were measured at the surface. An exception was the sediment profile of June 19 in which the highest incorporation rates of thymidine and acetate were determined in deeper sediment horizons (1 to 2 cm and 2 to 3 cm, respectively; see Fig. 4). Although the incorporation rates generally decreased with sediment depth, steadily decreasing activities were the exception. Dependent on the sample and the substrate, zonations in the activity were observed which indicate a stratification of microbial metabolism in the sediment profiles.

A comparison of the incorporation rates of dissolved organic substrates into microbial biomass measured at the 3 different periods (cf. Fig. 4 to 6) indicates an obvious seasonal influence: activities were highest in late summer. Generally, lower activities were recorded in fall, and the lowest activities were measured in late spring.

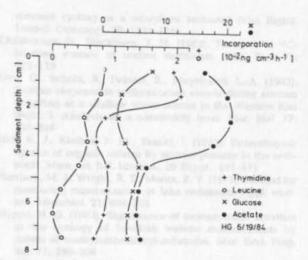The incorporation rates of leucine and thymidine

Fig. 4. Incorporation of ^{3}H-thymidine, leucine, glucose, and acetate. Sediment profile from Kiel Bight, 19 Jun 1984
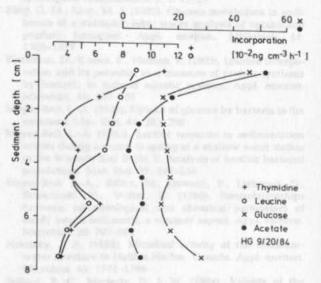

Fig. 5. Incorporation of ^{3}H-thymidine, leucine, glucose, and acetate. Sediment profile from Kiel Bight, 20 Sep 1984

revealed a similar depth-dependent variation pattern: activities differed at the surface but converged with sediment depth. If leucine incorporation represents protein synthesis (Kirchman et al. 1985) and thymidine incorporation represents DNA-synthesis (Pollard & Moriarty 1984), both activities should by synchronized at least during balanced growth.

From the sediment profile of June 19 (Fig. 4), it became especially obvious that the incorporation rates of glucose and acetate varied oppositely. In the 0 to 2.5 cm horizon, acetate incorporation increased with decreasing glucose incorporation. In the 2.5 to 4.5 cm horizon, however, acetate incorporation decreased whereas the incorporation of glucose increased. Below
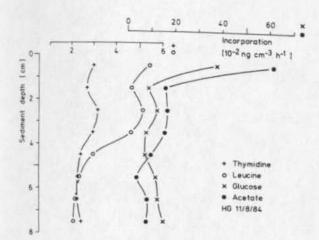
Fig. 6. Incorporation of ³H-thymidine, leucine, glucose, and acetate. Sediment profile from Kiel Bight, 8 Nov 1984

a sediment depth of 5 cm, both activities showed parallel curves. From the other sediment samples, only the profile of November 8 may give some indication for an inverse relation between the incorporation of glucose and acetate (see Fig. 5 & 6).

Because of the reasons stated above, the microbial incorporation of glucose is difficult to interpret, especially in anaerobic sediments. The incorporation of acetate, however, revealed a pronounced relation to the cycle of sulfate: maxima in sulfate reduction coincided with maxima in the acetate incorporation (Pollehne & Meyer-Reil unpubl. data). These observations underline the importance of acetate as a substrate for sulfate reducers in the sediments investigated.

A study is in progress to relate seasonal variations in hydrolytic activities and microbial incorporation of dissolved organic substrates to the input of organic material into sediments of the Kiel Bight. From this study further insights into the regulation of benthic microbial metabolism can be expected.

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

LUTZ-AREND MEYER-REIL

Marine Mikrobiologie, Institut für Meereskunde an der Universität Kiel, 2300 Kiel 1, Federal Republic of Germany

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 E_h 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
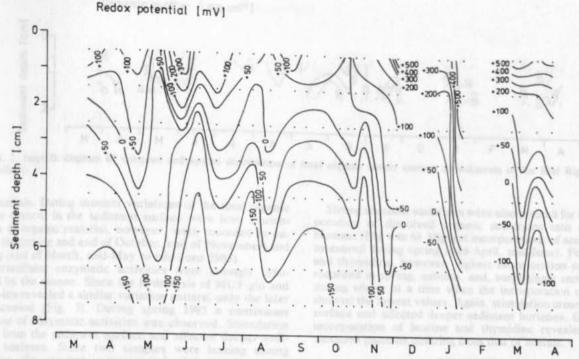
FIG. 1. Isopleth diagram of seasonal and spatial distribution of redox potential in sediments of the Kiel Bight (station Gabelsflach).

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: [^{3}H]acetate (42.7 mCi/mg), L-[4,5-^{3}H]leucine (880 mCi/mg), and [*methyl*-^{3}H]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 concen-

trations 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 with oxic 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
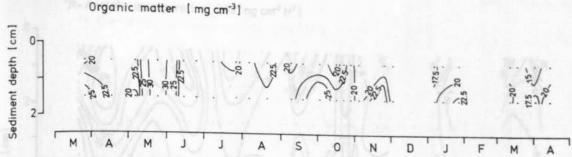
Organic matter [mg cm⁻³]

FIG. 2. Isopleth diagram of seasonal and spatial distribution of total organic matter content in sediments of the Kiel Bight (station Gabelsflach).

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 homogenously 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-

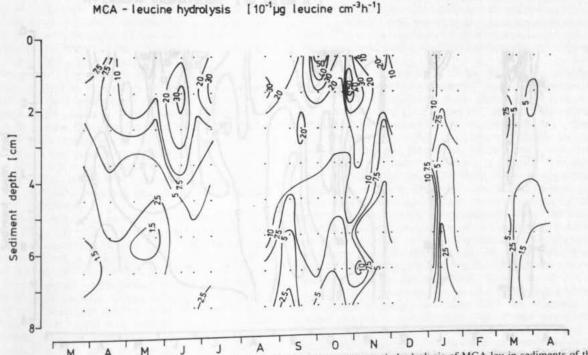MCA - leucine hydrolysis   [10⁻¹µg leucine cm⁻³h⁻¹]

FIG. 3. Isopleth diagram of seasonal and spatial distribution of extracellular enzymatic hydrolysis of MCA-leu in sediments of the Kiel Bight (station Gabelsflach).
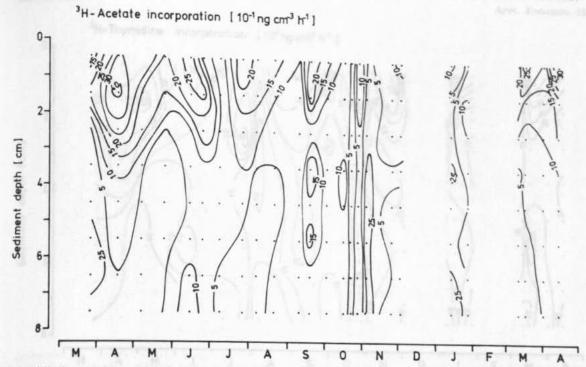
FIG. 4. Isopleth diagram of seasonal and spatial distribution of microbial incorporation of tritiated acetate in sediments of the Kiel Bight (station Gabelsflach).
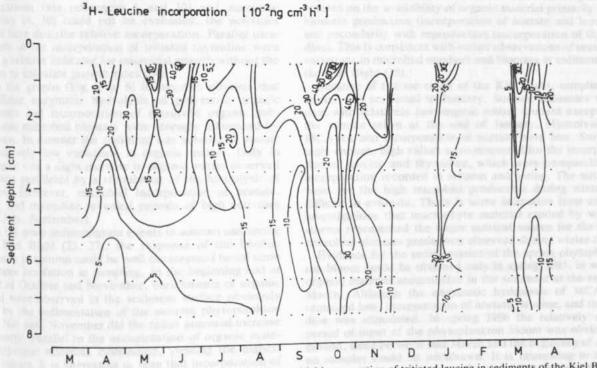

FIG. 5. Isopleth diagram of seasonal and spatial distribution of microbial incorporation of tritiated leucine in sediments of the Kiel Bight (station Gabelsflach).
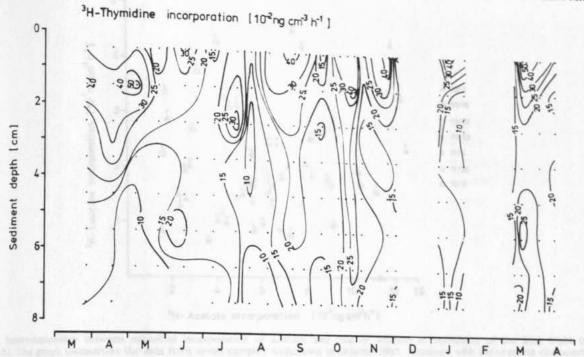
FIG. 6. Isopleth diagram of seasonal and spatial distribution of microbial incorporation of tritiated thymidine in sediments of the Kiel Bight (station Gabelsflach).

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 titriated 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.
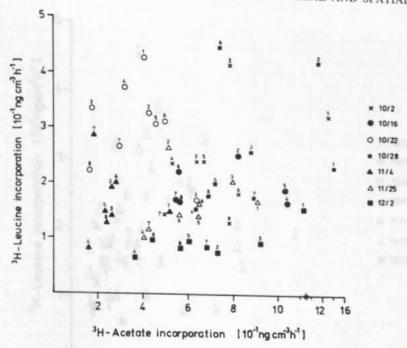
FIG. 7. Interrelationship between microbial incorporation of acetate and that of leucine in sediments of the Kiel Bight (station Gabelsflach). The graph summarizes the data from seven samples withdrawn in autumn 1985. Numbers with the symbols characterize the individual sediment horizons.

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 interdependences. 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 incor-

poration 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
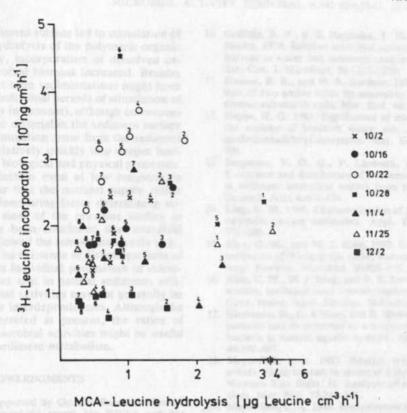
FIG. 8. Interrelationship between microbial incorporation of leucine and extracellular enzymatic hydrolysis of MCA-leu in sediments of the Kiel Bight (station Gabelsflach). For further explanations, see legend to Fig. 7.
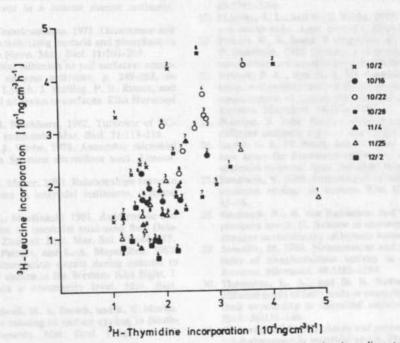

FIG. 9. Interrelationship between microbial incorporation of thymidine and that of leucine in sediments of the Kiel Bight (station Gabelsfalch). For further explanations, see legend to Fig. 7.

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.

ACKNOWLEDGMENTS

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