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Enzymatic decomposition of proteins and carbohydrates in marine sediments: methodology and field observations during spring*

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Abstract

Microbial biomass, concentrations and enzymatic decomposition rates of proteins and carbohydrates were analysed during spring 1980 from a 10 m sediment station in the Kiel Bight. During March, a considerable increase in total organic matter, proteins and carbohydrates was observed, followed by a decrease during April. The accumulation of organic matter in the sediment could be traced back to the breakdown of the algal spring bloom. Activity rates of α-amylase, β-D-glucosidase and proteolytic enzymes paralleled the variation pattern in concentrations of carbohydrates and proteins. During the decomposition processes, a drastic shift in the protein to carbohydrate ratio was observed. The dominating part of the enzymes involved in the decomposition of organic material obviously arose from autolysis of the algae cells themselves. After an initial depression, microbial biomass increased, obviously due to the availability of suitable substrates for microbial growth following the decomposition processes.

Introduction

In the decomposition of particulate organic matter in sediments, extracellular enzymes are involved, which are secreted from living cells (mainly microorganisms) as well as liberated during the lysis of cells (algae, microorganisms). Unless the enzymes are released in close contact to the substrate, their fate is uncertain. Processes like adsorption, decomposition and denaturation may greatly influence the efficiency of the enzymes released. According to studies carried out by BURNS (1980), some of these enzymes remain active in soil by the formation of humic-enzyme complexes. These active enzymes may contribute to the decomposition of organic material as well.

Proteolytic nzymes, α-amylase and β-D-glucosidase have been detected as secretion products of culturable marine periphytic bacteria (CORPE and WINTERS 1972) and as cell free enzymes in marine waters and sediments (KIM and ZoBELL 1974). The authors suggested the importance of these enzymes in the decomposition of particulate organic matter. However, our knowledge on relationships between concentrations and decomposition rates of organic matter in sediments is very limited. This paper presents information on the methodology of measuring enzymatic activities (α-amylase, β-D-glucosidase, proteolytic enzymes). In field experiments, concentrations and decomposition rates of proteins and carbohydrates were determined in brackish water sediments of the Kiel Bight following the breakdown of the algal spring bloom.

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Material and methods

Sampling: Sediment samples (0–2 cm horizon) were collected in plastic tubes (5.4 cm in diameter) by divers during spring 1980 from a 10 m station located in the brackish water of Kiel Bight. This area, know as ''Hausgarten'', is restricted to research and was subject to several investigations in recent years. At the station investigated, coarse sand of an average grain size of 0.7 mm is prevailing (WEFER and TAUCHGRUPPE KIEL 1974). Sediment for additional laboratory experiments was sampled in summer 1980 from sandy beaches of the Kiel Fjord, an area which has also undergone recent detailed investigation (MEYER-REIL et al. 1980).

Organic chemical analysis: Organic parameters were analysed from dried and ground sediment samples (mortar, Pulverisette 2; Fritsch GmbH). Protein was determined according to LOWRY et al. (1951) using bovine serum albumin (Boehringer Co) as a standard. Carbohydrates were assayed according to HANDA (1967) as discussed in detail by HENDRIKSON (1975) using soluble starch as a standard. Organic matter content was reported as the difference between the dry weight of the sediment and the residue left after combustion at 550°C.

Enzymatic activities: Fresh sediment samples (4 to 5 g of wet sediment) were incubated in 10 ml of filtered sterilized seawater in the presence of a suitable substrate. For the determination of the activity of α-amylase and proteolytic enzymes amylopectine azur (KIM and ZoBELL 1972) and hide powder azur (LITTLE et al. 1979) were added (approx. 25 mg). The activity of β-D-glucosidase was measured using p-nitrophenyl-β-D-glucosid dissolved in 10 ml of $NaHCO_3$ (25 mmol) buffer (MORRISON et al. 1977).
Samples (3 parallels) were incubated under shaking (150 rpm) at room temperature for 5 incubation periods. After centrifugation (10 min. 5000 rpm), the supernatant was analysed spectrophotometrically (PM 2K; Zeiss). Controls were run with sediment samples heated at 100°C for 30 min. Relative activity rates (changes in absorbance per 10 g of dry weight sediment per h) were determined from the slope of the activity curves calculated by linear regression. Relative activity rates were converted into units of enzyme equivalents per 10 g of sediment per h by means of standard curves prepared with commercially available enzymes (α-amylase, type III-A; protease, type V; Sigma Chemical Co.). In the case of β-D-glucosidase, relative activity rates were converted into μg of p-nitrophenol released per 10 g of sediment per h.

Microbial numbers, biomass: Sonicated (3 min. 50 Watt; Sonifier B 12, Branson Sonic Power) and diluted samples were filtered onto Nuclepore polycarbonate membranes, stained with acridine orange and analysed by epifluorescence microscopy. Biomass determinations were derived from size fractionations of the cells.

Results and discussion

Activity curves of β-D-glucosidase, α-amylase and proteolytic enzymes were linear during the incubation period (between 2 and 6 h for β-D-glucosidase and α-amylase; between 8 and 12 h for proteolytic enzymes). A typical activity curve (α-amylase as an example) is shown in Fig. 1.

As was to be expected, enzyme activities were strongly dependent on temperature. Within the temperature range studied (5, 10, 15, 20, 25, 30, 37°C), highest activity of β-D-glucosidase was obtained at 37°C. It is interesting to note that even at 5°C, approximately 30% of the activity measured at 20°C could be detected. The greatest increase in enzyme activity was recorded when the temperature was raised from 15 to 20°C, which obviously represents a critical temperature range at least for the

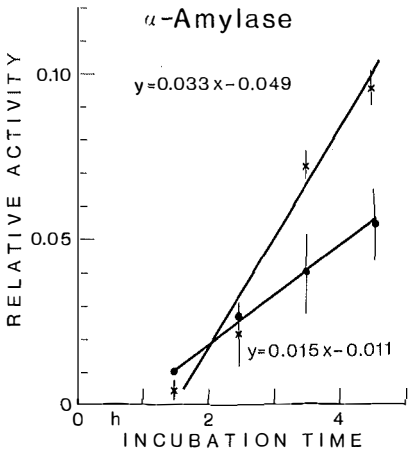

Figure 1.

Time-course development of α-amylase activity measured in untreated samples (x) and in samples treated with chloroform (•). Relative activity is expressed as changes in absorbance at 595 nm. Bars represent range of three observations.

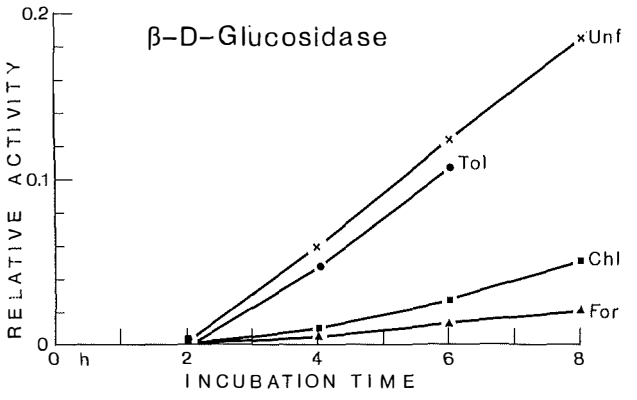

Figure 2.

Time-course of β-D-glucosidase. Effect of different inhibitors for preventing microbial growth on the activity measurements (Unf – untreated samples; Tol. Chl. For. – samples were treated with toluene, chloroform and formalin, respectively). Relative activity is expressed as changes in absorbance at 410 nm.

sediment systems studied. Within the salinity range investigated (0, 8, 16, 24, 32‰), highest enzymatic activity of β-D-glucosidase was measured between 0 and 8‰, although the activity was still high at 32‰ (80 % of the activity measured at the optimal salinity).

In the literature, different inhibitors have been used to prevent microbial activity during the enzyme tests. Fig. 2 illustrates the influence of different agents (added to prevent microbial activity) on the activity of β-D-glucosidase. Toluene, chloroform and formalin led to a decrease in enzymatic activity, an observation which is difficult to analyse. Processes like unmasking of enzymes (SKUJINS 1976), structural changes in the composition of enzymes as well as changes in the structure of microbial cell walls which become permeable to substrates and enzymatic products (OSHRAIN and WIEBE 1979), may be responsible for the overall decrease in the enzymatic activity observed. Furthermore, these processes may act differently from sample to sample. These unpredictable processes occurring in samples to which substances inhibiting microbial activity have been added make it extremely difficult to compare data from enzymatic activity determinations. Because of the relatively short incubation times at least for the determinations of α-amylase and β-D-glucosidase (cf. above), studies presented here were generally carried out with untreated samples.

Frequent sediment samples were taken between March 3 and May 5, 1980 from the "Hausgarten" area of the Kiel Bight. Sediments were analysed for organic matter content, concentrations and decomposition rates of proteins (proteolytic enzymes) and carbohydrates (α-amylase, β-D-glucosidase) as well as for microbial biomass. Results are illustrated in Fig. 3, 4. During March, a considerable increase in total organic matter, proteins and carbohydrates was observed. Maximum values were recorded at the end of March, followed by a drastic decrease during April. The almost identical variation pattern of the organic components studied should be pointed out (Fig. 3). During the investigation period, a drastic shift in the protein to carbohydrate ratio was observed. The ratio decreased from 1.5 (March 3, 13) to 1 (March 28, peak of the organic parameter); the ratio then increased to 2 (April 9) and afterwards reached a plateau of 1.2 to 1.1 (April 18, 23, 30; May 5).

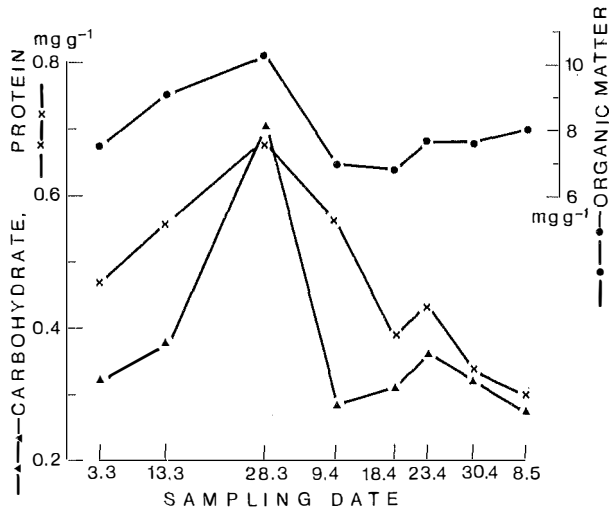

Figure 3.

Variations of organic matter content, proteins and carbohydrates measured during spring 1980 at a 10 m sediment station located in the „Hausgarten" area of the Kiel Bight.

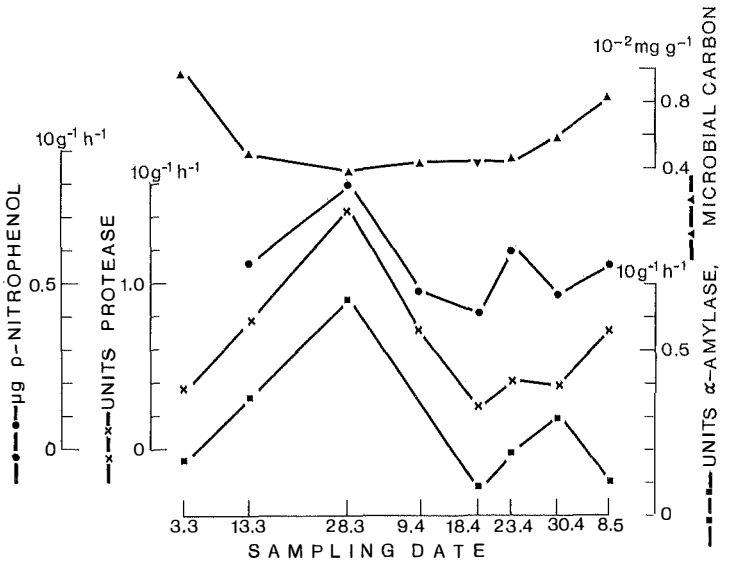

Figure 4.

Variations of microbial biomass, activity of β-D-glucosidase (μg of p-nitrophenol released per 10 g of sediment per h), α-amylase (units α-amylase equivalents per 10 g of sediment per h) and proteolytic enzymes (units protease equivalents per 10 g of sediment per h). Samples were withdrawn during spring 1980 at a 10 m sediment station located in the "Hausgarten" area of the Kiel Bight.

Decomposition rates of proteins (proteolytic enzymes) and carbohydrates (α-amylase, β-D-glucosidase) paralleled the variation pattern of total organic matter, proteins and carbohydrates (Fig. 4). Even the small peak in concentrations observed on April 23 corresponded to a peak in decomposition rates .Again, the almost identical variation pattern obtained for the activity rates of β-D-glucosidase, α-amylase and proteolytic enzymes should be pointed out.

The drastic increase in organic parameters in March can be traced back to the breakdown of a heavy plankton bloom (March 3–28) which at that time of the year almost totally sinks to the bottom due to the absence of zooplankton (v. BODUNGEN et al. 1975). The enrichment of organic matter in the sediment surface led to a considerable increase in the decomposition rates of proteins and carbohydrates. Thus it was possible to demonstrate for natural marine samples the close relationship between primary production of organic material in the water column, breakdown of primary producers and decomposition of the organic material in the sediment. It is difficult to decide where the enzymes responsible for the decomposition of the organic material arise from. According to investigations of BURNS (1980), part of the extracellular enzymes released by cells persists in soil and retains its activity by the formation of humic-enzyme complexes. These active enzymes may contribute to the decompositon of the organic material that sink to the sediment surface. Microbial biomass decreased during the enrichment of organic material in the sediment (Fig. 4), thus questioning the predominant role of microbial extracellular enzymes in the early stage of the decomposition process. However, microbial biomass increased

significantly towards the end of the observation period, obviously due to the liberation of suitable substrates for microbial growth following the decomposition of proteins and carbohydrates in the sediment. The dominating part of the enzymes obviously arose from autolysis of the plankton cells themselves, from which a high percentage reached the sediment surface as intact cells, as shown by a considerable increase in ATP during March corresponding to the increase in organic matter (GRAF, personal communication). The intact plankton cells may be responsible for the depression of microbial cells by the production of antimicrobial agents. In this connection it should be mentioned that meiofauna organisms which are closely linked to microorganisms (MEYER-REIL and FAUBEL 1980) obviously contribute significantly to the decomposition of particulate organic matter in sediments, as investigations of the activity rates of α-amylase and proteolytic enzymes in meiofauna organisms have shown (FAUBEL and MEYER-REIL in preparation).

Laboratory experiments are in progress to investigate the liberation of protein and carbohydrate decomposing enzymes during the autolysis of plankton cells as well as to study the induction of microbial extracellular enzyme production following the enrichment of organic material in sediments.

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