

## **FDA hydrolysis and resazurin reduction as a measure of microbial activity in sediments from the south-east Atlantic**

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**ABSTRACT:** Esterase and dehydrogenase activities were determined in depth profiles of sediments from the south-east Atlantic using fluorometric methods. The sensitivity of both methods was sufficient to record enzymatic activities in deep-sea clay and foraminiferous sand. Depending on water depth and location, significant differences between the depth profiles of enzyme activities could be determined. They appear to be dependent on the organic-matter input from sedimentation. Conclusions about the mode of microbial degradation of organic matter (oxic, anoxic, type of electron acceptors) are possible, using suitable chemical parameters. Under oxic conditions ( $E_h > 200$  mv) it was possible (using the method developed for dehydrogenase activities) to determine depth profiles similar to those of the esterase activities. Under suboxic or anoxic conditions, an appropriate separation between biological (dehydrogenase activity) and chemical resazurin reduction was not possible.

### INTRODUCTION

In oligotrophic biotopes, such as sediments in the deep-sea, sensitive methods for recording low-level microbial activities are necessary. To minimize alteration of the samples, methods are required which allow rapid treatment after sampling, and short analysis times. Both requirements are met by the determination of enzymatic activities by means of fluorescence spectrometry.

Dehydrogenases and esterases are promising enzyme groups for determining total microbial activities, because of their importance for microbial metabolism and the multitude of reactions they catalyze.

Dehydrogenases belong to the main group of oxidoreductases. On a cellular level they play a decisive role in both catabolism and anabolism. As integral components of intact cells (intracellular enzymes) (Ladd, 1978), they oxidize endogenous substrates by dehydrogenation, e.g. during glycolysis and in the tricarboxylic acid cycle, and channel substrate hydrogen in membrane-bound electron-transport chains. Dehydrogenases therefore reflect almost the total spectrum of oxidative microbial activities (Ladd, 1978) and fulfil a significant role in the oxidation of organic matter (Skujins, 1975).

The determination of dehydrogenase activity as a parameter for microbial activity has been frequently used in microbial ecology, especially in soil microbiology (cf. Alef, 1991). For spectrophotometric determinations, triphenyltetrazolium chloride (TTC) and idonitrotetrazolium violet (INT) usually serve as electron acceptors. The photometric

determination of resazurin reduction as a measure for dehydrogenase activity has been used for sediments (Liu & Strachan, 1979; Peroni & Rossi, 1986), to check bacteriological quality of seafood (Reddy et al., 1990; Kuemmerlin, 1982) and to control toxicity of activated sludges (Liu, 1981, 1983; Ewald et al., 1987; Ewald, 1989). Guilbault & Kramer (1964, 1965) described a fluorometric method based on resazurin reduction by pure enzymes.

The ester-cleaving enzymes are a subgroup of the main group hydrolases. A multitude of enzymes – esterases (e.g. lipases, phosphatases) and nucleases – are capable of cleaving ester bonds. Fluorescein esters, including fluorescein diacetate (FDA), were found to be hydrolysed by lipases and acylase, but not by acetylcholinesterase (Guilbault & Kramer, 1966). Esterases can be found extracellularly as well as intracellularly. The substrate FDA can be transported across cell membranes and deacetylated by non-specific esterases (Chrzanowski et al., 1984).

The determination of FDA hydrolysis has been used to estimate microbial biomass on coniferous needles (Swisher & Carroll, 1980), to measure total microbial activity in soil and litter (Schnürer & Rosswall, 1982), microbial activities in water samples (Holzapfel-Pschorn et al., 1987), and hydrolytic activity associated with biogenic structures in deep-sea sediments (Köster et al., 1991). According to Meyer-Reil (1991), however, it is not possible to distinguish between extra- and intracellular hydrolysis.

For our own investigations, dehydrogenase and esterase activities in sediments from the south-east Atlantic were examined within the scope of the DFG-promoted (Deutsche Forschungsgemeinschaft) Sonderforschungsbereich (SFB) 261 of the University of Bremen ("Der Südatlantik im Spätquartär: Rekonstruktion von Stoffhaushalt und Stromsystemen"). The results presented here were obtained on the "Meteor"-cruise M 20-2 (1991/1992, Abidjan-Dakar).

## MATERIAL AND METHODS

### Sediments

Sediment samples were investigated at, among other places, the Stations GeoB 1711, 1719, 1721 (continental slope in front of Namibia), GeoB 1724 (deep-sea plain of the Cape Basin) and GeoB 1726 (southern Walvis Ridge) (compare Fig. 1). The exact locations and water depths are given in Table 1.

Table 1. Locations and water depths of the examined GeoB Stations

GeoB Station	~ Wd [m]	Latitude	Longitude
1711	2000	23° 19' S	12° 23' E
1719	1000	28° 56' S	14° 10' E
1721	3000	29° 10' S	13° 05' E
1724	5100	29° 58' S	08° 03' E
1726	1000	30° 16' S	03° 16' E

Wd = water depth    S = south    E = east

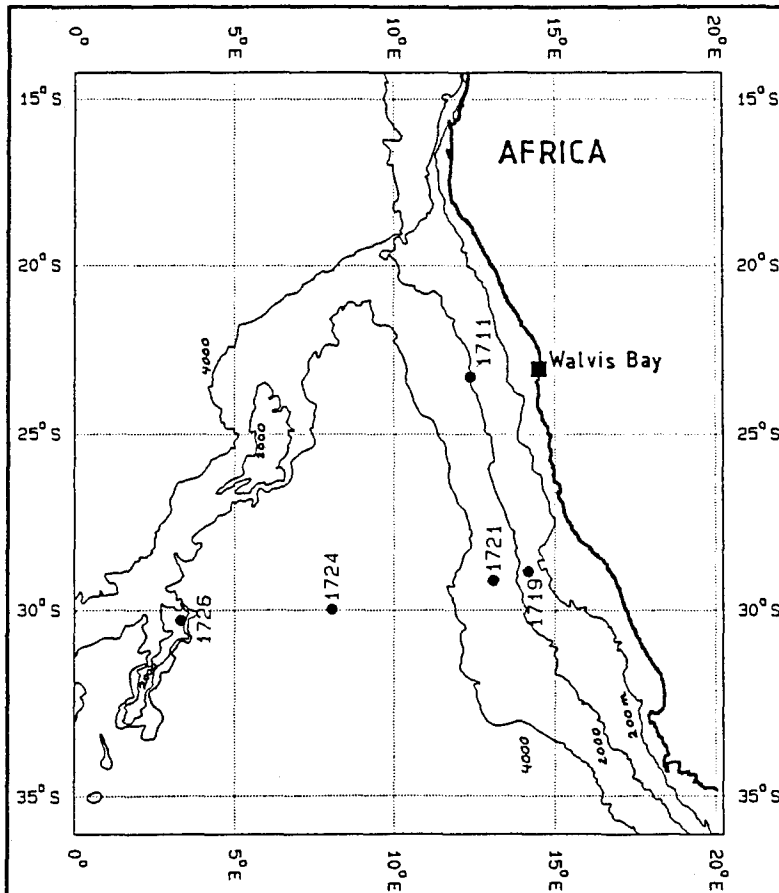


Fig. 1. Sampling sites for the determination of enzymatic activities – "Meteor" cruise M 20/2

### Enzymatic activities

Sediment samples were taken with syringes from multi-corers, box-corers and gravity-corers. Enzymatic activities were determined as rapidly as possible after sampling (within 24 h) on ship-board. Sediment suspensions were made by mixing sediment and sterile filtered sea-water in the ratio 1:4 (v:v).

#### *Esterase activity*

The determination of esterase activity is based on the hydrolysis of fluorescein diacetate (FDA) by nonspecific esterases and the fluorometric determination of the released fluorescein. The method was conducted, in the main, as described by Köster (1992). 25  $\mu$ l FDA solution (4.8 mM) were added to 1 ml of sediment suspension. The suspensions were incubated for 30 and 90 min. After addition of 3 ml sterile filtered seawater and centrifugation (3000 g for 10 min), the fluorescence of the supernatants was

determined immediately (ex 480, em 513 nm; slit maximum 5 nm), setting a 5  $\mu$ M fluorescein standard to 100 fluorescence units. Since FDA is subject to spontaneous hydrolysis (depending on the environmental conditions), controls with sediment suspension were autoclaved and treated in like manner. All work was done at 4 °C. The enzyme activity was calculated from the nmol fluorescein formed in the period 30 to 90 min after subtraction of the control values. The FDA hydrolysis was found to be linear up to 120 min of incubation, depending on the biological activity of the samples.

#### *Dehydrogenase activity*

The determination of dehydrogenase activity is based on the enzymatic reduction of resazurin ( $E_h + 0.05$  v) to resorufin in the course of the dehydrogenation of endogenous substrates and the fluorometric determination of the product resorufin (compare Guilbault & Kramer, 1965; Ewald, 1989).

50  $\mu$ l resazurin solution ( $1.6 \times 10^{-4}$  M) were added to 1 ml of sediment suspension. The mixture was incubated for 6 h at 20 °C. After addition of 0.5 ml borate buffer (0.2 M; ph 10.6), 3 ml sterile filtered sea-water and centrifugation (3000 g for 10 min), the fluorescence of the supernatants was determined (ex 560, em 590 nm; slit maximum 5 nm) setting a 1  $\mu$ M resorufin standard to 100 fluorescence units. Controls were treated with 25  $\mu$ l 25 % ethylmercurithiosalicyl acid to determine the abiotic resazurin reduction. Autoclaving leads to underestimation of the abiotic reduction, probably because it changes the chemical properties of the samples too much. The enzyme activity was calculated from the nmol resorufin formed in 1 h after subtraction of the control values. The resazurin reduction was found to be linear up to 8 h of incubation, depending on the sediment properties.

## RESULTS AND DISCUSSION

First, it should be noted that we determined enzymatic capacities with the methods described above, not in situ activities. Nevertheless, the enzymatic activities are thought to reflect the enzyme concentration present in the original sample, if a loss in activity and microbial proliferation is prevented. Furthermore, the enzymatic activities (especially in the upper sediment layers) can be attributed not only to microbial cells, but also to plant and animal cells. It was found by Köster et al. (1991), that the benthic fauna can contribute substantially to the measured esterase activities down to a sediment depth of 40 cm. It is not known if this is also the case in greater depths. Wetzel (1981) has shown that bioturbation only influenced the first 0.5 m of upwelling sediments from the north-west African continental slope. In all sediment layers, the possible contribution of accumulated extracellular enzymes (e.g. esterases) to the measured activities should not be disregarded. Enzymes can be stabilized, especially by association with humic matter, and persist in this way, even for geological periods (cf. Skujins, 1975).

Figure 2 shows two different depth profiles of esterase activities from the Stations GeoB 1721 and GeoB 1711. At Station 1721, high esterase activities are limited to the upper sediment decimeter. Below 10 cm, only an increase in activity at 90 cm is noticeable. At this depth, a gradient change in the phosphate content of the pore water (derived from concentration profiles) was found by the geochemical working group (Wefer & Schulz, 1993), which is also involved in the SFB. In contrast to Station 1721, Station 1711

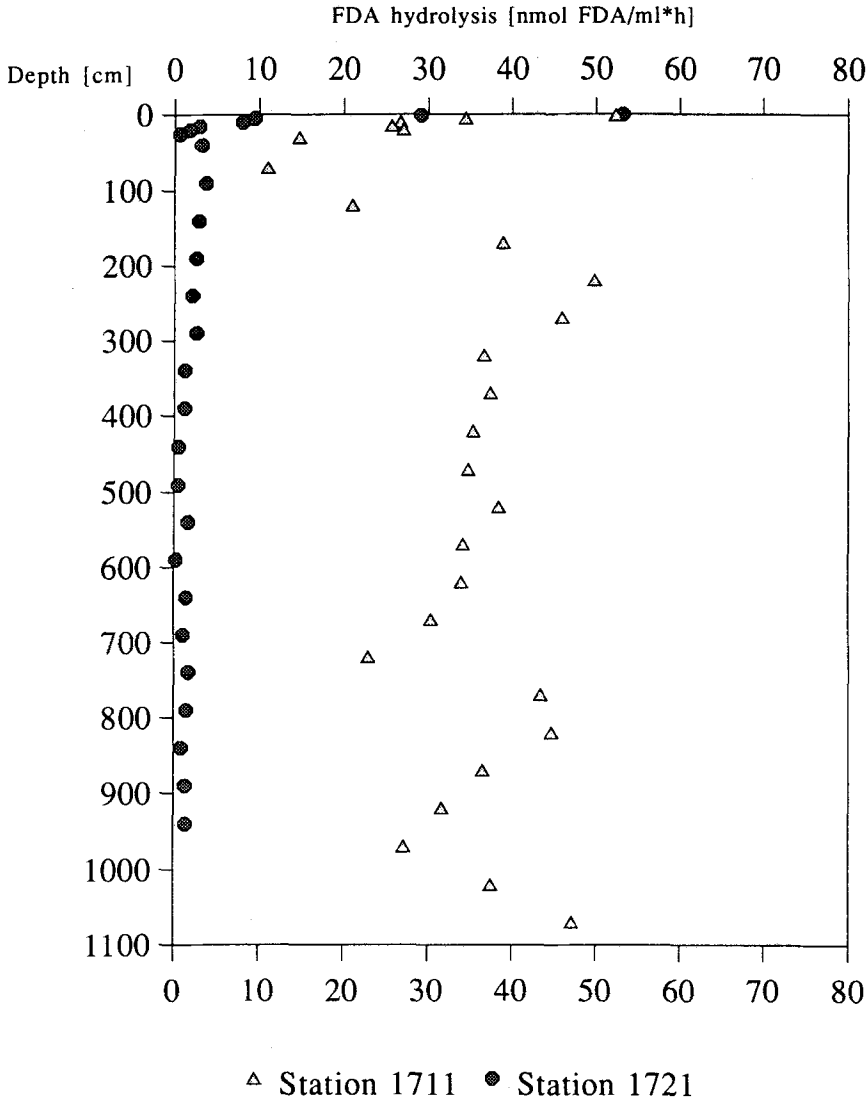


Fig. 2. Esterase activities in sediment samples from the Stations GeoB 1711 and 1721; water depth 2000 m and 3000 m, carbonate/clay sludge and carbonate sludge, respectively

shows high enzymatic activities at greater depths. Between 1 and 70 cm the esterase activity decreases, but at 2 m sediment depth the activity level prevailing at the surface is nearly attained. In the depth range of 2 m, the geochemical working group found gradient changes in regard to alkalinity and phosphate content of the pore water (ibid.). These are obviously caused by microorganisms. The high level of microbial activity at greater depths is accompanied by high sulphide concentrations in the pore water, especially at 10 m sediment depth (nearly 3000  $\mu\text{mol/l}$ ) (ibid.), indicating sulfate reduc-

tion. At this depth, a gradient change in regard to alkalinity, high ammonium concentrations in the pore water and a release of phosphate into the pore water were also found (ibid.). Derived from these findings, and the high enzymatic hydrolysis capacities from 2–11 m at Station 1711, we expect a high level of anaerobic decomposition of organic matter by microbial sulfate reduction as well as microbial fermentation.

The different depth profiles elucidate, moreover, that at Station 1711 there is more organic substance available for microbial degradation than at Station 1721. Both stations are located in the coastal upwelling area of the Benguela Coastal Current, which is

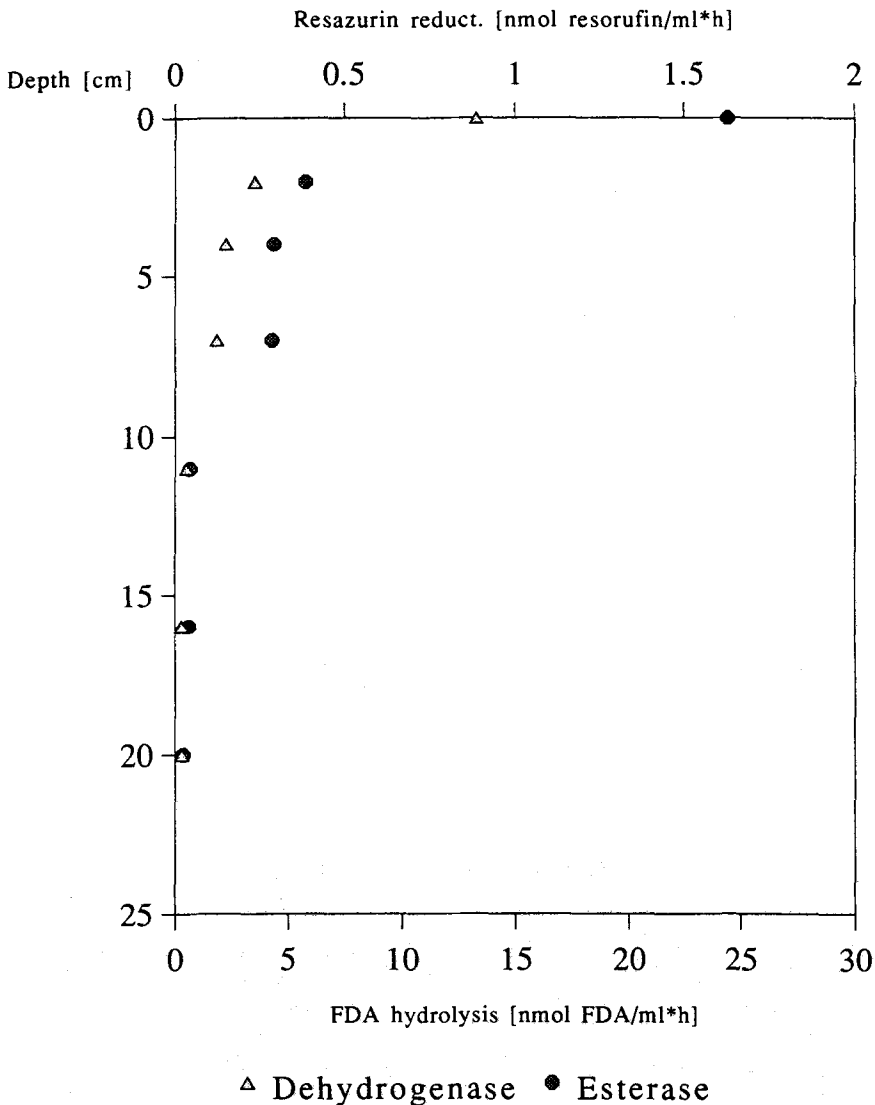


Fig. 3. Dehydrogenase and esterase activities in sediment samples from Station GeoB 1726; water depth 1000 m, foraminiferous sand

influenced by the south-east trade winds. In the upwelling area cold, nutrient-rich water reaches the surface, effecting high total annual primary production rates of more than  $100 \text{ g C m}^{-2} \text{ yr}^{-1}$  (Berger, 1989). But while Station 1711 has a water depth of 2000 m and lies presently in the central part of the coastal upwelling area, Station 1721 has a water depth of 3000 m and is situated at the southern boundary of the coastal upwelling area (cf. Oberhänsli, 1991).

Figures 3–6 show depth profiles of esterase activities, together with dehydrogenase activities.

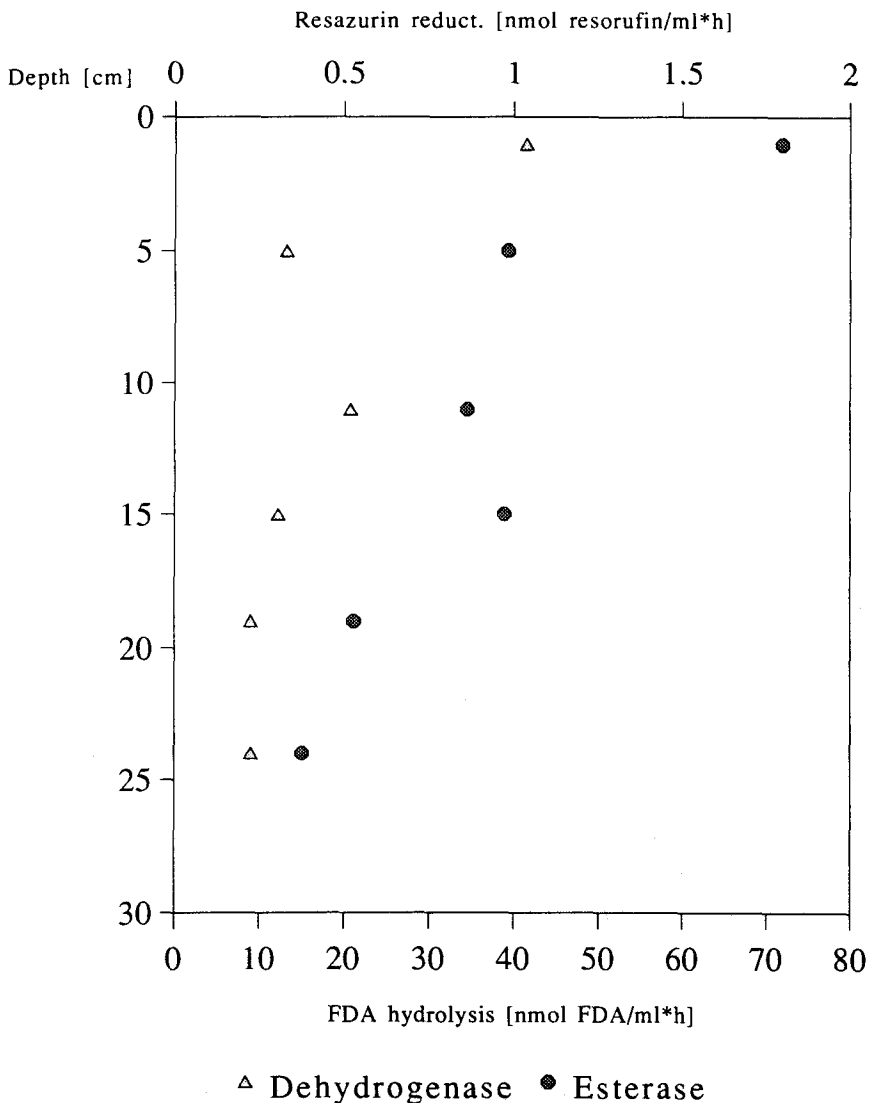


Fig. 4. Dehydrogenase and esterase activities in sediment samples from Station GeoB 1719; water depth 1000 m, carbonate/clay sludge

In Figures 3–5, both activity parameters show a similar trend. The same is valid for Station 1721 up to a sediment depth of about 90 cm (Fig. 6). In these areas a positive linear correlation of  $r = 0.846$  between both enzyme activities was determined. Because dehydrogenase are thought to be exclusively intracellular enzymes (within live cells) (Skujins, 1975, 1978), we believe these results indicate that in the upper sediment layers of the Stations 1719, 1721, 1724 and 1726 the esterase activities are also largely a result of enzymes of proliferating organisms, in contrast to accumulated enzymes (cf. Burns, 1982).

Below 90 cm (Fig. 6) the profiles for dehydrogenase and esterase activities differ very

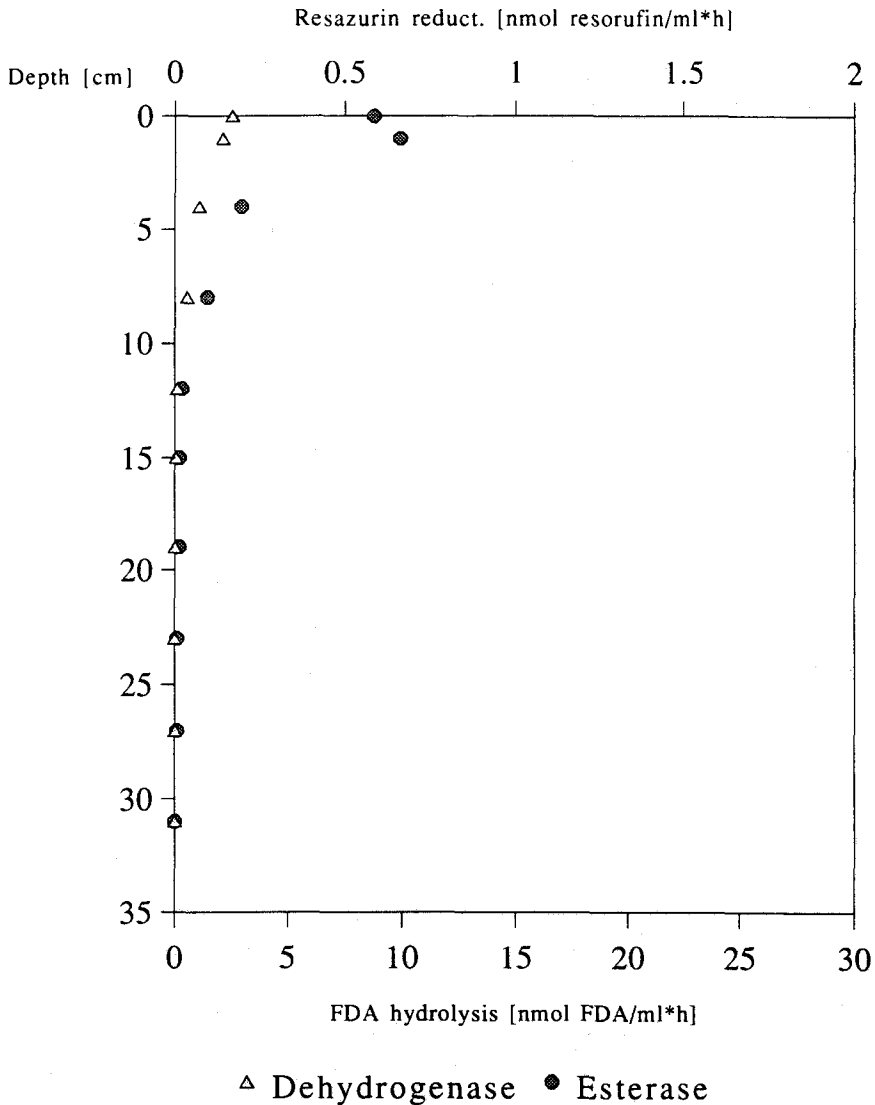


Fig. 5. Dehydrogenase and esterase activities in sediment samples from Station GeoB 1724: water depth 5100 m, deep-sea clay



much from one another. In contrast to the esterase activities, the dehydrogenase activities rise with increasing sediment depth. This would mean that the organic material is largely oxidized in the deeper sediment layers and not in the upper ones. Nevertheless, this conclusion is supported neither by the esterase activities nor chemical data (compare Wefer & Schulz, 1993). It was found that in the areas with high correspondence of both activity parameters, there were high  $E_h$ -values ( $> 200$  mv), but in the areas with low correspondence the  $E_h$ -values were below 200 mv (up to  $-88$  mv). According to Thalmann (1967), triphenyltetrazolium chloride-reduction as a measure for dehydrogenase

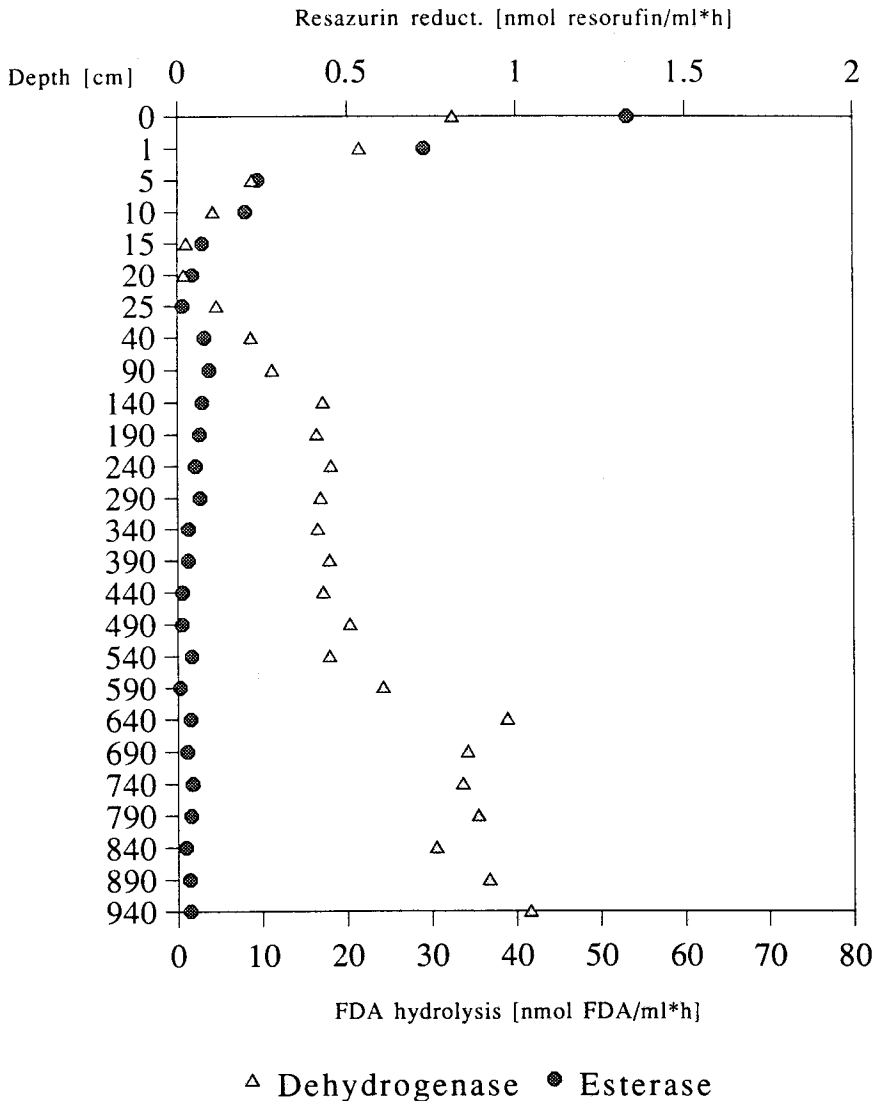


Fig. 6. Dehydrogenase and esterase activities in sediment samples from Station GeOB 1721; water depth 3000 m, carbonate-sludge

activity depends not only on the enzyme concentration, but also, among other things, on the presence of alternative electron acceptors and abiotic reducing compounds (e.g.  $\text{Fe}^{++}$ - and sulphide-compounds, nascent hydrogen). Therefore one must assume that the biological resazurin reduction cannot be determined under suboxic or anoxic conditions with the method described above, probably because an appropriate separation between chemical and biological reduction is not possible.

Table 2. Comparison of enzymatic activities,  $C_{\text{org}}$ - and  $N_{\text{total}}$ -content in the surface sediments (samples from box-corers, 1 cm depth)

Station GeoB	cm	Wd m	$C_{\text{org}}$ %	$N_{\text{total}}$ %	Esterase activities nmol FDA $\text{ml}^{-1} \text{h}^{-1}$	Dehydrogenase activities nmol resorufin $\text{ml}^{-1} \text{h}^{-1}$
1719-5	1	1024	2.41	0.32	72	1.04
1711-5	1	1964	1.74	0.24	52	0.85
1724-4	1	5102	0.51	0.1	10	0.14
1721-4	1	3079	0.38	0.07	29	0.54
1726-2	1	1006	0.1	0.02	6	0.24

C- and N-data (unpubl.) from P. Müller, FB Geowissenschaften, Universität Bremen  
 Wd = water depth  
 % as weight percent of the saltfree dry matter

Comparing the enzymatic activities at the sediment surface of all stations (1 cm depth; samples from box-corers) with each other, the following decreasing order can be determined: 1719, 1711, 1721 and, depending which activity is compared, Station 1724 or 1726 (compare Table 2). Furthermore, a relation to the organic carbon and total nitrogen content of the sediments can be stated. The enzymatic activities in the surface sediments rise with increasing C- and N-content, with the exception of Station 1721. But it should be noted that besides the quantity, the quality or availability of organic matter reaching the sea-floor also determines decomposition rates.

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