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POLYCHAETE TUBE WALLS AS ZONATED MICROHABITATS FOR MARINE BACTERIA

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ABSTRACT - The predominantly anoxic, sulfide-rich sandy sediment of a shallow lagoon at Kiel Fjord was densely inhabited by the polychaete worm *Nereis diversicolor* (280 cm^3 m^{-2} of biomass volume). Burrow walls which contrasted by their brown coloration of at least 1.5 mm thickness with the bulk of the reduced black sediment, made up for 6 % of the total volume of the 10 cm cores investigated. Only the uppermost mm of the internal surface layer contained detectable O_2. As a result of the build up of internal redox gradients, CO_2 dark fixation was activated in the burrow walls (by a factor of approximately 2). Contribution of epi- or endozoic activities associated with *Nereis diversicolor* to this kind of "perizoic" activation in the walls was negligible. Due to the relatively limited internal surface area produced by bioturbation, chemoautotrophic CO_2 fixation in burrow walls may not be very effective on a more global scale. On the other hand, reduced marine sediment devoid of a burrowing infauna showed considerable CO_2 dark fixation only at the oxidized sediment-water interface. Furthermore, assays of ribulose-biphosphate carboxylase activities and viable counts of potentially chemoautotrophic bacteria in different parts of the lagoon sediment suggested that burrow walls as the sites of maximal CO_2 fixation rates would not qualify as microbial barriers. This view of polychaete burrow walls as open enrichment systems for bacteria with key roles in the cycle of carbon was confirmed by additional determinations of heterotrophic (Fe^{3+}, and SO_4^{2-} reducing, proteolytic, chitinolytic, and agarolytic) bacteria.

Key words : CO dark fixation, thiosulfate, detritus, decomposition, sediment, bioturbation.

RÉSUMÉ - Le sédiment sableux riche en sulfures et à dominante anoxique d'une lagune peu profonde du fjord de Kiel, héberge une population dense d'un ver polychète : *Nereis diversicolor* (280 cm^3 m^{-2} en biomasse volume). Les parois des tubes qui contrastent par leur coloration brune d'au moins 1,5 mm d'épaisseur avec la masse de sédiment noir réduit, constituent jusqu'à 6 % du volume total des carottes de 10 cm. La présence d'O_2 n'est décelée que dans les tous premiers millimètres de la couche de surface interne. L'accroissement de la fixation de CO_2 (par un facteur de 2 environ) dans les parois des tubes est le résultat de l'augmentation du gradient rédox interne. La contribution des activités épi ou endozoïques associées à *Nereis diversicolor* dans ce genre d'activation «périzoïque» au niveau des parois est négligeable. La fixation chimioautotrophique de CO_2 dans les parois des tubes n'est pas vraiment importante à l'échelle plus générale, si la surface, limitée, touchée par la bioturbation est prise en référence. D'un autre côté, le sédiment réduit dépourvu d'une endofaune montre une forte fixation de CO_2 à l'obscurité seulement au niveau de l'interface oxydée eau-sédiment. De plus, les dosages de l'activité de la ribulose biphosphate carboxylase et les numérations des bactéries viables et potentiellement chimiotrophes dans les sédiments de la lagune suggèrent que les parois des tubes, comme sites des taux de fixation maximale de CO_2, ne constituent pas une barrière pour les bactéries. Cette hypothèse des parois des tubes de polychètes, comme système d'enrichissement ouvert aux bactéries jouant un rôle clé dans le cycle du carbone, est confirmé par des études supplémentaires sur les bactéries hétérotrophes (réduisant Fe^{3+} et SO_4^{2-}, proteolytiques, chitinolytiques et agarolytiques).

Mots clés : fixation de CO_2 à l'obscurité, thiosulfate, détritus, décomposition, sédiment, bioturbation.

INTRODUCTION

Bacteriological assays aiming at the zonation of different physiological groups in marine

hand, direct assays of key enzymes of the Calvin cycle such as RUBPCase would overcome heterotrophic interferences, but no longer allow to differentiate between photo- and chemoautotrophic activities.
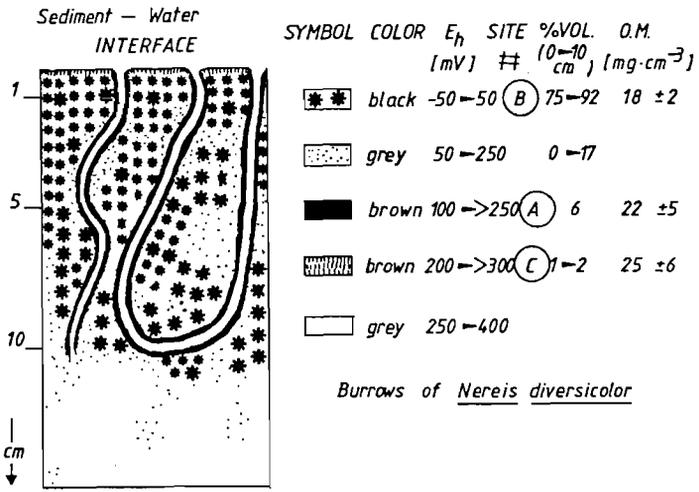

Figure 1 : Stein Lagoon : Schematic illustration of sampling sites A, B and C.
O.M. = organic matter content as ash free dry weight. % of total volume given for 10 cm cores.
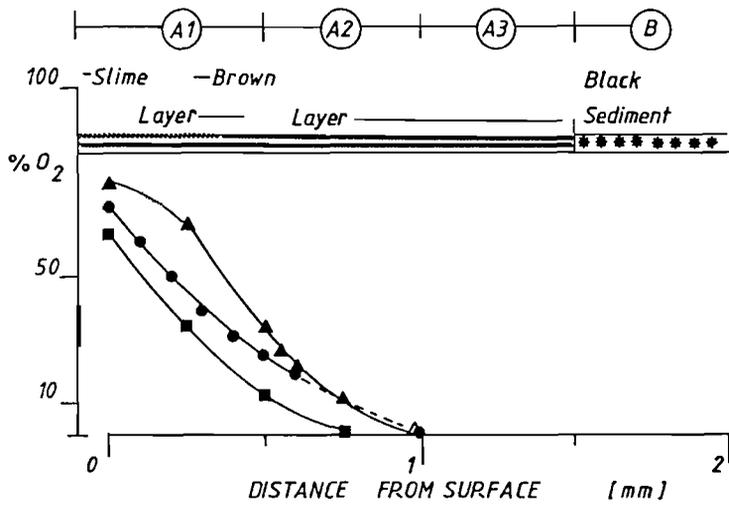

Figure 2 : Oxygen profiles of 3 burrow walls of the polychaete *Nereis diversicolor* from Stein Lagoon sediment. Sampling site A within the brown burrow wall is subdivided into compartments A1, A2 and A3.

In all sediment areas selected for this investigation (Fig. 1) RUBPCase levels showed only minor fluctuations, suggesting an almost evenly distributed capacity to fix carbon dioxide via photo- or chemoautotrophic pathways (Fig.3, upper part). On the other hand, patterns of CO_2 dark fixation *in vivo* were characterized by peaks in the burrow walls and, particularly, in their innermost layer (A3, Fig. 3, lower part).
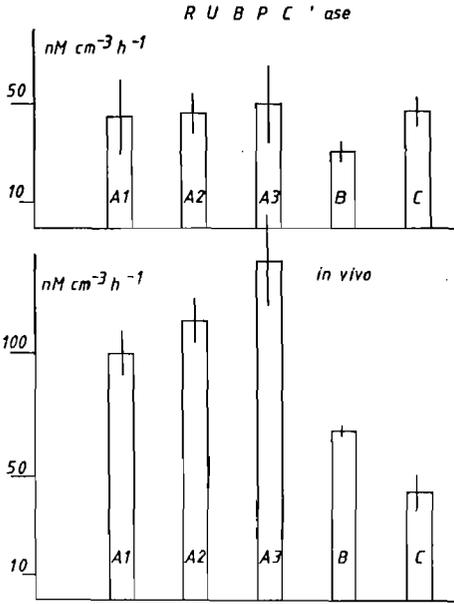
Figure 3 : Ribulose biphosphate carboxylase (RUBPC ase) activity vs. CO_2 dark fixation *in vivo* at different sampling sites in bioturbated sediment of Stein Lagoon.

In vertical profiles from other coastal sediments, CO_2 dark fixation peaked within the redox potential discontinuity (RPD) layers. These were located 2-3 cm below the surface, when the sediments were oxidized and bioturbated, but bound to the sediment surface, when anoxia had reached the uppermost cm and a burrowing macrofauna was absent (Fig. 4). Although no simple correlation existed between CO_2 dark fixation and Eh, the rates usually tended to decline rapidly below 100 mV, which is also evident from this example.
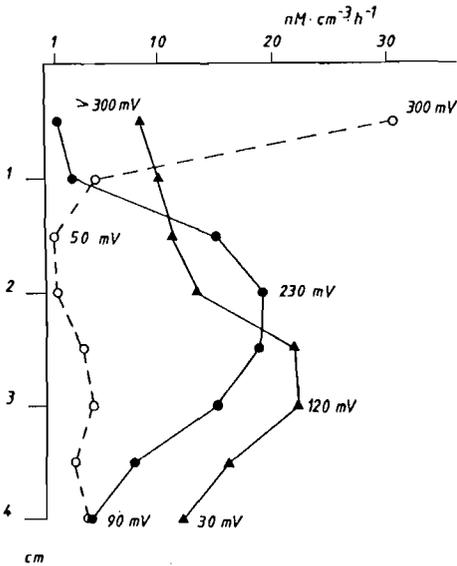

Figure 4 : CO_2 dark fixation in vertical profiles of sediments with and without bioturbation. ● = Kiel Bay at Boknis Eck, 18 m (bioturbated), O = Kiel Bay at Boknis Eck, 28 m (without burrowing infauna), Δ = Limfjorden at Bjornholm Bugt, 1.0 m (bioturbated). E_h values are shown for the depths with maximal and minimal CO_2 fixation rates obtained with core injection technique at *in situ* temperatures in April (O) and August (●, Δ).

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Was this predominance of CO_2 dark fixation at intermediate E_h values caused by gradients of inorganic H-donors for chemoautotrophic bacteria ? To check this, «activation assays» employing excess amounts of potential H-donors have first been used by Kepkay and Novitsky (1980). As shown in Figure 5, no stimulation by 8 mM of various H-donors occurred in the sulfide- rich bulk of the sediment from Stein Lagoon (B) at E_h values - 50 to + 50 mV, while limitation by thiosulfate was noted for the adjacent burrow walls. As stimulation in the thin, oxidized top layer (C) was achieved by different H-donors such as NH_4^+ and ferrous iron, chemoautotrophic CO_2 fixation in the burrow walls was apparently dominated by S-oxidizers.
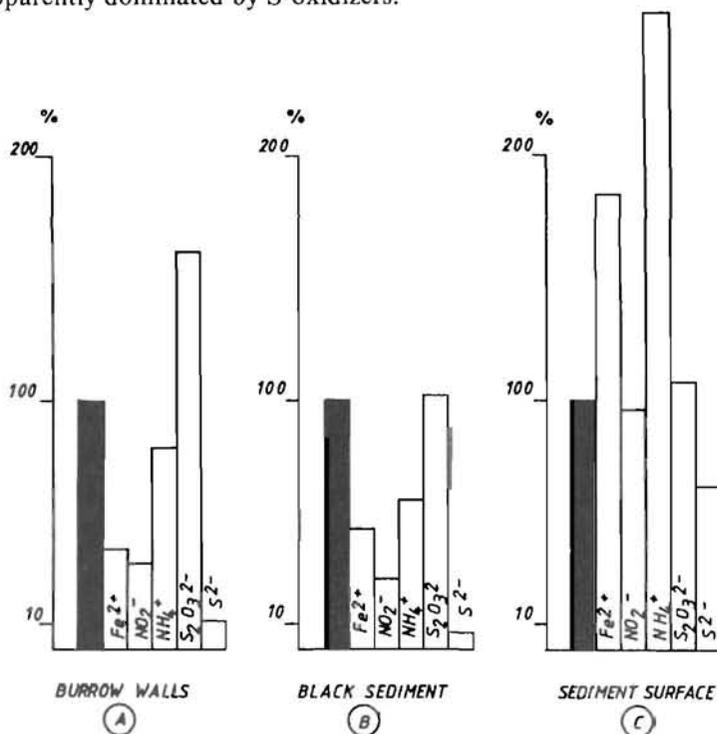

Figure 5 : Activation and inhibition of *in vivo* dark fixation of CO_2 by various potential H-donors (8mM) in burrow walls of *Nereis diversicolor* (A) as compared with adjacent anoxic parts (B) and top sediment (C) controls (Black columns) represent 100 % activity.

These results would confirm the assumption (Yingst and Roads, 1980) that thiobacilli find favorable conditions for growth at redox potential discontinuity layers that are formed by burrowing infauna. Thiosulfate-stimulation of CO_2 dark fixation has also been reported for shallow marine waters (Tuttle and Jannasch, 1977) and marine mud (Kepkay and Novitsky, 1980). In contrast to isolations of thiosulfate-oxidizing marine bacteria by Tuttle and Jannasch (1972), all the isolates obtained by MPN enrichment from Stein lagoon sediment produced acid as indicated by pH values decreasing to 2.6 - 3.5 ; see also Figure 8. As thiosulfate oxidizers may also utilize sulfide as H-donor (Tuttle and Jannasch, 1973), the apparent inhibition of CO_2 dark fixation by additional sulfide (and, less pronouncedly, also by ferrous iron and nitrite) may be interpreted as the result of an increased reducing power and suboptimal E_h according to Kepkay and Novitsky (1980).

Ammonia-stimulation of dark fixation in the oxidized top layer did not necessarily indicate a peak of bacterial nitrification ; for NH_4^+ ions are also known to increase dark CO_2 fixation by marine diatoms (Goldman and Dennett, 1983).

It should be stressed that stimulation of chemoautotrophic CO_2 fixation by potential H-donors provides a useful tool only, if concentrations of the compound added are limiting in the sample. In sediment samples with a low E_h even minor amendments of thiosulfate (1 mM) may cause the reverse effect, as demonstrated by thiosulfate inhibition of CO_2 fixation in sampling site B (Fig.6).
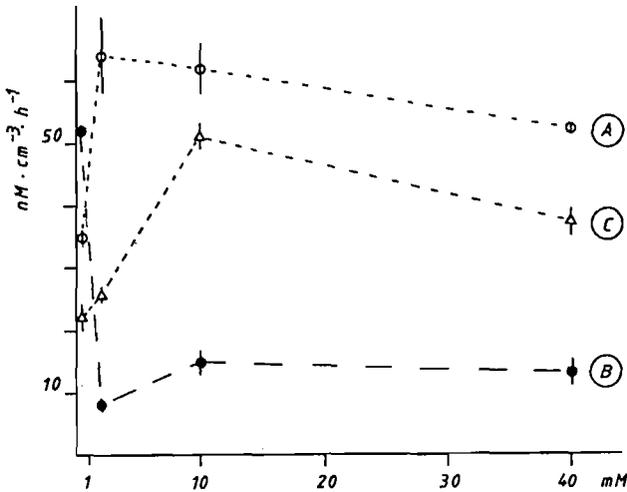

Figure 6 : Impact of increasing concentrations of $Na_2S_2O_2$ on CO_2 dark fixation rates in polychaete burrow walls (A), adjacent anoxic (B), and top surface sediment (C) from Stein Lagoon, August 6, 1984.

Lately, there has been increasing evidence for the existence of sulfur-oxidizing bacterial symbionts of marine invertebrates not only from deep sea hydrothermal vents (f.e. : Cavanaugh et al., 1981 ; Ruby et al., 1981), but also from coastal marine sediments (Cavanaugh, 1983 ; Giere et al., 1982 ; Ott et al., 1982). As yet, it would be premature to interpret the stimulation and enrichment of sulfur-oxidizing bacteria in polychaete burrow walls as evidence for a continuum of sulfur-based energy conservation extending from thiosulfate-oxidizing chemoautotrophs in the burrow walls to epi- and endozoic symbionts of a similar type.

There was no indication that the producer of the burrows, Nereis diversicolor, was carrying a substantial amount of epi-or endozoic chemoautotrophs (Fig.7). On an ash-free dry weight basis, CO_2 fixation by this polychaete worm amounted to less than 5 % of the activity detected in the burrow walls. It may be speculated that most of the CO_2 fixation by Nereis was channeled through heterotrophic pathways via anaplerotic enzymes (Hammen and Lum, 1964) and, in particular, via phosphoenolpyruvate car-boxykinase (Schöttler and Wienhausen, 1981). Fixation rates obtained from «perizoic» microbiota embedded in slime excretions or in the burrow walls were considerably greater than rates determined in isolated worms including their epi- and endozoic microfloras. (Tab. 1).
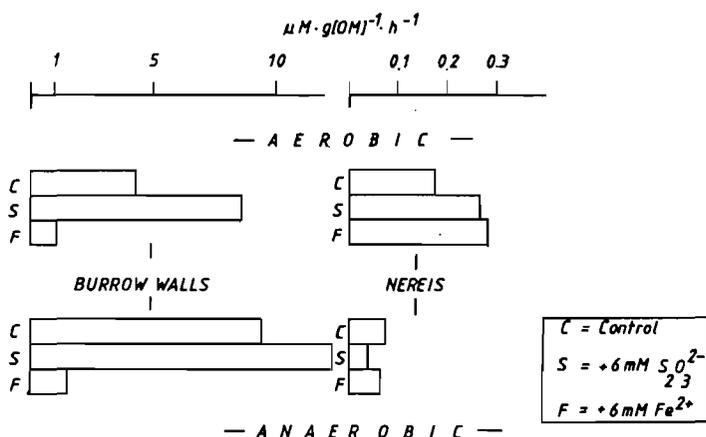
Figure 7 : "Perizoic" and epic-zoic CO_2 dark fixation on an ash free dry weight basis in burrows of the polychaete *Nereis diversicolor*. Impact of 6mM $FeSO_4$ and $Na_2S_2O_3$.

$\mu M\ g^{-1}h^{-1}CO_2$	
Epizoic:	
Pectinaria koreni	0.02 - 0.04
Nephtys hombergi	0.49
Nereis diversicolor	0.37 - 0.74
"Perizoic":	
Nereis diversicolor, adherent slime	3.97 - 6.46
Nereis diversicolor, burrow walls	3.58 - 9.40

Table 1 : CO_2 dark fixation by various epicoic and pericoic microbiota. Specific rates based on g of ash free dry weight.

Enhanced CO_2 fixation in oxidized infaunal burrow walls should be recognized as a rather widespread phenomenon. F.e., fixation rates in deep sea sediment from the Antarctic Ocean doubled in the area of polychaete burrow walls as compared with the reduced surroundings. (Reichardt, in prep.). However, instantaneous rates of CO_2 fixation along polychaete burrows would have to be much greater than twofold to merely equal CO_2 fixation in the reduced surroundings on a global, volumetric scale. (Just to cause a doubling of CO_2 fixation rates per unit of sediment volume, burrow walls from Stein lagoon representing 6 % of 10 cm cores would have to be about 15 times more active than the surrounding sediment). Despite of these limitations on a larger scale, polychaete burrows in sulfide-rich sediments may still be regarded as the center and starting point of various microbial activities that may easily spread out into surrounding areas. This assumption was supported by supplementary data on viable counts of certain bacteria and *in vitro* enzyme assays.

Viable counts

While boundary layers are expected to constitute an ecological niche for bacteria that depend on the supply of either reduced or oxidized inorganic ions, thiosulfate-oxidizers, ammonia - nitrifyers and manganese-oxidizers were not less abundant in other parts of Stein lagoon sediment than in the burrow walls (Fig. 8). Although these viable counts were no proof of activities *in situ*, their pattern of distribution may still indicate rapid

spreading (by meiofaunal carriers ?) or at least a long continued survival under adverse conditions.
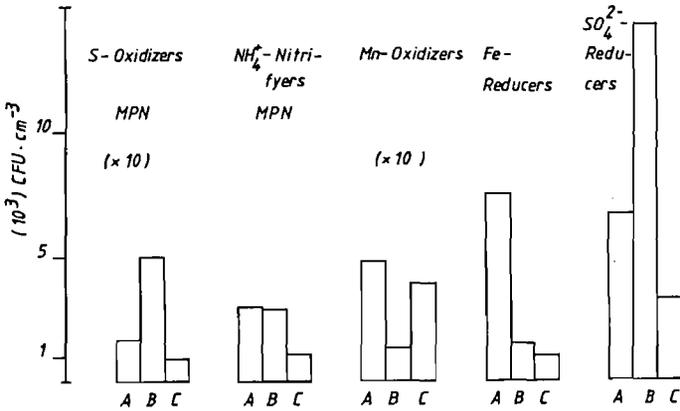

Figure 8 : Viable counts and MPN of selected groups of bacteria depending on reduced or oxidized inorganic ions in burrow walls and other parts of bioturbated sediment from Stein Lagoon. Example from August 6, 1984.
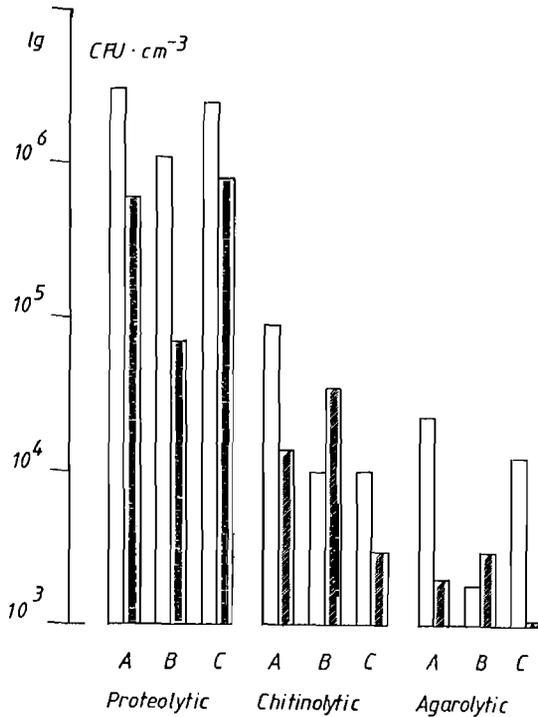

Figure 9 : Abundance (plate counts) of anaerobic bacteria (Fe^{3+} and SO_4^{2-} - reducers) and aerobic bacteria ($S_2O_3^{2-}$-oxidizers, NH_4^+-nitrifyers, and Mn-oxidizers) plotted as log of CFU in burrow walls (A) and other parts of bioturbated sediment from Stein Lagoon. Examples from August 15, 1984.

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Due to the feeding habit of *Nereis diversicolor,* particulate organic matter (POM) accumulate in their burrows. Again, strong enrichment of bacteria capable of decomposing this POM was anticipated. However, viable counts of some pertinent groups of bacteria from the burrow walls rarely exceeded those obtained from the reduced bulk sediment (sampling site B. Fig. 9). Possibly rapid mixing had prevented an excessive accumulation of decomposers. An almost even distribution of organic matter (ash-free dry weight) in all areas of the sediment investigated supports the idea that burrow walls were poor barriers for microorganisms.

It was concluded that polychaete burrows in predominantly anoxic, sulfide-rich marine sediments were able to create essential zonated niches for the development of bacteria that play key roles in either anabolic or catabolic pathways of the carbon cycle. In the case of Stein lagoon, a sulfide-rich sandy sediment, polychaete burrow walls turned out to be rather poor barriers for sediment bacteria and could be regarded therefore as an open enrichment system.

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