Microbial potential in deep-sea sediments

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Summary. In spite of high pressures and low temperatures in abyssal sediments of the North Atlantic Ocean, bacterial activity is evident and highest in the top 10 cm. At these locations the input of degradable organic material to the deep-sea bottom is low. Oxygen, therefore, remains the dominant oxidant in surface sediments. Although alternative electron acceptors like nitrate, oxidized manganese and sulfate are present in large amounts, they are not utilized in this natural habitat. In sediment cores which were collected from the site for laboratory perturbation studies, it was possible to stimulate microbially mediated processes which are dormant in situ. When the oxygen supply was cut off, nitrate and manganese reduction occurred. Denitrification was the major process observed in the upper anoxic layers, while nitrate-ammonification and manganese reduction occurred in deeper sediment strata (4–8 cm). This is evidence for the presence of a variety of different bacteria and of an anaerobic heterotrophic potential. Most of the activity is located in the top 10 cm of these sediments. The shift to anaerobiosis initiates microbial activities through which metals are converted into their mobile species at the lowered redox potential. Evaluation of the suitability of the deep sea as a repository for waste materials will have to account for the large dormant potential of microbial activities and the consequences of their release by changing the environmental conditions at the sea floor.
Key words. Bacterial activity; sediment; pore water; deep sea; electron acceptors; microcosms; nitrification; denitrification.

Introduction

Microbial activity in the sediments of deep-ocean basins is restricted in many ways. Nutrient limitation, low temperature and high hydrostatic pressure lead to low population densities and slow turnover rates of nutrient elements[26, 38]. Special physiological strategies for survival and growth are required to cope with these conditions[32, 35, 37, 47]. At in situ pressures, most deep-sea bacteria will survive with minimum metabolic activity. Most of them hardly express growth rates which one would expect for truly barophilic organisms[27].

The constant, non-limiting supply of oxygen to most sediments allows aerobic mineralization to proceed in the upper ten centimeters of the deposits[4, 23]. As oxygen is used up in deeper sediment strata, processes employing other electron acceptors such as NO_3^-, $\{Mn_{ox}\}$, $\{Fe_{ox}\}$ and SO_4^{2-} will become dominant, provided there are still oxidizable substrates available in these depths[18]. The relative importance of in situ oxygen reduction, nitrification, denitrification and organic matter mineralization in pelagic sediments has been investigated and modeled by several authors[3, 22, 23, 25, 44].

Bacterial density was found to be too low for direct observation by microscopic techniques and for accurate enumeration[5]. It was possible, however, to partially characterize the microorganisms in this ecosystem with enrichment cultures and through analyses of microbial marker molecules from phospholipid and lipopolysaccharide fractions collected from core samples[8]. Of the total viable biomass in the top 20 cm, 85% was located within the first 10 cm of the surface layer[8]. These organisms were mainly prokaryotic, and the presence of both aerobic *and* anaerobic bacteria was indicated.

It is the purpose of this study to better understand the biochemical changes which might take place in deep-ocean sediments under oxidant limiting conditions. The experiments mimic the situation where the sediment surface gets covered with a solid surface, impermeable to oxygen. This might apply, for example, to sediment regions which become covered by waste canisters.

Material and methods

Concept and experimental procedure

As a consequence of the lowered diffusion in the interstitial spaces and of the immobilization of microorganisms at solid-liquid interfaces sediments are highly stratified systems. They contain gradients of nutrients and biomass below the sediment-water interface. The shapes of depth profiles reveal catalytic capabilities which are located in different depth zones of the sediment. For our study, sediment samples were collected at various stations in the North Atlantic Ocean during the Dutch DORA mission on the MS *Tyro*[39] and the German NOAMP-III cruise in 1984 on the research vessel F.S. *Meteor* (table 1). Sediment cores (PVC tubes, 60 cm long, 8.6 cm I.D.) were subsampled from a box-core (0.25 m^3 sediment, max. 45 cm deep). For 'in situ profiles' porewater was extracted on board by squeezing 1–2 cm sediment slices (60–

Table 1. Sampling locations in the North Atlantic basin

Location	Position	Depth
Station 8	45.83°N/17.27°W	4725 m
Station 11	46.02°N/17.13°W	4725 m
Station 99	46.00°N/17.16°W	4700 m
Station 197	47.35°N/19.69°W	4100 m

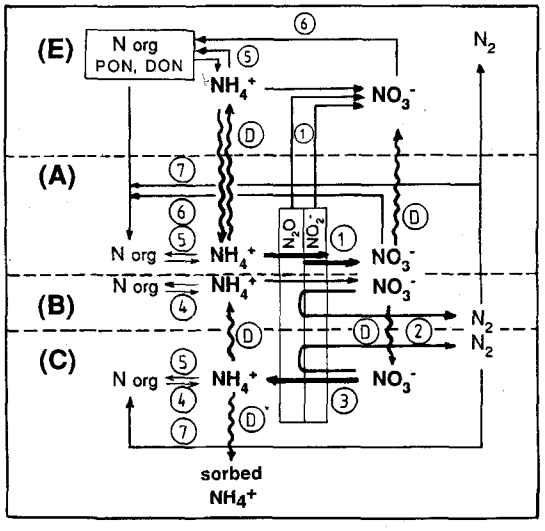

Figure 1. Conceptual model depicting the ecological coupling of different processes in nitrogen cycling across the redox transition zone in sediments. N-input through sedimentation of particle-bound and dissolved organic nitrogen (PON, DON). Major losses through gaseous intermediates or products of denitrification. Major zones: (A) oxic surface boundary layer; (B) suboxic sediment layer; (C) anoxic layer; (E) overlaying oxic water. Processes: (1) nitrification; (2) denitrification; (3) dissimilatory nitrate reduction, nitrate ammonification; (4) ammonification through mineralization; (5) ammonia assimilation; (6) nitrate assimilation; (7) nitrogen fixation; (D) diffusion.

120 cm^3) under a nitrogen atmosphere[5]. The porewater obtained (10–30 ml from each slice) was filtered through Whatman GF/C and Nuclepore filters (0.45 µm) and stored acidified (H_2SO_4, 0.05% (vol/vol) pH 2), or frozen at −20 °C in 5-ml polystyrene vials. Concentration-depth profiles were determined in porewater from freshly sampled cores from every station. Additional cores from station 197 were incubated in the laboratory for 260 days at 4 °C in the dark. During the entire incubation procedure the cores were kept sealed and no external substrates or oxidants were added. Transitions from oxic to anoxic conditions were allowed to occur, while the oxygen was being used up. The porewater depth profiles of organic carbon, ammonia, nitrate, nitrite and manganese were compared to the gradients of cores analyzed immediately after sampling onboard the ship. They represent concentrations as close as possible to in situ conditions. Sulfate was not measured because no signatures of sulfate reducing bacteria were found in previous studies[8]. A Mn-reduction stimulation experiment was performed with sediment from station 99 as described in the legend to figure 6.

A conceptual model which describes the possible coupling of the processes involved in the cycling of nitrogen through the redox transition layer (RTL) in sediments was used to explain the depth of the RTL in incubated microcosms (fig. 1 and table 2). It compiles information on the cycling of nitrogen from several sources[13,16,17,20,22,28–31,36,42–45]. Nitrate accumulates in the oxic top section of the oligotrophic sediment as a consequence of ammonia oxidation by nitrifying bacteria. Nitrate thus produced diffuses to deeper and higher sediment strata (fig. 2, B, D, G). Under the prevailing conditions it serves as oxidant for denitrification in deeper zones. Nitrate may also be reduced to ammonia by nitrate-ammonification under strictly anoxic conditions. Some of the ammonia is oxidized in the oxic sediment boundary layer. Since the reaction mechanisms differ among nitrifying and denitrifying bacteria from species to species a bacterial consortium will accumulate intermediate products in the porewater. Quantitatively the most sensitive one is nitrite. Although it is present in much smaller amounts than ammonia and nitrate, its turnover rate might be large enough to account for a quantitatively important coupling between reductive and oxidative parts of the N-cycle.

The postdepositional remobilization of manganese is a sensitive indicator for anoxic conditions[2]. Reductive dissolution of manganese oxides occurs when anoxic conditions advance into previously oxic sediments[14]. Microorganisms participate in the reductive dissolution of manganese either directly, by using manganese oxides as a terminal electron acceptor[11,33,34], or indirectly, through the production and excretion of reductants[12,19,40] capable of interacting with MnO_x. The dissolved manganese diffuses upward and will precipitate again in oxidized layers above. Thus manganese and other metals can be enriched at oxic/anoxic interfaces by continuous reduction, transport and precipitation[21,46]. The porewater analyses of cores sectioned on the site as well as the microcosm incubations in the laboratory were done under atmospheric pressure. It is not known how decompression of the sediment samples affected their geochemical composition. The effects of sediment-

Table 2. Correlation between changes of ammonia and nitrate concentrations and the potential for nitrification, denitrification and nitrate-ammonification in the conceptual ecosystem of figure 1.

Reaction type	(1)		(2)	(3)		(4)	Sum of (1)–(4)	
Concentration change	NH_4^+	NO_3^-	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NH_4^+	NO_3^-
Oxic layer	[−−]	[++]	0	0	0	[+]	[−]	[++]
Suboxic layer	[−−]	[++]	[−−]	0	0	[+]	[−]	0
Anoxic layer (a)	0	0	[−−]	0	0	[+]	[+]	[−−]
(b)	0	0	[−−]	[++]	[−−]	[+]	[+++]	[−−−−]

(1) Nitrification; (2) denitrification; (3) nitrate ammonification; (4) ammonification through organic N-mineralization; (a) anoxic layer with denitrification; (b) anoxic layer with concomitant denitrification and nitrate ammonification; [+]: slight accumulation; [+++] strong accumulation; [−]: slight decrease in concentration; [−−−]: strong decrease.

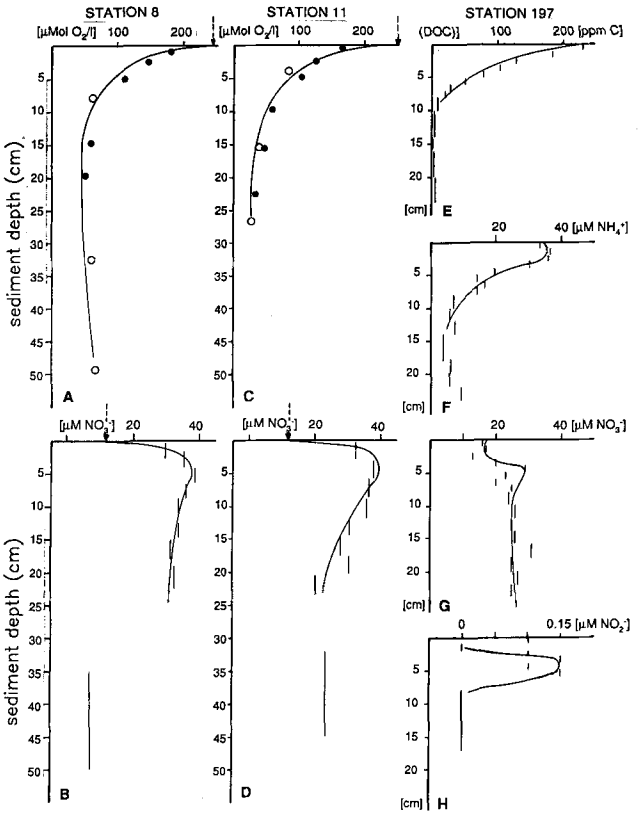

Figure 2. In situ porewater profiles. Oxygen and nitrate at station 8 (*A* and *B*) and station 11 (*C* and *D*) (Data from DORA mission provided by M. M. Rutgers van der Loeff, Texel). Symbol (→) represents concentrations in the water column. *E* dissolved organic carbon; *F* ammonia; *G* nitrate and *H* nitrite at station 197.

squeezing on porewater constituents, however, have been investigated and the results will be published elsewhere[6].

Analytical techniques

All methods for the chemical analysis of porewater and solid phase constituents were adapted to sample volumes of 1–3 ml. Absorbances of samples from colorimetric analyses were measured with an Uvikon 810 spectrophotometer (KONTRON AG, Zürich) in cells with a light path of 5 or 10 mm. Ammonia and nitrite were determined by the phenol-hypochlorite method and the azo-dye method respectively as described in Strickland and Parsons[41] with the sample volumes reduced to 1.3 ml for ammonia and to 2.4 ml for nitrite. Nitrate was determined by ultraviolet spectroscopy according to the method of Armstrong[1]. Since the nitrate peak shifted with varying chloride concentrations each assay was compared to a sample where the nitrate had been reduced completely by adding hydrazine sulfate (2.4 mM) and a sample with an internal standard (50 µM nitrate added). Since nitrate and nitrite gave almost identical spectra the measured concentrations had to be corrected by subtracting the nitrite concentration which was determined separately. Dissolved reduced manganese (DRM) was determined colorimetrically with a slight modification of the method described by Brewer and Spencer[10]. 240 µl of the mixed formaldoxime/ammonia-hydroxide reagent were added to 2.8 ml filtered sample and the absorbance was determined in a 10-mm cell at 450 nm within 30 min after mixing. This method showed an excellent reproducibility and a detection limit lower than that obtained with the atomic absorption method (less than 0.1 ppm). For the analysis of solid phase manganese (SM) 20–30 mg dried sediment were incubated in 2.5 ml hydroxylamine-HCl (1 M, pH 1.5) for 2–3 days. After centrifugation the chemically reducible manganese in the supernatant was measured by atomic absorption spectroscopy (BECKMAN Atomic Accessory with DB Spectrophotometer). Assays, where 5–10 mg of dry sediment were treated with the mixed ammonia/formaldoxime reagent[8], showed lower manganese concentrations indicating that not all of the chemically reducible manganese was reduced by this method. The reproducibility of both methods was strongly dependent on the particle size distribution of the dried sediment used for the assays. For the determination of dissolved organic carbon (DOC) 1-ml samples were acidified to pH 2 with HCl, combusted at 960 °C and the CO_2 thus produced was detected by infrared absorption spectrometry (DOC/TOC-Analyzer, PROCON AG, Zug). For the determination of the water content the sediment was dried to constant weight at 105 °C.

Results

In sediments of the North Atlantic nuclear waste disposal site[24, 39] organic carbon was present in significant amounts within the top 8 cm (fig. 2). Sediment from the boundary layer of a core taken at station 197 contained nearly 230 mg dissolved organic carbon/l porewater (equivalent to 121 µg/g sediment or 0.12‰ (w/w) when calculated with a water content of 52.4%). This is rather high for an ecosystem considered to be oligotrophic. As we know now this value of dissolved carbon was probably overestimated due to artifacts produced by the squeezing method. In a comparative study we have found that DOC-concentrations in squeeze-water were above the naturally occurring ones. Some carbon is released into the squeeze-water due to mechanical destruction of particulate organic matter[6]. Nitrate profiles showed subsurface concentrations which were 2–3 times as high as the ones in the overlaying water. This phenomenon is commonly observed with in situ profiles. Aerobic nitrification is considered to be the process responsible for the accumulation of nitrate in the oxygenated layer[20]. At all stations oxygen was not depleted within the top 50 cm. The major portion was consumed in the first 10 cm, presumably for the aerobic oxidation of organic matter.

Neither the chemical nature of the DOC-fraction nor the availability of its different compounds for microbial processes have been specified. It seems to be readily bioavailable since the dissolved organic carbon initially present in

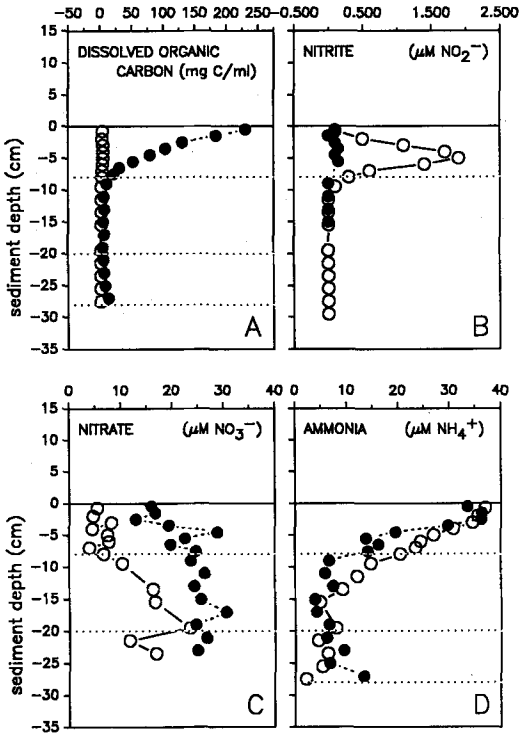

Figure 3. Chemical porewater profiles at station 197. *A–D* Vertical distribution of organic carbon, nitrite, nitrate and ammonia of two sediment cores. Porewater extracted at the beginning (core 197.1, •---•) and at the end (core 197.3, O---O) of incubation (260 d, 4 °C, dark; O_2 was excluded and no substrates were added).

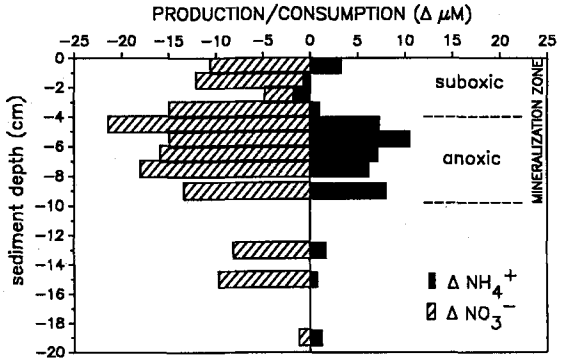

Figure 4. Net production and consumption of ammonia and nitrate during anaerobic incubation of a sediment microcosm (core 197.3). δNH_4^+ and δNO_3^- were calculated from the concentration differences in the ammonia and nitrate profiles of figure 3, C and D.

the porewater disappeared almost completely if the cores were incubated in the laboratory for several months (fig. 3, A). This indicates that biologically mediated DOC-oxidation took place. A small nitrite pool was formed during the incubation process (fig. 3, B). It cannot be decided whether the accumulation of nitrite in the zone of active carbon mineralization was due to nitrate reduction or to ammonia oxidation under microoxic conditions or to both. However, it demonstrated enhanced cycling of nitrogen under the laboratory conditions imposed. Considerable nitrate reduction in the organic rich layer at the top was evident. At the end of the incubation period of core 197.3 only 4–6 μmoles of nitrate per l of porewater were left within the top 8 cm (fig. 3, C). Ammonia remained rather constant in the top 4 cm but increased in deeper layers between 4 and 15 cm (fig. 3, D). The net concentration changes of ammonia and nitrate during the incubation period of sediment core 197.3 are plotted in figure 4. We have applied the conceptual nitrogen cycling model (fig. 1) to characterize the different processes responsible for the oxidation and reduction of inorganic nitrogen during the incubation. Generally the largest changes occurred in the organic rich layer at the top. The decrease of nitrate below 10 cm may result from diffusion upwards due to its extensive consumption below the sediment surface. The concept shown in figure 2 and table 3 is the basis for a characterisation of the different processes responsible for the oxidation and reduction of inorganic nitrogen during incubation. An overview in table 2 demonstrates that any process or a combination of two of them will cause different relative changes in interstitial nitrate and ammonia concentrations in the ecosystem. In addition, since each, nitrification, denitrification and nitrate ammonification, works under different redox conditions, conclusions about the redox state of the particular sediment depth are possible.

In all zones denitrification dominated. Nitrate consumption was always higher than concentration changes of ammonia indicating that nitrate was reduced to nitrogen gas. In the zones 1–3 cm and 4–8 cm other reactions proceeded simultaneously: nitrification in the upper zone (as concluded from the net decrease in the ammonia concentration) and nitrate ammonification in deeper zones (indicated by enhanced nitrate consumption and simultaneous ammonia production). Since the latter process requires anoxic conditions it was concluded that the redox transition zone moved upwards during incubation of core 197.3 as a consequence of complete oxygen depletion below 4 cm depth prior to the process of ammonification.

In the anoxic layer of core 197.3 manganese was mobilized (fig. 5). The peak in the concentration profile of soluble manganese seems to be related to a depletion zone of solid manganese between 3 and 8 cm depth. No such zone could be detected in any of the cores analyzed immediately after retrieval indicating that anaerobic conditions were created during incubation. The peak representing solid manganese may result from the reoxidation of reduced $Mn(II)^{2+}$ diffusing upward towards the oxic zones and subsequent redeposition of manganese oxides as modeled by Froelich et al.[18]. Similar cycling of manganese in the porewater of Lake Geneva sediment have been demonstrated[9]. They are considered to be a consequence of enhanced anaerobic metabolism due to experimental in situ stimulation of bacterial activity. For core 197.3 it implies that the anoxic zone stretched from 3 to 8 cm at the end of the incubation period. This finding is

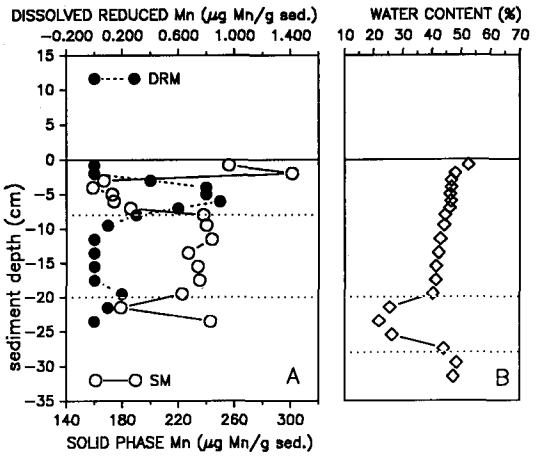

Figure 5. *A* Manganese reduction zones in an incubated sediment microcosm from station 197. Solid phase manganese was mobilized into the porewater due to anaerobic conditions occurring in the 'organic rich' surface layer during the course of incubation. *B* Water content of the sediment.

in good agreement with the data presented earlier in this paper. A second manganese reduction zone was observed below 20 cm where both the porosity and the color of the sediment were different from all other sediment regions. This was a particularity of the sediment at station 197 where the water content was only 22–26% between 20 cm and 28 cm compared to 40–50% in areas above and below (fig. 5). The higher carbon content at the same depth (fig. 2) may be an effect of higher sedimentation rates, changed composition of sedimenting organic particles or lowered mineralization capacity of the microbial community at this particular site several thousand years ago. This can be taken as evidence for possible changes in environmental conditions in the deep-sea over large time scales.

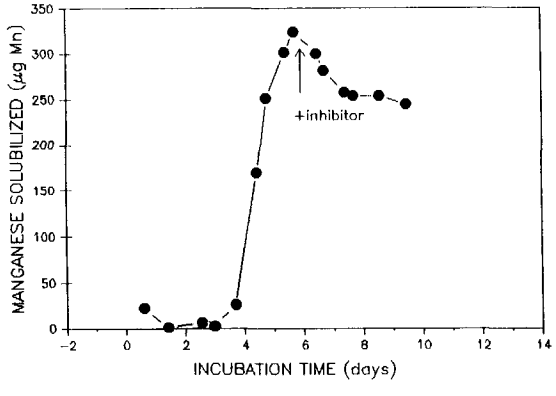

Figure 6. Stimulation of manganese reduction in a preincubated, nitrate depleted sediment core from station 99. The top 6 cm were removed and replaced by 500 ml culture medium[11]. Composition (per 500 ml): 10 g NaCl, 0.38 g KCl, 0.75 g $CaCl_2$, 5.1 g $MgCl_2$, 0.1 g yeast extract, 0.05 g peptone; 10 mM sodium succinate, 20 mM sodium acetate, 2 mM sodium bicarbonate, 10 mM TRIS buffer, pH 7.8. Nitrate, sulfate and fermentable organic substrates were omitted to prevent denitrification, sulfate reduction and fermentation respectively. Continuous bubbling with argon ensured mixing and oxygen-free conditions. Plot symbols represent the amount of Mn (in µg per 500 ml) solubilized into the culture medium from a sediment surface of 58.1 cm^2. Inhibitor (sodium azide) was added after 6 days, its concentration was 10 mM.

We were able to stimulate manganese reduction in an enrichment medium brought in contact with undisturbed sediment at 6 cm depth (fig. 6). Solubilization of natural manganese oxides was highest when the culture was bubbled with argon gas (3.6 µg Mn per day and cm^2 on day 5, after a lag of 4 days). In all assays a consortium of 3 different bacteria was enriched in the supernatant medium: long rod-shaped bacteria with terminal spores, smaller, non-motile rods and very small highly motile vibrio-type organisms. The Mn solubilization was completely inhibited by sodium azide (fig. 6).

Discussion

Most of the organic carbon that reaches the sea floor is degraded within the top 8 cm layer. This agrees with findings that 85% of the total viable bacterial biomass in the top 20 cm is located in this zone[8]. The amount of oxygen is in excess to allow aerobic respiration to proceed without depletion of this oxidant in situ. Other electron acceptors like nitrate, manganese or sulfate are present without being used up by anaerobic oxidation processes. Nitrate seems to be regulated by nitrification/denitrification within the top 8 cm. In contrast, oxidized manganese is buried unaffected by microbial processes as sedimentation proceeds. Under in situ conditions nitrate is predominantly accumulated by nitrification in the oxic top layer. In laboratory microcosms with limited oxygen supply, nitrate reduction was stimulated. Denitrification was the major process responsible for the nitrate depletion throughout the whole mineralization zone after oxygen became limiting. Nitrate ammonification was observed as a concomitant process in deeper sediment strata. Since this is a process requiring anoxic conditions, an anaerobic heterotrophic microbial potential must be present at these sediment depths. This conclusion is further supported by the observations that solid manganese was mobilized in sediments incubated under anoxic conditions and that fatty-acid analyses of freshly sampled sediments from station 11 indicated the presence of facultatively anaerobic, and anaerobic bacteria found in depths between 5 and 10 cm[8].

The data presented in this paper do not show whether other inorganic oxidants would also be used according to their position in the thermodynamic efficiency scale. From the analysis of phospholipids[8], however, we conclude that sulfate reduction will never dominate in these sediments as it commonly does in coastal sediments with high organic loads. It should also be pointed out that nitrate ammonification and manganese reduction are not expected to be effective in situ at undisturbed sites. There is no evidence, neither in the literature nor from our own results, which would indicate anoxic mineralization in the top layers of these pelagic deep-sea sediments of the

North Atlantic region. Our results clearly demonstrate, however, that the deep-sea sediments studied harbor the microbial potential necessary to perform anaerobic mineralization. The microorganisms are capable of utilizing the nutrients and oxidants naturally reaching the sea floor and they can be activated under laboratory conditions by simply limiting the oxygen supply.

Dayal et al.[15] found canisters at a former nuclear waste disposal site in 2800 m depth in the Atlantic. They were partially buried in the sediment (15–20 cm deep). The content of organic carbon and nitrogen in the surrounding area had increased. As shown in this paper these canisters must have reached the depth of the largest microbial biomass as well as the strata with the highest potential activity. There is evidence from our laboratory studies to suggest the presence of an anaerobic heterotrophic microbial potential which could metabolize greater amounts of organic substrates than are naturally brought to their habitat. It remains to be shown whether a stimulation would occur after feeding additional organic substrates in situ. Unless the environmental conditions of the deep sea can be simulated more closely, the conversion of our laboratory experiences into ecologically meaningful information remains a difficult task. From studies with pressure retaining equipment, the existence of barophilic bacteria with pressure optima identical to the in situ pressures could be demonstrated[49]. The maximum growth rates of even highly specialized organisms usually decrease with increasing pressure, and bacterial activity in the deep sea is generally low regardless of the occurrence of psychrophilic, barophilic and oligotrophic bacteria[27]. The limiting pressure is not known. Yayanos and Dietz[48] found bacteria actively metabolizing at 1000 atm pressure. But since elevated temperatures increase biological activity even under high pressures as is demonstrated most clearly in deep sea hydrothermal environments, there is reason to believe that the adapted heterotrophic activity could also be stimulated by an increased organic carbon load.

In summary we conclude from our results for waste disposal on deep-sea sediments:

- There are large dormant microbial potentials for aerobic *and* anaerobic processes in deep sea sediments of the North Atlantic.
- As waste canisters are buried in the sediment to a depth of 15–20 cm[15], they are in contact through a relatively large surface with the zone that contains the highest potential bacterial activity.
- An input of oxidizable organic matter to the sediment surface zone will most likely stimulate microbial activity. This could increase geochemical solubilization of various elements from the sediment and their redistribution in the ocean water.
- Waste canisters deposited on the sediment surface layer will cut off the oxygen supply from the ocean water and ease the shift to anaerobiosis. This initiates microbial activities through which metals are changed into their mobile species as a consequence of the altered environmental redox potential.
- The risk for steel corrosion mediated by hydrogen sulfide, which could be produced by sulfate reducing bacteria, is minimal since this physiological group is not active in the North Atlantic sediments examined. This does not exclude, however, corrosion by microbially mediated electron stripping from the metal of waste canisters.

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