Denitrification in the Central Baltic: evidence for H₂S-oxidation as motor of denitrification at the oxic-anoxic interface

Ingrid Brettar*, Gerhard Rheinheimer

Institut für Meereskunde an der Universität Kiel, Düsternbrooker Weg 20, W-2300 Kiel, Germany

ABSTRACT: Denitrification was investigated in August 1986 and July 1987 in the water column of the Gotland Deep, an anoxic basin in the Baltic Proper. Denitrification rates were determined by means of the acetylene blockage method in the whole water column with emphasis on the low-oxygen water above the H₂S-containing deep water. Denitrification was restricted to a layer of about 10 m at the oxic-anoxic interface (130 m depth), where NO_3^- and H₂S coexisted. Rates in 1986 and 1987 were 110 and 44 nmol N l^{-1} d^{-1} respectively. Samples from above the interface never showed denitrification, even after incubation periods of up to 12 d, obviously due to the combined effect of too high oxygen and too low carbon concentrations. Addition of nitrate to all samples enhanced denitrification only for H₂S-containing water samples from the anoxic deep water. The coincidence of electron donor availability and H₂S-concentration substantiated the hypothesis that reduced sulfur compounds could be valuable electron donors for denitrification by the bacterial community at the oxic-anoxic interface. Addition of sulfide or thiosulfate to water samples from just below the interface resulted in immediate denitrification of the added nitrate, with concomitant growth of the bacterial community. This indicates that denitrification in the oxic-anoxic interface could be efficiently driven by oxidation of reduced sulfur compounds provided by the anoxic deep water. Conceivable ecological implications of these findings are uncoupling of denitrification from carbon flux derived from phytoplankton primary production and strong dependence on mixing processes at the oxic-anoxic interface.

INTRODUCTION

Denitrification can play an important role in the nitrogen budget of marine environments. Deficiency in biologically utilizable nitrogen is the major limiting factor of primary production in the Central Baltic as well as in most marine environments (Wulff & Rahm 1988, Graneli et al. 1990). The Gotland Deep is a basin in the eastern part of the Baltic Proper, which represents the largest part of the Baltic Sea. The Baltic Sea as a whole can be considered as a large estuary (372 000 km²), strongly influenced by anthropogenic activity concerning phosphorus and nitrogen loads (Larsson et al. 1985). The Baltic Proper is characterized by a halocline at 60 to 90 m depth that inhibits vertical mixing. Below 70 m, water exchange relies on horizontal processes which are discontinuous and, especially for the

water masses below 130 m, rare events (Stigebrandt 1987). With prolonged stagnation periods the deep water displays oxygen deficiency and H₂S may accumulate from the sediment (Stigebrandt & Wulff 1987). Before our investigation there had been no major deep water exchange for 9 yr in the Gotland Deep.

The occurrence of denitrification in the low-oxygen and oxygen-deficient deep water was first suggested by Sen Gupta (1973) and measured by Rönner & Sörensson (1985). Their investigations in late spring showed a strong nitrate-limited denitrification in almost the whole water column below the halocline. Denitrification was considered not to have been limited by the availability of electron donors. In this case, as is generally accepted, organic carbon derived from phytoplankton primary production seemed to be the electron donor (Hattori 1983, Liu & Kaplan 1984). In this paper we report denitrification in a water column with H₂S-containing deep water where denitrification was restricted to a thin layer of the oxic-anoxic interface. The possible role of H₂S as an important electron

* Present address: Max-Planck-Institut für Limnologie, PO Box 165, W-2320 Plön, Germany

donor for denitrification was investigated and will be discussed.

MATERIAL AND METHODS

Sampling and field measurements. Samples were taken in the Gotland Deep aboard RV 'Poseidon' from 15 to 17 August 1986, and aboard RV 'Aranda' from 29 to 31 July 1987. The position of the sampling station (57°20.0′ N, 20°03.0′ E) in the central Baltic Sea is shown in Fig. 1. Water was collected in 5, 10 and 30 l Niskin PVC bottles. Salinity and temperature were determined by a CTD probe. Oxygen was measured by the Winkler method as described by Grasshoff (1983). Ammonium was determined by the indophenol blue method as modified by Koroleff (1983). Nitrate was reduced by a copper cadmium column to nitrite and

determined as nitrite as outlined by Grasshoff (1983). H_2S was determined photometrically by the methylene blue method as modified by Fonselius (1983). The above-mentioned methods are specified in Grasshoff et al. (1983). Parameters were analysed by the scientific crew of the ships directly after sampling.

Thiosulfate was determined titrimetrically as outlined by Grasshoff (1983). The method was rendered more sensitive by a factor of 10 by diluting the thiosulfate solution 10 times (final conc. 0.002 mol l^{-1}) and starting the titration after addition of only 1 ml of iodate standard instead of 10 ml. H_2S was precipitated by zinc acetate. The detection limit of the method was 7 µmol l^{-1}, the standard deviation 1 µmol l^{-1}. The method is rather unspecific and can give positive results not only in the presence of $S_2O_3^{2-}$ but also due to other iodine-oxidizable material. Therefore, it was used to demonstrate the absence of thiosulfate in the case of zero values, while
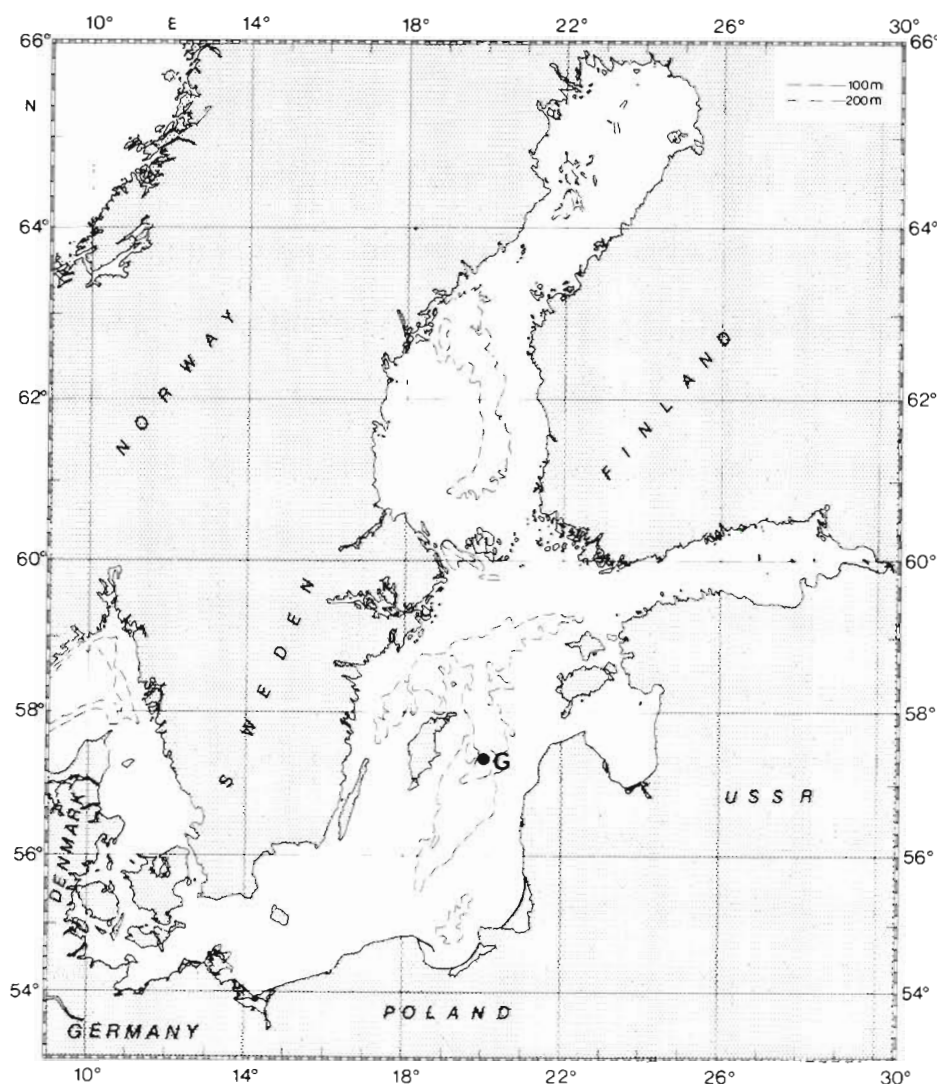

Fig. 1. Position of the 'Gotland Deep' sampling station (G) in the Baltic Proper

positive values will be interpreted carefully as thiosulfate and/or other iodine-oxidizable material.

Denitrification rate. Rates of denitrification were determined by the acetylene blockage method (Balderston et al. 1976, Yoshinari & Knowles 1976). The method used was a modification of those described by Rönner & Sörensson (1985) and Andersen et al. (1984). Samples were filled in 120 ml glass serum bottles directly after sampling, with overflowing to reduce oxygen contamination. Bottles were closed immediately with a teflon-coated butyl-rubber septum (3 mm) in an aluminium cap and crimped. This allowed a gas-bubble-free and air-tight containment of the sample. To create space for introduction of acetylene to the sample, 10 ml of water were replaced by the same volume of nitrogen [N_2, 5.0 (= >99.999 % purity)]. Then 18 ml of acetylene [C_2H_2 2.6 (= >99.6 % purity)] from cylinder, purified and checked for $PH_3 < 5$ ppm by the manufacturer (Messer-Griesheim, Duisburg), was added to each sample. Samples were incubated at 4.8 °C ± 0.5 °C in the dark for 2, 4, 6 and 12 d. Addition of substrates (NO_3^-, Na_2S, $Na_2S_2O_3$, Na-acetate) was always done before introduction of gases to the sample. For comparison, parallels were always run without acetylene addition.

Denitrification with substrate addition. To estimate the limiting effect of the available nitrate, nitrate was added to the water samples parallel to measurements of in situ denitrification. Additions were 50 μmol l^{-1} of NO_3^- to water samples from the oxic and the interface layer, and 100 μmol l^{-1} to samples from the anoxic deep water. Incubation was continued for up to 12 d in 1986, and up to 4 d in 1987.

To test the utility of reduced sulfur compounds as electron donors for denitrification by the interface bacterial assemblage, water samples (1987) from 5 to 10 m below the oxic-anoxic interface were spiked with NO_3^- (final conc. 100 μmol l^{-1}) and different electron donors. As reduced sulfur compounds, Na_2S and NaS_2O_3 (final conc. 50 μmol l^{-1}) were added. As a control, Na_2S and NO_3^- were added to autoclaved water samples that were subsequently incubated and analyzed in the same way as the regular samples. To compare the use of reduced sulfur compounds with that of organic material as electron donor, parallels with acetate (final conc. 100 μmol l^{-1}) and a mixture of acetate (50 μmol l^{-1}) and Na_2S (25 μmol l^{-1}) were run. Samples were preincubated for 1 to 2 d. Incubation was continued for 4 more days after substrate addition. Controls were run without any addition and with only nitrate and no electron donor addition.

For this experiment 2 samples were taken within half an hour from about 140 m depth, corresponding to a distance of 5 to 10 m below the oxic-anoxic interface. At sampling time both samples contained similar amounts of H_2S (10 to 12 μmol l^{-1}), no nitrate or nitrite, 2.0 to 2.5 μmol of NH_4^+ and no detectable oxygen. Since the chemical parameters were very similar, it was assumed that the samples had the same origin and were comparable.

For comparison, the same experiment with electron donor and nitrate addition was repeated with a sample taken from the sulfide-rich deep water (180 m). At sampling time this sample contained 61 μmol l^{-1} H_2S, 22 μmol l^{-1} NH_4^+, and no nitrate, nitrite or N_2O.

In these experiments, denitrification rates, concentrations of NO_3^-, NO_2^-, NH_4^+, H_2S, $S_2O_3^{2-}$ and bacterial numbers during preincubation and for a 4 d incubation period after substrate addition were followed.

N_2O measurement. For N_2O measurements gas samples were withdrawn from the head space by a gastight lockable syringe after equilibrating the sample for 15 min at 20 °C in a shaking water bath. Samples were immediately frozen after this procedure and stored for later analysis, e.g. NO_3^-, NO_2^-, NH_4^+. Gas samples were either directly injected in a gas chromatograph, or stored for later analysis in evacuated vials (4 ml, Becton Dickinson). N_2O concentrations were quantified on a gas chromatograph (model 438A, Packard Instr. Co., Inc., Rockville, MD, USA) with an electron capture detector (10 mCi ^{63}Ni) operated at 320 °C. The separation was done by injecting 1 ml of the gas sample on a stainless steel Poropak Q (80/100 mesh) column (3 m, 1/8″) at 60 °C and a gas flow of 18 ml min^{-1}. The carrier gas was an Ar/CH_4 (95:5, vol./vol.) mixture. The accuracy of the method for N_2O determinations after storage of the samples in evacuated vials improved with increasing concentrations: the standard deviation for concentrations above 30 nmol l^{-1} was better than 2 %, above 10 nmol l^{-1} better than 5 % and above 5 nmol better than 9 %. The detection limit was 3 nmol l^{-1}. The N_2O measurements were calibrated against standard N_2O-mixtures provided by Messer-Griesheim (Duisburg, Germany) and Alltech Europe (Brussels, Belgium). These gases were compared to standards provided by the Fraunhofer Institut für Atmosphärische Umweltchemie (H. Scheel, Garmisch-Partenkirchen, Germany). The N_2O concentrations in the water was calculated according to Weiss & Price (1980). For calculating the saturation value in the water samples a value of 300 ppbv N_2O as mean air value was used according to the mean air concentration close to the Baltic sea surface as determined by Rönner (1983).

Subsampling. Subsampling of incubated samples for determination of H_2S or $S_2O_3^-$ was always performed by first adding an overpressure of nitrogen gas and sampling with an N_2-flushed syringe.

Bacteria. Total counts of bacteria were made under an epifluorescence microscope after staining with

acridine orange according to Zimmermann et al. (1978). All samples were fixed in 2 % formaldehyde. Saprophytes were grown on yeast extract-peptone agar medium (ZoBell 2216E; Oppenheimer & ZoBell 1952) prepared with aged natural seawater diluted to a final salinity of 8 ‰. This standard medium was used so as to have a bacterial background parameter that allows comparison with other investigations.

RESULTS

In situ measurements

The depth profiles of physical and chemical parameters in the water column are summarized in Figs. 2 & 3. Profiles obtained in both years show pronounced similarities and are representative for the summer situ-
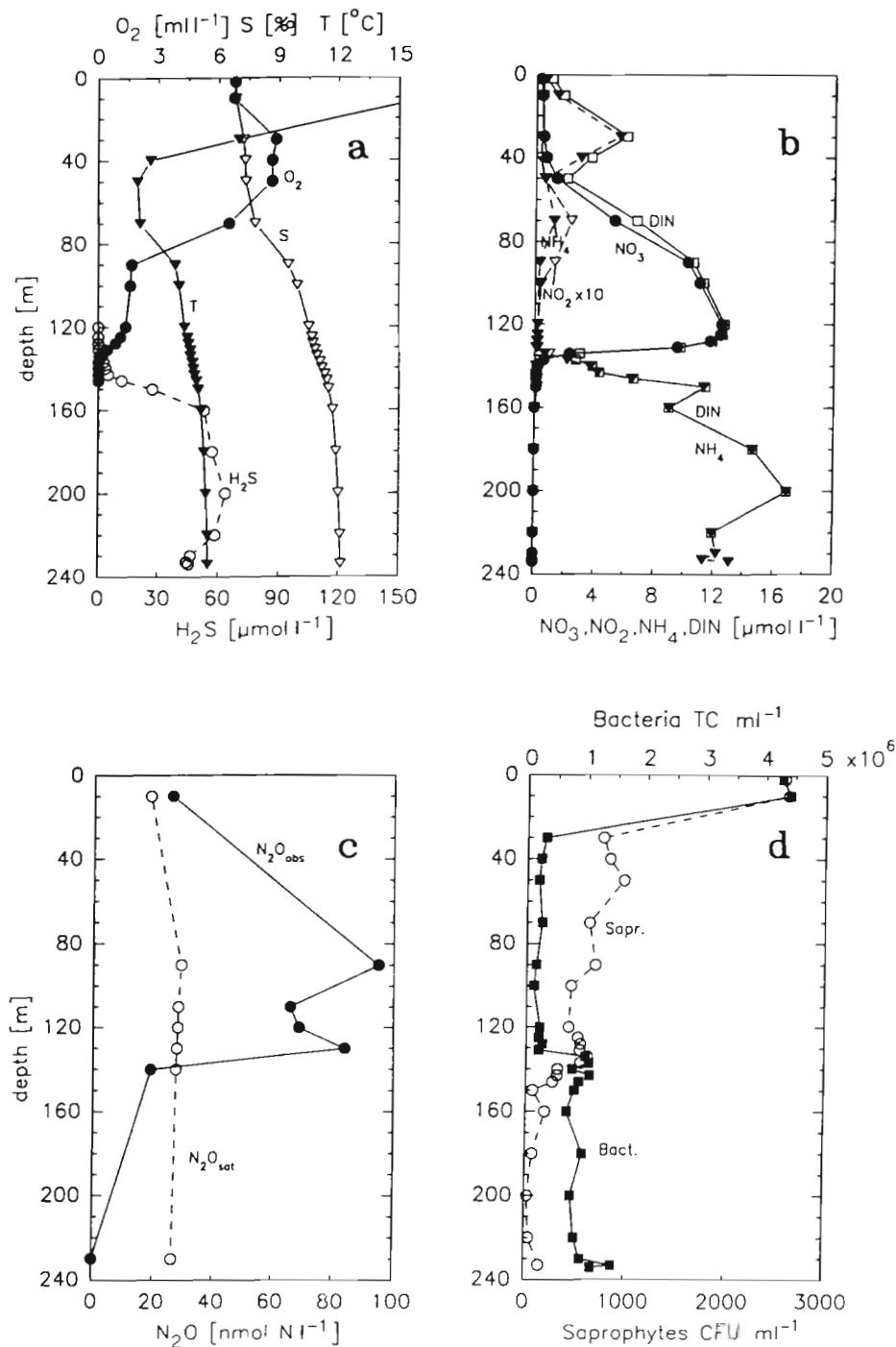

Fig. 2. Profiles in the water column of the Gotland Deep in summer 1986. (a) Temperature, salinity, oxygen and hydrogen sulfide; (b) vertical distribution of NO_3^-, NO_2^-, NH_4^-, dissolved inorganic nitrogen (= DIN = sum of NO_3^-, NO_2^-, NH_4^-); (c) observed N_2O concentration (N_2O_{obs}) and calculated saturation level (N_2O_{sat}); (d) distribution of bacteria [total counts of bacteria (TC) under epifluorescence microscope] and saprophytes grown on yeast extract peptone-agar
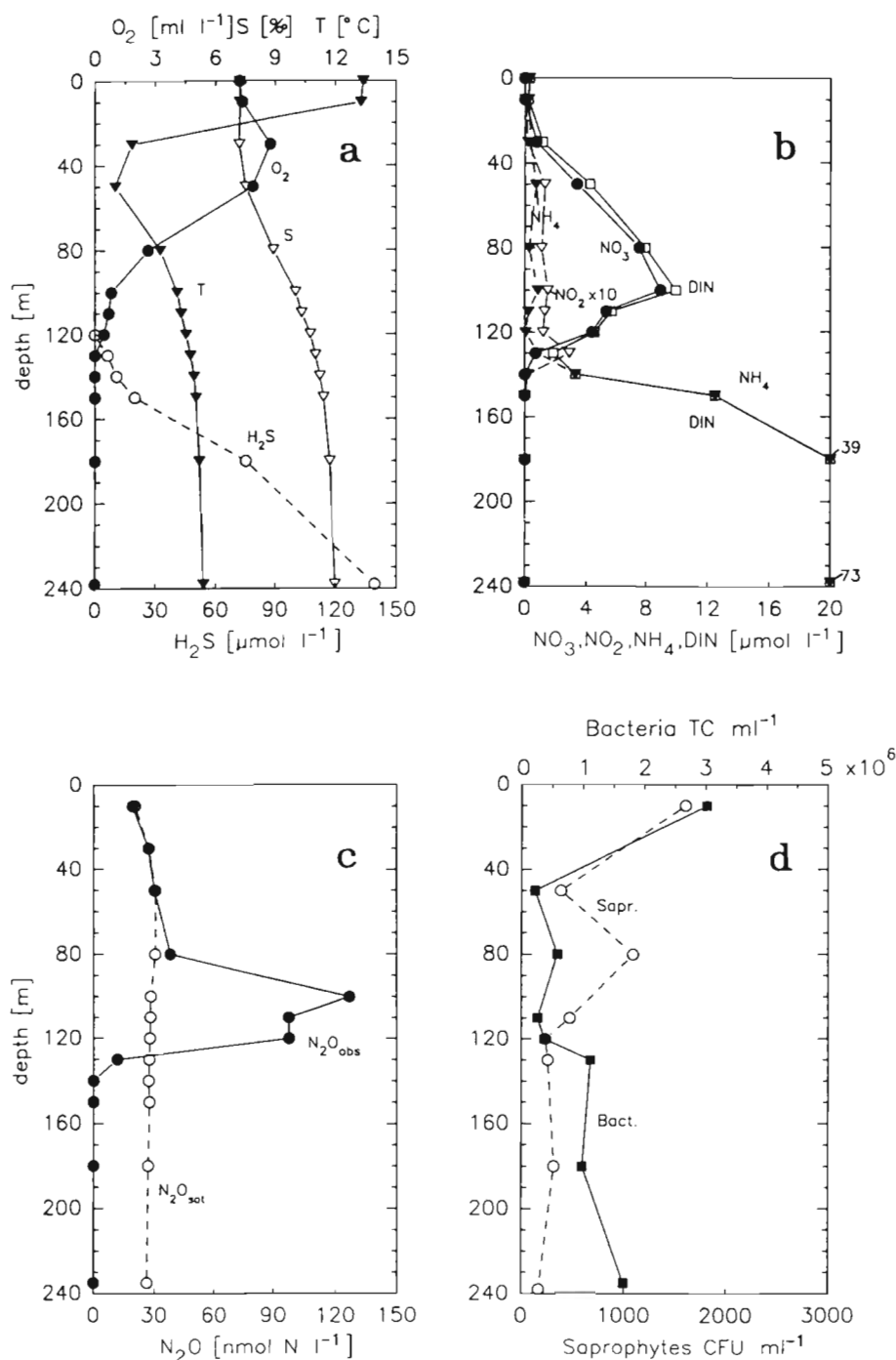
Fig. 3. Profiles in the water column of the Gotland Deep in summer 1987. For legend see Fig. 2

ation in the Gotland Deep (Rheinheimer et al. 1989). The Gotland Deep shows a salinity gradient increasing with depth. The steepest gradient is generally observed between 60 and 90 m; i.e. the halocline.

Oxygen was close to saturation level (> 80 %) in the water column above the halocline. O_2 decreased strongly within the halocline. Oxygen deficiency was only met in water samples containing H_2S. Lowest O_2 levels in water samples without H_2S were > 0.35 ml l^{-1}.

H_2S concentrations increased towards the sediment; in 1987 this increase was almost linear. Thiosulfate was measured only in summer 1987. It was undetectable in the oxic water column down to the oxic-anoxic interface. In the anoxic water column, the values showed an almost linear increase from the interface (130 m; 10 µmol l^{-1}) to the sediment-near water (238 m; 340 µmol l^{-1}). Due to the unspecifity of the titration method, these values cannot be ascribed with confi-

dence to thiosulfate. Therefore, only the fact that there was no accumulation of thiosulfate at the oxic-anoxic interface is deduced.

Nitrate was depleted in the upper 10 m and showed only small concentrations above the halocline. Within and below the halocline maximum nitrate concentrations up to 11 μmol l^{-1} were registered. Typically, a pronounced peak was observed above the oxic-anoxic interface followed by a strong decline in the interface layer. Nitrite was present only in low concentrations. Maximum values up to 0.2 μmol l^{-1} were reached in the halocline and the oxic-anoxic interface. The ammonium concentrations were usually very low in the oxic part of the water column. Higher concentrations occasionally showed up below the euphotic zone and in the halocline. High concentrations occurred in the anoxic part of the water column, where NH_4^+ showed an increase towards the sediment.

A more detailed view of the concentrations of NO_3^-, NO_2^-, NH_4^+, O_2 and H_2S in the vicinity of the interface is provided by Fig. 4.

The sum of NO_3^-, NO_2^- and NH_4^+, representing the major fraction of dissolved inorganic nitrogen (DIN), showed 2 minima: one in the surface layer, and one in the layer of the oxic-anoxic interface. Highest concentrations always occurred close to the sediment (see Figs. 2 & 3).

Nitrous oxide concentrations were close to the atmospheric saturation value in the water column above the halocline. Supersaturation was always found in the layer between the halocline and the oxic-anoxic interface. Maximum values of the nitrous oxide saturation were 450 % at 100 m depth in 1987, and 330 % at 90 m depth in 1986. Nitrous oxide was reduced to 43 and 70 % of saturation in the oxic-anoxic interface, and depleted in the anoxic deep water (Figs. 2c & 3c). Bacterial numbers reached highest values in the euphotic zone with up to 4.4 × 10^6 cells ml^{-1}. Less pronounced peaks were in the oxic-anoxic interface (about 1.6 × 10^6 cells ml^{-1}) and in the sediment-near water layer (about 1.8 × 10^6 cells ml^{-1}) (Figs. 2d & 3d). The aerobically grown saprophytes showed a pronounced maximum in the euphotic zone. Saprophyte numbers were always lowest in the anoxic part of the water column.

In situ denitrification

In situ denitrification as measured by the acetylene inhibition method was followed in 1986 in the whole water column with emphasis on the low oxygen water below the halocline. Denitrification could only be detected in samples from the oxic-anoxic interface with coexistence of H_2S and NO_3^-. These findings could be confirmed in 1987 (Fig. 5a, b). Rates of denitrification expressed as N_2O production were 110 nmol N l^{-1} d^{-1} (SD = 65 nmol N l^{-1} d^{-1}) in 1986 and 44 nmol N l^{-1} d^{-1} (SD = 8 nmol N l^{-1} d^{-1}) in 1987. Rates were linear up to 4 d with an increasing standard deviation with prolonged incubation time. In low oxygen samples from above the oxic-anoxic interface (10 m and 20 m above, with oxygen ranging from 0.4 to 1.3 ml l^{-1} at sampling time) denitrification could never be detected, even after incubation periods of up to 12 d.
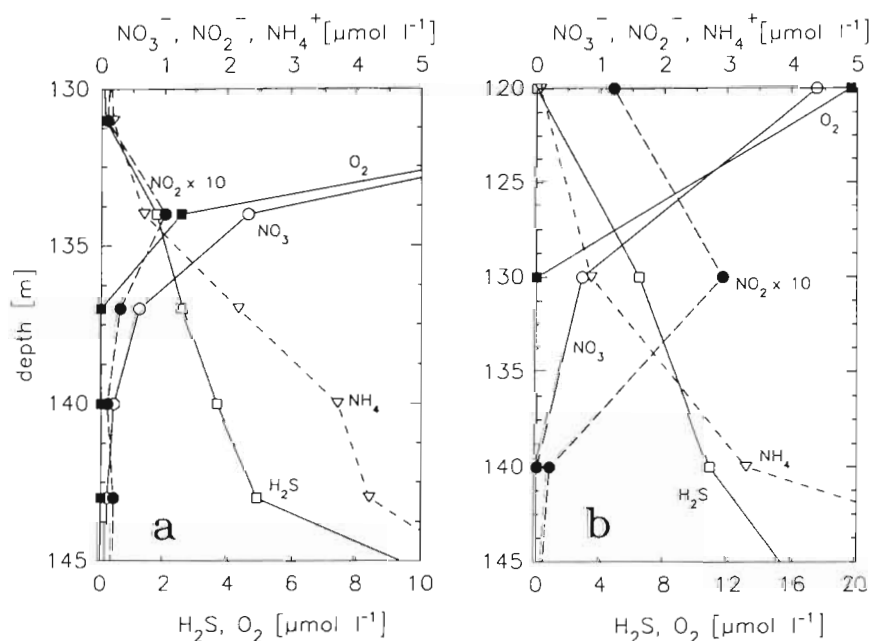

Fig. 4. Concentrations of NO_3^-, NO_2^-, NH_4^+, O_2 and H_2S in vicinity of the oxic-anoxic interface (detailed view of Figs. 2 & 3) in summer (a) 1986 and (b) 1987
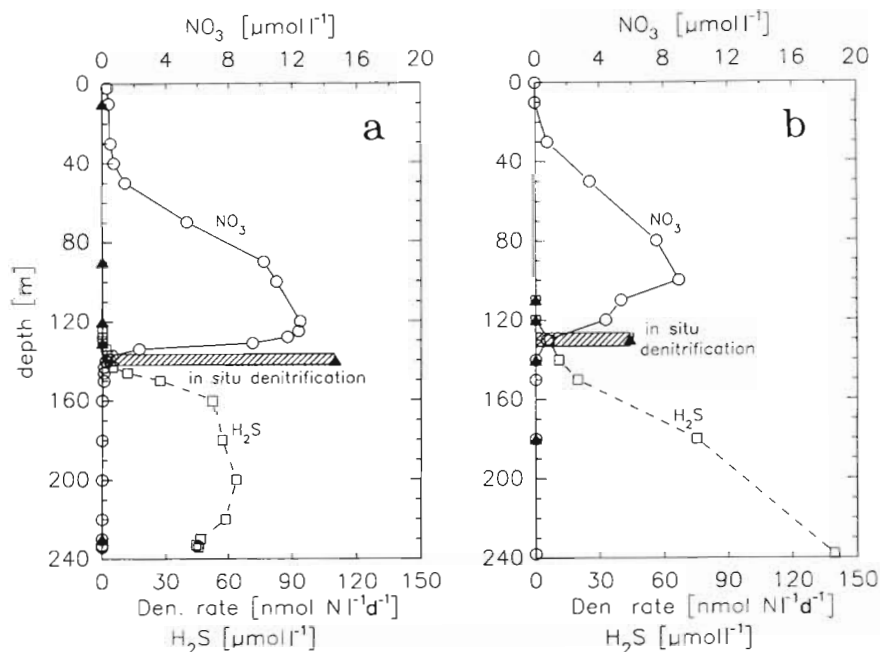
Fig. 5. In situ denitrification in the water column of the Gotland Deep in summer (a) 1986 and (b) 1987. (▲) Measured denitrification rate (connected to the x-axis by bars to emphasis the denitrification layer)

Denitrification after NO$_3^-$ addition

Nitrate addition neither increased the denitrification rates of the interface samples (Fig. 6), nor had it any effect on the water samples from above the interface. Even by incubating 1 l samples from above the inter-
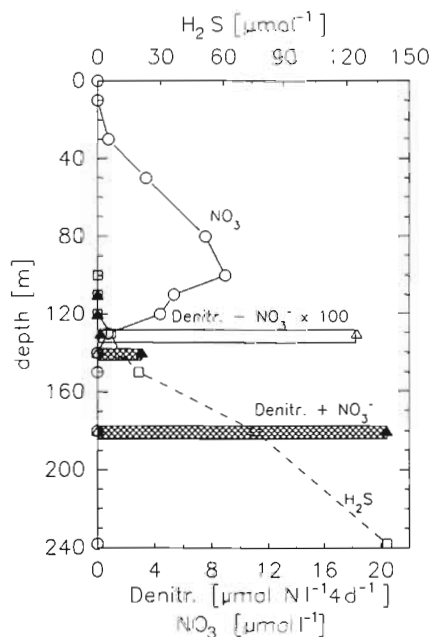

Fig. 6. Denitrification with and without nitrate addition after an incubation time of 4 d. Data shown are from the Gotland Deep 1987. (▲, △) Measured denitrification rates (connected to the y-axis by bars). (▲) With nitrate; (△) without nitrate (× 100)

face for 100 d, no denitrification could be detected. As only nitrate but no acetylene was added to these long-term incubations, changes in nitrogenous compounds were watched carefully. However neither decrease of nitrate nor increase of nitrite concentration was detected, that could have provided a hint of denitrification.

By contrast, water samples from the anoxic H$_2$S-containing deep water showed denitrification after addition of NO$_3^-$. This could be shown for water samples from 140 and 180 m in 1987 (see hatched bars in Fig. 6). The amount of denitrified nitrogen after 4 d of incubation increased with increasing H$_2$S-concentrations. Denitrification after addition of nitrate was also observed in samples from 230 m taken in 1986 (data not shown).

These results indicate that there was no limitation of denitrification by nitrate in the low oxygen and the interface layer, in contrast to the anoxic water layer. These findings substantiated the hypothesis that reduced sulfur compounds provided by the anoxic water could be valuable electron donors for denitrification.

Denitrification after addition of different electron donors

In this experiment, the utility of reduced sulfur compounds (H$_2$S, S$_2$O$_3^{2-}$) as electron donors for denitrifying bacteria of the oxic-anoxic interface (samples from 5 to 10 m below the interface) was observed. For comparison, acetate and a mixture of acetate plus H$_2$S were

provided. All samples were spiked with nitrate. Denitrification rates, and changes of nitrogenous compounds and of bacterial numbers were followed during a 4 d incubation period after substrate addition (Figs. 7 & 8).

The used water samples contained, immediately after sampling, small amounts of H_2S (about 10 μmol l^{-1}), no nitrate or nitrite, small amounts of NH_4^+ (about 2 μmol l^{-1}) and no detectable oxygen. To avoid the risk of the presence of undetectable oxygen, we preincubated the samples for 1 to 2 d. After this period H_2S had decreased to 0 to 0.5 μmol l^{-1} and oxygen was not detectable.

Samples from below the interface were preferred to interface samples, to make sure that there was no oxygen left that could interfere with the experiments, especially by oxidation of the added electron donors. We assumed that the bacterial community from that layer was still very well adapted to interface conditions.

After addition of nitrate alone about 3 μmol l^{-1} of N were denitrified. Denitrification was strongly enhanced by the addition of Na_2S and even more by the addition of NaS_2O_3. H_2S as well as $S_2O_3^{2-}$ were consumed within the first 2 d of incubation, while NO_3^- was denitrified. Denitrification was also enhanced by additions of Na_2S and acetate mixture, and of acetate alone.

The response to the addition of the latter compounds was a little slower. $S_2O_3^{2-}$ was never detected as an oxidation product of added H_2S. NH_4^+ had an initial concentration of 1.8 μmol l^{-1} and decreased during the 4 d incubation period by 1 to 1.5 μmol l^{-1}. NO_2^- showed usually low concentrations, below 1.5 μmol l^{-1}, with the exception of the assays with acetate addition. The autoclaved water samples did not show any change of the added H_2S and NO_3^- concentration during incubation under the same conditions.

The consumption of the reduced sulfur compounds as electron donors for denitrification was paralleled by growth of the bacterial assemblage (Fig. 8). Denitrification with $S_2O_3^-$ or with H_2S resulted in an increase of bacterial numbers. The increase was lowest after acetate addition. These data refer to experiments without acetylene addition to avoid any influence of acetylene on bacterial growth. Denitrification in samples without acetylene was assumed to be similar to samples with acetylene, as indicated by the same changes concerning NO_3^-, NO_2^-, NH_4^+, H_2S and $S_2O_3^{2-}$ (results not shown).

Similar results for denitrification and growth of the bacterial assemblage were obtained with water samples from 180 m with higher endogenous H_2S concentrations (61 μmol l^{-1}). The major differences compared
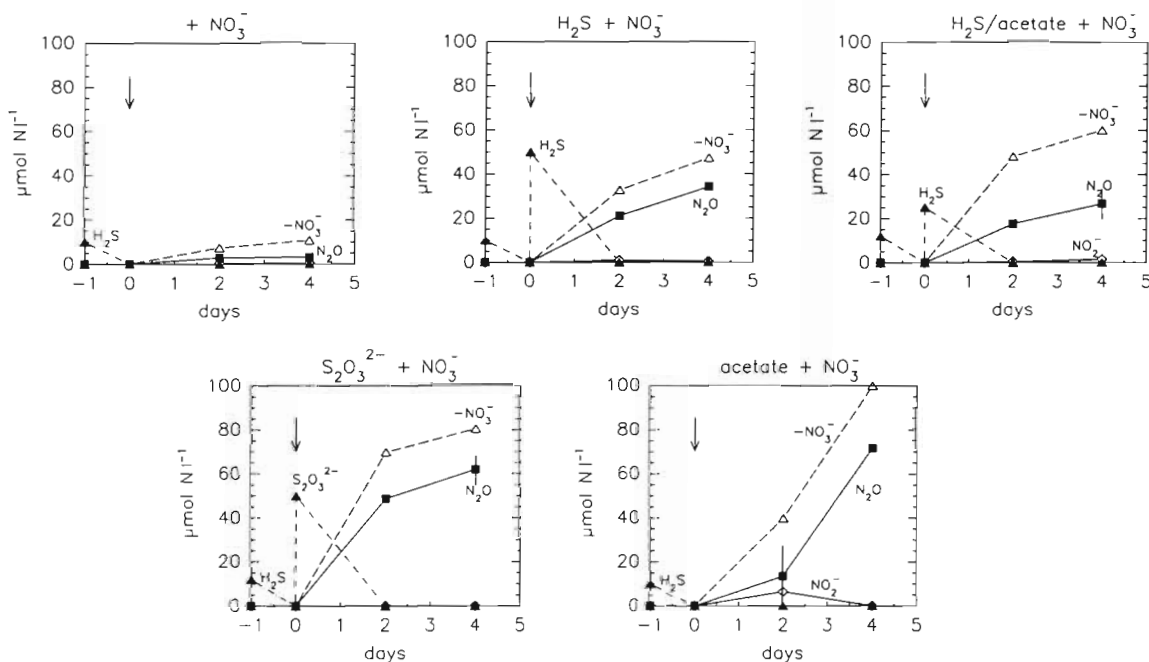

Fig. 7. Denitrification after addition of NO_3^- (100 μmol l^{-1}) and different electron donors to water samples from interface vicinity (5 to 10 m below). Denitrification was measured as N_2O production in presence of acetylene (bars indicate standard deviation for triplicates). Arrows indicate addition of 100 μmol l^{-1} NO_3^- plus electron donors indicated above panels. Added concentrations of electron donors: H_2S, $S_2O_3^{2-}$: 50 μmol l^{-1}; H_2S/acetate: 25 μmol l^{-1} H_2S + 50 μmol l^{-1} acetate; acetate: 100 μmol l^{-1} Consumption of NO_3^- ($-NO_3^-$), and production of NO_2^- and N_2O are given in μmol N l^{-1} NH_4^+ always showed decrease by about 1 to 1.5 μmol l^{-1} and is not shown. Consumption of initially present and added H_2S and $S_2O_3^{2-}$ is given in μmol l^{-1}
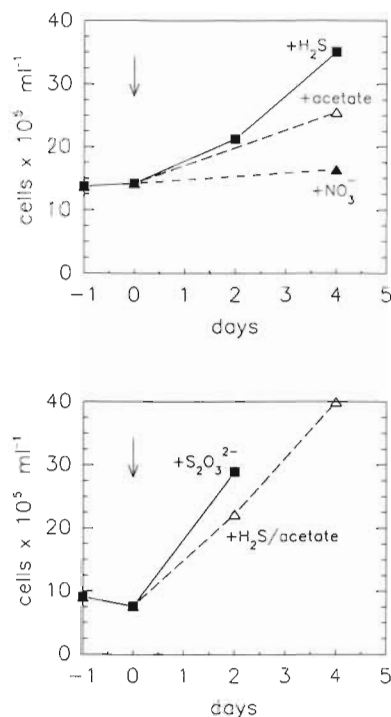
Fig. 8. Increase of bacterial numbers after addition of different electron donors and nitrate to water samples from interface vicinity. Arrows indicate addition of 100 µmol l^{-1} NO$_3^-$ plus the electron donor indicated. Sample origin and treatment was identical to samples shown in Fig. 7, except no acetylene was added

to the interface water samples were that (1) the response of the 180 m samples after addition of the respective electron donors and nitrate was slower, and (2) the added S$_2$O$_3^{-2}$ was not used, as far as could be determined by the titration method (for details see Brettar 1991). During all incubations with and without nitrate addition, an increase of NH$_4^+$ was never observed.

Comparison of changes of NO$_3^-$, NO$_2^-$, NH$_4^+$, H$_2$S and S$_2$O$_3^{2-}$ concentrations showed no significant differences between samples with and without acetylene addition for the interface samples. Acetylene had a retarding effect in the samples from 180 m. There was no evidence for utilization of acetylene during incubation periods up to 12 d.

In the presence of acetylene, a major fraction of the nitrate consumed was detected as N$_2$O. Without acetylene addition, only samples where both Na$_2$S and acetate were present displayed higher N$_2$O concentrations. For the assays with samples from the interface vicinity, in the presence of acetylene up to 84 % of the nitrate consumed could be detected as N$_2$O and NO$_2^-$, with NO$_2^-$ being of very minor importance. For sulfide and thiosulfate as electron donors, on average 74 and 78 %, respectively, of the nitrate consumed were detected as N$_2$O and NO$_2^-$. Addition of acetate had a lower recovery

rate (recovery rate = NO$_3^-$ consumed detectable as NO$_2^-$ plus N$_2$O) of 49 % after 2 d, but reached 72 % after 4 d. Lowest recovery rates were observed with acetate plus Na$_2$S addition: after 2 d 44 % and after 4 d 54 % were detected as N$_2$O and NO$_2^-$. In all cases, recovery rates were higher between the 2nd and 4th incubation day than during the first 2 days: between 84 and 124 % compared to the nitrate consumed between the 2nd and 4th day was detected as N$_2$O.

DISCUSSION

Relevance of reduced sulfur compounds as electron donors for denitrification

Denitrification could be shown to be restricted to the layer of the oxic-anoxic interface. Only water samples from this layer with the concomitant presence of H$_2$S and NO$_3^-$ showed detectable in situ denitrification. This layer showed a pronounced decrease of the concentration of dissolved inorganic nitrogen. Nehring (1987) attributed this decrease, usually observed at the oxic-anoxic interfaces of the Baltic proper, to denitrification.

It could be shown that H$_2$S and S$_2$O$_3^{2-}$ were valuable electron donors for the denitrifying bacterial community at the oxic-anoxic interface. Since denitrification and growth of this bacterial community with the reduced sulfur compounds occurred, it is likely that chemolithoautotrophic bacteria played a dominant role for the denitrification process at the oxic-anoxic interface. The finding that the availability of electron donors for denitrification followed the concentration of H$_2$S along the water column could be interpreted as suggesting the significance of reduced sulfur compounds as electron donors for denitrification.

According to Kuenen & Bos (1987) chemolithoautotrophic thiobacilli are likely to be competitive at oxic-anoxic interfaces. Since the ability to use both oxygen and nitrate as electron acceptors should be advantageous in this environment, an organism with similar physiological features to Thiobacillus denitrificans could be expected. It is conceivable that such an organism is mainly responsible for denitrification at the oxic-anoxic interface.

The presence of a highly active autotrophic microflora at the oxic-anoxic interface was shown by Gocke (1989) for the same day in August 1986 as that when denitrification was investigated. For that day Gocke reported a maximum CO$_2$ dark uptake rate of 55 µg C l^{-1} d^{-1}. In summer 1988 lower values, with a maximum of 15 µg C l^{-1} d^{-1}, were measured. In both years, the maxima were located in the layer of the oxic-anoxic interface and were much higher than values in the low-

oxygen water above (< 0.2 µg C l^{-1} d^{-1}). Maxima of CO_2 dark uptake have been reported for oxic-anoxic interfaces of comparable ecosystems: for the Cariaco Trench, Tuttle & Jannasch (1979) reported a maximum of 3 µg C l^{-1} d^{-1}; for the Black Sea, Sorokin (1972) measured around 6 µg C l^{-1} d^{-1}; for Saanich Inlet (B.C., Canada), Juniper & Brinkhurst (1986) found rates up to 24 µg C l^{-1} d^{-1}.

Another indication for a strong impact of bacterial autotrophic production on the carbon budget of the interface is the pronounced decrease of the C:N ratio of particulate material. The C:N ratio (mol C/mol N) decreased from its maximum of 9.1 in the lower part of the halocline (80 m) to the smallest value recorded of 6.8 in the denitrifying interface layer (130 m) (for data from the whole water column see Brettar 1991). According to Nagata (1986), planktonic bacteria show a low C:N ratio, on average around 5.0. It is therefore conceivable that the observed high chemosynthetic bacterial production lowered the C:N ratio. This would be due to increased bacterial biomass in the interface layer along with proliferation of organisms grazing on bacteria. A pronounced increase in the number of flagellates in this layer was observed (H. Galvao pers. comm.). Since flagellates also display a low C:N ratio (around 5.0, according to Newell & Linley 1984), their presence will contribute to a low C:N ratio of the particulate material.

Nevertheless, an additional role of organic carbon as electron donor for denitrification should not be excluded. The importance of organic carbon may vary according to the changing supply during the seasons due to changing sedimentation rates. In late spring and early summer, after the sedimentation of the spring bloom, when the water column shows the highest concentrations of organic carbon (Stigebrandt & Wulff 1987), the contribution of organic carbon may be enhanced. For a water column with stagnant anoxic deep water throughout the year, as is the case for the Gotland Deep, reduced sulfur compounds are likely to have a higher availability than organic carbon for most of the year Thus, H_2S-driven denitrification should be the predominant mechanism over the course of a year. The impact of organic carbon on denitrification is probably only of relevance during periods of high sedimentation rates.

Denitrification with reduced sulfur compounds as electron donors should play a role as soon as H_2S/NO_3^- interfaces occur These interfaces may occur when carbon-poor environments are supplied with H_2S. Thus, NO_3^- becomes available to autotrophic denitrifiers, and is not reduced beforehand by heterotrophic organisms. In sediments that are usually rich in organic carbon compared to the water column, H_2S-NO_3^- interfaces are very rare (Jørgensen 1988). By contrast, when

H_2S enters the water column H_2S/NO_3^- interfaces are often reported (Hashimoto et al. 1983, Sorokin 1964). Sorokin (1964) even demonstrated the potential of CO_2 dark uptake after addition of NO_3^- and S^{2-} to interface water samples from the Black Sea, the first suggestion of possible occurrence of autotrophic denitrification at oxic-anoxic interfaces in the water column.

Comparison of 'H₂S-denitrification' with reported rates in sediments and water column

A major problem in estimating the amount of nitrogen finally removed by 'H₂S-denitrification' is to judge how representative the measured rates are for denitrification at the oxic-anoxic interface. Since the measured rates were in the same order of magnitude in both years, for the following calculations it will be assumed that this order of magnitude can be taken as representative for the year. Due to variations in the strength of mixing at the interface the rate may show a very strong variability. It could be assumed that the bacterial assemblage present at the interface is very quickly able to consume new substrate supplied by mixing processes. This could be estimated from the experiments with H_2S and $S_2O_3^-$ additions.

Another consideration is that the true denitrification rate may have been underestimated due to alleviation of the acetylene blockage in the presence of H_2S. For strains of heterotrophic bacteria, soils and marine sediments, alleviation in presence of H_2S was reported (Tam & Knowles 1979, Adkins & Knowles 1986, Sørensen et al. 1980). It is difficult to elucidate the path of the nitrogen during in situ denitrification in interface samples, because of the difficulty in following and interpreting changes of very low concentrations of nitrogenous compounds. Therefore, the experiments with addition of nitrate and different electron donors are here evaluated for possible alleviation. The recovery rate of the nitrate consumed after addition of electron donors ranged on average from 54 to 78 % if we consider the whole incubation period of 4 d. The recovery rate was always lower for the first 2 d (44 to 70 %) than for the following 2 d (84 to 124 %) compared to the nitrate consumed in the same time period. The highest recovery rate was shown by the assay with thiosulfate, followed by the assay with sulfide addition. The lowest and most variable results were obtained from the assay with acetate plus sulfide addition.

The difference between the nitrate consumed and recovered may be caused mainly by 3 different processes: assimilation, NO production and N_2 production due to alleviation of acetylene blockage. The higher recovery rate of the nitrate consumed during the second 2 d compared to the first 2 d may be accounted for

in each process as follows: (1) assimilation is higher during the first phase, (2) intermediates at the oxidation state of NO are formed in the first phase and consumed during the second one, (3) the acetylene blockage is more effective in the second than in the first phase. The quantitative importance of each of these effects is difficult to estimate. For nitrate assimilation an upper estimate can be provided by calculating the biomass that may be produced by oxidation of the added thiosulfate. According to Justin & Kelly (1978), *Thiobacillus denitrificans* is able to fix 22.5 μmol C l^{-1} by oxidising 50 μmol l^{-1} $S_2O_3^{2-}$ to SO_4^- under denitrifying conditions, with the incorporation of 5.2 μmol N l^{-1} [calculated from a C:N ratio of 4.3 (mol/mol)]. As the microflora at the interface is likely to consist of different bacteria as well as grazing protozoa, N assimilation may differ from this calculated value. For the production of NO, no estimate can be given, because it was not measured during the experiments. Formation of NO as an intermediate in denitrification is not unlikely to occur since it was often reported especially in the presence of H_2S (Sørensen 1978).

We feel that N assimilation contributed to some extent to nitrate consumption. As estimated above, total usage should not exceed 5 μmol N l^{-1}, of which a maximum of 4 μmol NO_3^- l^{-1} should be used for assimilation; about 1 μmol N l^{-1} was already used from the NH_4^+ pool. Occurrence of NO is not unlikely and could account for the non-recovered nitrate. The third possibility is alleviation of the acetylene blockage. This may be of minor importance for autotrophic denitrification with $S_2O_3^{2-}$ or H_2S, as the recovery rate was higher for these assays, concomitant with a stronger increase of bacterial numbers. A higher rate of alleviation of the acetylene blockage is conceivable for denitrification in the presence of both acetate and sulfide.

Our suggestion is that autotrophic denitrification was not sensitive to alleviation of the acetylene blockage in the presence of H_2S, but heterotrophic denitrification was. This is consistent with the findings in the literature (Tam & Knowles 1979, Sørensen et al. 1980, Adkins & Knowles 1986), as only for heterotrophic bacteria and ecosystems with high availability of organic carbon was alleviation of the acetylene blockage reported. Tam & Knowles (1979) even mentioned that *Thiobacillus denitrificans* was not able to reduce N_2O in the presence of both sulfide and acetylene. The partial alleviation that cannot be excluded in samples spiked with $S_2O_3^{2-}$ or H_2S could also be attributed to a heterotrophic microflora living on the carbon liberated by flagellates from the autotrophic denitrifiers or excreted by the latter.

With these considerations in mind, it cannot be ruled out that the measured rates underestimate the true in situ rates. However, this underestimation is thought not to be a major source of error (probably not exceeding 30 % of the measured rate), and is assumed to be of minor importance compared to the influence of the variability of mixing processes and the resulting denitrification activities.

There are only a few measurements available on denitrification in the water column of the Baltic Proper, made by Rönner & Sörensson (1985) in late spring 1980 in the western part of the Baltic proper. They reported a mean rate per liter of 200 nmol N l^{-1} d^{-1}, and thus at least twice the rates we measured at the oxic-anoxic interface. Since the water layer showing denitrification extends much further than the interface, the total amount of nitrogen removed would have been considerably more. Shaffer & Rönner (1984) calculated the removal of nitrogen from sediment and water column based on a model of Baltic salt profiles. The calculated rates for the water column were only about 1/5 (36 nmol l^{-1} d^{-1}) of the measured ones. For the sediment they calculated a mean rate of 1.79 mmol m^{-2} d^{-1}. If we calculate N removal by denitrification at the oxic-anoxic interface assuming that the denitrification layer was 10 m thick and the denitrification rate ranged from half that measured in 1987 to double the higher rate observed in 1986, between 0.22 and 2.20 mmol N m^{-2} d^{-1} would be removed. According to these assumptions, denitrification at the oxic-anoxic interface could perform about as much N removal as sediment denitrification, although tending to be less. Denitrification in extended anoxic and low oxygen water layers as reported by Rönner & Sörensson (1985) has a much greater influence on the nitrogen bugdet. According to their results, about 3 and 90 mmol N m^{-2} d^{-1} were removed in the nitrate-limited water column of their 2 investigated stations respectively.

As a rough estimate, H_2S-driven denitrification should yield a lower N removal per m^2 than sediment denitrification in the Baltic Proper as calculated by Shaffer & Rönner (1984). The highest efficacy for nitrogen removal seems to be favoured by a low-oxygen, and carbon- and nitrate-rich water column. A situation of good carbon supply in the water column, in turn, is more likely in late spring and early summer than for the rest of the year.

Description of carbon flux and electron donor transport in a system with interface-denitrification

In a simplified way the coupling of carbon flux and denitrification in the water column of the Gotland Deep could be described by the following model (see Fig. 9):

While surface water and sediment-near water represent a carbon-rich environment, the water column in between is for most of the year relatively poor in utiliz-
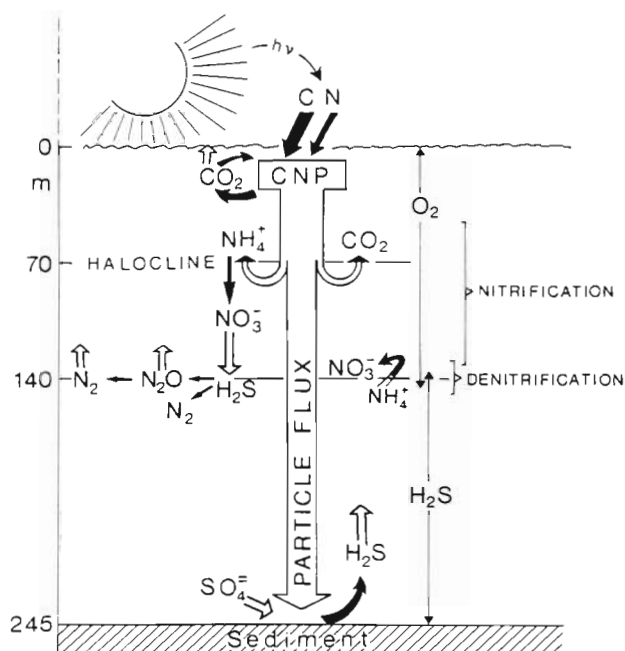
Fig. 9. Model of N-transformations at the oxic-anoxic interface in the water column of the Gotland Deep related to the overall particle flux. Solid arrows: transformation by organisms; hollow arrows: diffusive transport pathways. Arrows have only qualitative and no quantitative meaning

able organic carbon. At the sediment, organic material mineralized by sulfate reducers releases H_2S to the water column. At the oxic-anoxic interface H_2S is used as electron donor for denitrification of the NO_3^- and NO_2^- available here. Thus H_2S serves as a vector of electrons originally derived from the sedimented organic carbon. The accumulated H_2S represents an electron donor buffer, that is provided to the denitrifiers by exchange processes at the oxic-anoxic interface. Hashimoto et al. (1983) and Anderson (1984) assumed that the NH_4^+ for nitrification is also mainly provided by the anoxic deep water. Thus exchange processes at the oxic-anoxic interface enhance denitrification not only by mixing NO_3^- and H_2S, but also by supporting the ammonium supply to nitrifiers.

Ecological implications of 'H₂S-denitrification' and significance for the Baltic Proper

Denitrification at the oxic-anoxic interface in ecosystems with permanent anoxic deep water occurs without direct coupling to the flux of organic matter derived from phytoplankton primary production. We assume that the strongest influence on the denitrification rate will be provided by mixing processes at the oxic-anoxic interface. H_2S-driven denitrification at permanent interfaces should therefore be a process that occurs at

the same order of magnitude throughout year without showing a pronounced seasonality.

The relevance of this mechanism for the Baltic Proper should increase with the spread of H_2S-containing bottom water in the periods between deep water renewal (Stigebrandt & Wulff 1987). As the tendency towards decreasing oxygen concentrations in the Baltic Proper deep water since the beginning of the century is still continuing, it is likely that spreading of H_2S in the bottom water will be even more pronounced in the future (Andersin et al. 1979, Matthäus 1979), providing more extended oxic-anoxic interfaces in the water column.

Permanent interfaces for H_2S-denitrification will be provided at the top of the anoxic water layer. In case of deep water renewal, additional interfaces would be created within the anoxic layer. Occasional interleaving of O_2-rich water layers in the anoxic deep water should result in strong N-removal as indicated by denitrification after nitrate addition by interface samples as well as by samples from the anoxic deep water. Since interleaving provides large interface areas and enhanced mixing, denitrification should be very efficient in this case. A strong enhancement of denitrification due to deep water renewal and following mixing of oxic and anoxic water masses was already suggested by Nehring (1987).

LITERATURE CITED

Adkins, A. M., Knowles, R. (1986). Denitrification by *Cytophaga johnsonae* strains and by a gliding bacterium able to reduce nitrous oxide in the presence of acetylene and sulfide. Can. J. Microbiol. 32: 421–424

Andersin, A. B., Lassig, J., Sandler, H. (1979). Recent changes in the occurrence of soft-bottom macrofauna in the central Baltic proper. Comm. Meet. Int. Counc. Explor. Sea C.M.-ICES/L: 30

Anderson, J. J. (1984). The oxic-anoxic interface in Saanich Inlet. In: Proceedings of a multidisciplinary symposium on Saanich Inlet. Can. Tech. Rep. Hydrogr. Ocean Sci. 38: 17–23

Andersen, T. K., Jensen, M. H., Sørensen, J. (1984). Diurnal variation of nitrogen cycling in coastal, marine sediments. I. Denitrification. Mar Biol. 83: 171–176

Balderston, W. L., Sherr, B., Payne, W. J. (1976). Blockage by acetylene of nitrous oxide reduction in *Pseudomonas perfectomarinus*. Appl. environ. Microbiol. 31 504–508

Brettar, I. (1991). Denitrifikation in der zentralen Ostsee: Regulationsfaktoren und mikrobiologische Aspekte. Dissertation, Univ. Kiel. Ber Inst. Meeresk. Kiel 208

Fonselius, S. H. (1983). Determination of hydrogen sulphide.

In: Grasshoff, K., Ehrhardt, M., Kremling, K. (eds.) Methods of seawater analysis. Verlag Chemie Weinheim, New York, p. 73–80

Gocke, K. (1989). Bakterielle Stoffaufnahme im aeroben und anaeroben Milieu der Ostsee. Ber Inst. Meeresk. Kiel 188: 40–47

Graneli, E., Wallstrom, K., Graneli, W., Elmgreen, R. (1990). Nutrient limitation of primary production in the Baltic sea area. Ambio 19: 142–151

Grasshoff, K. (1983). Determination of nitrite, nitrate, oxygen, thiosulphate. In: Grasshoff, K., Ehrhardt, M., Kremling, K. (eds.) Methods of seawater analysis. Verlag Chemie Weinheim, New York, p. 139–142, 143–150, 61–72, 81–84

Grasshoff, K., Ehrhardt, M., Kremling, K. (1983). Methods of seawater analysis. Verlag Chemie Weinheim, New York

Hashimoto, L. K., Kaplan, W. A., Wofsy, S. C., McElroy, M. B. (1983). Transformation of fixed nitrogen and N_2O in the Cariaco Trench. Deep Sea Res. 30: 575–590

Hattori, A. (1983). Denitrification and dissimilatory nitrate reduction. In: Carpenter, E. J., Capone, D. G. (eds.) Nitrogen in the marine environment. Academic Press, New York, p. 191–232

Jørgensen, B. B. (1988). Ecology of the sulphur cycle: oxidative pathways in the sediments. In: Cole, J. A., Ferguson, S. J. (eds.) The nitrogen and sulfur cycle. 42nd Symp. Soc. J. Gen. Microbiol., Cambridge University Press, Cambridge, p. 31–63

Juniper, S. K., Brinkhurst, R. O. (1986). Water column dark CO_2-fixation and bacterial mat growth in intermittently anoxic Saanich Inlet, British Columbia. Mar Ecol. Prog. Ser. 33: 41–50

Justin, P., Kelly, D. P. (1978). Growth kinetics of *Thiobacillus denitrificans* in anaerobic and aerobic chemostat culture. J. gen. Microbiol. 107: 123–130

Koroleff, F. (1983). Determination of ammonia. In: Grasshoff, K., Ehrhardt, M., Kremling, K. (eds.) Methods of seawater analysis. Verlag Chemie Weinheim, New York, p. 150–157

Kuenen, J. G., Bos, P. (1987). Habitats and ecological niches of chemolitho(auto)trophic bacteria. In: Schlegel, H. G., Bowien, B. (eds.) Autotrophic bacteria. Brock/Springer Series in Contemporary Bioscience, FEMS Symp. 42, Berlin, p. 53–80

Larsson, U., Elmgren, R., Wulff, F. (1985). Eutrophication and the Baltic sea: causes and consequences. Ambio 14: 10–14

Liu, K. K., Kaplan, I. R. (1984). Denitrification rates and availability of organic matter in marine environments. Earth Planet. Sci. Lett. 68: 88–100

Matthäus, W (1979). Langzeitvariationen von Temperatur, Salzgehalt und Sauerstoffgehalt im Tiefenwasser der zentralen Ostsee. Beitr. Meeresk. 42: 41–93

Nagata, T (1986). Carbon and nitrogen content of natural planktonic bacteria. Appl. environ. Microbiol. 52: 28–32

Nehring, D. (1987). Temporal variations of phosphate and inorganic nitrogen compounds in central Baltic deep waters. Limnol. Oceanogr 32: 494–499

Newell, R. C., Linley, E. A. S. (1984). Significance of microheterotrophs in the decomposition of phytoplankton: estimates of carbon and nitrogen flow based on the biomass of plankton communities. Mar Ecol. Prog. Ser 16: 89–104

Oppenheimer, C. H., ZoBell, C. E. (1952). The growth and viability of sixty-three species of marine bacteria as influenced by hydrostatic pressure. J. mar Res. 11 10–18

Rheinheimer, G., Gocke, K., Hoppe, H.-G. (1989). Vertical distribution of microbiological and hydrographic-chemical parameters in different areas of the Baltic sea. Mar Ecol. Prog. Ser 52: 55–70

Rönner, U. (1983). Distribution, production and consumption of nitrous oxide in the Baltic Sea. Geochim. Cosmochim. Acta 47: 2179–2188

Rönner, U., Sörensson, F. (1985). Denitrification rates in the low-oxygen waters of the stratified Baltic proper. Appl. environ. Microbiol. 50: 801–806

Sen Gupta, R. (1973). A study on nitrogen and phosphorus and their interrelationships in the Baltic. Ph.D. thesis, Univ. Göteborg

Shaffer, G., Rönner, U. (1984). Denitrification in the Baltic proper deep water. Deep Sea Res. 31: 197–220

Sørensen, J. (1978). Occurrence of nitric and nitrous oxide in a coastal marine sediment. Appl. environ. Microbiol. 36: 809–813

Sørensen, J., Tiedje, J. M., Firestone, R. B. (1980). Inhibition by sulfide of nitric and nitrous oxide reduction by denitrifying *Pseudomonas fluorescens*. Appl. environ. Microbiol. 39: 105–108

Sorokin, Y I. (1964). On the primary production and bacterial activities in the Black Sea. J. Cons. int. Explor Mer 29: 41–60

Sorokin, Y I. (1972). The bacterial population and the processes of hydrogen sulphide oxidation in the Black Sea. J. Cons. int. Explor Mer 34: 423–454

Stigebrandt, A. (1987). A model for the vertical circulation of the Baltic deep water. J. phys. Oceanogr. 17: 1772–1785

Stigebrandt, A., Wulff, F. (1987). A model for the dynamics of nutrients and oxygen in the Baltic proper. J. mar. Res. 45: 729–759

Tam, T.-Y., Knowles, R. (1979). Effects of sulfide and acetylene on nitrous oxide reduction by soil and *Pseudomonas aeruginosa*. Can. J. Microbiol. 25: 1133–1138

Tuttle, J. H., Jannasch, H. W (1979). Microbial dark assimilation of CO_2 in the Cariaco Trench. Limnol. Oceanogr. 24: 746–753

Weiss, R. F., Price, B. A. (1980). Nitrous oxide solubility in water and sea water Mar. Chem. 8: 347–359

Wulff, F., Rahm, L. (1988). Long-term, seasonal and spatial variations of nitrogen, phosphorus and silicate in the Baltic: an overview. Marine environ. Res. 26: 19–37

Yoshinari, T., Knowles, R. (1976). Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochem. Biophys. Res. Comm. 69: 705–710

Zimmermann, R., Iturriaga, R., Becker-Birck, I. (1978). Simultaneous determination of the total number of aquatic bacteria and the number thereof involved in respiration. Appl. environ. Microbiol. 36: 926–935

This article was submitted to the editor