Microbial Ecology 5:105–115 (1979)

MICROBIAL ECOLOGY

# A Comparison of Oxygen, Nitrate, and Sulfate Respiration in Coastal Marine Sediments

Jan Sørensen, Bo Barker Jørgensen, and Niels Peter Revsbech

Institute of Ecology and Genetics, University of Aarhus, Ny Munkegade, DK-8000 Aarhus C, Denmark

**Abstract.** Aerobic respiration with oxygen and anaerobic respiration with nitrate (denitrification) and sulfate (sulfate reduction) were measured during winter and summer in two coastal marine sediments (Denmark). Both aerobic respiration and denitrification took place in the oxidized surface layer, whereas sulfate reduction was most significant in the deeper, reduced sediment. The low availability of nitrate apparently limited the activity of denitrification during summer to less than 0.2 mmoles $NO_3^-$ $m^{-2}$ $day^{-1}$, whereas activities of 1.0–3.0 mmoles $NO_3^-$ $m^{-2}$ $day^{-1}$ were measured during winter. Sulfate reduction, on the contrary, increased from 2.6–7.6 mmoles $SO_4^{2-}$ $m^{-2}$ $day^{-1}$ during winter to 9.8–15.1 mmoles $SO_4^{2-}$ $m^{-2}$ $day^{-1}$ during summer. The aerobic respiration was high during summer, 135–140 mmoles $O_2$ $m^{-2}$ $day^{-1}$, as compared to estimated winter activities of about 30 mmoles $O_2$ $m^{-2}$ $day^{-1}$. The little importance of denitrification relative to aerobic respiration and sulfate reduction is discussed in relation to the availability and distribution of oxygen, nitrate, and sulfate in the sediments and to the detritus mineralization.

## Introduction

The oxygen uptake of sediments has been used as a measure of "total community metabolism" (20). This assay includes both the aerobic respiration of organic matter and, e.g., the iron oxidation and the production of nitrate and sulfate by the processes of nitrification and sulfide oxidation. Nitrate and sulfate in turn serve as electron acceptors for the anaerobic respiratory processes of nitrate and sulfate reduction, and thus the assay of oxygen uptake should include both aerobic and anaerobic respiration of organic matter. However, the sedimentary metabolism may be underestimated by this assay if sulfide is released or is accumulated in the sediment (7), or it may be overestimated if the nitrification activity exceeds the activity of nitrate reduction.

The assay of oxygen uptake obviously provides no information on the relative importance of aerobic and anaerobic processes of detritus mineralization. The

0095–3628/79/0005–0105 $02.20

oxygen uptake of sediments has often been measured (5,14), but the contributory role of anaerobic mineralization, and of nitrate respiration in particular, has been less emphasized. Measurements of natural activities of denitrification (respiratory nitrate reduction to nitrogen gas) in marine sediments are few and scanty (4,15,19). The importance of sulfate reduction for the oxidation of organic detritus has previously been demonstrated (10).

In the present study, the rates of oxygen uptake, denitrification, and sulfate reduction were measured during summer and winter in two coastal marine sediments, and the relative importance of oxygen, nitrate, and sulfate respiration for detritus mineralization was estimated. The influence of temperature, faunal activity, and the availability of the electron acceptors, oxygen, nitrate, and sulfate are discussed.

## Materials and Methods

### *Sampling Sites*

The investigated areas were two estuaries on the east coast of Jutland, Denmark. The Randers Fjord is a 17 km long and 1–2 km wide fjord, whereas the Kysing Fjord is a small enclosed basin of 1.9 $km^2$ with a narrow entrance to the sea. Due to irregular water exchange there is a fluctuating salinity in the areas, but in general salinities are in the range of 15–20‰ (13).

In both areas, the sampling sites were located in shallow water (0.5 m) where the bottom was sandy due to wave action. Sediment cores, about 15 cm long, were taken by hand during winter (January 1978) and summer (June 1978) when the water temperature in situ was 3°C and 18°C.

All the assays were performed in the laboratory where the cores were reconditioned at the in situ temperature for more than 3 hours before any assay was initiated.

### *Texture and Redox Conditions*

The density and porosity of the sediments were determined in weighed core segments that were dried to constant weight at 105°C. Both parameters served for the conversion of measured concentrations and activities into appropriate dimensions.

Organic content (ignition loss) was determined by the weight loss of dried segments that were ignited at 450°C for 24 hours.

Redox potentials were recorded with a platinum electrode as described by Fenchel (6).

### *Assays of Oxygen Parameters*

The platinum needle electrodes of Baumgärtl and Lübbers (3) were shown to be applicable for a polarographic assay of dissolved oxygen in the sediments (N. P. Revsbech, in preparation). These electrodes consisted of a platinum wire that was mounted in an extended glass tube and covered at the tip with a collodion-polystyrene double membrane. The electrode was only 2–8 $\mu$m (O.D.) and allowed a fine resolution of the oxygen profiles in the sediments.

The oxygen electrode was calibrated in aerated seawater, and air saturation at 3°C and 18°C provided oxygen concentrations in the surface water (salinity 20‰) above the sediments of 365 $\mu$M and 245 $\mu$M, respectively [computed from solubility tables of Riley and Skirrow (16)].

The sediment cores for the assay of dissolved oxygen were submerged in a thermostated water bath of aerated seawater with modest circulation. The oxygen electrode was held in a vertical position above the core by a micromanipulator which also served to lower the electrode tip into the sediment. A silver/silver chloride reference electrode was placed in the surface water.

Oxygen concentrations were measured at 1-mm intervals in the sediments and profiles were recorded both in dark incubated cores and in cores that were illuminated on the sediment surface (700 W $m^{-2}$). An infrared filter restricted the light from a halogen lamp to the 400 to 800 nm range. The profiles were recorded after a stabilization period, which was several hours in the dark and 2 hours in the light.

The rate of oxygen uptake by the sediments was determined as described by Pamatmat (14) and Jørgensen (10). Sediment cores, in which the overlying water phase initially was air saturated, were stoppered and incubated in the dark for 1–1.5 hours at the in situ temperature. A small magnetic bar was held a few centimeters above the sediment surface by a wire through its short axis, and a rotation was provided by a stirrer outside the core. The stirring rate was established immediately below the suspension limit of the particulate material. Other cores were assayed without stirring, and the mean oxygen uptake, as measured by Winkler titration (17), of cores with a stagnant water phase and cores with a stirred water phase was used for calculation of the dark oxygen uptake. Generally, stirring increased the oxygen uptake by about 70%, but the calculated mean was anticipated to represent average in situ conditions.

### *Assays of Nitrogen Parameters*

Nitrate concentrations were measured in 1-cm segments of cores that were frozen immediately after reconditioning at the in situ temperature. Subsamples of about 5 g were thawed during centrifugation ($2000 \times g$) in tubes that also contained 2 ml of nitrate-free water to increase liquid volume and 200 $\mu$l of chloroform or 1 ml of saturated mercuric chloride solution to inhibit microbial activity during the assay (19). Two milliliters of supernatant was assayed by the method of Strickland and Parsons (17) in an automated analysis system (Chemlab).

Denitrification activity was determined by the rate of nitrous oxide accumulation when acetylene was applied to inhibit the reduction of nitrous oxide to dinitrogen (2,21). The present denitrification assay for application in sediments was previously described in detail (19). Acetylene-saturated water in quantities of 100 $\mu$l was injected into sediment cores at 1-cm intervals and the cores were then incubated for 5 hours in the dark at the in situ temperature. The injection was performed to provide a rapid and even distribution of the inhibitor in the cores. The amount of nitrous oxide accumulated during the incubation was determined in 1-cm segments of the cores in a combined gas extraction and gas chromatographic detection system. The gas extraction was performed in a helium-purged flask that contained the sediment segment for analysis and the liberated nitrous oxide was trapped in a liquid nitrogen-cooled loop prior to injection into the gas chromatograph.

### *Assays of Sulfur Parameters*

Sulfate was determined gravimetrically (1) in interstitial water that was obtained by pressure filtration through 0.45 $\mu$m membrane filters.

The rate of sulfate reduction was determined by the $^{35}S$-tracer technique described by Jørgensen (11). Labeled sulfate was injected in small aliquots (a few microliters) into the cores at 1–2-cm intervals and the amount of labeled sulfide produced after 5 hours of incubation was determined. Due to the heterogenous distribution of bacterial activity, the standard deviation of the mean (of five cores) was 35% (10).

## Results

At both localities, the sediment had an oxidized surface zone characterized by a brown coloration and positive redox potentials. The oxidized zone varied in thickness during summer and winter as shown in the first column of Figures 1 and 2. Only the average redox potential could be measured at a given depth since the

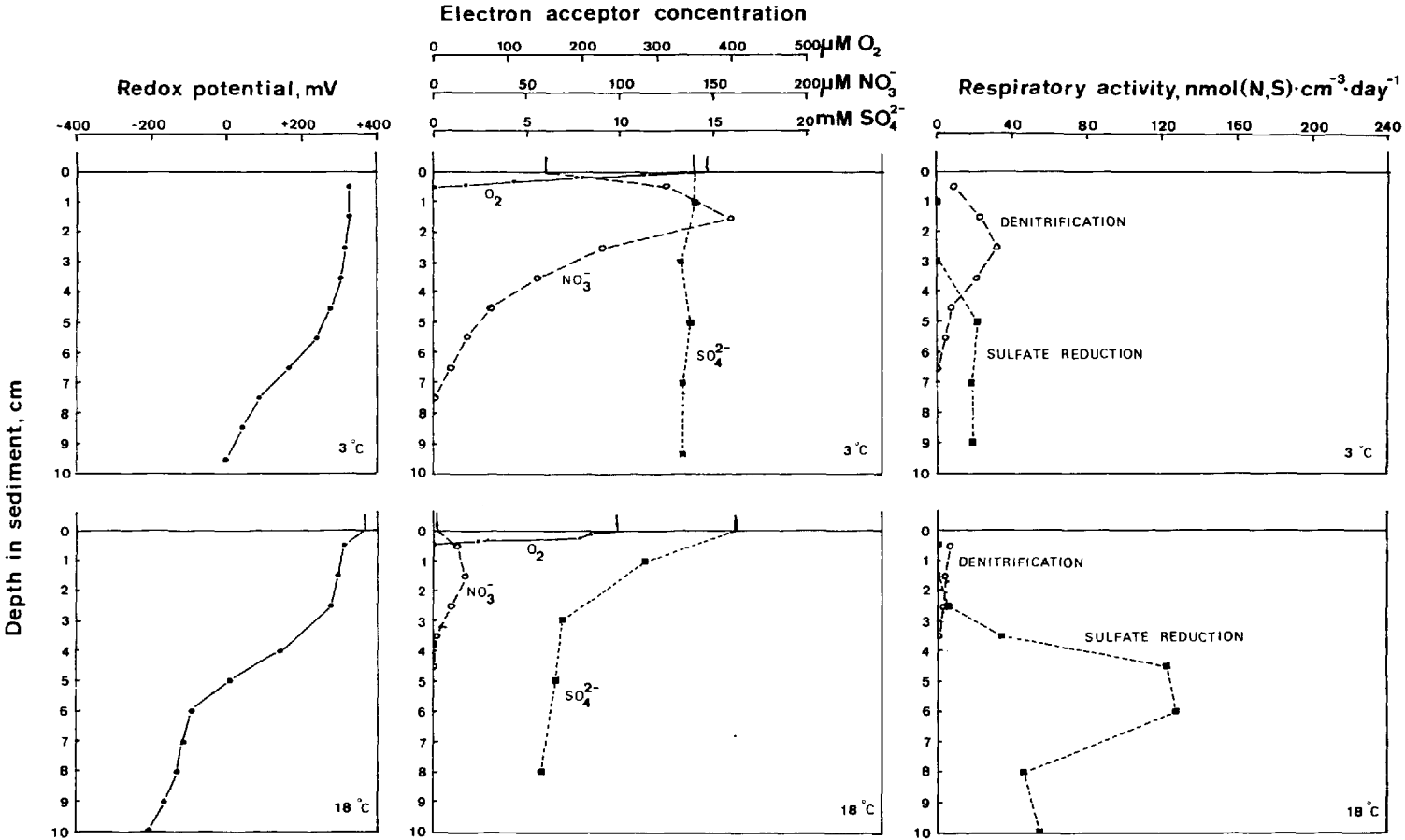


**Fig. 1.** Randers Fjord. Redox potentials, concentrations of oxygen, nitrate, and sulfate, and activity of denitrification and sulfate reduction during winter (3°C) and summer (18°C).

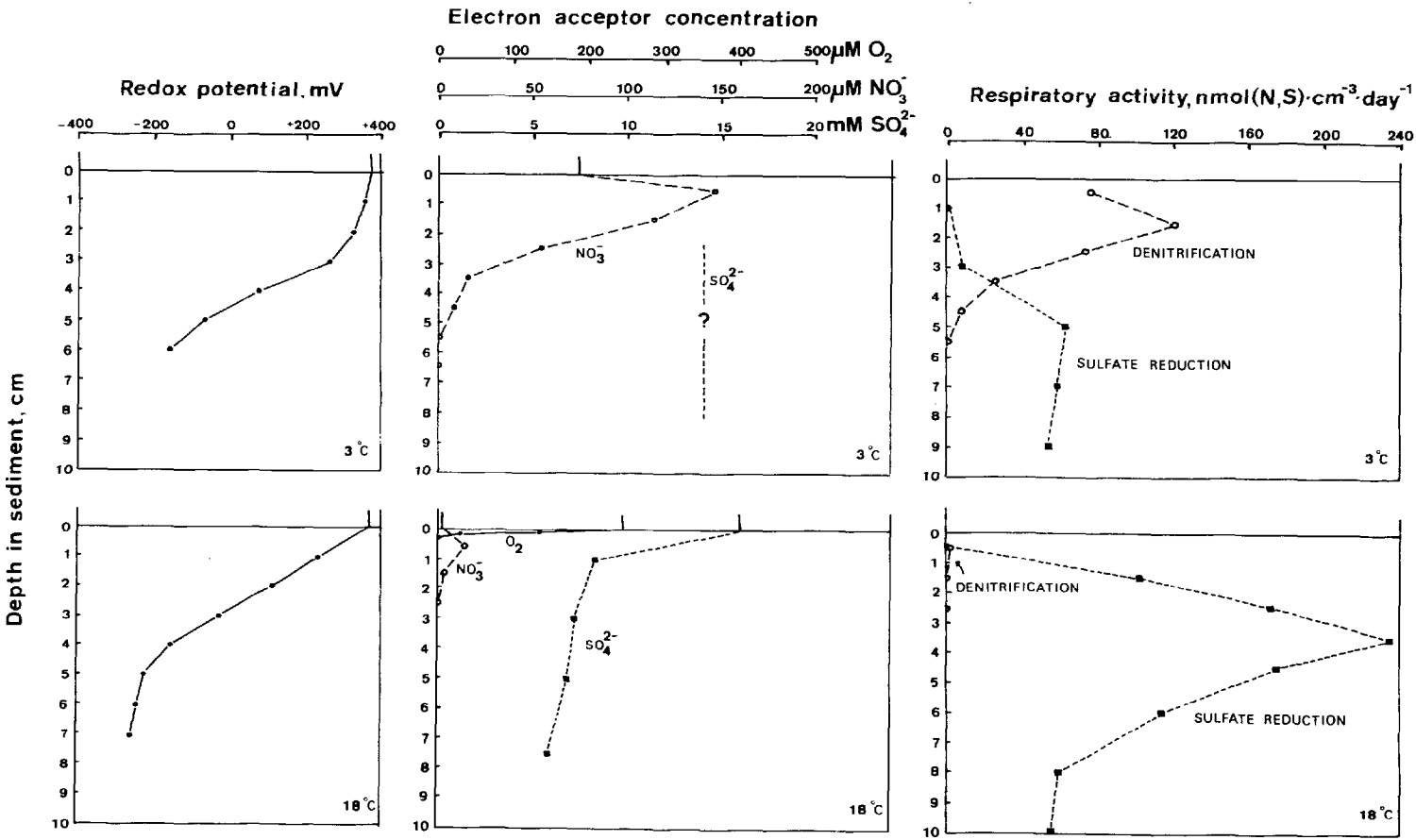


**Fig. 2.** Kysing Fjord. Redox potentials, concentrations of oxygen, nitrate, and sulfate, and activities of denitrification and sulfate reduction during winter (3°C) and summer (18°C). (Oxygen concentrations during winter were not measured.)

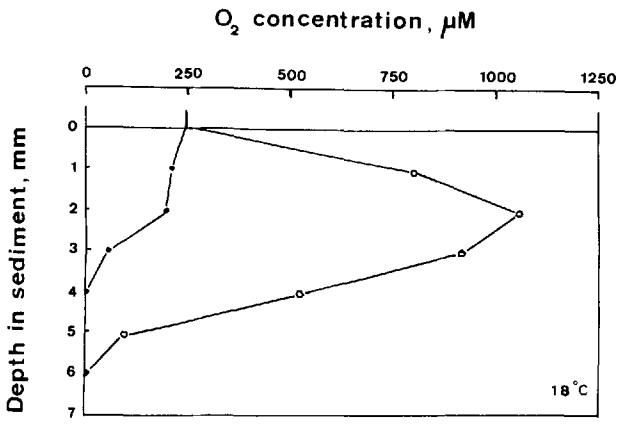


**Fig. 3.** Oxygen concentrations in a sediment core (summer, Randers Fjord) after (○) 2 hours of illumination (700 W/m²) of the sediment surface and after (●) 12 hours in dark.

size of the platinum electrode (a few millimeters) impeded the resolution of any microgradients.

The organic content of the sediments was low, about 1% (dry wt.) throughout the upper 10–15 cm.

*Distribution of Electron Acceptors*

The distribution of oxygen in the sediments showed a distinct change between light and dark. In the dark, the oxygen concentration decreased rapidly with depth to reach zero at 4 mm below the sediment surface (Fig. 3). When illuminated at natural light intensity, an oxygen peak developed at 2 mm depth. This was due to the photosynthetic activity of the benthic flora of diatoms and other microalgae in the uppermost 2–3 mm (8). The light-induced peak of oxygen caused the oxic zone to penetrate only 2 mm deeper into the sediment.

The restriction of oxygen to the uppermost 4–6 mm of the sediment is surprising in view of the general oxidizing conditions (positive redox potentials) down to 3–5 cm depth. A series of 30 depth profiles was measured in cores from the Randers Fjord during summer to show the variability of the oxygen penetration. Most of these showed the same simple distribution as in Figure 3, but frequently secondary peaks appeared deeper in the oxidized zone. Figure 4 shows an example where up to 30% of air saturation was reached at 10 mm depth. Of all the measured profiles, one-fourth showed such secondary peaks with oxygen concentrations reaching from 3% and even up to 90% air saturation. These secondary peaks were most probably associated with faunal burrows. Thus both amphipods (*Corophium* sp.) and polychaetes (*Nereis* sp.), which burrow in small oxygenated tubes in the sediment, were abundant. Due to their activity, oxygen may penetrate deeper into the sediment although in a heterogenous manner and thereby maintain the oxidized conditions in layers too deep to be reached by oxygen diffusion. Many other observations of chemical gradients in marine sediments support such an effect of bioturbation (9).

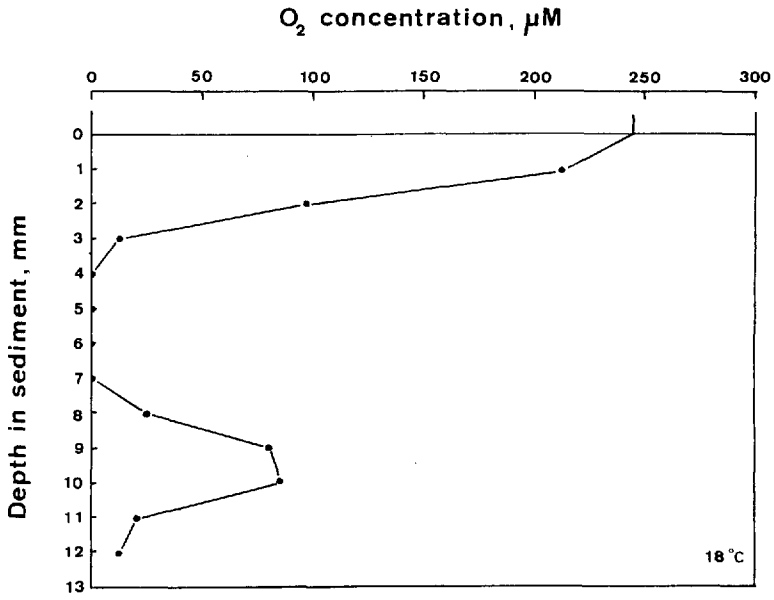


**Fig. 4.** Oxygen profile in the dark of a sediment core (summer, Randers Fjord) showing a secondary oxygen maximum associated with a faunal burrow.

The distribution of oxygen (dark profiles), nitrate, and sulfate in the sediments is shown in the second column of Figures 1 and 2. Whereas the main profile of oxygen was not markedly influenced by season, nitrate was strongly depleted during summer. Other nitrogen oxides were not considered in this context, although accumulations of nitrite, nitric oxide, and nitrous oxide may also be found in these sediments (Sørensen, in press). The peak concentrations of nitrate 1–2 cm below the sediment surface indicated that the activity of nitrification in the oxidized zone was the source of nitrate. There was a net transport of nitrate from the sediment surface, as indicated by the lower nitrate concentrations in the overlying waters. Oxygen for nitrification activity below the main oxygen profile was apparently supplied by the effect of bioturbation.

During winter the sediment from Randers Fjord (Fig. 1) showed high concentrations of sulfate throughout the upper 10 cm, whereas the concentrations decreased towards depth during summer. This was apparently caused by the increasing activity of sulfate reduction during summer, but it was most likely that a recent salinity change in the surface waters may have contributed to the steep sulfate gradient in the uppermost layer of the summer core. Winter measurements of sulfate concentrations in the Kysing Fjord (Fig. 2) are missing, but the profiles were probably not significantly different from those at Randers Fjord at the low temperature. At an intermediate temperature of 11°C during the previous fall, the sulfate concentrations in the Kysing Fjord sediment were 10–12 mM throughout the upper 10 cm (K. L. Brix, unpublished results) and winter concentrations of about 14 mM were anticipated.

**Table 1.** Oxygen uptake and anaerobic respiration on an areal basis (mmoles $O_2$, $NO_3^-$, $SO_4^{2-}$ $m^{-2}$ $day^{-1}$)

| | Randers Fjord | | Kysing Fjord | |
|---|---|---|---|---|
| | Winter | Summer | Winter | Summer |
| Oxygen uptake | 28[a] | 135 | 29[a] | 140 |
| Denitrification | 1.0 | 0.14 | 3.0 | 0.02 |
| Sulfate reduction[b] | 1.9 | 7.8 | 5.8 | 15.1 |

[a] Calculated from the summer values assuming a $Q_{10}$ of 3.2
[b] Upper 15 cm

*Respiratory Activity*

The vertical distribution of the anaerobic respiratory activity by denitrification and sulfate reduction is shown in the third column of Figures 1 and 2. In both sediments, denitrification activity was high during winter when the nitrate concentrations were significant, whereas the activity was at a minimum during summer when the nitrate concentrations were low. The inverse relationship between temperature and denitrification activity indicated that the availability of nitrate was limiting for the process.

Sulfate reduction, on the contrary, was obviously never limited by low sulfate concentrations, and the process was intensified at the high summer temperature. The bulk of the sulfate reduction was localized in the reduced sediment with negative redox potentials, but often the activity was significant also in the deeper part of the oxidized zone where the redox potential was between 0 and +200 mV, apparently associated with reduced microsites or larger, reduced patches in this zone.

Unfortunately, no method was available to determine the vertical distribution of the aerobic respiration in the sediments. Only the data on dark oxygen uptake on an areal basis could be applied to evaluate the importance of aerobic respiration. The results are shown in Table 1, where also the cumulative rates of denitrification and sulfate reduction have been calculated. In both sediments, the dark oxygen uptake and the sulfate reduction were highest during summer whereas the opposite was true for denitrification. The cumulative estimates of sulfate reduction were based only on the measured activities in the upper 15 cm. A previous study which also included sandy coastal sediments (10) showed similar rates of sulfate reduction, and only 20% of the total activity was found in deeper layers below 15 cm.

## Discussion

*Distribution of Respiratory Activity*

It was ascertained that aerobic respiration, denitrification, and sulfate reduction might all proceed concurrently in the lower part of the oxidized surface zone

when oxygen and nitrate were both available. The presence of secondary oxygen maxima in association with faunal burrows indicated that oxygen was supplied by bioturbation to the oxidized layers below the main oxygen profile, but the extent to which aerobic respiration was significant here as compared to denitrification was apparently dependent on the availability of nitrate for the latter process. During winter, when nitrate concentrations were high, the activity of denitrification most probably exceeded the respiratory oxygen consumption in the lower part of the oxidized zone, whereas the opposite apparently was true during summer when the nitrate concentrations were low. It was not clear why the nitrate production was low during summer.

Respiration was exclusively accomplished by sulfate reduction in the deeper, reduced layers of the sediments. Sulfate was still present in high concentrations during summer, and the availability of suitable carbon sources probably limited the activity of this process in the reduced layers of the sediments.

### *Significance of Respiration*

The measurement of dark oxygen uptake did not discriminate between the respiratory consumptions by single groups of organisms, e.g., the faunal element, and the bacteria. Further, the oxygen uptake included also microbial (and chemical) oxidations such as nitrification and sulfide oxidation.

Nitrification activity in the sediments was not measured, but Figures 1 and 2 indicate that nitrification exceeded the nitrate reduction in situ since there was an apparent export of nitrate from the sediment surface by diffusion. However, on the evidence of little increase of the nitrate pool during the incubations of the denitrification assay, it seems unlikely that the nitrate production by nitrification was excessively higher than the nitrate reduction.

In addition to denitrification activity, the coastal marine sediments may also show a significant activity of dissimilatory reduction of nitrate to ammonia (12,-18). Any significance of this process in situ would also imply that nitrification must exceed denitrification. The study of Koike and Hattori (12) indicated, however, that this dissimilatory nitrate reduction may be inferior to the activity of denitrification in sandy coastal sediments with a low organic content.

For a rough estimate of the oxygen consumption by ammonia oxidation, it may be assumed that nitrification and denitrification balanced each other in the sediments under study. Thus a nitrification activity during summer of 0.02 mmoles N $m^{-2}$ $day^{-1}$ in the Kysing Fjord (Table 1) would require 0.04 mmoles $O_2$ $m^{-2}$ $day^{-1}$, according to

$$NH_3 + 2\ O_2 \rightarrow HNO_3 + H_2O \qquad (1)$$

Likewise, the oxidation of sulfide, which was produced at a rate of 15.1 mmoles S $m^{-2}$ $day^{-1}$, would require 30.2 mmoles $O_2$ $m^{-2}$ $day^{-1}$, according to

$$H_2S + 2\ O_2 \rightarrow H_2SO_4 \qquad (2)$$

The accumulation of sulfide in the sediments as pyrite was neglected since this fraction was only 10% of the sulfide production in similar sandy sediments from the Limfjorden (Jørgensen, unpublished results).

The calculation shows that out of the oxygen uptake of 140 mmoles $O_2$ $m^{-2}$

day$^{-1}$, about 20% was used for sulfide oxidation and a little for ammonia oxidation. The rest was used for aerobic respiration of organic substrates and possibly for oxidations of methane and metals, e.g., ferrous iron. The significance of the latter oxidations could not be ascertained, and thus the remaining approximately 80% of the oxygen uptake, or 110 mmoles $O_2$ m$^{-2}$ day$^{-1}$, may serve only as a rough estimate of the aerobic respiration of organic substrates.

Each mole of oxygen, nitrate, and sulfate oxidizes 1, 1.25, and 2 moles of organic carbon, according to

$$(CH_2O) + O_2 \rightarrow CO_2 + H_2O \quad (3)$$

$$5\,(CH_2O) + 4\,HNO_3 \rightarrow 5\,CO_2 + 7\,H_2O + 2\,N_2 \quad (4)$$

$$2\,(CH_2O) + 7H_2SO_4 \rightarrow 2\,CO_2 + 2\,H_2O + H_2S \quad (5)$$

In the example referred to above, the relative contributions by aerobic respiration, denitrification, and sulfate reduction would be 110 (at maximum), 0.05, and 30.2 mmoles C m$^{-2}$ day$^{-1}$, respectively, if it is assumed that the respiratory processes take place at the expense of organic substrates of carbohydrate composition. This calculation indicates that denitrification is of little importance for the mineralization of organic matter during summer in these coastal sediments.

During winter, the relative importance of the respiratory processes changes significantly. Unfortunately, dark oxygen uptake was not measured during winter, but if the $Q_{10}$ of 3.2 for oxygen uptake found in the sediments from the Limfjorden (10) is applied, then the figures of dark oxygen uptake during winter would be about 20% of the summer values (Table 1). Thus sulfate reduction would account for an increased fraction of the total respiration during winter and the relative importance of denitrification would also be increased. According to Table 1 and Equations 4 and 5, the oxidation of organic carbon by denitrification was one-third of the carbon oxidation by sulfate reduction during winter.

Although denitrification is of minor importance for the mineralization of organic matter, the process may play an important role as a reductive pathway in the sedimentary nitrogen transformations. As examplified by the winter cores, the peak activities of denitrification and sulfate reduction were almost similar, but a much faster turnover of the nitrate pool as compared to the sulfate pool was apparent from the pool sizes. This liability of the nitrate pool illustrates the dynamic character of that part of the sedimentary nitrogen cycle where the processes of nitrification and denitrification are involved.

*Acknowledgments.* The skillful assistance of Preben G. Sørensen is gratefully acknowledged. The first author was supported by Grant 521/11 from the Danish Natural Science Research Council.

## References

1. American Public Health Association: Standard Methods for the Examination of Water and Wastewater, 13th ed., Washington, D.C. (1971)
2. Balderston, W. L., B. Sherr, and W. J. Payne: Blockage by acetylene of nitrous oxide reduction in *Pseudomonas perfectomarinus*. Appl. Environ. Microbiol. **31**, 504–508 (1976)
3. Baumgärtl, H., and D. W. Lübbers: Platinum needle electrode for polarographic measurements of oxygen and hydrogen. In M. Kessler, D. F. Bruley, L. C. Clark, Jr., D. W. Lübbers, I. A.

Silver, and J. Strauss (eds.): Oxygen Supply, pp. 130–136. Urban and Schwarzenberg, Munich (1973)
4. Bender, M. L., K. A. Fanning, P. N. Froelich, and V. Maynard: Interstitial nitrate profiles and oxidation of sedimentary organic matter in the eastern equatorial Atlantic. Science **198,** 605–609 (1977)
5. Edberg, N., and B. Hofsten: Oxygen uptake of bottom sediments studied in situ and in the laboratory. Water Res. **7,** 1285–1294 (1973)
6. Fenchel, T.: The ecology of marine microbenthos.4. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities with special reference to the ciliated protozoa. Ophelia **6,** 1–182 (1969)
7. Fenchel, T., and B. B. Jørgensen: Detritus food chains of aquatic ecosystems: the role of bacteria. In M. Alexander (ed.): Advances of Microbial Ecology, vol. 1, pp. 1–58. Plenum Press, New York (1977)
8. Fenchel, T., and B. J. Straarup: Vertical distribution of photosynthetic pigments and the penetration of light in marine sediments. Oikos **22,** 172–182 (1971)
9. Goldhaber, M. B., R. C. Aller, J. K. Cochran, J. K. Rosenfeld, C. S. Martens, and R. A. Berner: Sulfate reduction, diffusion, and bioturbation in Long Island Sound sediments: report of the FOAM group. Am. J. Sci. **277,** 193–237 (1977)
10. Jørgensen, B. B.: The sulphur cycle of a coastal marine sediment (Limfjorden, Denmark). Limnol. Oceanogr. **22,** 814–832 (1977)
11. Jørgensen, B. B.: A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. I. Measurements with radiotracer techniques. Geomicrobiol. J. **1,** 11–28 (1979)
12. Koike, I., and A. Hattori: Denitrification and ammonia formation in anaerobic coastal sediments. Appl. Environ. Microbiol. **35,** 278–282 (1978)
13. Muus, B. J.: The fauna of Danish estuaries and lagoons. Distribution and Ecology of Dominating Species in the Shallow Reaches of the Mesohaline Zone. Høst and Søn, Copenhagen (1967)
14. Pamatmat, M. M.: Oxygen consumption by the seabed.4. Shipboard and laboratory measurements. Limnol. Oceanogr. **16,** 536–550 (1971)
15. Richards, F. A., and W. W. Broenkow: Chemical changes, including nitrate reduction in Darwin Bay, Galapagos, over a two-month period, 1969. Limnol. Oceanogr. **16,** 758–765 (1971)
16. Riley, R. P., and G. Skirrow: Chemical Oceanography, vol. 1, 2nd ed. Academic Press, London (1975)
17. Strickland, J. D. H., and T. R. Parsons: A practical handbook of sea water analysis. Fish. Res. Bd. Can., Bull. 167, Ottawa (1972)
18. Sørensen, J.: Capacity for denitrification and reduction of nitrate to ammonia in a coastal marine sediment. Appl. Environ. Microbiol. **35,** 301–305 (1978)
19. Sørensen, J.: Denitrification rates in a marine sediment as measured by the acetylene inhibition technique. Appl. Environ. Microbiol. **36,** 139–143 (1978)
20. Teal, T. M., and J. Kanwisher: Gas exchange in a Georgia salt marsh. Limnol. Oceanogr. **6,** 388–399 (1961)
21. Yoshinari, T., and R. Knowles: Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochem. Biophys. Res. Commun. **69,** 705–710 (1976)