Journal of Industrial Microbiology & Biotechnology (1999) 22, 439–448
© 1999 Society for Industrial Microbiology 1367-5435/99/$12.00
http://www.stockton-press.co.uk/jim

Invitational ONR Lecture

Mud, Microbes, and Mineralization

TOM M. FENCHEL

Department of Ecology and Genetics, University of Aarhus, DK-8000 Aarhus C, Denmark

A number of studies on the quantitative importance of different microbial processes in Danish off-shore and estuarine sediments are summarized. In off-shore sediments, about 50% of the mineralization is catalyzed through sulfate reduction, whereas denitrification only accounts for 1–5% of the total respiratory activity. The vertical distribution of the different microbial processes leading to the mineralization of organic matter can be explained as the result of competition for electron acceptors and by the energetic yield of the different kinds of respiration processes. The relative quantitative importance of the different processes is explained by the availability of the different electron acceptors. The influence of light, season, and spatial heterogeneity on the chemical and microbial zonation patterns in the sediment is demonstrated. Finally, the effect of sedimentary microbial processes, in particular denitrification and the reduction and oxidation of sulfur compounds, is discussed in terms of global element cycling.

INTRODUCTION

Knowledge on the diversity of prokaryote metabolic processes has steadily increased during the last century. A quantitative understanding of the role of different microbial processes in the biosphere and the interactions between different processes has been achieved only much more recently, and this knowledge is still far from being complete. The reason for the recent advances within this field is first of all the development of suitable techniques, in particular, the use of isotopes for the study of element cycling.

During the last decades, it has become clear that microbial activity has a significant influence on the qualitative and quantitative composition of the atmosphere and hydrosphere (Broda 1975; Cloud 1976); it was probably first clearly pointed out by Hutchinson (1954). One example is the maintenance of dinitrogen in the atmosphere. As pointed out by Sillén (1966), the process

$$N_2 + 5/2\ O_2 + H_2O \leftrightharpoons 2\ HNO_3$$

is exergonic. In spite of the high activation energy of the N_2 molecule, its oxidation takes place slowly, directly in the atmosphere during electrical discharges and indirectly through biological and industrial nitrogen fixation followed by the microbial oxidation of NH_4^+ to NO_3^-. The only known pathway by which N_2 is returned to the atmosphere is microbial denitrification. In numerous other ways, microbial processes control the environmental chemistry; the availability of plant nutrients, mineral deposition and dissolution, and many other important processes. The quantification of microbial processes in nature is, therefore, a central theme for microbial ecology.

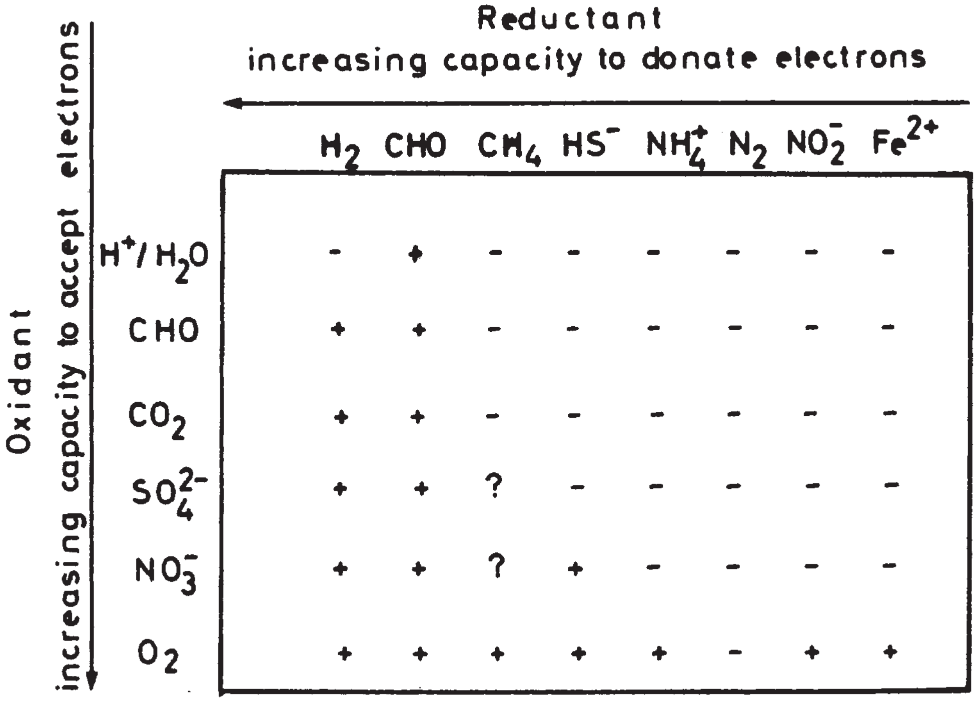
This presentation reviews some aspects of several studies on microbially catalyzed processes in the sediments of the inner Danish waters. Emphasis is on the general and fundamental properties of the prokaryote communities in sediments and the pathways by which organic material is mineralized.

DISCUSSION

Mineralization in Mud: Competition for Electron Acceptors

During some period in Precambrian times, prokaryotes were responsible for all bioenergetic processes. In the extant biosphere, eukaryote photosynthesis is by far the most important process by which organic material is produced (Bolin 1970). However, in most ecosystems only a fraction of this primary production is utilized by herbivorous animals (Fenchel and Blackburn 1979: Fenchel and Jørgensen 1977). The larger part, and in particular the tissue of higher plants, is deposited as dead organic material (detritus and litter) which is mineralized by microorganisms, in terrestrial environments by fungi and bacteria, and in aquatic environments predominantly by bacteria. The microbial bioenergetic processes are, therefore, driven by the potential chemical energy of the reduced carbon of the dead organic material.

In order to achieve an understanding of the relative importance of the different mineralization processes, it is fruitful to start with a general discussion on bacterial energy metabolism. Energy metabolism may be phototrophic or chemotrophic. Chemotrophic (dissimilatory) energy metabolism involves redox processes. Figure 1 shows oxidants (substrates) and reductants (terminal electron acceptors) involved in different



	H_2	CHO	CH_4	HS^-	NH_4^+	N_2	NO_2^-	Fe^{2+}
H^+/H_2O	-	+	-	-	-	-	-	-
CHO	+	+	-	-	-	-	-	-
CO_2	+	+	-	-	-	-	-	-
SO_4^{2-}	+	+	?	-	-	-	-	-
NO_3^-	+	+	?	+	-	-	-	-
O_2	+	+	+	+	+	-	+	+

FIG. 1. Potential and realized chemotrophic redox reactions sustaining bacterial growth. The free energy yield of the different processes increase toward left and downward. The anaerobic oxidations of CH_4 by NO_3^- and by $SO_4^=$ are controversial.

bioenergetical processes. In terms of free energy, an electron acceptor as O_2 is favorable compared to NO_3^- which again is more favorable than $SO_4^=$ and CO_2. Since O_2, NO_3^-, and $SO_4^=$ in a sediment are transported to the deeper layers through diffusion from the oxic or photic surface layers, the three electron acceptors are depleted from the surface in the mentioned order. Going downward in a sediment, one would therefore expect to pass, in succession, zones of oxygen respiration, nitrate respiration, sulfate respiration, and finally carbon dioxide respiration (methanogenesis). Since sulfate reducers and methanogenic bacteria depend on H_2 or low molecular fatty acids such as acetate or lactate (Fenchel and Blackburn 1979; Widdel and Pfennig 1977), fermenting bacteria are found to initiate the mineralization beneath the oxic zone.

Some of the above predictions are demonstrated in Fig. 2, which shows the chemical gradients and respiration processes in an estuarine sediment. Methanogenesis is detectable in these sediments (Blackburn, unpubl. observations) but may not be of quantitative importance since sulfate first reaches limiting concn at a considerable depth in the sediment where only little, mainly refractory, organic material remains. Methane does not build up in interstitial water in the presence of sulfate; this is possibly due to

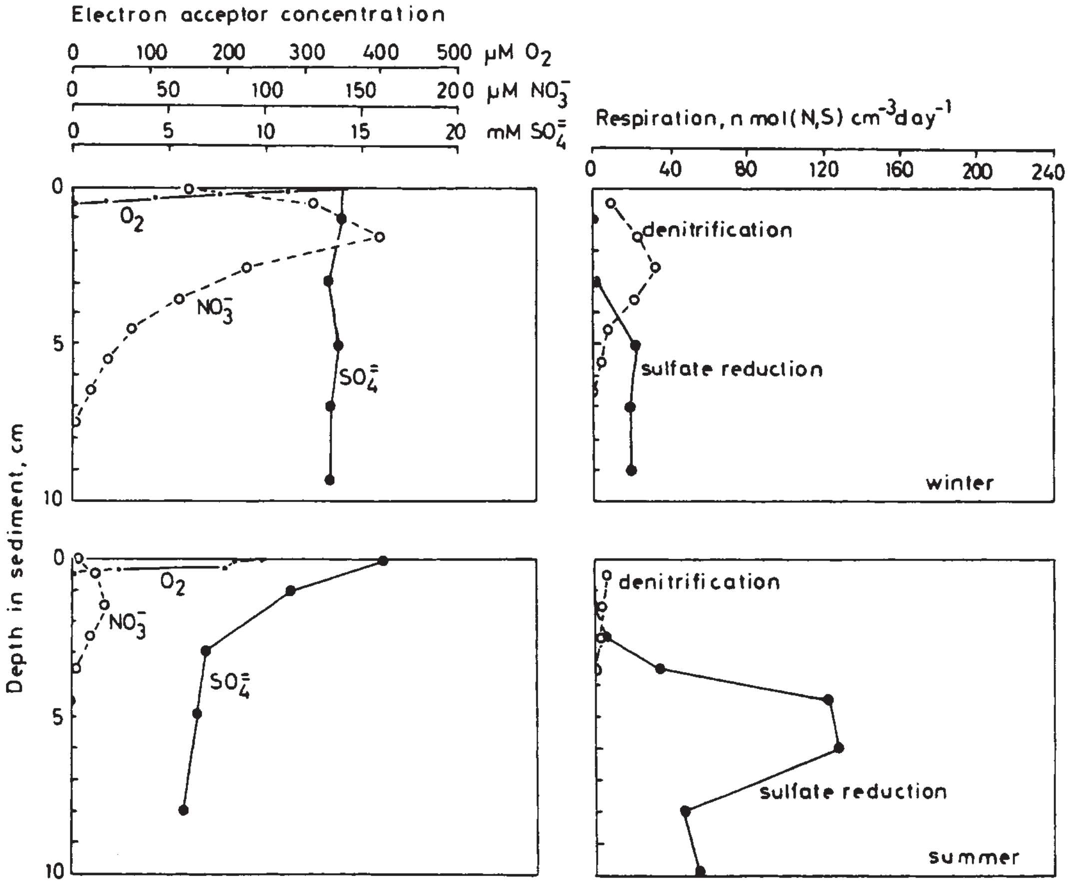


FIG. 2. The vertical distribution of electron acceptors and of nitrate and sulfate respiration in a Danish estuarine sediment during winter and during summer. Data from Sørensen et al. (1979).

anaerobic methane oxidation by sulfate (Cappenberg and Prins 1974; Martens and Berner 1974).

The above mentioned processes lead to the production of low molecular reducing compounds. The end product of dinitrification is in principle N_2, although some N_2O is also formed, and some fermenting bacteria may mediate the hydrogenation of nitrate to form NH_4^+ (Sørensen 1978a,c). Ammonia is also an end product of the mineralization of amino acids. Sulfate reduction leads to the formation of HS^- and CO_2 reduction, to CH_4. These compounds move upward in the sediment along diffusion gradients and are successively oxidized as they reach the oxic zone. Sulfide may also become oxidized by bacteria with nitrate as oxidant, and it is likely that methane may be oxidized by sulfate and perhaps nitrate. In the surface of shallow water sediments, the oxidation of reduced sulfur compounds also may take place through bacterial photosynthesis.

As a result of these processes, more or less closed element cycles are found in the sediment. The dissolved compounds participating in the cycles move vertically along diffusion gradients, and the reduced forms of the principle elements involved—C, N, and S—act as energy carriers from the lower, chemically reduced part of the sediment to the oxidized surface. The cycling of sulfur and of nitrogen will be considered separately.

The Sulfur Cycle

A schematic and somewhat simplified presentation of the sedimentary sulfur cycle is shown in Fig. 3, left. Among several methods employed to estimate transfer rates, tracer methods with ^{35}S-labeled compounds have yielded the most reliable figures (Jørgensen 1977a, 1978; Jørgensen and Fenchel 1974). Figure 3, right, shows a quantitative description of the sulfur cycle in an off-shore sediment. It is seen that the amount of sulfide contributed from the fermentation of sulfur-containing amino acids is very small compared to the amount of sulfide produced through dissimilatory sulfate reduction. The sulfur cycle is nearly in a steady state; interstitial sulfate has a turnover time of

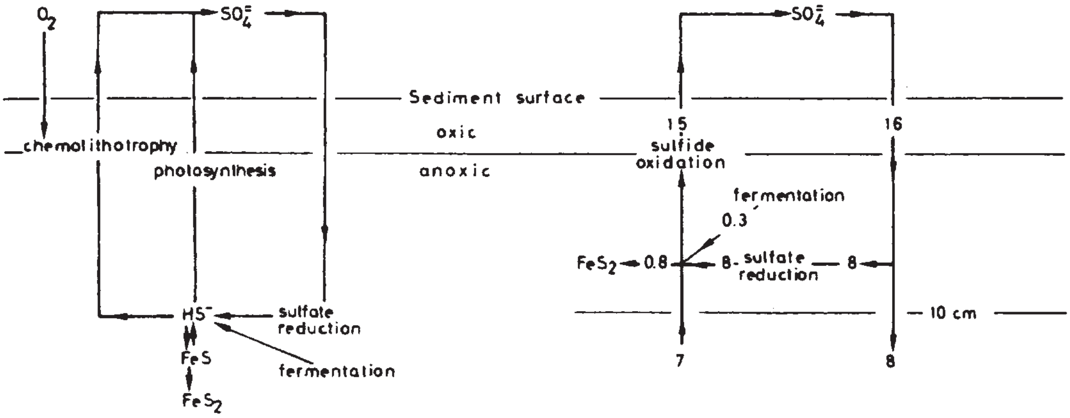


FIG. 3. A schematic representation of the microbial sulfur cycle in sediments. Some intermediate oxidation steps of sulfur, notably S^o, are omitted for simplicity. To the right, the transfer rates of sulfur (annual averages given as mmol S m^{-2} day^{-1}) are shown for the upper 10 cm of the sediment (Limfjorden, Denmark). Data from Jørgensen (1977a).

4–5 mo in the upper 10 cm of the sediment while sulfide is turned over every 1–5 days. Some 7% of the reduced sulfur is deposited as pyrite; this may be considered as a fossilization of potential energy which, at least over a shorter time span, is lost to the system.

An appreciation of the quantitative importance of sulfate reduction can be obtained by comparisons with the total oxygen uptake of the sediment. Typically, the uptake of oxidation equivalents of oxygen is about twice that of sulfate. Since only a small fraction of the formed sulfide is accumulated (as pyrite), the remaining must be oxidized. This means, again, that about half the oxygen uptake of a sediment represents the reoxidation of sulfide which may be considered a kind of "oxygen debt" of the sediment. The other half of the oxygen uptake then represents all other types of aerobic metabolism by bacteria and animals. From this we can conclude that about half of the mineralization of organic material is catalyzed by sulfate reduction. The importance of sulfate respiration in marine sediments is due to the high availability of sulfate in seawater. In oxygen-saturated seawater, sulfate represents about 200 times more oxidation equivalents than does oxygen.

The reoxidation of sulfide is complex. It may occur spontaneously in the presence of oxygen, the end products being mainly thiosulfate and sulfate. Sulfide (and S^0, and $S_2O_3^=$) oxidation also may be mediated by two types of bacterial processes. In the presence of oxygen, chemolithotrophic sulfur bacteria oxidize reduced sulfur compounds with elemental sulfur or sulfate as end products. In the presence of light, but generally under anaerobic conditions, purple and green sulfur bacteria and some types of cyanobacteria utilize reduced sulfur compounds as electron donors in a photosynthetic process with elemental sulfur or sulfate as end products (Fenchel and Blackburn 1979; Cohen et al 1975).

The oxidation of reduced sulfur is studied most easily in lakes with a chemocline and where the hypolimnion is anoxic and sulfide-containing. In such systems, it can be demonstrated that sulfide and oxygen coexist in a dynamic steady state in a layer along the chemocline, maybe 10–20 cm thick. Jørgensen et al. (1979a) studied the chemocline in the hyperhaline "Solar Lake" in the Sinai Desert in detail. They demonstrated peaks of thiosulfate as well as of elemental sulfur. The thiosulfate probably originates from abiotic oxidation of sulfide while the elemental sulfur probably derives from photosynthetic sulfide oxidation below the oxic zone. Only 5–10 cm above the chemocline these substances disappear due to chemolithotrophic oxidation. In this lake, the whole chemocline shows a diurnal vertical migration pattern due to photosynthetic sulfide oxidation in the light. Thus, the anoxic boundary layer is submerged some 10 cm during late afternoon as compared to the morning.

In sediments, similar vertical zonation patterns also are found, although they are much more difficult to study since steep gradients occur over distances of a few millimeters. In shallow water sediments where the anoxic zone is close to the sediment surface, diurnal vertical migrations of the chemical gradients of the microbial processes and of the microorganisms themselves can be demonstrated (Blackburn et al. 1975; Jørgensen et al. 1979b; see also Fig. 5). In accordance with the penetration of light in sediments (Fenchel and Straarup 1971), the zone affected by these vertical migrations measures < 5 mm. In an organic sediment, Jørgensen et al. (1979b) found about 0.5 mM oxygen in the interstitial water during the day; during the night, the same horizon was completely anoxic and contained > 2 mM sulfide.

In most sediments, however, the idealized vertical zonation patterns described above are complicated by spatial heterogeneity. Jørgensen (1977b) demonstrated that microbial sulfate reduction and sulfide oxidation may occur at the same depth in a sediment and ascribed this to the presence of reduced microniches. This term refers to detrital particles or invertebrate fecal pellets which sustain interior anaerobic conditions in oxic environments. Reasonable assumptions with respect to diffusion coefficients of oxygen and with respect to the level of microbial activity inside such particles show that the minimum size of particles which can sustain an interior anoxic environment in oxygen-containing water is 0.2–2 mm. Similar complications of the zonation patterns arise from burrows of worms and crustaceans which extend the oxidized zone down into the sediment. Therefore, one may expect and does find that opposite or competitive processes occur at the same depth in a sediment: nitrification and denitrification, sulfate reduction and sulfide oxidation, and sulfate reduction and methanogenesis.

The Nitrogen Cycle

The microbial nitrogen cycle is simpler than the sulfur cycle in the sense that no photosynthetic organism which uses NH_4^+ as an electron donor seems to exist. In other respects, the nitrogen cycle is more difficult to study. From a methodological point of view, this is due to the absence of an easily available radioactive isotope of nitrogen. Since several of the key compounds of the nitrogen cycle (such as NO_3^-) occur at very low concn, but with a high turnover rate in nature, it means that real tracer experiments with compounds labeled with the stable isotope ^{15}N are very difficult or even impossible. The study of the microbial nitrogen cycle is also complicated by the fact that the assimilatory metabolism of nitrogen is quantitatively important. This is in contrast to the sulfur cycle in which, for example, the assimilatory reduction of sulfate plays a quantitatively modest role as compared to dissimilatory sulfate reduction, and may be ignored in many contexts.

A schematic presentation of the sedimentary nitrogen cycle is shown in Fig. 4. The key process is the production of ammonia from decomposing amino acids. The total production, the concurrent bacterial assimilation of NH_4^+, and thus the net production

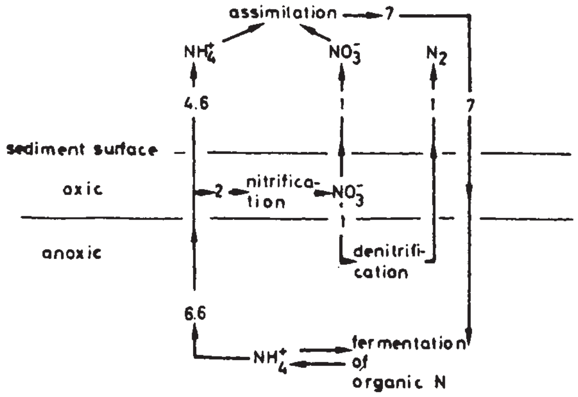


FIG. 4. The microbial nitrogen cycle in an off-shore sediment (Limfjorden, Denmark). Transfer rates are given as mmol N m^{-2} day^{-1} and represent annual averages. Data compiled from Blackburn (1979b) and from Blackburn and Henriksen (unpubl. data).

of ammonia can be quantified by a $^{15}N-NH_4^+$ dilution technique (Blackburn 1979a). The excess ammonia, which diffuses upward in the sediment, is then available for nitrification. Considerations based on nitrate profiles in the sediment and on quantifications of *in situ* denitrification rates (Oren and Blackburn 1979; Sørensen 1978b; Sørensen et al. 1979) show that only a part of the ammonia is nitrified. The remaining part of the ammonia enters the water column above the sediment or is assimilated by microalgae or bacteria at the sediment surface (Henriksen and Blackburn, unpubl. data). The fraction of ammonia which is nitrified increases with the increasing thickness of the oxidized surface layer. During winter, when the oxidized layer is relatively thick, most of the reactive nitrogen which enters the water from the sediment is in the form of nitrate, whereas the opposite is the case during summer. The activity of burrowing invertebrates also enhances nitrification (Henriksen, unpubl. observations).

Comparison between Figs. 3 and 4 shows that compared to sulfate, nitrate is relatively unimportant as an electron acceptor and denitrification constitutes only some 2% of the total respiratory activity in the studied sediment. The main importance of denitrification is that it leads to a loss of reactive nitrogen since its most important end product is N_2. This loss, and also some fossilization of organic nitrogen in the sediment, is compensated for by nitrogen fixation and by the nitrate contained in the freshwater run-off from land.

Sediments and Atmospheric Sulfur

Sulfur in the atmosphere has recently drawn attention since the combustion of fossil fuels has proven to be an environmental nuisance on a local scale, due to the acidification of rainwater. An evaluation of a potential global effect requires a quantitative understanding of the natural sources of atmospheric sulfur. In the atmosphere, sulfur shows a spatial and temporal heterogenous distribution. It occurs as $SO_4^=$ (in aerosols), as SO_2, in trace amounts as organic compounds (dimethyl sulfide, mercaptans), and as H_2S. Natural sources are seawater spray (which may be only of local importance), volcanic emission, and volatile organic sulfur compounds derived from terrestrial environments (moist soils, swamps, decaying plant material). The isotope composition of atmospheric sulfur suggests that biogenic origin is important (Margulis and Bandy 1977; Svensson and Söderlund 1976). The last potential source of atmospheric sulfur, with which we will be concerned here, is the microbial sulfate reduction in marine sediments.

Enormous quantities of sulfate are reduced annually to sulfide in marine sediments. By far the most of this sulfide is reoxidized or stored in the sediment. However, even if a very small fraction of the sulfide could escape to the atmosphere as H_2S, this could mean a very substantial contribution to atmospheric sulfur. Since dissolved sulfide ($H_2S + HS^- + S^=$) has a very short half-life in oxygenated water (Chen and Morris 1972; Jørgensen et al 1979a), sulfide is unlikely to escape from sediments to the atmosphere if the sediments are covered by only about 0.5 m of oxygenated water. However, the escape of sulfide from the sediments of extensive estuaries, salt marshes, and tidal mud flats characteristic of many shore lines remains a possibility.

By the continuous passing of the air contained in inverted plexiglass boxes placed on exposed sediments through H_2S-traps, it has been possible to quantify the release of H_2S from such sediments (Hansen et al. 1978). Figure 5 shows an example from a locality (a partly exposed sediment with a high content of decaying sea grass) with an

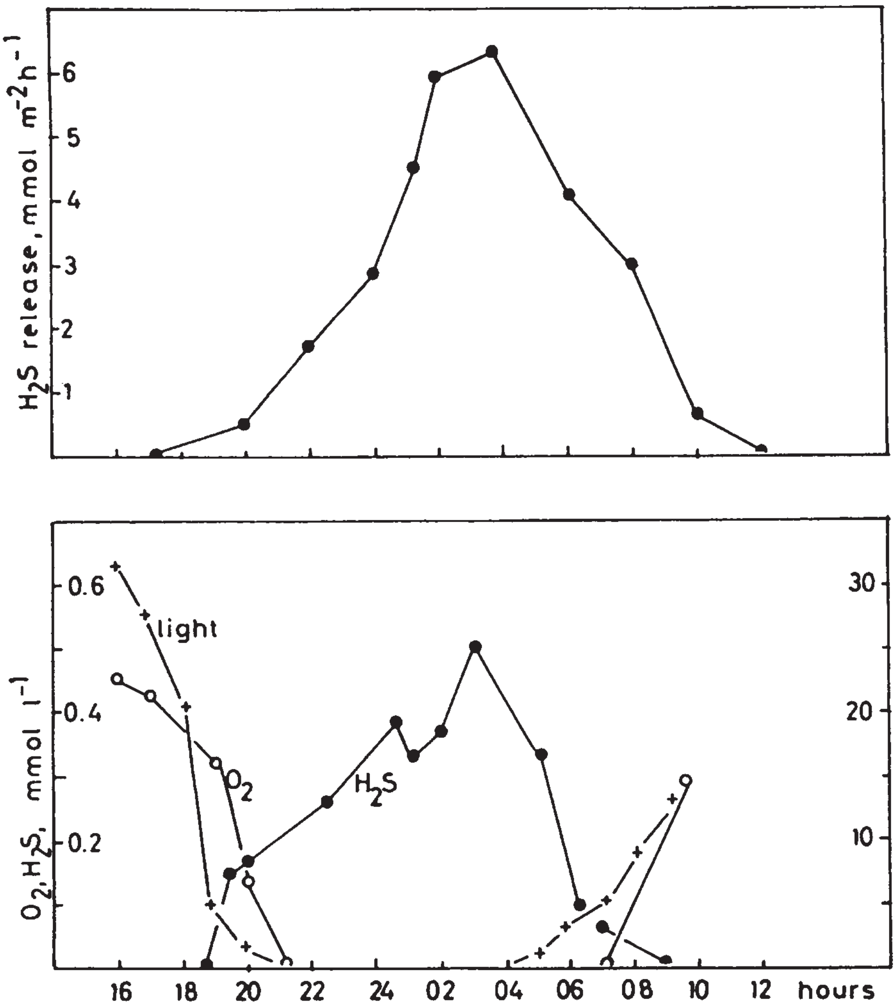


FIG. 5. Release of H_2S to the atmosphere, light conditions, and the contents of H_2S and O_2 immediately above sediment during a summer day. Data from Hansen et al. (1978).

unusually high emission rate of H_2S. The figure clearly shows that all H_2S is emitted during the night. During the day, photosynthetic sulfur bacteria constitute an efficient sulfide filter and, therefore, control the emission of H_2S to the atmosphere.

Experiments such as the one shown in Fig. 5 indicate that microbially generated sulfide in shallow water sediments may indeed constitute an important source of atmospheric sulfur. It is, however, still not possible to evaluate these results in terms of the global sulfur cycle. There are two reasons for this. The intensity of H_2S emission has proven to be patchily distributed even within a small area and it will be a hard task to integrate the measurements over a larger geographical scale with any reasonable degree of accuracy. Another serious difficulty is that the fate of the H_2S in the air immediately over the estuarine sediments is difficult to predict. Most of the H_2S will autooxidize rapidly in the air and much of it may be deposited as sulfate in raindrops quite locally rather than become a constituent of the atmosphere for any length of time.

However, at this point the problem is no longer that of the microbial ecologist but has become one of atmospheric science.

LITERATURE CITED

Blackburn, T. H. 1979a. Method for measuring rates of NH_4^+ turnover in anoxic marine sediments, using a $^{15}N-NH_4^+$ dilution technique. *Appl. Environ. Microbiol.* 37:760–765.

———. 1979b. Seasonal variations in the rate of organic–N mineralization in anoxic marine sediments. Colloque International C.N.R.S. Biogeochimie de la Matiere Organique a l'interface Eau-Sediment Marin. Marseille 25–27 April, 1979.

Blackburn, T. H., P. Kleiber, and T. Fenchel. 1975. Photosynthetic sulfide oxidation in marine sediments. *Oikos* 26:103–108.

Bolin, B. 1970. The carbon cycle. *Sci. Am.* 223 (3):124–132.

Broda, E. 1975. *The Evolution of the Bioenergetic Process*. Pergamon Press, Oxford, 211 p.

Cappenberg, T. E., and R. A. Prins. 1974. Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a freshwater lake. III. Experiments with ^{14}C–labelled substrates. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 40:457–469.

Chen, K-Y., and J. C. Morris. 1972. Kinetics of oxidation of aqueous sulfide by O_2. *Environ. Sci. Technol.* 6:529–537.

Cloud, P. 1976. Beginnings of biospheric evolution and their biochemical consequences. *Palaeobiology* 2:351–387.

Cohen, Y., B. B. Jørgensen, E. Padan, and M. Shilo. 1975. Sulfide-dependent anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. *Nature* 257:489–492.

Fenchel, T., and T. H. Blackburn. 1979. *Bacteria and Mineral Cycling*. Academic Press, London, 211 p.

Fenchel, T., and B. B. Jørgensen. 1977. Detritus food chains of aquatic ecosystems: the role of bacteria. *Adv. Microb. Ecol.* 1:1–57.

Fenchel, T., and B. J. Straarup. 1971. Vertical distribution of photosynthetic pigments and the penetration of light in marine sediments. *Oikos* 22:172–182.

Hansen, M. H., K. Ingvorsen, and B. B. Jørgensen. 1978. Mechanisms of hydrogen sulfide release from coastal marine sediments to the atmosphere. *Limnol. Oceanogr.* 23:68–76.

Hutchinson, G. E. 1954. The biochemistry of the terrestrial atmosphere. Pages 371–433 *in* G. Kuiper, ed. *The Solar System*. Vol. 2. The University of Chicago Press, Chicago.

Jørgensen, B. B. 1977a. The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark). *Limnol. Oceanogr.* 22:814–832.

———. 1977b. Bacterial sulfate reduction within reduced microniches of oxidized marine sediments. *Mar. Biol.* 41:7–17.

Jørgensen, B. B., and T. Fenchel. 1974. The sulfur cycle of a marine sediment model system. *Mar. Biol.* 24:189–201.

Jørgensen, B. B., J. G. Kuenen, and Y. Cohen. 1979a. Microbial transformations of sulfur compounds in a stratified lake (Solar Lake, Sinai). *Limnol. Oceanogr.* 24:799–822.

Jørgensen, B. B., N. P. Revsbech, T. H. Blackburn, and Y. Cohen. 1979b. Diurnal cycle of oxygen and sulfide microgradients and microbial photosynthesis in an organic sediment. *Appl. Environ. Microbiol.* 38:46–58.

Margulis, P. J., and A. R. Bandy. 1977. Estimate of the contribution of biologically produced dimethyl sulfide to the global sulfur cycle. *Science* 196:647–648.

Martens, C. S., and R. A. Berner. 1974. Methane production in the interstitial waters of sulfate-depleted marine sediments. *Science* 185:1167–1169.

Oren, A., and T. H. Blackburn. 1979. Estimation of sediment denitrification rates at in situ nitrate concentrations. *Appl. Environ. Microbiol.* 37:174–176.

Sillén, L. G. 1966. Regulation of O_2, N_2 and CO_2 in the atmosphere: thoughts of a laboratory chemist. *Tellus* 18:198–206.

Sørensen, J. 1978a. Capacity for denitrification and reduction of nitrate to ammonia in a coastal marine sediment. *Appl. Environ. Microbiol.* 35:301–305.

———. 1978b. Denitrification rates in a marine sediment as measured by the acetylene inhibition technique. *Appl. Environ. Microbiol.* 36:139–143.

———. 1978c. Occurrence of nitric and nitrous oxides in a coastal marine sediment. *Appl. Environ. Microbiol.* 36:809–813.

Sørensen, J., B. B. Jørgensen, and N. P. Revsbech. 1979. A comparison of oxygen, nitrate, and sulfate respiration in coastal marine sediments. *Microb. Ecol.* 5:105–115.

Svensson, B. H., and R. Söderlund, eds. 1976. Nitrogen, phosphorus and sulfur—global cycles. *Ecol. Bull.* 22. Swedish Natural Science Council, Stockholm. 192p.

Widdel, F., and N. Pfennig. 1977. A new anaerobic, sporing, acetate oxidizing, sulfate reducing bacterium, *Desulfotomaculum* (emend.) *acetoxidans*. *Arch. Microbiol.* 112:119–122.