Marine Geology, 113 (1993) 3–25
Elsevier Science Publishers B.V., Amsterdam

Microbial mats: A joint venture

Hans van Gemerden
Department of Microbiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

(Revision accepted April 28, 1993)

ABSTRACT

Van Gemerden, H., 1993. Microbial mats: A joint venture. In: R.J. Parkes, P. Westbroek and J.W. de Leeuw (Editors), Marine Sediments, Burial, Pore Water Chemistry, Microbiology and Diagenesis. *Mar. Geol.*, 113: 3–25.

Microbial mats characteristically are dominated by a few functional groups of microbes: cyanobacteria, colorless sulfur bacteria, purple sulfur bacteria, and sulfate-reducing bacteria. Their combined metabolic activities result in steep environmental microgradients, particularly of oxygen and sulfide.

The driving force of most microbial mats is photosynthesis by cyanobacteria and algae. Subsequently, sulfate-reducing bacteria, using excretion-, lysis-, and decomposition products of cyanobacteria, produce sulfide by the dissimilatory reduction of sulfate. The sulfide can be reoxidized to sulfate by colorless and purple sulfur bacteria.

Colorless sulfur bacteria are chemotrophic organisms primarily oxidizing sulfide and other reduced forms of sulfur with oxygen to obtain energy. The oxidation of reduced sulfur species also provides reducing equivalents for the reduction of carbon dioxide to cellular carbon. The final product of sulfide oxidation is sulfate, with elemental sulfur, deposited extracellularly, as the principal intermediate.

Purple sulfur bacteria primarily are anaerobic phototrophic organisms using sulfide and other reduced forms of sulfur exclusively as the electron donor for the reduction of CO_2 to cellular carbon. Usually, sulfur is temporarily stored intracellularly. The final product of the oxidation of reduced forms of sulfur is sulfate.

The niches for these metabolically different groups of microbes in ecosystems with steep, often non-overlapping, gradients of oxygen and sulfide appear to be spatially separated. However, maximum viable counts of colorless sulfur bacteria and purple sulfur bacteria were both found in the top 5–10 mm of mats. Unexpectedly, viable counts of sulfate-reducing bacteria also peaked at the same depth horizon.

Sulfide is inhibitory for most oxygenic phototrophs. Sulfide production immediately underneath the layer of cyanobacteria might inhibit their growth, and, consequently, that of the entire ecosystem. In microbial mats this effect is minimized by the combined action of colorless and purple sulfur bacteria. Colorless sulfur bacteria generally have a much higher affinity for sulfide than purple sulfur bacteria, however, in microbial mats, their activity is hampered by low oxygen supply rates. As shown by pure culture studies with colorless sulfur bacteria, sulfide is incompletely oxidized when oxygen is short in supply, resulting in the production of potential electron donors for purple sulfur bacteria, such as sulfur, thiosulfate and polysulfides. In the absence of purple sulfur bacteria, colorless sulfur bacteria would not be able to maintain a low sulfide concentration due to shortage of oxygen, which in turn would result in increased inhibition of oxygenic photosynthesis.

It thus appears that the combined action of all four groups of functional microbes mentioned effectively results in optimal growth of these recent "stromatolites".

1. Introduction

Microbial mats are vertically laminated organo-sedimentary structures developing on solid surfaces. They typically have steep gradients of oxygen and sulfide, and characteristically are dominated by only a few functional groups of microbes: cyanobacteria, colorless sulfur bacteria, purple sulfur bacteria, and sulfate-reducing bacteria. Aerobic heterotrophic organisms are functionally important as their activity leads to oxygen depletion, and fermentative organisms provide growth substrates for sulfate-reducing bacteria. Other, numerically less important, groups are nitrifying and denitrifying bacteria, and methanogenic bacteria. The lamination of the biologically active layers often is preserved in older (deeper), already degrading, layers. Microbial mats probably are the oldest

0025-3227/93/$06.00

structured ecosystems on earth. Microfossils in lithified ancient microbial mats (stromatolites) have been dated ≈3.5 billion years old. Because of morphological similarities, recent microbial mats are considered to be analogues of Precambrian stromatolites (Margulis et al., 1980).

Laminated microbial sediment ecosystems develop under a wide range of environmental conditions, and can be found in hypersaline coastal lagoons, hot springs, alkaline lakes, and marine intertidal flats (Cohen, 1984, 1989; Jørgensen and Cohen, 1977; Javor and Castenholz, 1981, 1984; Jørgensen et al., 1983; Bauld, 1984; Stal et al., 1985; De Wit and Van Gemerden, 1988; Visscher and Van Gemerden, 1991).

Our knowledge of structure, function, and dynamics of recent microbial mats has increased over the past years due to the development of suitable techniques that enabled the detailed analysis of these small-scaled ecosystems, and to laboratory studies of isolated organisms under environmentally relevant conditions. The availability of microsensors for oxygen, sulfide, redox potential, pH, and light has allowed fine-scale measurements to be made, and, in combination with careful electron microscopy, has revealed steep and fluctuating gradients, particularly of oxygen and sulfide, in which organisms interact (Jørgensen et al., 1983; Revsbech and Ward, 1984; Pierson et al., 1987; Nicholson et al., 1987; De Wit and Van Gemerden, 1988; De Wit et al., 1989; Lassen et al., 1992). This contribution focuses on microbial interactions in benthic marine microbial mats as they develop in intertidal flats. Understanding the common effort of functionally different groups of microbes in mat building, may help to understand ancient stromatolite formation.

2. Developmental stages of benthic marine microbial mats

Microbial mats develop in time as the result of microbial growth and activity, sediment trapping and binding in the organic matrix, and sedimentation (Margulis et al., 1980). Important environmental parameters for the development of these ecosystems, as formulated by Walter (1976) are grain size of the substratum, capillary attraction of water, penetration of light, sedimentation and erosion rates, and grazing pressure.

As a rule, the grain size of the substratum is strikingly uniform (Stal et al., 1985). On Scapa Beach (Orkney Islands, Scotland) the dominant particle size class is 180–250 μm (Van Gemerden et al., 1989), which effectively enables capillary attraction of water. Light absorption by cyanobacteria is in part complementary to that of purple sulfur bacteria, enabling the latter to develop underneath the oxygenic phototrophs. Also, reflection of light by the quartz surfaces provides for sufficient light, even in deeper layers (Jørgensen and Des Marais, 1986; Jørgensen, 1988, 1989; Lassen et al., 1992).

Sediments in intertidal environments are exposed to water movement and wind as eroding forces. The critical friction velocity, defined as the lowest water/air movement resulting in detachment and transport of sediment particles, is increased by mat formation, and, compared to non-inhabited sediments is 1.7–8.6 times higher for cyanobacteria-dominated mats on Mellum (Germany) (Führböter and Manzenrieder, 1987) and maximally is 5 times higher for mats of purple sulfur bacteria at Pinella Point (Tampa Bay, Florida, USA) (Grant and Gust, 1987). Microcolonies of the purple sulfur bacterium *Thiocapsa roseopersicina*, colonizing beaches on the Orkney Islands, cement individual sand grains to each other and the resulting aggregates can withstand severe wave action (Van Gemerden et al., 1989). Microbial mats thus increase the stability of sediments.

Most microbial mats develop under conditions that exclude, or at least limit the abundance of grazing organisms (Cohen, 1989). Especially anaerobic protozoa are potential predators in the largely anoxic mat environment (Javor and Castenholz, 1984). However, the gradients of oxygen show day/night shifts and these organisms are generally very sensitive even to low concentrations of oxygen (Fenchel and Finlay, 1990a), and presumably also have low growth efficiencies (Fenchel and Finlay, 1990b). In addition, sulfide toxicity and fluctuations in salinity have been suggested as factors restraining massive development of anaerobic pro-

tozoa (Javor and Castenholz, 1984). It is important to realize that mat building depends on the suppression of invertebrate grazers that otherwise would consume the cyanobacterial top layer (Awramik, 1984).

In benthic microbial mats reactions are carried out by a cascade of (micro)organisms. The following developmental stages can be recognized:

(1) Organisms capable of colonizing nutrient-poor sand flats invariably are N_2-fixing cyanobacteria, performing oxygenic photosynthesis (Table 1, eqs. 1.1 to 1.3). Their gliding ability allows them to move upwards after being covered by sand. Growth and survival of other organisms depends on excretion and lysis products of cyanobacteria. In young mats, enrichment with nitrogenous compounds is of particular importance, whereas in mature mats excretion of carbon sources is more relevant. Cyanobacteria are the driving force of most microbial mats, as they provide growth substrates for other organisms as well as physical strength. Pioneer species such as *Oscillatoria* are replaced in later stages by trichome-forming organisms such as *Microcoleus chthonoplastes*. The latter is often dominant in marine microbial mats.

(2) Soluble organic compounds are aerobically respired by a variety of heterotrophic organisms. Particularly during the night this results in anoxia. During the day aerobic respiration by heterotrophic bacteria may result in reduced oxygen microenvironments around oxygenic phototrophs (Marshall, 1989), thus possibly preventing photooxidative death (Eloff et al., 1976).

(3) The sediment is enriched with organic molecules due to excretion and lysis (Bateson and Ward, 1988), and fermentation. A substantial amount of low molecular weight organic products may originate from the dark metabolism by cyanobacteria (Stal et al., 1989; Stal, 1991) (Table 1, eqs. 1.7 to 1.9).

(4) Subsequently, these compounds are degraded, partially or complete, by the anaerobic dissimilatory sulfate-reducing bacteria (i.e. organisms carrying out an anaerobic respiration with sulfate as the terminal electron acceptor), resulting in the production of sulfide (Table 1, eqs. 4.1 to 4.9).

(5) Colorless sulfur bacteria and purple sulfur bacteria, often sandwiched in between the layer of cyanobacteria and sulfate-reducing bacteria, oxidize the obnoxious sulfide back to sulfate. Colorless sulfur bacteria (such as *Thiobacillus* and *Beggiatoa*) utilize sulfide both as the energy source and as the electron donor for CO_2 reduction. These organisms oxidize sulfide (and other reduced inorganic sulfur species) with oxygen or nitrate to obtain energy for growth and survival (Table 1, eqs. 2.1 to 2.3). In addition, the oxidation of sulfide provides the electrons needed for the reduction of CO_2 to cellular organic carbon (Table 1, eqs. 2.4 to 2.7). Purple sulfur bacteria (such as *Thiocapsa* and *Chromatium*) use light as a source of energy and use sulfide (and other inorganic reduced sulfur species) exclusively for the reduction of CO_2 to cellular carbon (Table 1, eqs. 3.1 to 3.5). These two groups of organisms are responsible for the fact that less than 1% of the H_2S produced in dissimilatory sulfate reduction reaches the atmosphere (data for Danish estuaries) (Ingvarson and Jørgensen, 1982, cf. Trüper, 1984).

Due to oxygen penetration during the day, green sulfur bacteria, which are obligately anaerobic organisms, usually do not proliferate in microbial mats. However, under continuously anoxic conditions, their effective use of extremely low light intensities allows them to grow underneath the population of purple sulfur bacteria (Nicholson et al., 1987; Pierson et al., 1987).

A schematic representation of a mature intertidal benthic mat, based on the information provided above, is shown in Fig. 1.

(6) As a result of growth, particle trapping, and accretion, the annual elevation of the surface of the mat may range from 1–2 mm (Visscher, 1992) to as much as 10 mm (Javor and Castenholz, 1981), depending on erosion rates. At a certain stage the capillary attraction of water becomes insufficient and rooted plants (starting with *Salicornia* sp.) take over; eventually the microbial mat becomes a salt marsh.

3. Biological cycling of sulfur

Ecosystems dominated by interconversions of sulfur compounds are referred to as "sulphureta"

TABLE 1

Some relevant reactions carried out by microorganisms in microbial mats

1.a Cyanobacteria: oxygenic photosynthesis		
energy source: light e^- donor: H_2O carbon source: CO_2		
e^- donor for CO_2 fixation	$2H_2O \rightarrow O_2 + 4[H]$	1.1
CO_2-fixation	$CO_2 + 4[H] \rightarrow \langle CH_2O \rangle + H_2O$	1.2
Total	$CO_2 + H_2O \rightarrow \langle CH_2O \rangle + O_2$	1.3
1.b Cyanobacteria: anoxygenic photosynthesis		
energy source: light e^- donor: H_2S carbon source: CO_2		
e^- donor for CO_2 fixation	$2H_2S + 3H_2O \rightarrow H_2S_2O_3 + 8[H]$	1.4
CO_2-fixation	$2CO_2 + 8[H] \rightarrow 2\langle CH_2O \rangle + 2H_2O$	1.5
Total	$2CO_2 + 2H_2S + H_2O \rightarrow 2\langle CH_2O \rangle + H_2S_2O_3$	1.6
1.c Cyanobacteria: fermentation, sulfur respiration		
energy source: glycogen, trehalose e^- acceptor: S^0		
Total	$C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + CH_3CH_2OH + CO_2$	1.7
	(approximation) $C_6H_{12}O_6 + H_2O \rightarrow 2CH_3COOH + 2CO_2 + 3H_2 + 2[H]$	1.8
	$2[H] + S^0 \rightarrow H_2S$	1.8
Total	(approximation) $C_6H_{12}O_6 + H_2O + S^0 \rightarrow 2CH_3COOH + 2CO_2 + 3H_2 + H_2S$	1.9
2 Colourless sulfur bacteria: chemosynthesis		
energy source: H_2S/O_2 principal e^- donor: H_2S principal carbon source: CO_2		
	$3H_2S + 1\frac{1}{2}O_2 \rightarrow 3S^0 + 3H_2O$	2.1
	$3S^0 + 4\frac{1}{2}O_2 + 3H_2O \rightarrow 3H_2SO_4$	2.2
energy	$3H_2S + 6O_2 \rightarrow 3H_2SO_4$	2.3
	$H_2S \rightarrow S^0 + 2[H]$	2.4
	$S^0 + 4H_2O \rightarrow H_2SO_4 + 6[H]$	2.5
e^- donor for CO_2-fixation	$H_2S + 4H_2O \rightarrow H_2SO_4 + 8[H]$	2.6
CO_2-fixation	$2CO_2 + 8[H] \rightarrow 2\langle CH_2O \rangle + 2H_2O$	2.7
Total	$2CO_2 + 4H_2S + 6O_2 + 2H_2O \rightarrow 2\langle CH_2O \rangle + 4H_2SO_4$	2.8
3.a Purple sulfur bacteria: anoxygenic photosynthesis		
energy source: light principal e^- donor: H_2S principal carbon source: CO_2		
	$H_2S \rightarrow S^0 + 2[H]$	3.1
	$S^0 + 4H_2O \rightarrow H_2SO_4 + 6[H]$	3.2
e^- donor for CO_2-fixation	$H_2S + 4H_2O \rightarrow H_2SO_4 + 8[H]$	3.3
CO_2-fixation	$2CO_2 + 8[H] \rightarrow 2\langle CH_2O \rangle + 2H_2O$	3.4
Total	$2CO_2 + H_2S + 2H_2O \rightarrow 2\langle CH_2O \rangle + H_2SO_4$	3.5
3.b Purple sulfur bacteria, chemosynthesis		
energy source: H_2S/O_2 principal e^- donor: H_2S principal carbon source: CO_2 See eqns. 2.1 to 2.8		
4 Sulfate-reducing bacteria: dissimilatory sulfate reduction, sulfate respiration		
energy source: organic C, H_2 e^- donor: organic C, H_2 carbon source: organic C		
4.a incomplete oxidation[1]	$2CH_3CHOHCOOH + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 8[H]$	4.1
e^- acceptor	$H_2SO_4 + 8[H] \rightarrow H_2S + 4H_2O$	4.2
Total	$2CH_3CHOHCOOH + H_2SO_4 \rightarrow 2CH_3COOH + 2CO_2 + H_2S + 2H_2O$	4.3
4.b complete oxidation[2]	$2CH_3CHOHCOOH + 6H_2O \rightarrow 6CO_2 + 24[H]$	4.4
e^- acceptor	$3H_2SO_4 + 24[H] \rightarrow 3H_2S + 12H_2O$	4.5
Total	$2CH_3CHOHCOOH + 3H_2SO_4 \rightarrow 6CO_2 + 3H_2S + 6H_2O$	4.6
4.c e^- donor[3]	$4H_2 \rightarrow 8[H]$	4.7
e^- acceptor	$H_2SO_4 + 8[H] \rightarrow H_2S + 4H_2O$	4.8
Total	$4H_2 + H_2SO_4 \rightarrow H_2S + 4H_2O$	4.9

[1] Incomplete oxidation to acetate also observed for propionate, butyrate, and other not branched fatty acids.
[2] Complete oxidation to CO_2 also observed for acetate and ethanol.
[3] Most hydrogen-utilizing sulfate-reducing bacteria can use formate as well.

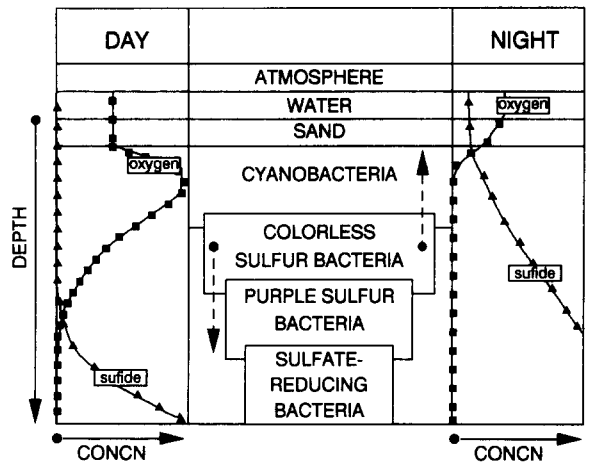

Fig. 1. Simplified scheme of a laminated microbial sediment ecosystem (microbial mat) showing the diel fluctuations of oxygen and sulfide concentrations in relation to the vertical distribution of functional groups. The dashed arrows indicate the possible migration of motile colorless sulfur bacteria to exploit the shifting gradients of sulfide and oxygen. Major reactions carried out by the microbial groups indicated are listed in Table 1.

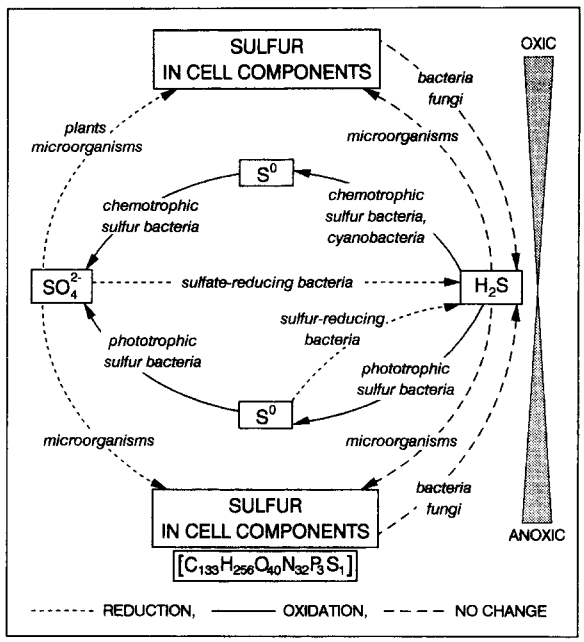

Fig. 2. Biogeochemical sulfur cycle in a sedimentary ecosystem with oxic/anoxic compartmentation.

(Baas Becking, 1925). Marine microbial mats can be regarded as ideal model systems to study sulfur cycling, because organic matter is primarily of autochthonous origin, metazoan grazing is minimal, and bioturbation is insignificant (Cohen, 1989). Since production and consumption of organic matter are spatially separated over μm to mm distances, microbial mats can be regarded as relatively closed systems in terms of nutrient cycling.

A simplified model of the biological cycling of sulfur in an ecosystem with oxic ("aerobic") and anoxic ("anaerobic") compartments is visualized in Fig. 2. Cycling between elemental sulfur and sulfide commonly is referred to as the "small sulfur cycle" to differentiate this process from the "large sulfur cycle" in which sulfur is cycled between sulfide and sulfate (Trüper, 1984).

Sulfur is an essential element for all organisms due to its vital role in the synthesis of organic sulfur compounds e.g. sulfur-containing amino acids. However, the cellular content of sulfur is only 1–2% of the dry weight. Due to the fact that the oxidation state of sulfur may vary in between −2 (as in sulfide) and +6 (as in sulfate), different sulfur species can act as electron donor (and become more oxidized) or as electron acceptor (and become more reduced). These processes are predominantly mediated by microbes. It is for this reason that the role of bacteria in the sulfur cycle is quantitatively so important.

The reduction of sulfate and the oxidation of sulfide are decisive processes in the sulfur cycle; in the carbon cycle photosynthesis and respiration are the most important processes, whereas in the nitrogen cycle nitrogen-fixing organisms play a key role in the colonization of intertidal sand flats. It should be realized that cycles of elements are closely linked, though they usually are treated separately.

4. Vertical distribution of sulfur bacteria in a microbial mat

Due to differences in type of chlorophyll and accessory pigments of cyanobacteria and anoxygenic phototrophic bacteria, and the blackening of deeper layers due to FeS precipitation, microbial mats clearly have a laminated appear-

ance when observed with the naked eye (Nicholson et al., 1987; Pierson et al., 1987; D'Amelio et al., 1989). Pigment lamination often is preserved after burial in deeper sediment layers (Palmisano et al., 1989).

However, not only are colorless sulfur bacteria named so for obvious reasons, also populations of sulfate-reducing bacteria in itself are colorless, and purple sulfur bacteria may lack photopigments (see below) resulting in faintly colored or even colorless cultures. Therefore, the macroscopical vertical coloration of the mats may not reflect the actual distribution of the different groups of organisms.

Most probable number (MPN) counts of three groups of sulfur bacteria in a microbial mat on the Frisian Island of Texel (The Netherlands) not only showed that the maximum population densities of purple sulfur bacteria and colorless sulfur bacteria are found in the top 5 mm of the mat, but also that the population density of sulfate-reducing bacteria peaks in the top 10 mm (Fig. 3, note log scale) (Visscher et al., 1992). All three groups of sulfur bacteria increased in number during the growth season. Anoxygenic phototrophs and colorless sulfur bacteria increased by 2–3 orders of magnitude, while sulfate reducers increased twofold. It is of interest to note that purple sulfur bacteria, colorless sulfur bacteria, and sulfate-reducing bacteria together accounted for up to 40% of the total bacterial population (Visscher and Van Gemerden, 1993). High population densities of sulfate-reducing bacteria in surface layers of sediments have been reported previously by Jørgensen and Bak (1991) and Bak and Pfennig (1991).

MPN counts reveal the number of organisms able to grow under the conditions offered. For purple sulfur bacteria the counts include both phototrophically growing (and thus pigmented) cells, as well as chemotrophically growing cells showing little or no pigmentation. Hence, the vertical distribution of MPN counts of purple sulfur bacteria may not be coincident with the conspicuous red layer underneath the green cyanobacterial layer.

Colorless sulfur bacteria normally require oxygen, and would be expected to peak in the top layers, while motile representatives may migrate to exploit the shifting gradients of sulfide and oxygen (see Fig. 1).

The similar vertical distribution of sulfate-reducing bacteria to other sulfur bacteria indicates that they are not killed in the presence of oxygen during the day, however it does not exclude the possibility that the organisms are only active (i.e. produce sulfide) during the night when the sediment is virtually anoxic.

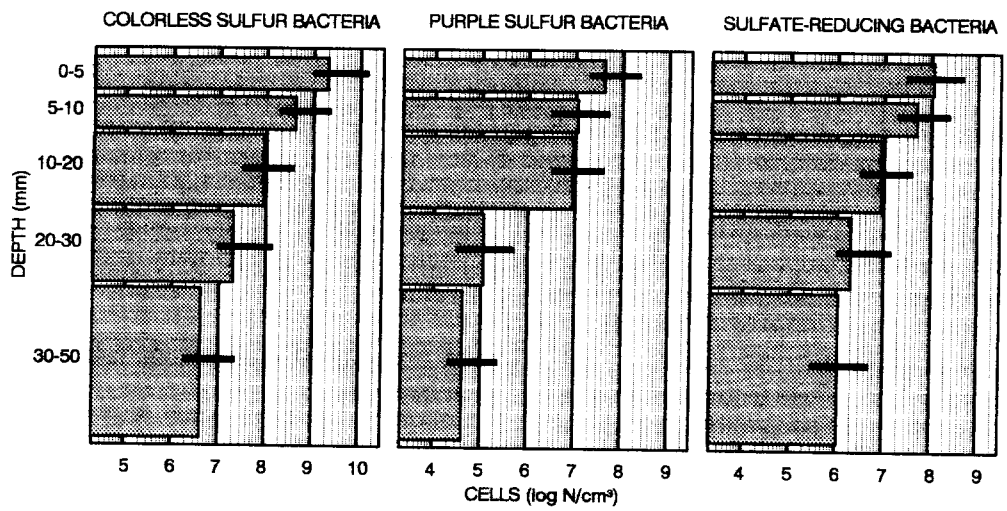

Fig. 3. Most Probable Number (MPN) counts of colorless sulfur bacteria (left panel), purple sulfur bacteria (center panel), and sulfate-reducing bacteria (right panel) in a microbial mat on the Frisian island of Texel, The Netherlands. Enumerations were made in August 1989. Error bars indicate 95% confidence interval. Redrawn from Visscher and Van Gemerden (1993).

5. Vertical distribution of biological activities

5.1. Oxygenic photosynthesis

The application of glass microsensors for oxygen with a tip diameter of 2–10 μm has enabled Danish scientists to estimate quantitatively rates of gross oxygenic photosynthesis in small-scaled ecosystems (Revsbech and Jørgensen, 1983, 1986; Revsbech et al., 1989; Jørgensen and Des Marais, 1986; Jensen and Revsbech, 1989). The rate of photosynthesis in a layer is determined from the initial rate of decrease of the concentration of O_2 after darkening of the sediment layer. This elegant method has one drawback: in layers with zero oxygen concentration, one cannot conclude whether or not photosynthesis was occurring, because oxygen production might have been equalled by oxygen consumption (Castenholz et al., 1991). The same reasoning applies to the occurrence of sulfate reduction in layers with zero sulfide concentrations.

During active oxygenic photosynthesis, often elevated pH values are observed in addition to increased O_2 concentrations (Revsbech and Ward, 1984; D'Amilio et al., 1989). The vertical distribution of chlorophyll reflects the distribution of oxygenic phototrophic organisms, and in a Texel microbial mat showed a maximum of 0.825 mg cm^{-3} sediment in the 2.5–5 mm layer (Visscher and Van Gemerden, 1991). At 8 a.m. the *p*H was around 7.5 throughout the mat, while at 6 p.m. it had increased to 10.5 at the 5 mm depth horizon, but still remained about 7.5 at the surface of the sediment and at depths > 10 mm. This phenomenon is commonly observed, and is explained by the occurrence of CO_2 fixation in habitats having limited buffering capacity (shortage of carbonate).

5.2. Sulfate reduction

As stated above, MPN counts of sulfate-reducing bacteria cannot be considered conclusive evidence for sulfide production, due to the possibility of limited metabolic activity of these microbes in the presence of oxygen. Circumstantial evidence for the depth horizon at which sulfide production occurs is obtained from the vertical distribution of total free sulfide ($H_2S/HS^-/S^{2-}$) (Fig. 4A). When sulfate reduction occurs primarily in the deeper layers of the sediment, one would expect that the sulfide concentration would increase with increasing depth, with maximum concentrations well below the layer of purple sulfur bacteria. Instead, the maximum sulfide concentration ($\approx$0.5 mmol l^{-1}) was observed at a depth of 10 mm, while concentrations of less than 0.05 mmol l^{-1} were observed below 20 mm and above 5 mm depth (Fig. 4A). These data suggest that sulfate reduction is most prominent in the 5–10 mm layer.

With respect to the availability of substrates for sulfate-reducing and other heterotrophic bacteria, conditions in the top layers are most favorable: a maximum organic matter content of 0.32 g g^{-1} sediment was found in the top 5 mm, and rapidly decreased to become less than 0.05 g g^{-1} sediment below the 15 mm depth horizon (Visscher et al., 1992a). Excellent growth substrates for sulfate-reducing bacteria (lactate, acetate, ethanol, H_2; Table 1, eqs. 4.1 to 4.9) (Widdel, 1988) are also produced by dark fermentation of the directly overlying cyanobacteria (Stal et al., 1989; Stal, 1991) (Table 1, eqs. 1.7 to 1.9).

Actual sulfate-reduction rates in sediments can be measured with radiotracers (Jørgensen, 1978); sulfate-reduction rates calculated from sulfide depth profiles often do not correlate with radiotracer data (Revsbech et al., 1989) for as yet unknown reasons.

Because dissimilatory sulfate reduction is considered to be an obligately anaerobic process, it has been proposed that sulfate reduction in oxic environments takes place in anoxic microniches (Jørgensen, 1977a), but this hypothesis was not supported by micro-electrode measurements (Jørgensen and Bak, 1991). Cores taken from the Texel mat, after being injected with $^{35}SO_4^{2-}$, were incubated either in complete darkness or in the light (800 μE m^{-2} s^{-1}). Dark incubation resulted in shallow oxygen penetration (Fig. 5A), while in the light oxygen, produced by oxygenic phototrophs in the surface layers, penetrated to a depth of 5 mm (Fig. 5B). Sulfate-reduction rates in the 5–10 mm depth layer were virtually identical under dark and light incubation. In the 0–5 mm

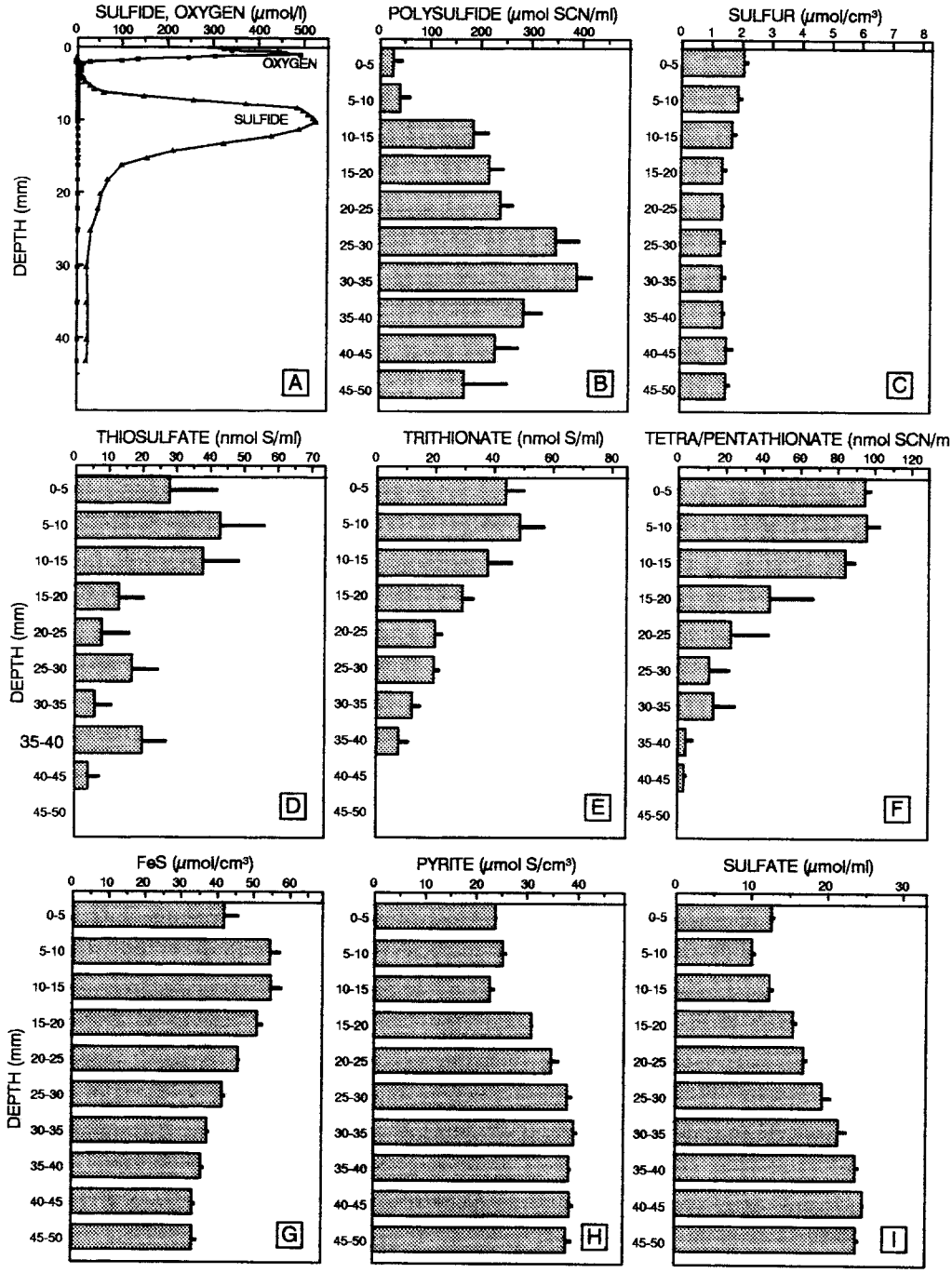

Fig. 4. Depth profiles of oxygen and inorganic sulfur compounds in the top 50 mm of a microbial mat on the Frisian island of Texel, The Netherlands. Concentrations of water-soluble species are expressed per volume interstitial water, while concentrations of insoluble compounds are expressed per volume sediment. The water content of the surface layers was 0.3–0.4 ml cm^{-3} (Visscher et al., 1992). Concentrations of polysulfide and polythionates are given as sulfane-sulfur concentrations, measured as SCN^-, because the exact chain length is unknown. Small black bars indicate standard deviation ($n=3$). Redrawn from Visscher and Van Gemerden (1993).

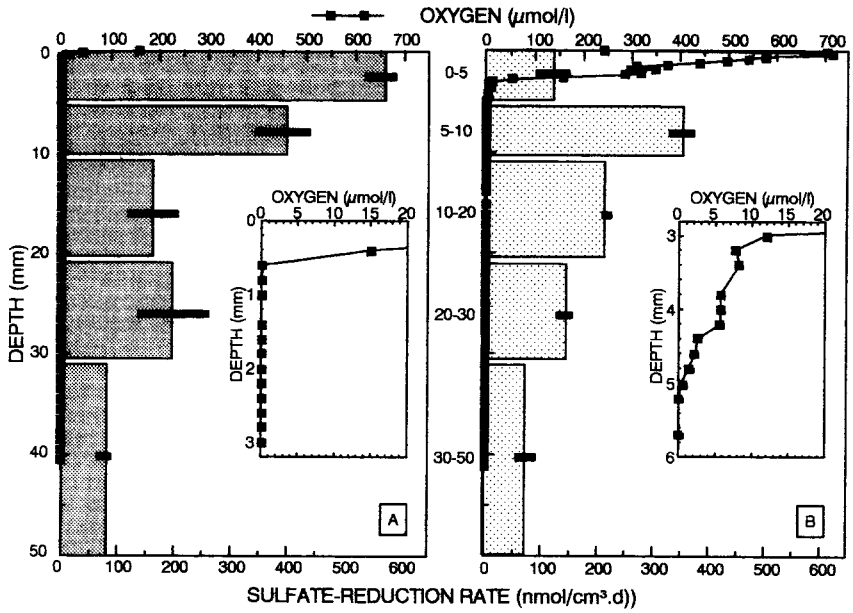

Fig. 5. Sulfate-reduction rates (bars) measured in the top 50 mm of a microbial mat on the Frisian island of Texel, The Netherlands. Measurements were carried out in cores injected with $^{35}SO_4^{2-}$ and incubated in the dark (A), or in the light (B). Reduced ^{35}S products measured include S^{2-}, polysulfides, S^o, polythionates, FeS, and FeS_2, but not thiosulfate. Oxygen profiles were measured with needle electrodes at the end of the incubation period. Error bars indicate standard deviation ($n=3$). Redrawn from Visscher et al. (1992).

depth layer the rate in the light still was 22% of that in the dark. It thus appears that sulfate reduction occurs in the presence of oxygen. High rates of sulfate reduction (>1000 nmol cm^{-3} day^{-1}) in the presence of oxygen have been reported previously for the top layer of microbial mats in the Sinai (Cohen, 1984; Skyring et al., 1989), Mexico (Canfield and Des Marais, 1991), and Australia (Skyring, 1984) and for the water column of the Cariaco Trench (Hastings and Emerson, 1988).

Some species of sulfate-reducing bacteria are less sensitive to oxygen than others. Good survival of several strains has been reported after prolonged incubation in the presence of oxygen (Cypionka et al., 1985; Dilling and Cypionka, 1990; Marschall et al., 1993).

6. Sulfur speciation in a mature benthic microbial mat

The vertical distribution of inorganic and organic sulfur species, and microorganisms in the Texel showed similar seasonal patterns over the years. The data discussed below were collected in August 1989.

6.1. Free sulfide and oxygen

The distribution of free sulfide (H_2S, HS^-, S^{2-}) in relation to the site of sulfate reduction has been discussed previously. Other important features are the vertical distribution of sulfide in relation to the profile of oxygen, and fluctuations of sulfide and oxygen as they occur over a day/night cycle.

Often an overlap of sulfide and oxygen is observed in microbial mats (Revsbech et al., 1983; Ward et al., 1989; D'Amelio et al., 1989; Visscher et al., 1991). The simultaneous presence of detectable concentrations of sulfide and oxygen, results in high rates of consumption of both parameters at the oxic/anoxic interface (Revsbech et al., 1989). When no overlap of oxygen and sulfide is observed, the depth stratum devoid of both oxygen and sulfide is referred to as the sub-oxic layer, or, because of the possible participation of iron and manganese in the transfer of electrons (Ghiorse, 1988), as the Fe–Mn zone (Sørensen and

Jørgensen, 1987). The possibility of identical rates of production and consumption of oxygen and sulfide, resulting in zero concentrations, could apply to this zone.

Secondly, for obvious reasons, sulfide and oxygen profiles shift towards the surface of the mat during the night (Revsbech et al., 1989; De Wit et al., 1989; Van Gemerden, 1990; also visualized in Fig. 1). Motile organisms are able to migrate along with the moving interface, but benthic microbes are confronted with changing environmental conditions. In addition, particularly with respect to oxygen, fluctuations often occur within short times. The depth profile of oxygen is the combined result of production and consumption. In microbial mats, illuminated in the laboratory, so-called steady states are established upon prolonged illumination. At best, such conditions mimic the light climate in permanently blue-skyed areas, but they are certainly unrealistic for microbial mats in temperate zones. Oxygen concentrations at a fixed depth in a microbial mat on the Island of the Grey Monks (The Netherlands) changed from 300 to 50 $\mu mol\, l^{-1}$ within 10 minutes as the result of clouds passing by (R. de Wit and H. van Gemerden, unpubl. data).

6.2. Other inorganic forms of sulfur

Standing concentrations of inorganic sulfur species in the Texel mat are shown in Fig. 4B–I. Polysulfides (including organic forms), elemental sulfur, FeS, and FeS_2 were present in millimolar (m*M*) concentrations, while thiosulfate, tri-, tetra-, and pentathionate were found at micromolar (μM) levels. The concentrations of sulfide, thiosulfate, and tetra/pentathionate all increased during the growth season, while those of polysulfide and elemental sulfur decreased. These changes were most paramount in the top layers where also the highest population densities of sulfur bacteria were observed, suggesting that microbial activities play an important role in the establishment of these profiles. Polysulfide can be used as electron donor by the dominant purple sulfur bacterium *Thiocapsa roseopersicina* (Visscher et al., 1990), and also FeS and elemental sulfur are potential electron donors. This indicates that sulfide depletion not necessarily does imply electron donor depletion. Relatively low polysulfide concentrations (compared to deeper layers) were observed in the top 10 mm of the microbial mat (Fig. 4B). Circumstantial evidence of microbial polysulfide utilization is obtained from the fact that concentrations decreased significantly after the start of the growth season (May: 0–5 mm 122, 5–10 mm 192; August: 0–5 mm 26, 5–10 mm 40, values in $\mu mol\, ml^{-1}$); in deeper strata less significant changes were observed. Significant amounts of polysulfide also have been reported to occur in salt marshes (Lord et al., 1983; Luther et al., 1988) and other microbial mats (Aizenshtat et al., 1983; Van Gemerden et al., 1989). Polysulfides are known to react rapidly with organic matter (Luther et al., 1986; Vairavamurthy and Mopper, 1987, 1989; Kohnen et al., 1989; Vairavamurthy et al., 1992) and they link inorganic to organic sulfur cycling (Aizenshtat et al., 1983; Vairavamurthy et al., 1992; J.W. de Leeuw and J.S. Sinnenghe Damsté, pers. commun., 1992).

The profile of FeS typically peaked in the 5–15 mm layer (Fig. 4G), while pyrite increased with depth (Fig. 4H). Elemental sulfur was distributed uniformly throughout the sediment (Fig. 4C). Over the growth season significant decreases were observed, particularly in the top 10 mm of the mat (May: 0–5 mm 4.8, 5–10 mm 6.2; August: 0–5 mm 2.0, 5–10 mm 1.8, values in $\mu mol\, cm^{-3}$). Sulfate concentrations in the top layers were below that of seawater (Fig. 4I)—which is explained by the fact that replenishment with seawater typically occurred twice a month only—and increased with depth.

The sulfur cycle, as we observe it, is the combined result of (micro)biological activities and physico-chemical processes. On a biological time scale, much attention is paid to compounds with high turnover rates and small pool sizes, compared to compounds with large pool sizes such as sedimentary sulfides (FeS, FeS_2) and sulfates ($CaSO_4.2H_2O$, $MgSO_4.7H_2O$). Still too little is known about fluxes of sulfur species, and related biological activities. Nevertheless, measurement of pool sizes and enumeration of bacteria allows a better understanding of S cycling. Interconversions of inorganic sulfur compounds as they may occur in laminated sedimentary ecosystems with oxic/

anoxic compartmentation are shown in Fig. 6, in the text below, bold numbers in parentheses refer to the reactions indicated in Fig. 6. A number of relevant equations is listed in Table 1.

Sulfide (H_2S, HS^-, S^{2-}) in marine sediments is generated principally (97–99%) as a result of dissimilatory sulfate reduction (**1**) (Jørgensen, 1977b), although sulfur reduction (**2**) may contribute. Without the participation of O_2, purple sulfur bacteria oxidize sulfide to zero-valent sulfur ("elemental sulfur", S°)—stored intracellularly (iS°) (**3**), or deposited outside the cells (eS°) (**4**)—which, eventually, is oxidized to sulfate without detectable intermediates (**5**,**6**). Some cyanobacteria (e.g. *Oscillatoria limnetica*) oxidize sulfide to S° (**4**) (Cohen et al., 1975a), others (e.g. *Microcoleus chthonoplastes*) to thiosulfate (**7**) (De Wit and Van Gemerden, 1987b; De Wit et al., 1988). In the

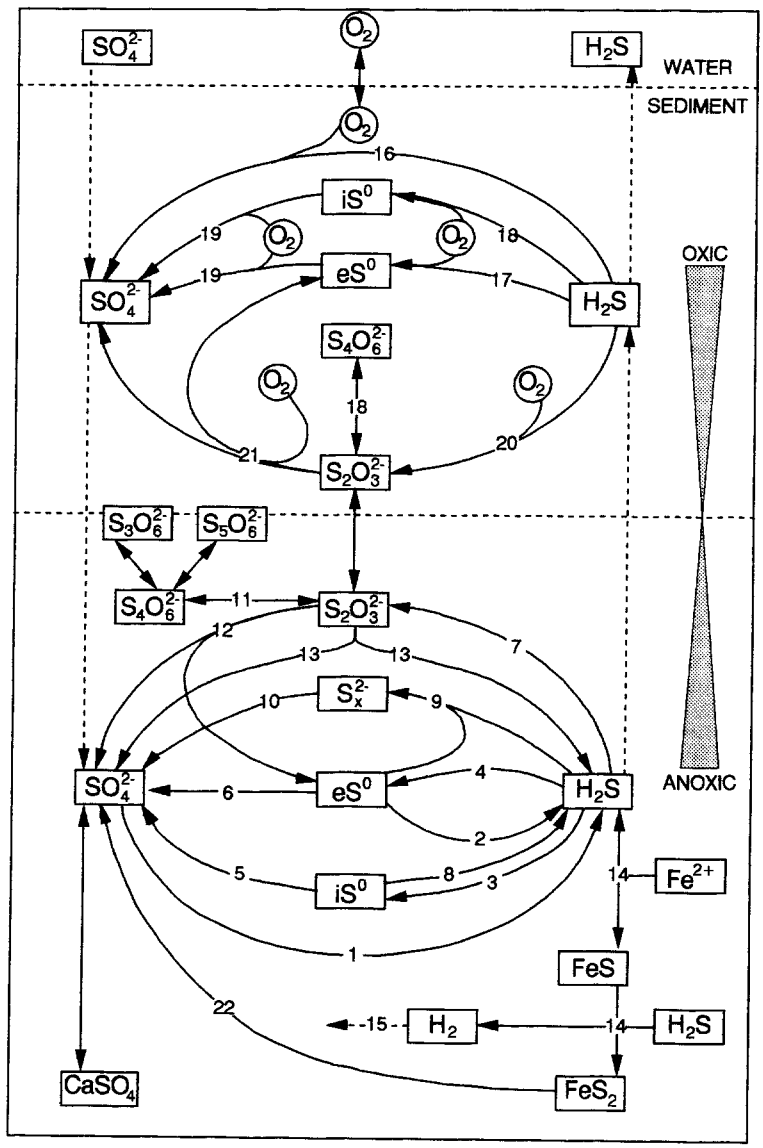

Fig. 6. Inorganic sulfur cycling in a sedimentary ecosystem with oxic/anoxic compartmentation. The numbered reactions are mentioned in the text. Major reactions carried out by colorless sulfur bacteria, purple sulfur bacteria and sulfate-reducing bacteria are listed in Table 1.

dark purple sulfur bacteria produce small amounts of sulfide by dissimilatory sulfur reduction (**8**) (Van Gemerden, 1968), and a similar reaction is performed by many cyanobacteria (**2**) (Oren and Shilo, 1979; Stal, 1991).

Sulfide reacts chemically with S° to form polysulfides (S_x^{2-}) (**9**). These compounds are more stable at high pH and have been detected in cultures of alkaliphilic purple sulfur bacteria belonging to the genus *Ectothiorhodospira* (Then and Trüper, 1983), but were also found in cultures of green sulfur bacteria grown at moderate pH (Visscher and Van Gemerden, 1988). Green and purple sulfur bacteria oxidize polysulfides to sulfate (**10**), S° being an intermediate (not shown in Fig. 6) (Visscher and Van Gemerden, 1988; Visscher et al., 1990).

Thiosulfate is formed by some green sulfur bacteria as an intermediate in sulfide oxidation (**7**), the final product being sulfate (**12**, **6**) (Steinmetz and Fischer, 1982). Low concentrations of thiosulfate were also detected in cultures of the purple sulfur bacterium *Chromatium vinosum* (Steudel et al., 1990).

Thiosulfate can serve as an electron donor for most chemotrophic and phototrophic sulfur bacteria. In cultures, often thiosulfate is used instead of sulfide, in order to avoid problems related to the toxicity of sulfide and the chemical reaction between sulfide and oxygen. Oxidation of thiosulfate by anoxygenic phototrophic bacteria may result in the formation of tetrathionate (**11**) (Then and Trüper, 1981) or the formation of S° and SO_4^{2-} (**12**). Also, thiosulfate may be split in S° and SO_3^{2-} (not shown in Fig. 6), whereafter both moieties are oxidized to sulfate (Smith, 1966, cf. Fischer, 1989).

Thiosulfate is also used by some sulfate-reducing bacteria (*Desulfovibrio sulfodismutans*) in an energy-conserving disproportionation reaction yielding sulfate and sulfide (**13**) (Bak and Cypionka, 1987; Bak and Pfennig, 1987). ^{35}S-labeling proved this pathway to be very important in certain marine environments (Jørgensen, 1990; Jørgensen and Bak, 1991).

An important sulfur sink is the formation of pyrite (**14**). In Fig. 6, FeS_2 formation is assumed to take place as postulated by Drobner et al. (1990). Removal of H_2 (**15**) by sulfate-reducing bacteria or methanogens could pull this reaction in a thermodynamically favorable direction. At extremely low S^{2-} concentrations, pyrite can be formed directly from inorganic polysulfide and ferrous iron without the participation of FeS, according to:

$S_x^{2-} + Fe^{2+} \rightarrow S_{(x-1)}^{2-} + FeS_2$ (not shown in Fig. 6). This reaction was reported to occur faster than the reaction between FeS and elemental sulfur according to $FeS + S^0 \rightarrow FeS_2$ (not shown in Fig. 6). These reactions can be purely chemical or mediated by microbial activity (Howarth, 1979; King, 1983; Luther et al., 1986; Luther, 1991).

In the presence of oxygen, sulfide can be oxidized to sulfate by colorless sulfur bacteria (**16**), thiobacilli form zero-valent sulfur as intermediate which is deposited outside the cells (eS°) (**17**), whereas *Beggiatoa* and other large thiobacteria form intracellular sulfur (iS°) (**18**). Unless oxygen is limiting (see section 7.3), the end product of sulfide oxidation by colorless sulfur bacteria is sulfate (**19**). Colorless sulfur bacteria can oxidize sulfide to thiosulfate (**20**) (Kelly, 1988), a reaction which also occurs chemically (Jørgensen et al., 1979a,b). The end product of microbial thiosulfate oxidation is sulfate (**21**, **19**). Pyrite is oxidized to sulfate by colorless sulfur bacteria (**22**), but its oxidation not necessarily requires the presence of oxygen (Luther et al., 1986).

Phototrophic bacteria with the ability of chemotrophy oxidize sulfide to S°, which is stored intracellularly (**18**) (De Wit and Van Gemerden, 1987b), with sulfate as end product (**19**).

6.3. Organic sulfur compounds

Organic sulfur compounds may be formed abiotically in reactions between inorganic polysulfides and organic molecules. Relatively low molecular weight organic S-compounds result as well from the microbially mediated breakdown of protein and dimethyl-sulfoniopropionate [DMSP, $(CH_3)_2$–S–CH_2–CH_2–COOH] (Kiene and Taylor, 1988a; Kiene et al., 1990). When degraded, sulfur-containing amino acids (cysteine, methionine) yield methanethiol (MSH, CH_3–SH), dimethyl sulfide (DMS, CH_3–S–CH_3), and dimethyl disulfide (DMDS, CH_3–S–S–CH_3). DMSP, which is pre-

sent in many strains of marine phytoplankton (Kelly, 1988) and presumably acts as an osmolyte (Reed, 1983), yields DMS and acrylate ($CH_2=CH_2COOH$), or 3-mercaptopropionate (3-MPA, $HS-CH_2-CH_2-COOH$) and MSH upon bacterial degradation (Kiene and Visscher, 1987; Kiene and Taylor, 1988b; Kiene et al., 1990). Recently, evidence was obtained for DMSP-lyase activity in axenic cultures of the phytoplanktonic species *Phaeocystis* sp. (Stefels and Van Boekel, 1993) and *Emiliania huxleyi* (J. Stefels, pers. commun., 1993), suggesting that some oceanic DMS formation may not be mediated by bacteria. DMSP is also formed by the dominant cyanobacterium in mature microbial mats *Microcoleus chthonoplastes*. Upon a down-shift in salinity, mimicking severe rainfall, 50% of the cellular DMSP was excreted (Visscher and Van Gemerden, 1991b), which likely resulted in the formation of DMS.

Potential DMS consumers are sulfate-reducing bacteria and methanogens (Kiene and Visscher, 1987), colorless sulfur bacteria (Smith and Kelly, 1988; Visscher et al., 1991), and phototrophs (Zeyer et al., 1987; Visscher and Van Gemerden, 1991a).

The vertical distribution of organic sulfur compounds in the Texel microbial mat revealed high concentrations of DMS and DMSP in the top 10 mm (220 and 260 nmol cm^{-3}, respectively) coinciding with the presence of *Microcoleus* (Visscher and Van Gemerden, 1991b). DMSP concentrations in the top layer increased from 180 nmol cm^{-3} in May to 260 nmol cm^{-3} in November. Maximum concentrations of DMS in microbial mats typically are 20,000–40,000 times higher than observed in near-shore waters (Turner et al., 1988). Part of the DMS in the mats could have arisen from chemical degradation of DMSP as a result of *p*H values > 10.5 encountered in the late afternoon (Visscher and Van Gemerden, 1991b); at lower *p*H values DMSP is stable (half-life at *p*H 8.2 and 10°C is eight years, Dacey and Blough, 1987). Concentrations of DMS and DMSP rapidly decreased with depth and were below the limit of detection at depths > 30 mm.

MSH (maximum 40 nmol cm^{-3}) peaked in the 30–40 mm layer, while DMDS (maximum 90 nmol cm^{-3}) was not observed below 30 mm, which might indicate that DMDS was formed by MSH oxidation, a process reported to occur rapidly in sediments (Kiene and Capone, 1988).

7. Experiments with isolated organisms

Experimental work with pure cultures of organisms isolated from microbial mats is highly relevant due to the dominance of a few functional groups of microbes each with a low species diversity. Some illustrative examples are given below.

To simulate the low nutrient concentrations often encountered in natural habitats, continuous cultivation, in which part of the culture is continuously replaced by fresh medium, is often preferred over batch cultivation, in which no medium replenishment occurs. The chemostat technique is also very suitable for applying regimes, e.g. light/dark, or oxic/anoxic conditions. To facilitate a proper interpretation of the data, a short description of a continuous culture is included.

The essential parts of a continuous culture (chemostat) are: (1) A reservoir bottle, containing the growth medium in which all nutrients are present in excess except one. (2) A culture vessel with constant volume, which is stirred to ensure homogeneity. (3) A metering pump, which continuously and at a constant rate adds fresh medium to the culture, thus replacing part of the culture fluids, including cells. The dilution rate D is defined as the ratio of flow rate to culture volume and has the dimension h^{-1}.

Growth can be mathematically described as dx/dt, in which x is the population density expressed in an appropriate parameter (e.g. protein), and t is time. The growth rate per unit biomass is $1/x \times dx/dt$, commonly referred to as the specific growth rate μ. The relation between μ and the nutrient concentration (s) is adequately described by $\mu = \mu_{max}\ s/(K_s + s)$, in which μ_{max} is the maximum specific growth rate—determined by the intrinsic properties of the organism—and K_s is the nutrient concentration at which $\mu = \frac{1}{2}\mu_{max}$. The affinity for a nutrient is expressed as $\mu_{max}/(K_s + s)$. Since organisms usually compete for a mutual nutrient at (very) low concentrations of s, affinities commonly are calculated as μ_{max}/K_s.

The relation between μ and the doubling time t_d is $\mu = \ln 2/t_d$.

After some time, when inoculated with an organism capable of growth in the medium supplied, a continuous culture reaches a so-called steady state, in which μ equals D. During steady-state conditions, biological and chemical parameters remain constant in time. In practice this means that an organism can be grown at any $\mu > D_{critical}$, where $D_{critical}$ is close to μ_{max}. When growth is not possible (e.g. phototrophic organisms in the dark, or aerobic organisms in media devoid of oxygen), washout occurs at a rate dictated by D.

The essential differences between a batch culture and a continuous culture thus relate to the substrate concentration in the culture and the specific growth rate of the organism: in batch culture the concentration of s is high for most of the time, and, consequently, the organism grows with μ_{max}. In continuous culture, the organism can be grown at limiting concentrations of s at any μ between 0 and $D_{critical}$.

7.1. Sulfide utilization by Microcoleus chthonoplastes

Cyanobacteria, having photosystems I and II, primarily are oxygenic phototrophs using H_2O as the electron donor (Table 1, eqs. 1.1 to 1.3). However, many species also are able to perform an anoxygenic photosynthesis in which sulfide acts as the electron donor (Cohen et al., 1975a,b; Garlick et al., 1977; De Wit and Van Gemerden, 1987a, 1988; De Wit et al., 1988; Stal, 1991). *Oscillatoria limnetica* switches off oxygenic photosynthesis at sulfide concentrations as low as 0.1–0.2 m*M*, and, after an induction period of 2 h, switches on anoxygenic photosynthesis, now using sulfide instead of water (Cohen et al., 1975a,b). In *Microcoleus chthonoplastes*, commonly the dominant cyanobacterium in microbial mats, sulfide concentrations <0.2 m*M* stimulate oxygenic photosynthesis. At higher concentrations the organism gradually shifts to anoxygenic photosynthesis (Cohen, 1984). It takes about 3 h before sulfide utilization starts, thereafter sulfide is stoichiometrically converted to thiosulfate (De Wit and Van Gemerden, 1987, Table 1, eqs. 1.4 to 1.6). Unlike *Oscillatoria*, *Microcoleus* is hampered in its growth by the absence of O_2. In the presence of 3(3,4-dichlorophenyl)1,1-dimethylurea (DCMU), which selectively inhibits photosystem II, growth does not occur, unless oxygen is supplied externally (De Wit and Van Gemerden, 1988). *Microcoleus* thus is an obligately aerobic organism. Conceivably, the oxygen is required for the synthesis of polyunsaturated fatty acids (Padan, 1979; De Wit and van Gemerden, 1988). *Microcoleus* is severely inhibited by sulfide: in the absence of DCMU the addition of 0.3 m*M* sulfide results in a 50% reduction of the specific growth rate, and complete inhibition of growth is observed at sulfide concentrations exceeding 1 m*M* (De Wit and Van Gemerden, 1988).

7.2. The impact of oxygen on growth of purple sulfur bacteria

Purple sulfur bacteria primarily are anoxygenic phototrophic organisms using sulfide and other reduced forms of sulfur as the electron donor for the reduction of CO_2 to cellular carbon (Table 1, eqs. 3.1 to 3.5). During phototrophic growth these organisms do not require oxygen. Some species can withstand low to moderate O_2 concentrations, whereas for others oxygen is lethal. Because of the small vertical dimensions of microbial mats, oxygen, produced by the directly overlying layer of cyanobacteria, penetrates into the layer of purple sulfur bacteria.

Thiocapsa roseopersicina, commonly the dominant purple sulfur bacterium in mature microbial mats, has a number of metabolic characteristics that enable it to thrive under rather harsh conditions. An important feature is the ability to survive and grow both phototrophically and chemotrophically with sulfide or thiosulfate (De Wit and Van Gemerden, 1987). Other relevant properties are the utilization of polysulfides (Visscher et al., 1990) and DMS (Visscher and Van Gemerden, 1991a). Chemotrophy, and the impact of fluctuations of oxygen and light are discussed below.

During chemotrophic growth, in which sulfide is respired with oxygen in order to fulfill energy requirements, not all reducing equivalents are used for the reduction of CO_2 to cellular carbon, as in

phototrophic growth (Table 1, eqs. 2.1 to 2.8, and 3.1 to 3.5, respectively). Chemotrophy inevitably results in lower yields than phototrophy, and a ratio of 1 to 3 has been reported (De Wit and Van Gemerden, 1987). The chemotrophic yield of *Thiocapsa* is similar to that found in thiobacilli (Kelly, 1982).

In *Thiocapsa* oxygen has an inhibitory effect on the synthesis of photopigments but not on growth, and prolonged incubation in the presence of oxygen results in completely colorless cells. However, the capacity to synthesize bacteriochlorophyll (Bchl) and carotenoids is retained, even after 10 generations (De Wit and Van Gemerden, 1990a).

It was observed tha the protein yield of *Thiocapsa*, incubated in the light under regimes ranging from 4 h oxic/20 h anoxic to 21 h oxic/3 h anoxic conditions, was similar to that found in fully phototrophically growing cells, despite the fact that the content of BChl was progressively lowered due to lack of synthesis during the oxic periods of increasing length. During incubation at a 21 h oxic/3 h anoxic regime, the specific Bchl content is only 20% of that reached during continuously anoxic conditions. Apparently, this is sufficient because the organism does not grow chemotrophically, as can be judged from the high yield (De Wit and Van Gemerden, 1990a).

Incubations at a regimen 23 h oxic/1 h anoxic, mimicking the environmental conditions faced by individuals growing close to the surface of the mat, revealed a yield intermediate between values obtained during fully phototrophic growth and fully chemotrophic growth. It can therefore be concluded that *Thiocapsa* is capable of simultaneously using its chemotrophic and phototrophic growth potential (Schaub and Van Gemerden, 1993).

In microbial mats, oxygen is present during day times, and absent during nights. Thus, the synthesis of photopigments is hampered during the day due to the presence of oxygen, whereas during the night synthesis of photopigments would be expected not to be possible due to the lack of a source of energy. However, incubation of *Thiocapsa* in a 14 h oxic and light/10 h anoxic and dark regime, resulted in a time-dependent equilibrium in which the yield was the same as observed during continuously light and anoxic incubation conditions (De Wit and Van Gemerden, 1990b). During the oxic–light periods, synthesis of photopigments was not observed: this is illustrated by the fact that the time course of the Bchl concentration is virtually identical to the theoretical washout rate of BChl (i.e., zero rates of biosynthesis and degradation). Thus, the decrease in the concentration of photopigments was entirely due to the dilution of the culture with fresh medium. However, in the anoxic–dark periods, BChl was synthesized. This phenomenon enabled *Thiocapsa* to grow phototrophically in the subsequent oxic–light period. The synthesis of BChl and carotenoids in the anoxic–dark periods can be explained by the fact that during the preceding oxic–light period, glycogen had been accumulated intracellularly. It was calculated that the synthesis of BChl and spirilloxanthin, the major carotenoid in *Thiocapsa*, could be accounted for by the degradation of no more than 17% of the glycogen. Degradation of the remaining glycogen thus could account for the observed growth in the dark (De Wit and Van Gemerden, 1990b).

It appears that with these metabolic capacities the purple sulfur bacterium *Thiocapsa roseopersicina* is able to cope effectively with the encountered chemical and physical conditions. *Thiocapsa* also is able to competitively exclude those purple sulfur bacteria having higher affinities for mutual substrates, such as sulfide. An example of the latter category is *Ectothiorhodospira shaposhnikovii*. This organism, isolated from a microbial mat, outcompetes *Thiocapsa* under constant environmental conditions, but is unable to do so at similar regimes as described above (Van Gemerden and De Wit, 1986).

7.3. Sulfide oxidation by Thiobacillus thioparus

Colorless sulfur bacteria are chemotrophic organisms primarily oxidizing sulfide and other reduced forms of sulfur with oxygen to obtain energy (Table 1, eqs. 2.1 to 2.3). The oxidation of reduced sulfur species also provides reducing equivalents for the reduction of carbon dioxide to cellular carbon (Table 1, eqs. 2.4 to 2.7). The final

product of sulfide oxidation is sulfate, with elemental sulfur, deposited extracellularly, as the principal intermediate.

From the highest serial dilutions of the top 5 mm of the Texel mat a *Thiobacillus thioparus* strain was isolated. This organism shows rapid growth on CO_2, with sulfide, thiosulfate, or DMS as the sole source of energy.

In microbial mats, regardless whether or not an overlap of sulfide and oxygen occurs (see section 6.1), oxidation of sulfide appears to be primarily carried out at low concentrations of oxygen and sulfide. In order to mimic these environmental conditions, chemostat cultures of *T. thioparus* were subjected to conditions ranging from severe sulfide limitation to severe oxygen limitation. The head space of the culture vessel was flushed continuously with air/N_2 mixtures at ratios ranging from 100/0 to 20/80, the total flow being identical in each case. The oxygen concentration in the cultures was monitored with an electrode. Data on sulfur species, oxygen, and protein are shown in Table 2 (Van den Ende and Van Gemerden, 1993).

Applying gas mixtures containing 100–70% air, at which decreasing concentrations of oxygen in the culture were detected, resulted in virtually complete oxidation of sulfide to sulfate. Using gas mixtures containing 60–30% air, resulting in oxygen concentrations below the limit of detection. Sulfide still could not be detected, but the concentration of other sulfur species increased with decreasing oxygen availability. Quantitatively the most important sulfur species was elemental sulfur, followed by thiosulfate, tetrathionate, and polysulfide (chain length unknown). Only after the air in the gas mixture was reduced to 20%, free sulfide could be detected in the culture, albeit at concentrations of <0.04 mmol l^{-1}. Decreasing oxygen availability, results in a decrease in the total rate of electron donor utilization, and, consequently, in a decrease in the concentration of biomass (protein) (Van den Ende and Van Gemerden, 1993).

It is anticipated that a similar partial sulfide oxidation occurs in microbial mats at the oxygen/sulfide interface.

8. Mat building: a joint venture

The organisms in a microbial mat thrive in such close proximity that diffusion becomes an important factor in regulating material transport. Under these conditions organisms mutually will affect each other in a number of ways. Interactions range from cooperation to antagonism, although the over-all effect is very positive.

The expected interactions in microbial mats between cyanobacteria, colorless sulfur bacteria,

TABLE 2

Oxidation of sulfide at various rates of oxygen supply by *Thiobacillus thioparus* strain T5

Air/N_2 in headspace (%)	Oxygen in culture (μmol l^{-1})	Sulfur (mmol l^{-1})	Thiosulfate (mmol S l^{-1})	Tetrathionate (mmol S l^{-1})	Polysulfide (mmol S l^{-1})	Sulfide (mmol l^{-1})	Sulfate (mmol l^{-1})	Protein (mg l^{-1})
100/0	84.8	0.253	0.004	0.034	–	–	6.779	23
90/10	57.8	0.321	0.004	0.032	–	–	6.733	24
80/20	32.0	0.234	0.002	0.037	–	–	6.827	23
70/30	7.6	0.210	0.002	0.033	–	–	6.875	23
60/40	–	0.978	0.126	0.040	0.001	–	5.975	20
50/50	–	1.973	0.314	0.051	0.001	–	4.731	18
40/60	–	3.089	0.416	0.048	0.003	–	3.544	15
30/70	–	4.090	0.932	0.068	0.009	0.001	1.870	12
20/80	–	5.201	1.222	0.146	0.044	0.034	0.493	8

– = not detectable. Chemostat data obtained at a dilution rate of 0.1 h^{-1} (steady state), the maximum specific growth rate (μ_{max}) of *Thiobacillus thioparus* is 0.35 h^{-1}. The concentration of sulfide in the inflowing medium (S_R) was 7.090 mmol l^{-1}. Source: Van den Ende and Van Gemerden (1993).

purple sulfur bacteria, and sulfate-reducing bacteria, are visualized in Fig. 7. Cyanobacteria dominate the top layer, while colorless sulfur bacteria, purple sulfur bacteria, and sulfate-reducing bacteria harbor the underlying layers. Few sulfur bacteria were found at deeper horizons of the Texel mat, and the microbiology of these layers largely remains obscure. In Fig. 7 arrows point to the positively of negatively affected group(s). In the text below, bold numbers in parentheses refer to reactions indicated in Fig. 7. The principal reactions carried out by the dominant groups of microbes are summarized in Table 1.

Factors of crucial importance are the concentrations of oxygen and sulfide, but the presence of other sulfur compounds is relevant as well. Sulfate-reducing bacteria depend on cyanobacteria for carbon- and energy sources (**1**) but at the same time they are slowed down by the oxygen produced by the latter group (**2**). In turn, growth of the cyanobacteria is inhibited by sulfide (**3**). Colorless sulfur bacteria need oxygen and for that reason largely depend on the cyanobacteria (**4**). In the purple sulfur bacteria photopigment synthesis is inhibited by oxygen (**5**), and this may force these organisms to turn to chemotrophy resulting in much lower yields. On the other hand, both types of sulfide-oxidizing microbes are stimulated by excretion products of cyanobacteria (**6**)(**7**). Finally, colorless and purple sulfur bacteria compete for mutual electron donors (**8**). Oxygenic photosynthesis carried out by the cyanobacteria, is the driving force in most microbial mats, but the involvement of other microbial groups is by no means trivial.

In theory, mats harboring no other organisms than cyanobacteria, may continue to grow at the surface of the sediment. Deposition of sand would result in healthy gliding cells to move upwards,

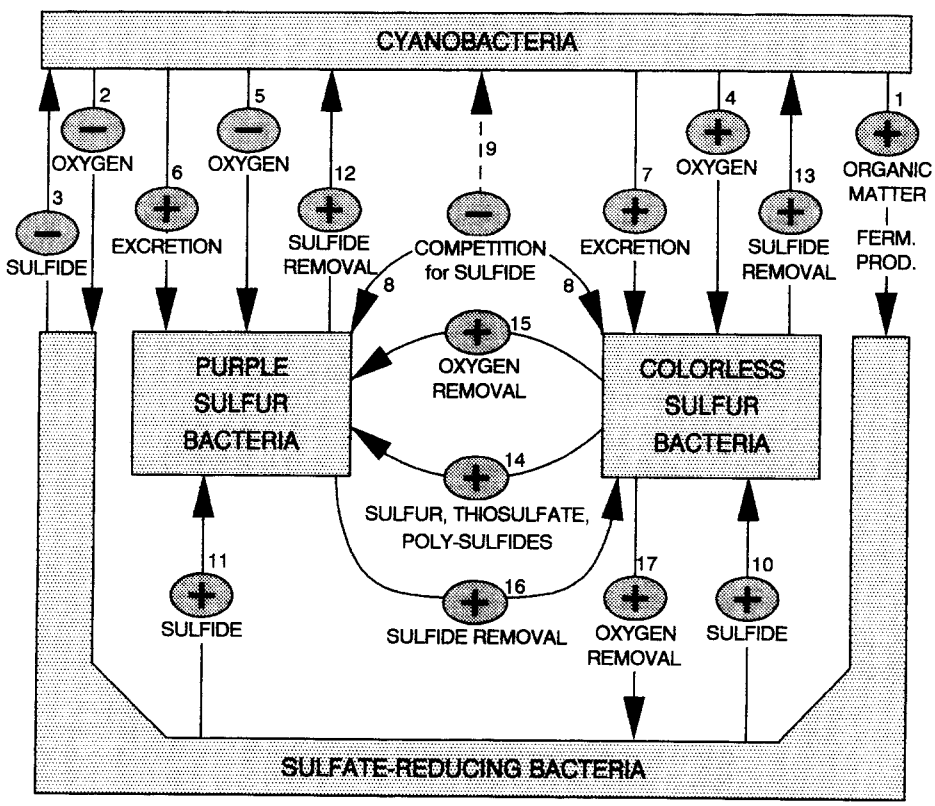

Fig. 7. Interactions between functional groups of microbes in a microbial mat. The top layer is dominated by cyanobacteria, the layer underneath harbors colorless sulfur bacteria, purple sulfur bacteria, and sulfate-reducing bacteria. Arrows point to the group affected, positive and negative interactions are indicated by + and −, respectively. The reactions performed by specific groups of organisms are discussed in the text and have been summarized in Table 1.

leaving behind less vital individuals, which would die after their internal storage resources had been depleted. Oxygenic photosynthesis and aerobic respiration are complementary processes. In theory, sufficient oxygen is produced during the day to allow for complete aerobic degradation of photosynthates and storage compounds during the night. However, due to the small vertical dimensions of microbial mats, a substantial amount of oxygen produced during the day will escape to the overlying water or the atmosphere. Even mats exclusively composed of oxygenic phototrophic organisms can be expected to have an oxygen deficit some time after sunset. For their energy metabolism in the dark, cyanobacteria in microbial mats must rely on fermentation or anaerobic respiration, rather than on aerobic respiration.

In reality, microbial mats are communities composed of phototrophic, chemotrophic and heterotrophic organisms. The organic remains, resulting from cyanobacterial productivity, form an exquisite energy source for other microorganisms, and the presence of aerobically respiring organisms will shorten the time span during which oxygen is available. In the nights, some oxygen will diffuse from the air or overlying water into the mat. However, this process probably is of marginal importance as during the day the high levels of oxygen supersaturation (200–400%) drive much larger oxygen fluxes from the mat to the air. The oxygen deficit in the mat as community is further increased as during the growth season nights are short and days are long.

Excess degradable carbon-left over from previous year(s) or produced nightly by fermentation in the overlying cyanobacteria—in combination with anoxic conditions, result in sulfide production by sulfate-reducing bacteria. The shallow oxygen penetration may slow down sulfate reduction in the very top layers, but continuous sulfide production in deeper layers is inevitable.

For the mat-building *Microcoleus* the ecological relevance of the ability to utilize sulfide as the electron donor in anoxygenic photosynthesis appears to be detoxification rather than growth. Under these conditions growth of cyanobacteria is slow compared to that of purple sulfur bacteria, moreover, sulfide utilization requires induction. More importantly, the affinity of cyanobacteria for sulfide is very low compared to other sulfide-oxidizing microorganisms (Van Gemerden and De Wit, 1986), and when used, thiosulfate is formed (Table 1, eqs. 1.4 to 1.6). For growth, the cyanobacteria rely on the powerful mechanism of oxygenic photosynthesis and not on the oxidation of sulfide (**9**). The bulk of the sulfide is oxidized by colorless and purple sulfur bacteria (Table 1, eqs. 2.1 to 2.8, and 3.1 to 3.5, respectively). Their effective removal of the sulfide, as it diffuses upwards from deeper production sites or is produced in situ, not only supports their own growth (**10**)(**11**), but it is also beneficial to the overlying cyanobacteria (**12**)(**13**).

Apart from their collective effort in sulfide removal, colorless sulfur bacteria and purple sulfur bacteria have little in common and will compete for this reduced sulfur compound (**8**). The extent to what H_2S is oxidized by either group very much depends on the availability of oxygen. With no oxygen available, sulfide will be exclusively oxidized by purple sulfur bacteria, provided light is available. With excess oxygen, virtually all sulfide will be oxidized by colorless sulfur bacteria, despite the fact that purple sulfur bacteria are capable of chemotrophic growth. This is explained by the fact that colorless sulfur bacteria have much higher affinities for sulfide than purple sulfur bacteria (Visscher et al., 1992b). In microbial mats most of the sulfide is oxidized at the oxygen/sulfide interface at subsaturating oxygen concentrations. The sulfide affinity of *Thiocapsa* is insufficiently high to enable this organism to compete successfully with *Thiobacillus*. However, the latter can not fully oxidize all sulfide to sulfate because of oxygen deficiency. The production of somewhat less reduced sulfur compounds by *Thiobacillus* does provide *Thiocapsa* with sufficient electron donors (**14**). As the oxygen is effectively removed by *Thiobacillus* (**15**), pigment synthesis in *Thiocapsa* is no longer inhibited, which allows it to grow phototrophically resulting in maximum yields. The expected result is coexistence between chemotrophically growing *Thiobacillus* and phototrophically growing *Thiocapsa*, operating in concert to remove sulfide, which was indeed observed during oxygen limitation (F. P. van den Ende and H. van

Gemerden, unpubl. data). High oxygen concentrations, although in itself advantageous to thiobacilli (**4**), would inhibit sulfate-reducing bacteria (**2**), resulting in decreased rates of sulfide formation. Utilization of oxygen by thiobacilli thus not only promotes their own growth, but is also advantageous for sulfate reducers (**17**).

Thiocapsa thus benefits from the presence of *Thiobacillus*. It can be argued that vice versa colorless sulfur bacteria benefit from the presence of purple sulfur bacteria. In the absence of the latter, sulfide removal by colorless sulfur bacteria would not be complete due to lack of oxygen. In the absence of purple sulfur bacteria, sulfide would reach the layer of cyanobacteria, possibly resulting in severe inhibition of growth of cyanobacteria and cessation of oxygen production. Bearing in mind that the cyanobacterium *Microcoleus chthonoplastes* is the driving force of these microbial mats, the result would be disastrous. In theory, *Microcoleus* could be replaced by less sulfide sensitive cyanobacteria like *Oscillatoria* sp., but these do not build the characteristic leathery mats which provide physical protection to organisms growing underneath. The solution to this ecological and evolutionary problem is sulfide removal without the participation of oxygen, and that is exactly what purple sulfur bacteria can do (**12**).

In summary, the driving force of microbial mats are the cyanobacteria. However, their continued oxygenic photosynthesis is made possible by the beneficiary effects of colorless and purple sulfur bacteria in the functional removal of the sulfide produced by sulfate-reducing bacteria after oxygen-dependent heterotrophs and fermentative bacteria have degraded the organic compounds originating from lysis and excretion. Colorless and purple sulfur bacteria operate in concert because the former excrete electron donors for the purple sulfur bacteria when oxygen is short in supply. The shortage of oxygen is the consequence of the small vertical dimension of microbial mats, resulting in oxygen escaping to the atmosphere during daytime. Mat building thus is a joint venture of functionally different groups of microbes.

Acknowledgements

I like to thank Rutger de Wit, Pieter T. Visscher, Frank P. van den Ende and Bartholomeus E.M. Schaub for their careful experimental work and for the numerous stimulating discussions on microbial interactions on a millimeter scale. Special thanks go to Jan "Electrode" Beukema for the design and skilful construction of needle sensors. The comments of Rodney A. Herbert, Peter Westbroek and John Parkes on an earlier version of the manuscript are acknowledged.

References

Aizenshtat, Z., Stoler, A., Cohen, Y. and Nielsen, H., 1983. The geochemical sulphur enrichment of recent organic matter by polysulfides in the Solar-Lake. In: M. Bjoroy, P. Albrecht, C. Cornford, K. de Groot, G. Eglington, E. Galimov, D. Leythaeuser, R. Pelet, J. Rullkotter and G. Speers (Editors), Advances in Organic Geochemistry. Wiley, Chicester, pp. 279–288.

Awramik, S.M., 1984. Ancient stromatolites and microbial mats. In: Y. Cohen, R.W. Castenholz and H.O. Halvorson (Editors), Microbial Mats: Stromatolites. Alan Liss, New York, pp. 1–21.

Baas Becking, L.G.M., 1925. Studies on the sulphur bacteria. Anal. Bot., 39: 613–651.

Bak, F. and Cypionka, H., 1987. A novel type of energy metabolism involving fermentation of inorganic sulphur compounds. Nature, 326: 891–892.

Bak, F. and Pfennig, N., 1987. Chemolithotrophic growth of *Desulfovibrio sulfodismutans* sp. nov. by disproportionation of inorganic sulfur compounds. Arch. Microbiol., 147: 184–189.

Bateson, M.M. and Ward, D.M., 1988. Photoexcretion and fate of glycolate in a hot spring cyanobacterial mat. Appl. Environ. Microbiol., 54: 1738–1743.

Bauld, J., 1984. Microbial mats in marginal marine environments: Shark Bay, Western Australia and Spencer Gulf, South Australia. In: Y. Cohen, R.W. Castenholz and H.O. Halvorson (Editors), Microbial Mats: Stromatolites. Alan Liss, New York, pp. 39–58.

Canfield, D.E. and Des Marais, D.J., 1991. Aerobic sulfate reduction in microbial mats. Science, 251: 1471–1473.

Castenholz, R.W., Jørgensen, B.B., D'Amilio, E. and Bauld, J., 1991.Photosynthetic and behavorial versatility of the cyanobacterium *Oscillatoria boryana* in a sulfide-rich microbial mat. FEMS Microbiol. Ecol., 86: 43–58.

Cohen, Y., 1984. Oxygenic photosynthesis, anoxygenic photosynthesis and sulfate reduction in cyanobacterial mats. In: M.J. Klug and C.A. Reddy (Editors), Current Perspectives in Microbial Ecology. ASM, Washington, DC, pp. 435–441.

Cohen, Y., 1989. Photosynthesis in cyanobacterial mats and its relation to the sulfur cycle: a model for microbial sulfur interactions. In: Y. Cohen and E. Rosenberg (Editors), Microbial Mats. Physiological Ecology of Benthic Microbial Communities. ASM, Washington, DC, pp. 22–36.

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

Cohen, Y., Padan, E. and Shilo, M., 1975b. Facultative anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. J. Bacteriol., 123: 855–861.

Cypionka, H., Widdel, F. and Pfennig, N., 1985. Survival of sulfate-reducing bacteria after oxygen stress and growth in sulfate-free oxygen-sulfide gradients. FEMS Microbiol. Ecol., 31: 39–45.

Dacey, J.W.H. and Blough, N., 1987. Hydroxide decomposition of DMSP to DMS. J. Geophys. Res. Lett., 14: 1246–1249.

D'Amelio, E.D., Cohen, Y. and Des Marais, D.J., 1989. Comparative functional ultrastructure of two hypersaline submerged cyanobacterial mats: Guerrero Negro, Baja California Sur, Mexico and Solar Lake, Sinai, Egypt. In: Y. Cohen and E. Rosenberg (Editors), Microbial Mats. Physiological Ecology of Benthic Microbial Communities. ASM, Washington, DC, pp. 97–113.

De Wit, R. and Van Gemerden, H., 1987a. Chemolithotrophic growth of the phototrophic sulfur bacterium *Thiocapsa roseopersicina*. FEMS Microbiol. Ecol., 45: 117–126.

De Wit, R. and Van Gemerden, H., 1987b. Oxidation of sulfide to thiosulfate by *Microcoleus chthonoplastes*. FEMS Microbiol. Ecol., 45: 7–13.

De Wit, R. and Van Gemerden, H., 1988. Interactions between phototrophic bacteria in sediment ecosystems. Hydrobiol. Bull., 22: 135–145.

De Wit, R. and Van Gemerden, H., 1990a. Growth of the phototrophic purple sulfur bacterium *Thiocapsa roseopersicina* under oxic/anoxic regimens in the light. FEMS Microbiol. Ecol., 73: 69–76.

De Wit, R. and Van Gemerden, H., 1990b. Growth and metabolism of the purple sulfur bacterium *Thiocapsa roseopersicina* under combined light/dark and oxic/anoxic regimens. Arch. Microbiol., 154: 459–464.

De Wit, R., Jonkers, H.M., Van den Ende, F.P. and Van Gemerden, H., 1989. In situ fluctuations of oxygen and sulphide in marine microbial sediment ecosystems. Neth. J. Sea Res., 23: 271–281.

De Wit, R., Van Boekel, W.H.M. and Van Gemerden, H., 1988. Growth of the cyanobacterium *Microcoleus chthonoplastes* on sulfide. FEMS Microbiol. Ecol., 53: 203–209.

Dilling, W. and Cypionka, H., 1990. Aerobic respiration in sulfate-reducing bacteria. FEMS Microbiol. Lett., 71: 123–128.

Drobner, E., Huber, H., Wächterhäuser, G., Rose, D. and Stetter, K.O., 1990. Pyrite formation linked with hydrogen evolution under anaerobic conditions. Nature, 346: 742–744.

Eloff, J.N., Steinityz, Y. and Shilo, M., 1976. Photooxidation of cyanobacteria in natural conditions. Appl. Environm. Microbiol., 31: 119–126.

Fenchel, T. and Finlay, B.J., 1990a. Oxygen toxicity, repiration and behavioural responses to oxygen in free-living anaerobic ciliates. J. Gen. Microbiol., 136: 1953–1959.

Fenchel, T. and Finlay, B.J., 1990b. Anaerobic free-living protozoa: growth efficiencies and the structure of anaerobic communities. FEMS Microbiol. Ecol., 74: 269–276.

Fischer, U., 1989. Enzymatic steps and dissimilatory sulfur metabolism by whole cells of anoxyphotobacteria. In: E.S. Saltman and W.J. Cooper (Editors), Biogenic Sulfur in the Environment. (ACS Symp. Ser., 393.) Washington DC, pp. 262–279.

Führböter, A. and Manzenrieder, H., 1987. Biostabilisierung von Sandwatten durch Mikroorganismen. In: G. Gerdes, W.E. Krumbein and H.-E. Reineck (Editors), Porträt einer Insel. Waldemar Kramer, Frankfurt a.M., pp. 123–140.

Garlick, S., Oren, A. and Padan, E., 1977. Occurrence of facultative anoxygenic photosynthesis among filamentous and unicellular cyanobacteria. J. Bacteriol., 129: 623–629.

Ghiorse, W.C., 1988. Microbial reduction of manganese and iron. In: A.J.B. Zehnder (Editor), Biology of Anaerobic Microorganisms. Wiley, New York, pp. 305–331.

Grant, J. and Gust, G., 1987. Prediction of coastal sediment stability from photopigment content of mats of purple sulphur bacteria. Nature, 330: 244–246.

Hastings, D. and Emerson, S., 1988. Sulfate reduction in the presence of low levels of oxygen in water column of the Cariaco Trench. Limnol. Oceanogr., 33: 391–396.

Howarth, R.W., 1979. Pyrite: its rapid formation in a salt marsh and its importance in ecosystem metabolism. Science, 203: 49–51.

Javor, B.J. and Castenholz, R.W., 1981. Laminated microbial mats, Laguna Guerrero Negro, Mexico. Geomicrobiol. J., 2: 237–273.

Javor, B.J. and Castenholz, R.W., 1984. Invertebrate grazers of microbial mats. In: Y. Cohen, R.W. Castenholz and H.O. Halvorson (Editors), Microbial Mats: Stromatolites. Alan Liss, New York, pp. 85–94.

Jensen, J. and Revsbech, N.P., 1989. Photosynthesis and respiration of a diatom biofilm cultured in a new gradient growth chamber. FEMS Microbiol. Ecol., 62: 29–38.

Jørgensen, B.B., 1977a. Bacterial sulfate reduction within reduced microniches of oxidized marine sediments. Mar. Biol., 41: 7–17.

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

Jørgensen, B.B., 1978. A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. I. Measurement with radiotracer techniques. Biogeochem. J., 1: 11–27.

Jørgensen, B.B., 1988. Ecology of the sulphur cycle: oxidative pathways in sediments. In: J.A. Cole and S.J. Ferguson (Editors), The Nitrogen and Sulphur Cycles. Cambridge Univ. Press, pp. 31–63.

Jørgensen, B.B., 1989. Light penetration, absorption and action spectra in cyanobacterial mats. In: Y. Cohen and E. Rosenberg (Editors), Microbial Mats: Physiological Ecology of Benthic Microbial Communities. ASM, Washington, DC, pp. 123–137.

Jørgensen, B.B., 1990. A thiosulfate shunt in the sulfur cycle of marine sediments. Science, 249: 152–154.

Jørgensen, B.B. and Bak, F., 1991. Pathways and microbiology of thiosulfate transformations and sulfate reduction in a marine sediment (Kattegat, Denmark). Appl. Environ. Microbiol., 57: 847–856.

Jørgensen, B.B. and Cohen, Y., 1977. Solar Lake (Sinai). 5. The sulfur cycle of the benthic cyanobacterial mats. Limnol. Oceanogr., 22: 657–666.

Jørgensen, B.B. and Des Marais, D.J., 1986. A simple fiberoptic microprobe for high resolution light measurements: application in marine sediments. Limnol. Oceanogr., 31: 1376–1383.

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

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

Jørgensen, B.B., Revsbech, N.P. and Cohen, Y., 1983. Photosynthesis and structure of benthic microbial mats: microelectrode and SEM studies of four cyanobacterial communities. Limnol. Oceanogr., 28: 1075–1093.

Kelly, D.P., 1982. Biochemistry of the chemolithotrophic oxidation of inorganic sulphur. Philos. Trans. R. Soc. London, Ser. B, 298: 499–529.

Kelly, D.P., 1988. Oxidation of sulphur compounds. In: J.A. Cole and S.J. Ferguson (Editors), The Nitrogen and Sulphur Cycles. Cambridge Univ. Press, pp. 65–98.

Kiene, R.P. and Capone, D.G., 1988. Microbial transformations of methylated sulfur compounds in anoxic salt marsh sediments. Microbial Ecol., 15: 275–291.

Kiene, R.P. and Taylor, B.F., 1988a. Biotransformations of organosulphur compounds in sediments via 3-mercaptopropionate. Nature, 332: 148–150.

Kiene, R.P. and Taylor, B.F., 1988b. Demethylation of dimethylsulfoniopropionate and production of thiols in anoxic marine sediments. Appl. Environ. Microbiol., 54: 2208–2212.

Kiene, R.P. and Visscher, P.T., 1987. Production and fate of methylated sulfur compounds from methionine and dimethylsulfoniopropionate in anoxic salt marsh sediments. Appl. Environ. Microbiol., 53: 2426–2434.

Kiene, R.P., K.D. Malloy and Taylor, B.F., 1990. Sulfur-containing amino acids as precursors of thiols in anoxic coastal sediments. Appl. Environ. Microbiol., 56: 156–161.

King, G.M., 1983. Sulfate reduction in Georgia salt marsh soils: an evaluation of pyrite formation by use of ^{35}S and ^{55}Fe tracers. Limnol. Oceanogr., 28: 987–995.

Kohnen, M.E.L., Sinnenghe Damsté, J.S., Ten Haven, H.L. and De Leeuw, J.W., 1989. Early incorporation of polysulfides in sedimentary organic matter. Nature, 341: 640–641.

Lassen, C., Ploug, H. and Jørgensen, B.B., 1992. A fibre-optic scalar irradiance microsensor: application for spectral light measurements in sediments. FEMS Microbiol. Ecol., 86: 247–254.

Lord, C.J., III and Church, T.M., 1983. The geochemistry of salt marshes: sedimentary ion diffusion, sulfate reduction and pyritization. Geochim. Cosmochim. Acta, 47: 1381–1391.

Luther, G.W., III, 1991. Pyrite synthesis via polysulfide compounds. Geochim. Cosmochim. Acta, 55: 2839–2851.

Luther, G.W., III and Church, T.M., 1988. Seasonal cycling of sulfur and iron in porewaters of a Delaware salt marsh. Mar. Chem., 23: 295–309.

Luther, G.W., III, Church, T.M., Scudlark, J.R. and Cosman, M., 1986. Inorganic and organic sulfur cycling in salt-marsh pore waters. Science, 232: 746–749.

Margulis, L., Barghoorn, E.S., Ashendorf, D., Banerjee, S., Chase, D., Francis, S., Giovannoni, S. and Stolz, J., 1980. The microbial community in the layered sediments at Laguna Figueroa, Baja California, Mexio: does it have Precambrian analogues? Precambrian Res., 11: 93–123.

Marschall, C., Frenzel, P. and Cypionka, H., 1993. Influence of oxygen on sulfate reduction and growth of sulfate-reducing bacteria. Arch. Microbiol., 159: 168–173.

Marshall, K.C., 1989. Cyanobacterial–heterotrophic bacterial interactions. In: Y. Cohen and E. Rosenberg (Editors), Microbial Mats: Physiological Ecology of Benthic Microbial Communities. ASM, Washington, DC, pp. 239–245.

Nicholson, J.A., Stolz, J.F. and Pierson, B.K., 1987. Structure of a microbial mat at Great Sippewissett Marsh, Cape Cod, Masschusetts. FEMS Microbiol. Ecol., 45: 343–364.

Oren, A. and Shilo, M., 1979. Anaerobic heterotrophic dark metabolism in the cyanobacterium *Oscillatoris limnetica*: sulfur respiration and lactate fermentation. Arch. Microbiol., 122: 77–84.

Padan, E., 1979. Impact of facultatively anaerobic phototrophic metabolism on ecology of cyanobacteria (blue-green algae). In: M. Alexander (Editor), Adv. Microbial Ecol. Plenum, New York, pp. 1–48.

Palmisano, A.C., Cronin, S.E., D'Amelio, E.D., Munoz, E. and Des Marais, D.J., 1989. Distribution and survival of lipophilic pigmnets in a laminated microbial mat community near Guerrero Negro, Mexico. In: Y. Cohen and E. Rosenberg (Editors), Microbial Mats: Physiological Ecology of Benthic Microbial Communities. ASM, Washington, DC, pp. 138–152.

Pierson, B.K., Oesterle, A. and Murphy, G.L., 1987. Pigments, light penetration and photosynthetic activity in the multilayered microbial mats of Great Sippewissett Salt Marsh, Masschusetts. FEMS Microbiol. Ecol., 45: 365–376.

Reed, R.H., 1983. Measurement and osmotic significance of ß-dimethylsulfoniopropionate in marine microalgae. Mar. Biol. Lett., 4: 173–178.

Revsbech, N.P. and Jørgensen, B.B., 1983. Photosynthesis of benthic microflora measured with high spatial resolution by the oxygen microprofile method: capabilities and limitaions of the method. Limnol. Oceanogr., 28: 749–756.

Revsbech, N.P. and Jørgensen, B.B., 1986. Microelectrodes: their use in microbial ecology. In: K.C. Marshall (Editor), Advances in Microbial Ecology, 9. Plenum, New York, pp. 293–352.

Revsbech, N.P. and Ward, D.M., 1984. Microelectrode studies of interstitial water chemistry and photosynthetic activity in a hot spring microbial mat. Appl. Environ. Microbiol., 48: 270–275.

Revsbech, N.P., Jørgensen, B.B., Blackburn, T.H. and Cohen, Y., 1983. Microelectrode studies of the photosynthesis and O_2, H_2S and pH profiles in a microbial mat. Limnol. Oceanogr., 28: 1062–1074.

Revsbech, N.P., Christensen, P.B. and Nielsen, L.P., 1989. Microelectrode analysis of photosynthetic and respiratory processes in microbial mats. In: Y. Cohen and E. Rosenberg (Editors), Microbial Mats: Physiological Ecology of Benthic Microbial Communities. ASM, Washington, DC, pp. 153–162.

Schaub, B.E.M. and Van Gemerden, H., 1993. Simultaneous phototrophic and chemotrophic growth in the purple sulfur bacterium *Thiocapsa roseopersicina*. FEMS Microbiol. Ecol., in press.

Skyring, G.W., Lynch, R.M. and Smith, G.D., 1989. Quantitative relationship between carbon, hydrogen and

sulfur metabolism in cyanobacterial mats. In: Y. Cohen and E. Rosenberg (Editors), Microbial Mats: Physiological Ecology of Benthic Microbial Communities. ASM, Washington, DC, pp. 170–179.

Skyring, G.W., 1984. Sulfate reduction in marine sediments associated with cyanobacterial mats in Australia. In: Y. Cohen, R.W. Castenholz and H.O. Halvorson (Editors), Microbial Mats: Stromatilites. Liss, New York, pp. 265–275.

Smith, N.A. and Kelly, D.P., 1988. Isolation and physiological characterization of autotrophic sulphur bacteria oxidizing dimethyl disulphide as sole source of energy. J. Gen. Microbiol., 134: 1407–1417.

Sørensen, J. and Jørgensen, B.B., 1987. Early diagenesis in sediments from Danish coastal waters: microbial activity and Mn–Fe–S geochemistry. Geochim. Cosmochim. Acta, 51: 1583–1590.

Stal, L.J., 1991. The metabolic versatility of the mat-building cyanobacteria *Microcoleus chthonoplastes* and *Oscillatoria limosa* and its ecological significance. Algol. Stud., 64: 453–467

Stal, L.J., Heyer, H., Bekker, S., Villbrandt, M. and Krumbein, W.E., 1989. Aerobic–anaerobic metabolism in the cyanobacterium *Oscillatioria limosa*. In: Y. Cohen and E. Rosenberg (Editors), Microbial Mats. Physiological Ecology of Benthic Microbial Communities. ASM, Washington, DC, pp. 255–276.

Stal, L.J., Van Gemerden, H. and Krumbein, W.E., 1985. Structure and development of a benthic marine microbial mat. FEMS Microbiol. Ecol., 31: 111–125.

Stefels, J. and Van Boekel, W.H.M., 1993. Production of DMS from dissolved DMSP in axenic cultures of the marine phytoplankton species *Phaeocystis* sp. Mar. Ecol. Progr. Ser., in press.

Steinmetz, M.A. and Fischer, U., 1982. Cytochromes of the green sulfur bacterium *Chlorobium vibrioforme* f. *thiosulfatophilum*. Purification, characterization and sulfur metabolism. Arch. Microbiol., 131: 19–26.

Steudel, R., Holdt, G., Visscher, P.T. and Van Gemerden, H., 1990. Search for polythionates in cultures of *Chromatium vinosum* after sulfide incubation. Arch. Microbiol., 153: 432–437.

Then, J. and Trüper, H.G., 1981. The role of thiosulfate in sulfur metabolism of *Rhodopseudomonas globiformis*. Arch. Microbiol., 130: 143–146.

Then, J. and Trüper, H.G., 1983. Sulfide oxidation in *Ectothiorhodospira abdelmalekii*. Evidence for the catalytic role of cytochrome c-551. Arch. Microbiol., 135: 254–258.

Trüper, H.G., 1984. Microorganisms and the sulfur cycle. In: A. Müller and B. Krebs (Editors). Sulfur. Its Significance for Chemistry, for the Geo-, Bio- and Cosmosphere and Technology. Elsevier, Amsterdam, pp. 367–382.

Turner, S.M., Malin, G., Liss, P.S., Harbour, D.S. and Holligan, P.M., 1988. The seasonal variation of dimethylsulfoniopropionate concentrations in near-shore waters. Limnol. Oceanogr., 33: 364–375.

Vairavamurthy, A. and Mopper, K., 1987. Geochemical formation of organosulphur compounds (thiols) by addition of H_2S to sedimentary organic matter. Nature, 329: 623–625.

Vairavamurthy, A. and Mopper, K., 1989. Mechanistic studies of organosulfur (thiol) formation in marine sediments. In: E.S. Saltman and W.J. Cooper (Editors), Biogenic Sulfur in the Environment. (ACS Symp. Ser., 393.) ACS, Washington, DC, pp. 231–242.

Vairavamurthy, A., Mopper, K. and Taylor, B.F., 1992. Occurrence of particle-bound polysulfides and significance of their reaction with organic matters in marine sediments. Geophys. Res. Lett., 19: 2043–2046.

Van den Ende, F.P. and Van Gemerden, H., 1993. Sulfide oxidation under oxygen limitation by a *Thiobacillus thioparus* isolated from a marine microbial mat. FEMS Microbiol. Ecol., in press.

Van Gemerden, H., 1968. On the ATP generation of *Chromatium* in darkness. Arch. Mikrobiol., 64: 118–124.

Van Gemerden, H., 1990. Immobilized anoxygenic phototrophic bacteria in tidal aereas. In: J. de Bont, J. Visser and J. Tramper (Editors), Physiology of Immobilized Cells. Elsevier, Amsterdam, pp. 37–48.

Van Gemerden, H. and De Wit, R., 1986. Strategies of phototrophic bacteria in sulphide-containing environments. In: R.A. Herbert and G.A. Codd (Editors), Microbes in Extreme Environments. Academic Press, London, pp. 111–127.

Van Gemerden, H., Tughan, C.S., De Wit, R. and Herbert, R.A., 1989. Laminated microbial ecosystems on sheltered beaches in Scapa Flow, Orkney Islands. FEMS Microbiol. Ecol., 62: 87–102.

Visscher, P.T., 1992. Microbial sulfur cycling in laminated marine ecosystems. Ph.D. Thesis, Univ. Groningen.

Visscher, P.T. and Van Gemerden, H., 1988. Growth of *Chlorobium limicola* f. *thiosulfatophilum* on polysulfides. In: J.M. Olson, J.G. Ormerod, J. Amesz, E. Stackebrandt and H.G. Trüper (Editors), Green Photosynthetic Bacteria. Plenum Press, New York, pp. 287–295.

Visscher, P.T. and Van Gemerden, H., 1991a. Photoautotrophic growth of *Thiocapsa roseopersicina* on dimethyl sulfide. FEMS Microbiol. Lett., 81: 247–250.

Visscher, P.T. and Van Gemerden, H., 1991b. Production and consumption of dimethylsulfoniopropionate in marine microbial mats. Appl. Environ. Microbiol., 57: 3247–3242.

Visscher, P.T. and Van Gemerden, H., 1993. Sulfur cycling in laminated marine microbial ecosystems. In: R.S. Oremland (Editor), Biogechemistry of Global Change: Radiatively Active Trace Gases. Chapman and Hall, New York, pp. 672–690.

Visscher, P.T., Nijburg, J.W. and Van Gemerden, H., 1990. Polysulfide utilization by *Thiocapsa roseopersicina*. Arch Microbiol., 155: 75–81.

Visscher, P.T., Quist, P. and Van Gemerden, H., 1991. Methylated sulfur compounds in microbial mats: in situ concentrations and metabolism by a colorless sulfur bacterium. Appl. Environ. Microbiol., 57: 1758–1763.

Visscher, P.T., Prins, R.A. and Van Gemerden, H., 1992a. Rates of sulfate reduction and thiosulfate consumption in a marine microbial mat. FEMS Microbiol. Ecol., 86: 283–294.

Visscher, P.T., Van den Ende, F.P., Schaub, B.E.M. and Van Gemerden, H., 1992b. Competition between anoxygenic phototrophic bacteria and colorless sulfur bacteria in a microbial mat. FEMS Microbiol. Ecol., 101: 51–58.

Walter, M.R., 1976. Stromatolites. (Developments in Sedimentology, 20.) Elsevier, Amsterdam, 790 pp.

Ward, D.M., Weller, R., Shiea, J., Castenholz, R.W. and

Cohen, Y., 1989. Hot spring microbial mats: anoxygenic and oxygenic mats of possible evolutionary significance. In: Y. Cohen and E. Rosenberg (Editors), Microbial Mats: Physiological Ecology of Benthic Microbial Communities. ASM, Washington, DC, pp. 3–15.

Zeyer, J., Eicher, P., Wakeham, S.G. and Schwarzenbach, R.P., 1987. Oxidation of dimethyl sulfide to dimethyl sulfoxide by phototrophic purple bacteria. Appl. Environ. Microbiol., 53: 2026–2032.