Vol. 128: 161-170, 1995 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published November 23

Mats of colourless sulphur bacteria. I. Major microbial processes

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ABSTRACT: Mats of colourless sulphur bacteria from 2 marine sediments were studied with respect to sulphide and oxygen fluxes, rates of key microbial processes and spatial and temporal heterogeneity. In a relatively protected habitat dominated by *Beggiatoa* species, about 70% of the sediment O_2-consumption could be accounted for by the oxidation of S^{2-} to SO_4^{2-}, about 15% by food chains fuelled by chemoautotrophic production, while the remainder was due to aerobic processes not directly associated with the sulphur cycle. In this sediment about 85% of the allochthonous and autochthonous (phototrophic and chemotrophic production) organic carbon was mineralised anaerobically. The other habitat was a shallow bay which is often subject to wave-induced transport and erosion of the sediment. From autumn to spring, sediment surface communities of colourless sulphur bacteria were patchy in time and space and they represented different successional stages with respect to the composition of the biota and to process rates. Here the relative significance of chemoautotrophic sulphide oxidation was highly variable. During summer the surface microbial communities of this sediment were dominated by phototrophs and the colourless sulphur bacteria constituted a less important component.

KEY WORDS: Microbial mats · Chemoautotrophic production · Colourless sulphur bacteria · Oxic-anoxic interphase

INTRODUCTION

Dense layers of colourless sulphur bacteria may form conspicuous white patches ranging in size from a few millimetres to several metres diameter on the surface of marine sediments. The phenomenon is known from many different benthic habitats including deep sea and shallow hydrothermal vents (Powell et al. 1983, Karl 1987, Jannasch et al. 1989), below productive upwelling areas (Fossing et al. 1995, Gallardo et al. 1995), and permanently or occasionally in various productive shallow water areas (Fenchel 1969, Ankar & Jansson 1973, Jørgensen 1977b, Juniper & Brinkhurst 1986). These communities are dominated by large bacteria which can be recognised morphologically and include filamentous and colonial forms such as *Beggiatoa* and *Thioploca* as well as unicellular forms such as *Thiobacillus*, *Thiovulum*, *Thiospira* and *Macromonas* (e.g. La Rivière & Schmidt 1992); in reflected light these bacteria appear white due to inclusions of elemental sulphur. The organisms use sulphide as substrate and oxidise it to elemental sulphur or to sulphate; excepting the case of geothermal vents, the sulphide derives from dissimilatory sulphate reduction in the underlying anaerobic sediment.

In sediments with a 'suboxic zone' (that is, a micro- or anaerobic zone without detectable free sulphide), microbial sulphide oxidation is not confined to a narrow zone (Jørgensen 1977a). This may also apply to geothermal vents where advection creates centimetre-thick zones in which sulphide and oxygen coexist (Jannasch et al. 1989, P. Dando pers. comm.). However, when low oxygen and low sulphide concentrations coexist within the chemocline and when the vertical fluxes of these compounds depend only on molecular diffusion, the sulphur bacteria will form distinct 200 to 600 µm thick layers and the metabolic activity of the bacteria maintains extremely steep gradients of sulphide and oxygen (Jørgensen & Revsbech 1983, Nelson et al. 1986b). If the zone of overlap between sulphide and oxygen is situated at (or above) the sedi-

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ment surface, the bacterial mats become visible. In sediments with photosynthetic activity the layer of white sulphur bacteria (together with the associated biota) performs diurnal vertical migrations and it appears on the surface only during darkness; in the light it is found beneath a layer of phototrophic microorganisms (e.g. Fenchel 1969, Garcia-Pichel et al. 1994, Fenchel & Bernard 1995).

Many aspects of these bacteria including their diversity, metabolism, growth yield, and chemosensory motile behaviour have previously been studied in detail (e.g. Jørgensen & Revsbech 1983, Møller et al. 1985, Nelson et al. 1986a, b, Kuenen 1989, Nelson 1989, La Rivière & Schmidt 1992, Fenchel 1994). With respect to the role of colourless sulphur bacteria for phagotrophic food chains and the composition of the associated biota, it is mainly exotic habitats (such as deep sea hydrothermal vents) which have drawn attention. In spite of some earlier work (Fauré-Fremiet 1951, Fenchel 1969) shallow water mats of colourless bacteria have mainly been considered as a sign of environmental deterioration and ecological studies have been very limited. Notwithstanding that anthropogenic eutrophication may lead to an expansion of areas covered by such mats, these do represent natural, complex and diverse communities.

The present paper describes the role of mats of colourless sulphur bacteria in the carbon cycling of 2 types of sediments, interactions with other microbial components and the trophic role of chemoautotrophic production. A following paper (Bernard & Fenchel 1995) will describe the biota and successional patterns.

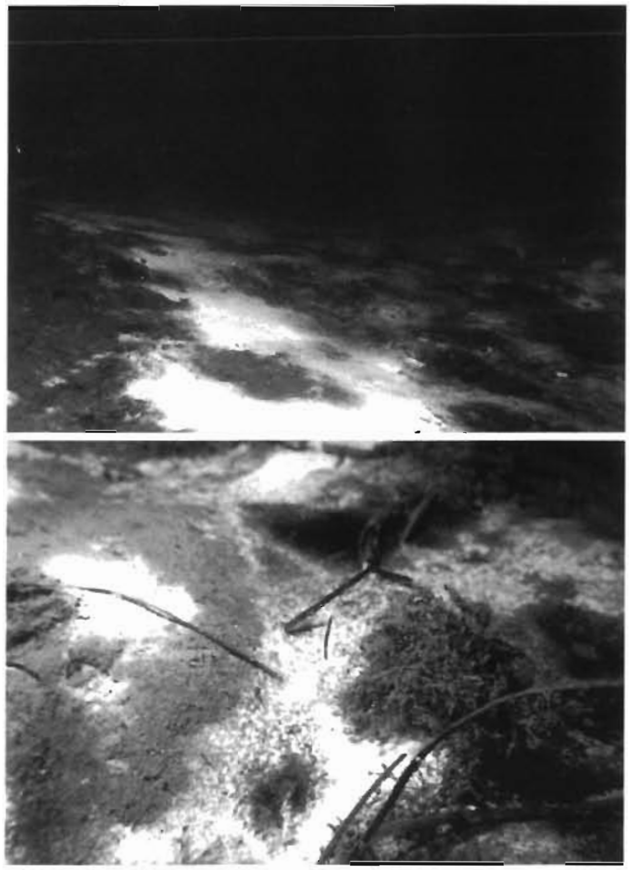

Fig. 1 *Beggiatoa* mats at 6.5 m depth in North Harbour, Helsingør, Denmark. In the close up (below) the eelgrass leaves are about 0.5 cm wide. Photograph by I. Aagaard

MATERIAL AND METHODS

Sediment cores (diameter 5 cm) were collected by SCUBA diving in the outer basin of the North Harbour in Helsingør, Denmark, during the period November 1994 to April 1995. The sediment consists of poorly sorted fine sand with a high content of organic material (~15% of dry wt) mainly in the form of debris of macroalgae and seagrass tissue and with a porosity of about 80%. Large patches of white sulphur bacteria dominated by large *Beggiatoa* filaments and often measuring several metres in diameter are evident throughout the year (Fig. 1); between these white patches the layer of sulphur bacteria is covered by a layer of cyanobacteria and/or diatoms. In periods with strong wind exposure the upper few millimetres of the sediment become fully oxidized and the sulphur bacteria are not visible on the surface; conversely, during long calm periods the water column immediately above the sediment may become anoxic and the water is then cloudy from suspended sulphur bacteria.

Samples were also collected in the innermost part of Nivå Bay about 15 km south of Helsingør at water depths from 0.1 to 0.5 m. The sediment consists of well-sorted sand with a median grain size of about 250 µm; organic contents were not measured. From autumn to spring patches of white sulphur bacteria (usually smaller than 20 cm) are evident except immediately after windy periods when the surface layers are oxidised to a depth of several millimetres to centimetres. The composition of the sulphur bacteria varies according to the age of the patch, the younger ones being dominated by *Thiospira* and/or *Thiovulum* and more mature patches by *Beggiatoa* (see Bernard & Fenchel 1995 for details). During summer, white sulphur bacteria are usually only visible on the surface during night and early morning; in the light they migrate down into microbial mats dominated by diatoms, cyanobacteria and purple sulphur bacteria. The Nivå Bay locality is described in more detail in Fenchel (1969, 1993). Samples were collected by hand by pressing plexiglass tubes (inner diameter 4.2 or 7 cm) into the sediment. Samples were collected from April to July 1994 and in autumn and winter 1994/95. Finally, a few cores were collected in shallow water in the innermost part of the Helsingør North Harbour. In the laboratory the collected cores were kept at room temperature (~20°C) in dim daylight and the overlying water was continuously bubbled with air.

Oxygen, sulphide and pH profiles were measured in the laboratory at ~20°C on the day of sampling or on the following day. As shown below, the O_2^- and S^{2-} gradients were reversibly affected by changes in light intensity and in the intensity of bubbling; otherwise the gradients remained unchanged for at least 4 d in the laboratory. We used O_2-microelectrodes constructed according to Revsbech & Jørgensen (1986) and a picoammeter and a polarisation voltage of −0.75 V. During measurements the electrodes were calibrated at the surface of the bubbled water [100% atmospheric saturation (atm. sat.)] and about 1 mm below the sediment surface (0%). For pH measurements we used a 'needle electrode' (MI-407, Microelectrodes, Inc., Londonderry, NH, USA) together with a pH meter (Radiometer, Copenhagen). Sulphide electrodes were made by etching the end of thin silver wire with nitric acid to a diameter of 50 to 100 µm. The wire was then mounted in glass capillaries with araldite and the exposed silver tip was coated electrolytically with Ag_2S. Potentials were read against a calomel electrode using a pH meter. The electrodes were initially calibrated in sulphide solutions and total sulphide was later calculated from the measured potential and corresponding measurements of pH. Total sulphide (S^{2-} + HS^- + H_2S) is referred to as 'sulphide' or S^{2-} in the following. Oxygen will be expressed as P_{O_2} (% atmospheric pressure; 100% atm. sat. = 21.2 kPa), but for flux estimates the values were converted to molar concentrations on the basis of salinity and temperature. All other concentrations are expressed as mol l^{-1}.

The profiles were measured with the electrodes mounted in a micromanipulator and measurements were taken at depth intervals of 50 or 100 µm. Zero depth was determined as the point where the electrode tips just touched the surface of the sediment or of the bacterial mat as observed with a dissection microscope. Typical results are shown in Figs. 2 (~600 µm thick *Beggiatoa* mat) and 3 (~200 µm thick *Thiovulum* film). Flux was estimated as the slope of the linear part of the profile, assuming that linearity reflects that the substance is conservative in this region and that diffusion coefficients are constant with depth. Flux can then be calculated from Fick's law as $J = D\frac{dC}{dz}$ where D represents diffusion coefficients (taken from Cussler 1989), C is concentration and z is depth. Since the slope of O_2 gradients did not change when passing from the water and into the mat (Fig. 2) and since porosity of the bacterial mat is at least ~90% (see Bernard & Fenchel 1995), we did not correct for porosity in the superficial <1 mm top of the sediment. The effect of the vertically descending electrode on the diffusive boundary layer above the sediment (and hence

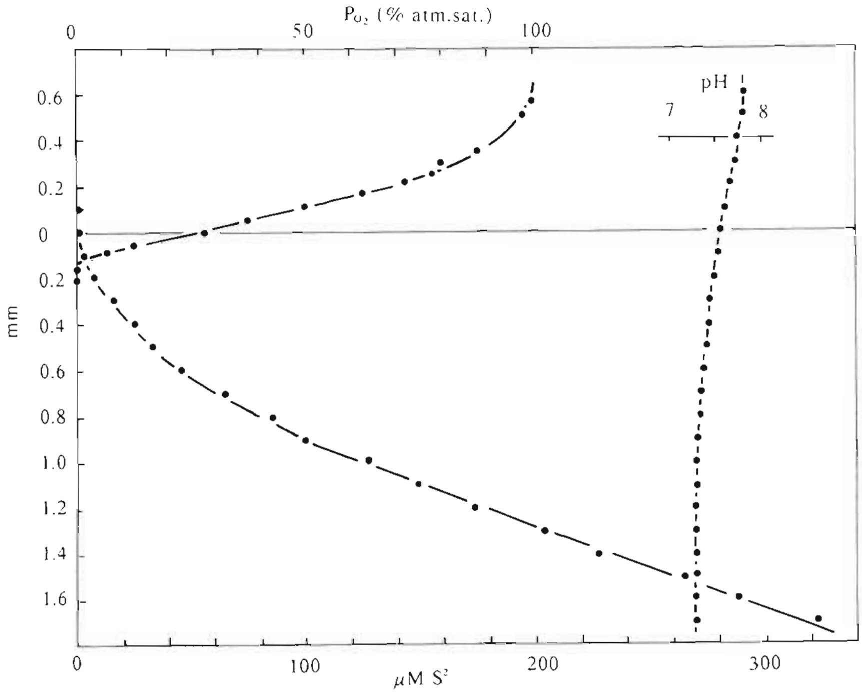

Fig. 2. O_2^-, S^{2-} and pH profiles in a ~600 µm thick *Beggiatoa* mat

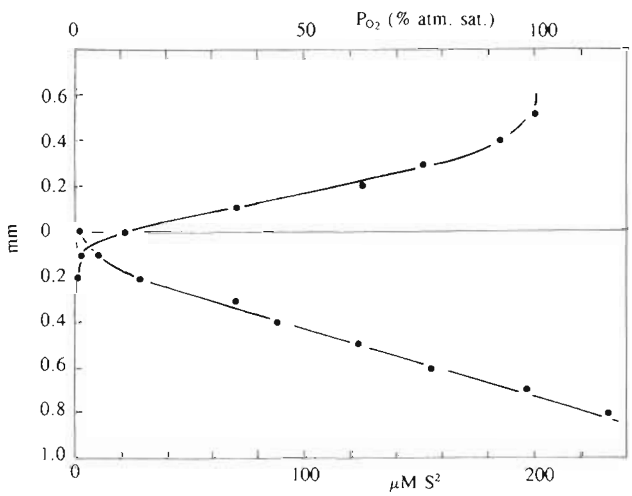

Fig. 3. O_2^- and S^{2-} profiles in a sediment surface (Nivå Bay, Denmark) with a ~200 µm thick *Thiovulum* film

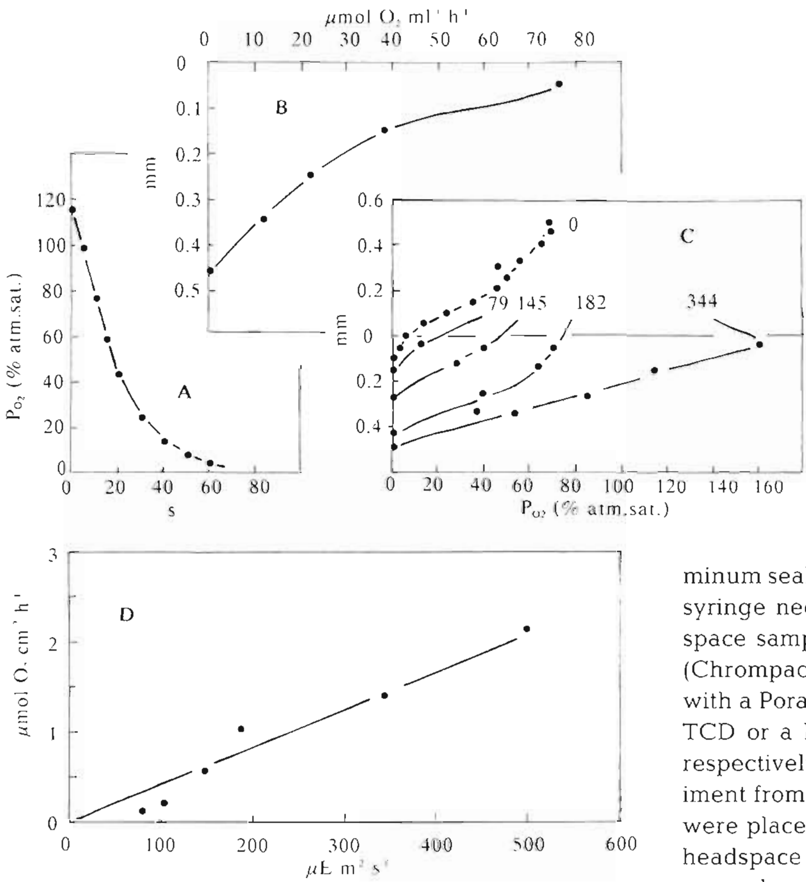

Fig. 4. Oxygenic photosynthesis in a *Beggiatoa* mat. (A) Decrease in P_{O_2} at a depth of 200 µm after light had been turned off (initial surface illumination: 344 µE m^{-2} s^{-1}); the initial slope is the photosynthetic rate at this depth. (B) Photosynthetic rate as function of depth. (C) Steady state O_2^- profiles at different light intensities (expressed as µE m^{-2} s^{-1}). (D) Photosynthetic rate per unit area as a function of surface illumination

on the O_2 profile) demonstrated by Glud et al. (1994) was ignored for technical reasons (it was not possible to introduce the electrodes from beneath). For crude estimates of CO_2, CH_4 and SO_4^{2-} fluxes (see below) we did compensate for porosity following the empirical equation of Rasmussen & Jørgensen (1992). Photosynthetic rates were measured following the technique of Revsbech et al. (1981). Briefly, O_2 electrodes were placed at a given depth in a core which was exposed with a known surface light intensity for a sufficiently long period (~15 min) to reach a steady state O_2 tension. The light was then turned off and the initial linear decrease in O_2 tension was then a measure of photosynthetic rate at the position of the electrode tip. Repeating this for several depths and integrating the resulting curve yields an estimate of the photosynthetic rate per unit area (Fig. 4)

To measure dissolved CO_2, CH_4 and SO_4^{2-}, 1 ml interstitial water was drawn from the side of cores through 1 mm holes filled with silicone rubber at 0.5 or 1 cm intervals. Sulphate was determined on filtered samples by turbidometry following precipitation with $BaCl_2$ (American Public Health Association 1975). For CH_4 and CO_2 measurements, samples were placed in 15 ml serum bottles with rubber stoppers and aluminum seals and 1 ml 0.01 N HCl was added through a syringe needle. After vigorous shaking, 100 µl headspace samples were injected in a gas chromatograph (Chrompack CP9000, Middelburg, The Netherlands) with a Porapak Q column with N_2 as carrier and with a TCD or a FID detector for measuring CO_2 and CH_4, respectively. For measuring methanogenesis 1 ml sediment from different depths and 1 ml O_2-free seawater were placed in 15 ml stoppered serum bottles and the headspace was flushed with O_2-free N_2. The bottles were placed on a shaking table and after 1 h the headspace was again flushed with N_2 in order to remove residual CH_4. Thereafter 100 µl gas samples were taken at 1 h intervals and analysed for CH_4 and the linear increase with time was used as a measure of methanogenesis.

RESULTS AND DISCUSSION

Spatial and temporal heterogeneity

Effect of temperature

Since measurements were otherwise all carried out at ~20°C, we measured O_2 profiles on 3 cores collected simultaneously in the harbour locality and kept for 20 h at 4, 8 and 20°C, respectively. This yielded a Q_{10} of about 1.8 for this range. This allowed for a crude conversion of measured rates to temperatures measured in the field.

Effect of turbulence, advection and P_{O_2} of the overlying water

As previously demonstrated by Jørgensen & Des Marais (1990), O_2 uptake of sediments with a high respiration rate is influenced by advective and turbulent

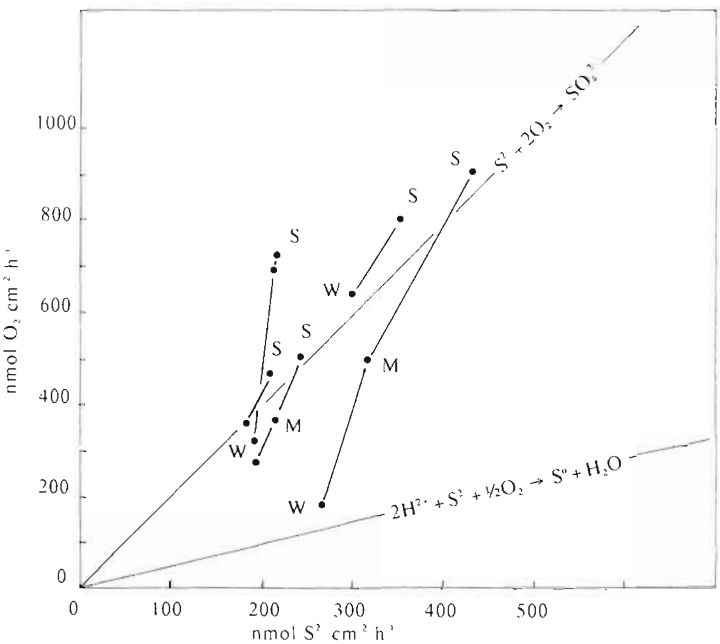

Fig. 5. Fluxes of S^{2-} and O_2 of *Beggiatoa* mats as a function of turbulence [intensity of bubbling: (S) strong, (M) medium, (W) weak] in the overlying water column

Bay samples (see Fig 8). When samples were collected, spots with conspicuous covering of white sulphur bacteria were chosen. Whether the sulphur bacteria occur at the surface, however, depends not only on the intensity of sulphide flux from the underlying sediment, but also on the turbulence of the overlying water; collection on very calm days could therefore result in samples with a relatively low O_2 and S^{2-} uptake rates as compared to samples from more windy periods.

Spatial heterogeneity

Spatial heterogeneity was especially evident in Nivå Bay. Fig. 6 shows the O_2 isopleths along a 5 mm transect including a stretch of about 2 mm long where colourless sulphur bacteria were evident on or slightly above the surface. It also shows that the O_2 flux along the transect varied by a factor of almost 3. The reason for this was probably the heterogenous distribution of decomposing organic material in the sediment (there were no signs of larger burrowing animals in the immediate vicinity of the transect). This clearly shows that fluxes of O_2 and of S^{2-} measured at one point cannot be taken as an average value for a finite area of the sediment

water movement since this affects the thickness of the diffusive boundary layer, which again limits the flux of O_2 from the water. This effect was studied in cores from the harbour by changing the (unquantified) intensity of air bubbling in the overlying water (Fig. 5). When the bubbling was decreased to a low intensity, the oxic-anoxic boundary layer migrated upwards by up to 400 µm (sometimes ending 200 to 300 µm above the surface) within minutes. The *Beggiatoa* filaments responded by extending up into the water so that the mat achieved a woolly appearance. At the same time the O_2 flux decreased. It is apparent from Fig. 5 that the S^{2-} flux did not decrease accordingly so that the O_2 flux/S^{2-} flux ratio decreased with decreasing O_2-availability. The explanation is probably that the O_2-limited bacteria preferably oxidise S^{2-} incompletely to S^0 and so store elemental sulphur. In mats dominated by free-swimming sulphur bacteria (such as *Thiovulum*) the cells are capable of following the O_2 gradients up into the water column (Fenchel 1994). A similar change in the ratio of S^{2-} and O_2 with changing water flow is therefore not expected, but this was not investigated.

For all measurements shown in Fig. 8 a vigorous bubbling was used, but since this could not be standardised very well the effect explains some of the variation in O_2 flux estimates. The effect may also have contributed indirectly to the variance of O_2-uptake, especially evident in the case of the Nivå

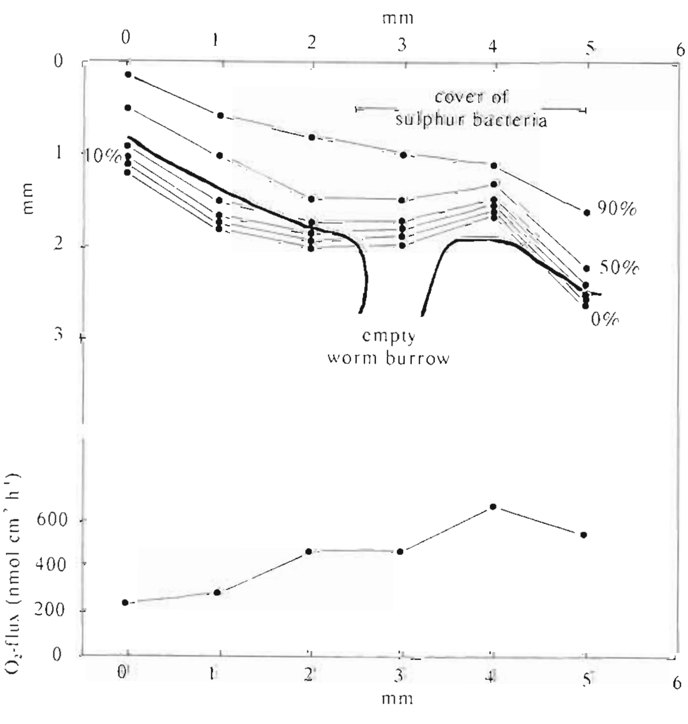

Fig. 6. O_2 isopleths and O_2 flux along a 5 mm long transect of the surface of a sediment (Nivå Bay)

surface. In addition, and as previously observed by Jørgensen & Des Marais (1990), the O_2 isopleths tend to trace the irregular topography of the sediment surface; this results in a systematic underestimation of the sediment O_2 uptake on an areal basis if it is calculated from O_2 profiles.

An additional cause of heterogeneity of the Nivå Bay samples is that they derive from patches which undergo successional changes. Occasional exposure to waves and currents results in sediment transport and erosion; the surface layers of the sediment are then oxidised and at the same time fresh organic material is buried. In following calm periods sediment patches with a higher amount of buried organic material and thus a more intense sulphate reduction develop a cover of colourless sulphur bacteria; these mat communities undergo characteristic successional stages over a period of 1 to 2 wk. This is described in more detail in Bernard & Fenchel (1995); see also Fenchel (1993).

Major microbial processes and the role of chemoautotrophic production

Fig. 7 shows an example of concentration profiles of S^{2-}, SO_4^{2-}, CO_2 and CH_4 as well as a profile of the rate of methanogenesis to a depth of 14 cm in the 6.5 m harbour site. The concentration gradients can only be used for a very crude estimate of process rates due to vertical heterogeneity and because over distances of several centimetres the gradients may not represent a steady state situation. However, 3 measured gradients of CO_2 in the upper centimetres of the sediment corresponded to a flux of 600 to 800 nmol CO_2 cm^{-2} h^{-1}, which is consistent with estimates of sulphate reduction rates of 300 to 400 nmol cm^{-2} h^{-1} (cf. Fig. 8), provided that sulphate reduction is the dominating terminal mineralisation process. There was a small concentration peak of CH_4 and of the rate of methanogenesis at 3 to 6 cm depth, but high concentrations and production rates were evident only when SO_4^{2-} was depleted at a depth of 8 to 10 cm. Below this depth, gas pockets were observed in the sediment. Integrated CH_4-production (down to 14 cm) was about 25 nmol cm^{-2} h^{-1} corresponding to <10% of the total anaerobic carbon mineralisation. The concave-upward shape of the CH_4 around 8 cm suggests that the bulk of the methane produced is oxidised anaerobically through sulphate reduction (e.g. Iversen & Blackburn 1981, Iversen & Jørgensen 1985) although it is also possible that some methane escapes through ebullition.

Fig. 8A shows corresponding values of O_2 and S^{2-} fluxes to the *Beggiatoa* mat. The values are consistent with those previously found for a similar sediment (Jør-

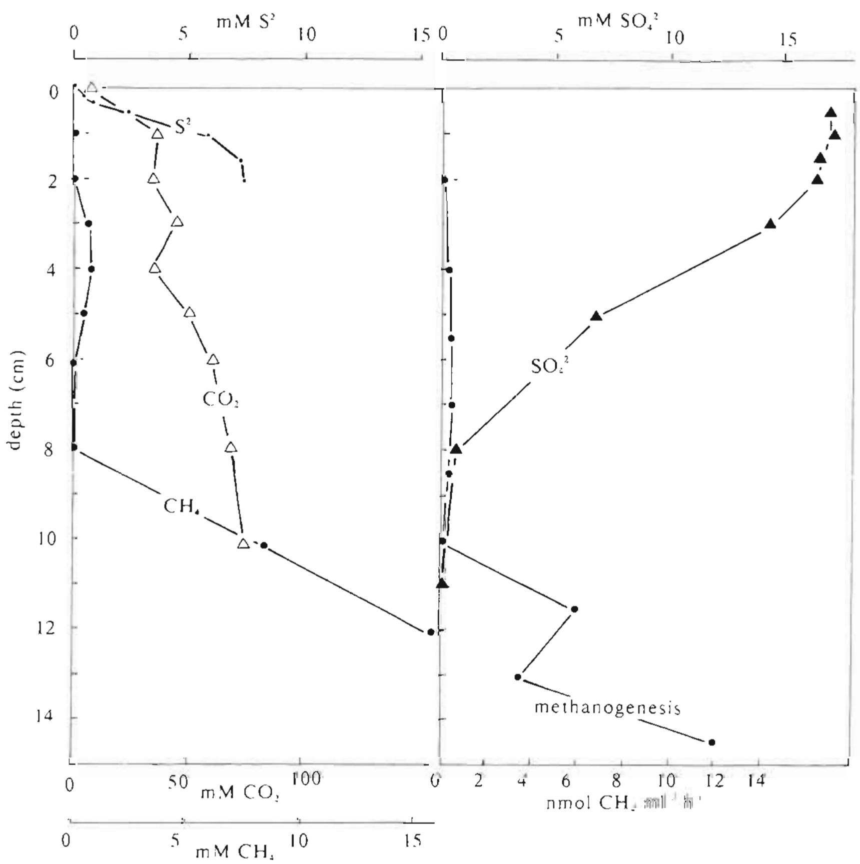

Fig. 7 Profiles of CO_2, S^{2-}, CH_4, SO_4^{2-} and of the rate of methanogenesis in the sediment beneath a *Beggiatoa* mat. The sulphate profile derives from a separate sediment core

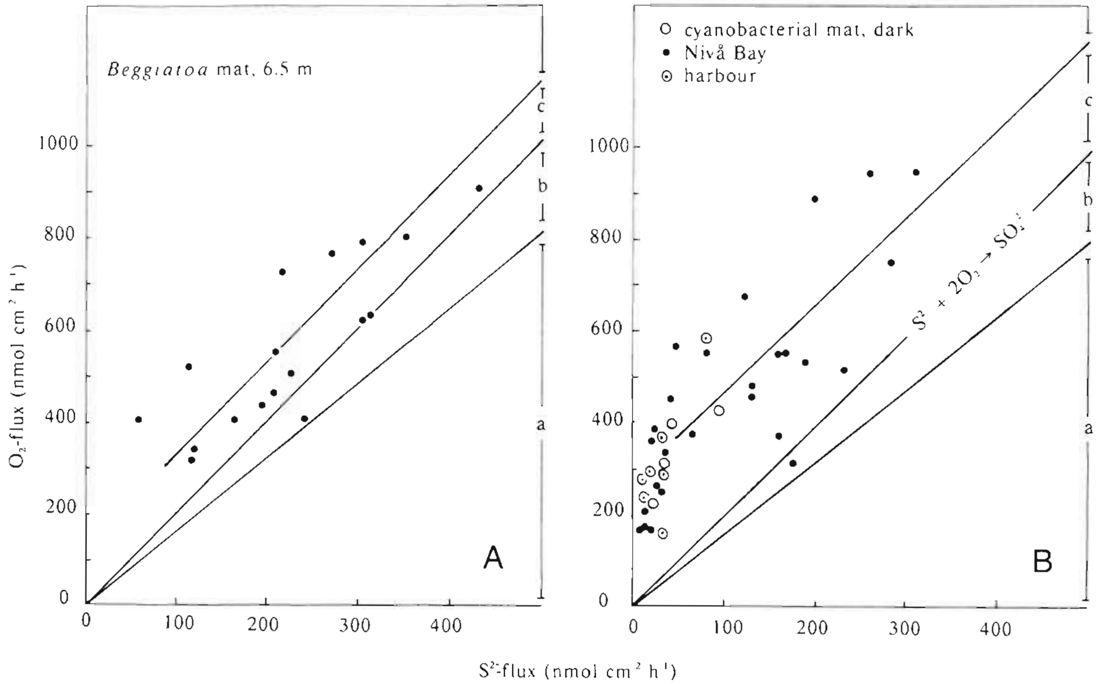

Fig. 8. Corresponding fluxes of S^{2-} and O_2 (A) of the *Beggiatoa* mat from the North Harbour at 6.5 m and (B) from patches with various types of colourless sulphur bacteria or cyanobacterial mats (in the dark) from Nivå Bay and from the shallow site in the harbour. For further explanation see text

gensen 1977b) and for other active biofilms (Kühl & Jørgensen 1992). With the assumption that the mat is in a steady state (no net change in biomass) then the flux estimates can be used to estimate the fraction of the O_2 uptake due to S^{2-} oxidation, the chemoautotrophic production by sulphur bacteria and the fraction of the O_2 uptake which is not directly associated with the S cycle. The slope of O_2 versus S^{2-} flux (2:1) suggests that complete oxidation to SO_4^{2-} took place. In Fig. 8, $a + b$ corresponds to $S^{2-} + 2O_2 \rightarrow SO_4^{2-}$. However, since the sulphur bacteria grow by assimilatory reduction of CO_2, some of the consumed sulphide is used as an electron donor for C reduction rather than for O_2 reduction (Nelson et al. 1986a). The molar yield of sulphide-oxidising bacteria (with SO_4^{2-} as the principal end product) has been measured to be within the range of 6 to 10 g dry wt organic material mol^{-1} S^{2-} oxidised (Kuenen 1989); we here use the value of 0.4 mol C mol^{-1} S^{2-} found by Nelson et al. (1986b) in a pure culture of *Beggiatoa*; this corresponds to b in Fig. 8. Thus $a + b + c$ corresponds to the total O_2 uptake of the sediment, a represents the part of the O_2 uptake directly used for S^{2-} oxidation, b is the production of sulphur bacteria (in C equivalents or in O_2 equivalents when this biomass is eventually mineralised aerobically). Finally, c represents the part of the sediment respiration which is not directly associated with the sulphur cycle (e.g. respiration of phototrophs in the dark, respiration of various heterotrophic aerobes not consuming or degrading sulphur bacteria, and the respiration of nitrifiers and methylotrophic bacteria). Thus for a typical sulphide flux, about 70% of the sediment respiration (= input of organic C to the system – burial of fossil organic C and of pyrite) is accounted for by the oxidation of S^{2-} to SO_4^{2-}, about 15% by the mineralisation of chemoautotrophic production (through food chains or heterotrophic bacteria) and about 15% by other aerobic processes.

The *Beggiatoa* mat in the harbour contained a substantial amount of filamentous cyanobacteria and sometimes substantial numbers of diatoms and green euglenoids, and when exposed to full daylight in the laboratory, the sulphur bacteria migrated beneath the phototrophs, leaving a brownish-green surface layer. The rate of photosynthesis as a function of light intensity is shown in Fig. 4. Direct measurements showed that <5% of incident surface light penetrates to 6.5 m depth so that the photosynthetic potential exceeds realised *in situ* values. Even under favourable conditions during summer, the phototrophs are not capable of covering the O_2 and organic C demands of the sediment and the main carbon supply must derive from accumulated organic debris (mainly tissue of eelgrass and macroalgae).

Fig. 9 summarises the flow of materials and energy in the upper 14 cm of the harbour site sediment and its

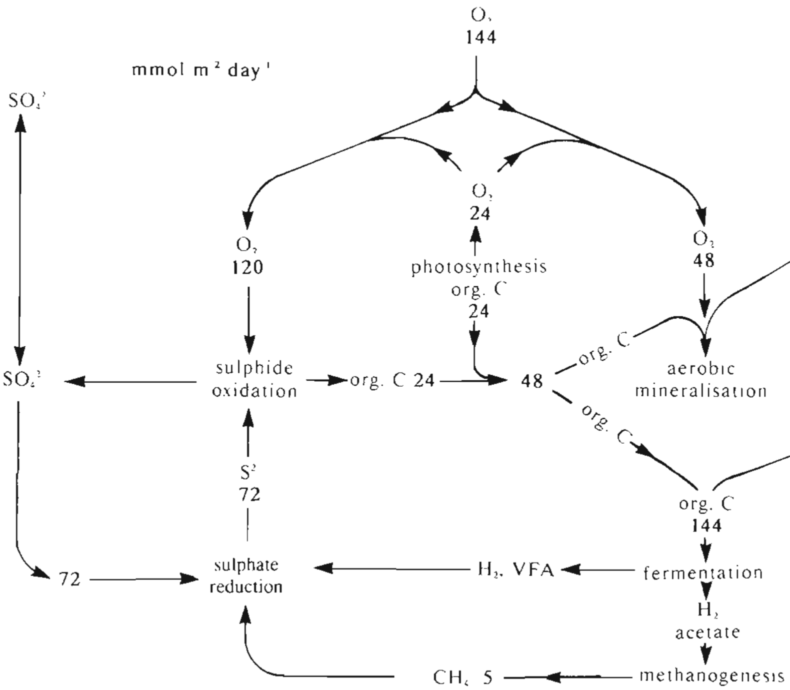

Fig. 9. The quantitatively most important microbial processes of the *Beggiatoa* mat community and the underlying 14 cm of anaerobic sediment (VFA: volatile fatty acids)

microbial mat. The figures are not to be considered as an average over time or space, but as representations of an example based on a S^{2-} flux of 300 nmol cm^{-2} h^{-1} and a dark O_2 consumption of 700 nmol cm^{-2} h^{-1}. The photosynthetic rate is a crude estimate based on a typical photon flux at 6.5 m depth during daytime in spring and an extrapolation of the data shown in Fig. 4D.

There are 2 more serious reservations with respect to Fig. 9. The cores from the harbour never showed visible signs of phototrophic (purple or green) sulphur bacteria, not even if the sides of cores were exposed to light for several days. It is known, however, that many cyanobacteria are both tolerant to sulphide and capable of anoxygenic photosynthesis with S^{2-} as an electron donor (Cohen et al. 1986). In addition, filamentous cyanobacteria were observed to congregate in the sulphidic zone on the illuminated side of cores. It is therefore possible that some phototrophic (anoxic) sulphide oxidation took place in the light.

It is also possible that some sulphide oxidation took place with nitrate rather than oxygen as the electron acceptor according to Fig. 10; it has been shown that at least some colourless sulphur bacteria may be denitrifiers (Kuenen 1989). The nitrate would derive from nitrification in the lower part of the oxic zone, based on NH_4^+ diffusing upwards from the anoxic zone, and some of the formed NO_3^- could diffuse downwards and serve for sulphide oxidation via denitrification. If this mechanism were important, then the overall stoichiometry of sulphide oxidation by O_2 would be different than suggested in Fig. 9. This is because in nitrification NH_4^+ is oxidised to NO_3^-. In denitrification, however, NO_3^- is reduced only to N_2. The oxidation of 1 unit of S^{2-} would therefore require 3.2 rather than 2 units of O_2 if it takes place via nitrification-denitrification. Although nitrification-denitrification was not studied, it is possible to set an upper bound for its quantitative role. Assuming a C:N ratio of 6 for the anaerobically mineralised organic material, an upwards NH_4^+ flux of about 100 nmol cm^{-2} h^{-1} is predicted. Complete nitrification would then require 17% of the total O_2 consumption. It is, however, known that denitrification is incomplete in sediments with a reducing zone close to the surface (Vanderborght & Billen 1975). Furthermore, it is unlikely that all the produced NO_3^- would become available for denitrification and it is therefore unlikely that the pathway described in Fig. 10 is quantitatively important for sulphide oxidation. The sul-

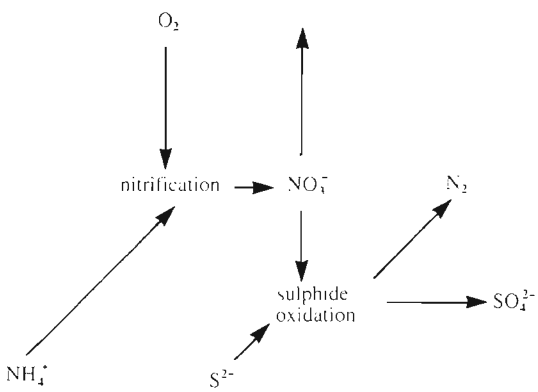

Fig. 10. Nitrification-denitrification as a possible intermediate in the overall oxidation of S^{2-} by O_2 to SO_4^{2-}

phide profiles in *Beggiatoa* mats suggest that S^{2-} oxidation took place within the entire (500 to 600 µm thick) mat although O_2 could only be detected in the upper 150 to 200 µm. Denitrification could be a contributing explanation for this. Thus Fossing et al. (1995) found that nitrate is a major electron acceptor for *Thioploca* mats of the upwelling areas off the coast of Chile, and that the bacteria store NO_3^- in vacuoles and transport it down into the sulphidic zone by vertical migration. This mechanism, however, requires high NO_3^- concentrations in the overlying water, a condition which does not apply in the present case. Also, the K_s for O_2-uptake of sulphur bacteria is very low (~0.5% atm. sat. according to Kuenen 1989). This is at or below the detection limit of the O_2-electrodes, so very low and undetectable amounts of O_2, although still sufficient to sustain some aerobic activity, may have been present slightly deeper than indicated in Fig. 2. The individual *Beggiatoa* filaments are in constant motion and are therefore subject to a varying P_{O_2}.

Fig. 8B shows corresponding values of S^{2-} and O_2 fluxes of Nivå Bay samples (including a few summer samples with cyanobacterial mats which were measured in the dark) and of samples from the shallow site in the harbour. Most of these samples were dominated by free-swimming colourless sulphur bacteria (mainly *Thiovulum*); these form thinner films (~200 µm) which avoid complete anoxia (Fig. 3; see also Fenchel 1994, Bernard & Fenchel 1995). These samples showed a much larger variation in terms of S^{2-} flux and in terms of the ratio between S^{2-} and O_2 flux, reflecting the transient nature of the patches. In these shallow water samples phototrophic S^{2-} oxidation plays a substantial role from spring to autumn. As seen in Fig. 8, chemotrophic sulphide oxidation often accounted for <50% of the total respiration and chemoautotrophic production for only 10 to 30% of the C demand of aerobic heterotrophs.

Acknowledgements. The study was supported by grants from the Danish Natural Science Research Council (0088-1) and from the European Union (MAST Programme, 93-0058). We are grateful to Mr Ib Aagaard for collecting samples by SCUBA diving and to Ms Jeanne Johansen for technical assistance.

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This article was submitted to the editor

Manuscript first received: May 9, 1995
Revised version accepted: June 9, 1995