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Ecology of the Bacteria of the Sulphur Cycle with Special Reference to Anoxic-Oxic Interface Environments [and Discussion]

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Ecology of the bacteria of the sulphur cycle with special reference to anoxic–oxic interface environments

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H_2S is produced as a main end-product of anaerobic mineralization in anoxic, sulphate-rich environments by a diverse population of sulphate-reducing bacteria. The sulphate reducers can carry out an almost complete oxidation of detrital organic matter to CO_2. The H_2S consequently becomes an important electron carrier from the anoxic to the oxic world. Thiobacilli and other colourless sulphur bacteria have the potential to oxidize the H_2S at the oxic–anoxic interface in sediments or stratified waters, but their role is still poorly understood. A comparison of sulphide oxidation processes in the chemoclines of the Black Sea, the Solar Lake and in a *Beggiatoa* mat indicated that depth scales and retention times of coexisting O_2 and H_2S regulate the bacterial involvement in the sulphide oxidation. The H_2S specialists, *Beggiatoa* and *Thiovulum*, are optimally adapted to compete with the autocatalytic oxidation of H_2S by O_2. Microelectrode measurements show retention times of O_2–H_2S in the bacterial mats or veils of less than 1 s. In photic chemoclines of stratified waters or sulfureta, the phototrophic sulphur bacteria or cyanobacteria interact with the sulphide oxidation at the O_2–H_2S interface. Short cycles between H_2S and intermediate oxidation products, S^0 or $S_2O_3^{2-}$, are created. The bacteria of the sulfuretum are highly adapted to the diurnal rhythm of light, O_2 and H_2S.

Introduction

The biological transformations of sulphur compounds in natural ecosystems are closely coupled to the formation of living biomass and to the subsequent decomposition and remineralization of the biomass. The living organisms contain about 1 % sulphur of their dry mass. This percentage fixes the stoichiometric coupling between the sulphur and the carbon flow through aerobic food chains.

In the anoxic parts of the world, the sulphate-reducing bacteria constitute a specialized group of organisms that use the external sulphur source as an electron acceptor rather than for biosynthetic purposes. By their dissimilatory sulphate reduction these bacteria uncouple the carbon and the sulphur flow in anaerobic, bacterial food chains away from the assimilatory stoichiometry. They also uncouple the carbon and the energy flow, as the sulphide produced carries a significant fraction of the detrital energy. This energy may be exploited by other types of bacteria with a chemotrophic or phototrophic energy metabolism.

The sulphate-reducing bacteria thereby drive an important dissimilatory sulphur cycle in which inorganic sulphur compounds serve as extracellular electron carriers. Such a sulphur cycle was even more important during the early Precambrian evolution of the biosphere before molecular oxygen was evolved by oxygenic phototrophs and began to accumulate on Earth. Today, the functional role of sulphur has largely been taken over by oxygen, but in ecosystems that are partly anoxic, the dissimilatory sulphur cycle still plays an important role.

The bacteria of the sulphur cycle are therefore interesting from several points of view. They are of significant quantitative importance for the element cycles and nutrient balance of many ecosystems. They are modern representatives of bacterial communities that inhabited the Earth during most of the Precambrian period. Finally, they comprise many unique examples of bacterial physiology and biochemistry.

In this paper I shall briefly introduce some of the bacteria of the sulphur cycle and then give examples of how they interact with the environment through their metabolic processes and how they adapted to a life at the anoxic–oxic interface. The examples are strongly biased towards the studies at our own laboratory and little attempt is made to give an exhaustive coverage of the literature.

Sulphide production in the anoxic world

The sulphate-reducing bacteria

The dissimilatory reduction of sulphate to sulphide is the energy-conserving process in a number of strictly anaerobic bacteria. The classical members of these sulphate reducers belong to the genus *Desulfovibrio*. They occur widely distributed in marine, limnic and terrestrial environments that have sulphate available and are sufficiently reducing. Many new isolates of sulphate reducers were recently obtained from such anaerobic environments by F. Widdel and N. Pfennig (Widdel 1980). They are divided into seven genera and include, for example, the short, rod-shaped *Desulfobacter postgatei*, the ovoid *Desulfobulbus propionicus*, the filamentous, gliding bacteria *Desulfonema limicola*, as well as new members of the genus *Desulfovibrio*. Their isolation has widely broadened the known spectrum of organic substrates that can be used as carbon and energy sources by sulphate reducers. Alone or in syntrophy these bacteria have the potential to oxidize the main products of bacterial fermentations, including fatty acids, alcohols, dicarboxylic acids and some aromatic compounds, completely to CO_2. An important physiological difference between the individual species is their ability to carry out a complete or only a partial substrate oxidation. While the classical *Desulfovibrio* species excrete acetate as the end-product of their incomplete substrate oxidation, most of the new types oxidize organic compounds completely to CO_2. The species *Desulfobacter postgatei* is even specialized to use acetate, which serves as its sole carbon and energy source.

Studies on the sulphate-dependent oxidation of fermentation products in anaerobic sediments have confirmed the presence and activity of these new isolates in natural environments. Laanbroek & Pfennig (1981) found three genera to be common in brackish-water sediments: the lactate-oxidizing *Desulfovibrio*, acetate-oxidizing *Desulfobacter*, and propionate-oxidizing *Desulfobulbus*, which all occurred in numbers of 10^3–10^4 colonies per cubic centimetre. Although such counts may yield important comparative data, the total number of sulphate reducers must be at least 1000-fold higher (Jørgensen 1978). Studies on substrate utilization by sulphate reducers in marine sediment slurries have shown the quantitative contribution by different fermentation products (Sørensen *et al.* 1981). Acetate, propionate and butyrate accounted for about 50, 15 and 5%, respectively, while H_2 added another 10–15% to the electron donors for the sulphate-reducing bacteria. Lactate, ethanol, C_5–C_{18} fatty acids, etc. may be partly metabolized by the hydrogen-producing acetogenic bacteria rather than being oxidized by sulphate reducers.

Bacteria that use elemental sulphur, but not sulphate, as an electron acceptor were first

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isolated from marine, sulphide-rich muds and from co-cultures with green sulphur bacteria growing on H_2S and acetate (Biebl & Pfennig 1978; Pfennig & Biebl 1976). These bacteria, e.g. *Desulfuromonas acetoxidans*, seem to occur commonly in anaerobic marine environments, although their quantitative role is still unknown.

Sulphide production in sediments

The environment of the sulphate-reducing bacteria in Nature is sandwiched between the oxidized zone of soils, lakes and seas on the upper side and the deep, methanogenic sediment zones on the lower side. The oxidized state of sulphate is maintained and regenerated in the oxic world. From here, the sulphate is transported down into anoxic areas of sediments and water where bacteria gradually reduce it to H_2S. The depth of the sulphate reduction zone depends on the initial concentration of sulphate and on the intensity of reduction. In lake sediments, sulphate is already depleted at a few centimetres depth, while in the sea bottom, sulphate may reach many metres down.

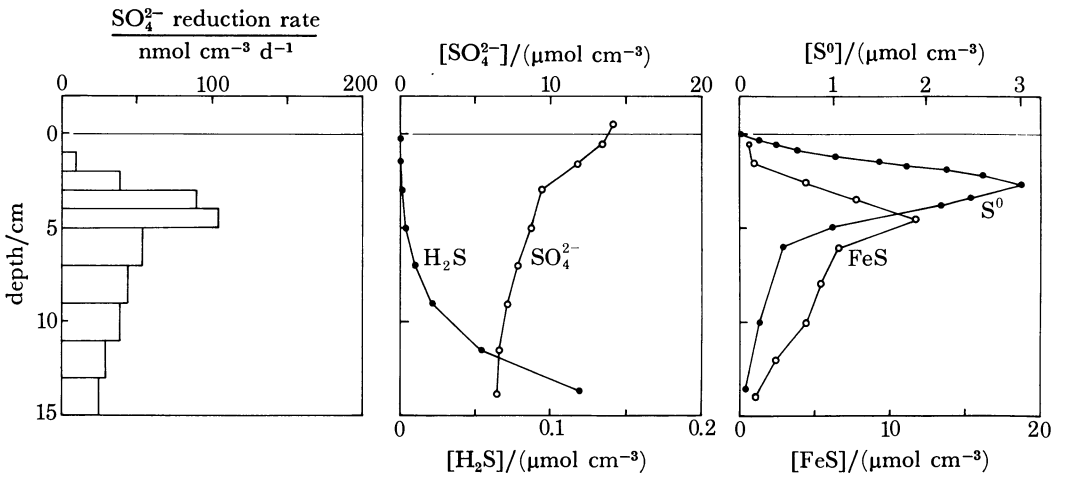

FIGURE 1. Distribution of sulphate-reducing activity and of inorganic sulphur compounds in a coastal, marine sediment, Kysing Fjord, Denmark, Sulphate reduction rates were measured by ^{35}S tracer technique. (Data from Troelsen & Jørgensen (1982).)

The intensity of sulphate reduction in anoxic environments is governed by the influx of organic matter. Over 90 % of the organic detritus that precipitates on the sea floor is mineralized again, while only a small percentage becomes permanently buried. A survey of oxygen uptake and sulphate reduction rates in shelf sediments from the coast to 200 m water depth has demonstrated the important role of sulphate-reducing bacteria for mineralization (Jørgensen 1982). In organic-rich sediments, the sulphate reducers oxidized half of all the incoming detritus to CO_2. This implies that organic matter buried below the oxic surface can be oxidized almost quantitatively at the expense of sulphate. Sulphate-reducing bacteria therefore constitute the true terminal step in the anaerobic microbial food chain of these sediments.

A typical example of the sulphate reduction activity in a coastal sediment is shown in figure 1. Maximum rates of metabolism were found a few centimetres below the sediment surface in the uppermost part of the sulphide zone. Sulphate was slightly depleted with depth and had a long residence time of 3–5 months as calculated from the reduction rates and the pool size. The distribution of H_2S shows that sulphide must have been oxidized, or at least

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disappeared, at a sediment depth of a few centimetres. Measurements of oxygen distribution in such a sediment generally showed a penetration to only a few millimetres depth (Revsbech *et al.* 1979, 1980), and there was consequently no overlapping between O_2 and H_2S. The sulphide may have been transported up rapidly by bioturbation due to the benthic fauna. It may also have reacted with ferric iron in the sediment to produce ferrous iron and elemental sulphur. The ferrous iron will precipitate with further H_2S to produce FeS, which slowly reacts with the S^0 to form pyrite, FeS_2 (Berner 1970; Goldhaber & Kaplan 1974).

Mass balance calculations for sulphide production and accumulation in coastal sediments have shown that large amounts of H_2S are reoxidized to sulphate near the sediment surface and that O_2 must be the ultimate electron acceptor (Jørgensen 1977). It is a general observation, however, that oxygen and sulphide are separated in these sediments by a zone a few centimetres deep in which neither of the two compounds can be detected. How does the oxidation then take place? Bioturbation may perhaps provide a part of the explanation but it is difficult to quantify in relevant terms. There may also be an electron carrier such as iron involved that is reduced by sulphide and oxidized by oxygen. At the present stage it is an embarrassing fact that we neither understand the chemical mechanism of sulphide oxidation in typical marine sediments nor the quantitative involvement of bacteria in this process. Research on H_2S oxidation has instead been focused on the less typical environments on Earth where oxygen and sulphide do meet and coexist in detectable concentrations. It should be remembered, however, that these environments are exceptions and that studies of the typical environments of sulphide oxidation are greatly needed.

There are three main types of such O_2–H_2S interface environments. One is the chemocline of stratified water bodies such as the Black Sea and the Cariaco Trench or of meromictic lakes. The other is the sulfureta of shallow marine or fresh water sediments where the accumulation of organic matter has led to intensive sulphide production and to mass development of sulphide-oxidizing bacteria. A third, important type, which was discovered and investigated only in the most recent years, is the hydrothermal vents of, for example, the Galapagos rifts. Here sea water percolates through deep sediment strata and is again expelled through cracks and chimneys on the bottom. By then the water is hot and rich in sulphide and it supports the growth of free-living or symbiotic chemoautotrophic bacteria that obtain energy from the oxidation of sulphide (Cavanaugh *et al.* 1981; Felbeck 1981; Jannasch & Wirsen 1979, 1981). The bacteria seem to form the nutritional basis for rich benthic communities of worms, bivalves, crabs, etc. These vent ecosystems will be further discussed by J. G. Kuenen and J. R. Postgate at this symposium.

An example of the extremely high sulphate reduction rates, which lead to the formation of a sulfuretum, is shown in figure 2. In comparison with figure 1, the maximum reduction rates were here over 10-fold higher and were strongly focused towards the sediment surface where the large sulphate pool had a residence time of only 5 days. The steep gradient of H_2S showed that it diffused up to the very surface of the sediment before it was oxidized. The high and sharp peak of elemental sulphur at the surface was produced by the dense coatings of sulphur bacteria and is typical of sulfureta. Extracellular S^0 may be used as an electron acceptor by bacteria such as *Desulfuromonas*, which was isolated from this sediment (N. Pfennig, personal communication).

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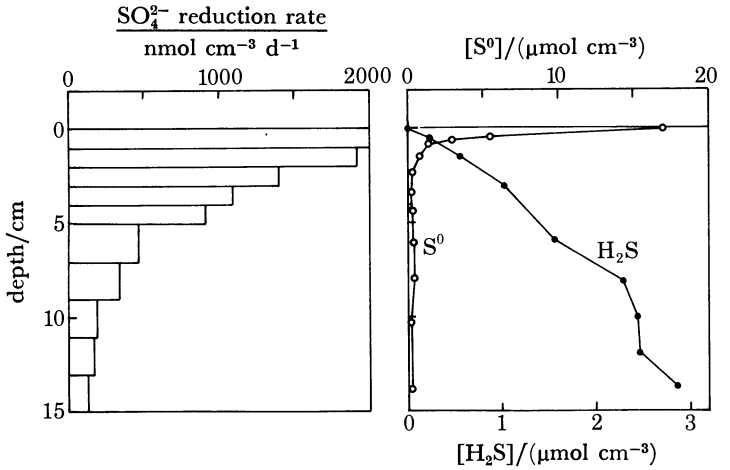

FIGURE 2. Distributions of sulphate reducing activity, dissolved H_2S and elemental sulphur in a marine sulfuretum from Kalø Vig, Denmark. The organic content was 30–40 % dry mass of the sediment. (Data from Troelsen & Jørgensen (1982).)

AEROBIC SULPHIDE OXIDATION

Colourless sulphur bacteria

The potential to oxidize reduced, inorganic sulphur compounds with oxygen or nitrate as the electron acceptor is a common feature for all the colourless sulphur bacteria. The group, however, covers a wide range of morphological, physiological and ecological types. The microbiology of the colourless sulphur bacteria is reviewed by Kuenen & Beudeker (this symposium).

Among the thiobacilli, which have been the most studied of all the representatives, there are obligate chemolithotrophs that synthesize their cell carbon from external CO_2 via the Calvin cycle and that derive energy for cellular processes from the oxidation of reduced sulphur compounds. *Thiobacillus thioparus* and *Thiomicrospira pelophila* are examples of this group. The facultative chemolithotrophic thiobacilli, e.g. *T. intermedius* and *Thiobacillus* A2, can grow as pure heterotrophs on a range of organic molecules but they can also conserve energy from the oxidation of sulphur compounds. When growing chemolithotrophically some *Thiobacillus* species can assimilate a mixture of organic substrates and CO_2 for cell carbon synthesis. The ratio between organic and inorganic carbon incorporated will then depend on the relative concentrations of the organic substrate and the sulphur compound (Gottschal & Kuenen 1980; Gottschal *et al.* 1979).

In addition to sulphide, the thiobacilli can oxidize elemental sulphur, thiosulphate and other reduced sulphur compounds that are common intermediates during sulphide oxidation in Nature. There are other bacteria that are more specifically dependent on H_2S. These organisms are closely associated with the oxygen-sulphide interface environments where the two compounds coexist between opposing gradients. Such gradient bacteria include the filamentous genera *Beggiatoa* and *Thioploca*, the spherical *Thiovulum*, and others. The life between oxygen and sulphide has required special adaptations in the bacteria, of which a well developed chemotaxis and the formation of internal sulphur droplets are most conspicuous.

It is not yet clear whether the gradient bacteria are mainly heterotrophs or whether they can grow chemolithotrophically on H_2S and use CO_2 for cell synthesis. This is partly due to the

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difficulties in growing the bacteria in pure culture. Isolates of *Beggiatoa* grow mostly heterotrophically on acetate (Strohl & Larkin 1978). They seem to have a low capacity to fix CO_2 but the growth yield may increase by the addition of sulphide to the heterotrophic growth medium (Güde *et al.* 1981; Strohl *et al.* 1981). There is evidence of autotrophic growth in *Thiovulum* when it forms veils at the oxygen–sulphide interface, so in this organism sulphide may possibly serve as the main energy source (Wirsen & Jannasch 1978).

The oxidation of sulphur compounds can also serve other purposes than the donation of electrons. Under anaerobic conditions, *Beggiatoa* may use the accumulated sulphur in the cells as an electron acceptor and reduce it back to H_2S (Nelson & Castenholz 1981). For many heterotrophic bacteria that are only 'occasional oxidizers' of reduced sulphur compounds, the role of the oxidation is less clear (Kuenen 1975). A large number of strains have, for example, been isolated from marine waters that could oxidize thiosulphate incompletely to tetrathionate (Tuttle & Jannasch 1972).

An important ecological aspect of the bacterial oxidation of sulphide is the linkage of the sulphur and carbon cycles by the autotrophic formation of cell carbon during the oxidation of sulphide. The growth yield of autotrophic sulphide oxidizers is, however, rather low, even in the specialized thiobacilli. These organisms transfer only up to 10–15% of the electrons from H_2S or $S_2O_3^{2-}$ to CO_2, which corresponds to the formation of 5–13 g dry mass of cell material per mole of substrate (Beudeker *et al.* 1982; Kuenen 1979). The sulphide oxidation by the chemolithotrophic heterotrophs does not provide new synthesis of organic carbon from CO_2. It may any how play an important role in carbon balance because more organic matter is preserved as cell material instead of being respired.

Experimental approaches and their limitations

Three different approaches have been used to study the activities of bacteria in the chemocline of stratified lakes, fjords and marine basins in order to demonstrate their possible role in the oxidation of reduced sulphur compounds:

(1) isolation and enumeration of bacteria that were either obligate or facultative oxidizers of sulphide and thiosulphate;

(2) measurement of bacterial CO_2 fixation and studies of its dependence on the concentrations of oxygen and reduced sulphur compounds;

(3) experimental studies of H_2S oxidation kinetics and products in relation to the presence or absence of living bacteria.

True thiobacilli, which could grow autotrophically with reduced sulphur compounds as the only energy source, have been sought but are usually not found in the chemoclines of stratified, marine basins such as the Cariaco Trench or the Black Sea (Tuttle & Jannasch 1973, 1977; Kuenen 1975). Instead, enrichments on sulphide or thiosulphate have yielded a range of heterotrophic bacteria. These evidently live on the organic compounds that are released in the oxygen–sulphide boundary layer or are transported up from the deep anoxic water masses below. The bacteria have the potential to oxidize thiosulphate either to tetrathionate or completely to sulphate. At least some of them may conserve energy from the process (Tuttle & Jannasch 1973; Kuenen 1975).

The presence of thiobacilli has been reported from the chemocline of several lakes. Sorokin (1970) attributed the CO_2 fixation maximum at the oxygen–sulphide interface of Lake Belovod

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and Lake Gek Gel in Russia to autotrophic thiobacilli. From the chemocline of the hypersaline Solar Lake in Sinai, Jørgensen *et al.* (1979) enriched sulphide- and thiosulphate-oxidizing bacteria in the chemostat on a purely inorganic medium. Although such enrichments or isolations may demonstrate the occurrence of thiobacilli, it is difficult to draw conclusions about their numbers or even their activity.

An activity measurement can be obtained from studies of CO_2 fixation in the dark directly in the chemocline. Since most thiobacilli may obtain all or a significant part of their organic carbon from CO_2, their growth on reduced sulphur compounds would be expected to yield a peak of CO_2 fixation at the oxygen–sulphide interface. Such a peak is indeed found in most chemoclines and it has been tempting to conclude that autotrophic thiobacilli were operating here. The little success in isolating the thiobacilli suggests that more versatile organisms with a generally heterotrophic metabolism may be responsible for the CO_2 fixation. This does not mean that the CO_2 fixation is independent of the sulphide oxidation. There are several examples showing that fixation rates are stimulated by the addition of reduced sulphur compounds (Jørgensen *et al.* 1979; Tuttle & Jannasch 1977).

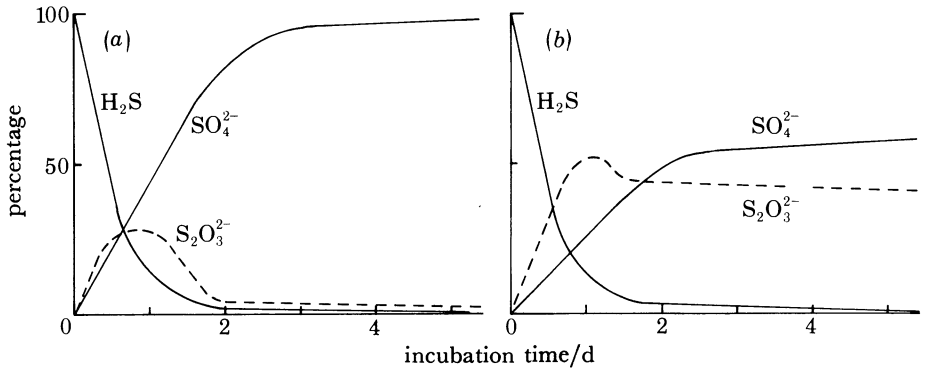

FIGURE 3. Transformation of ^{35}S-labelled H_2S to $S_2O_3^{2-}$ and SO_4^{2-} in oxic chemocline samples from the Black Sea. The relative distribution of label is shown against incubation time. (*a*) Untreated sample; (*b*) chloroform-killed sample. (Redrawn from Sorokin (1972).)

A quantification of the contribution by bacteria to sulphide oxidation requires chemical studies of the oxidation kinetics. In the presence of oxygen in natural sea water or fresh water, H_2S will have a half-life in the order of one or a few hours (Almgren & Hagström 1974, and references therein). The kinetics are rather complex as the oxidation depends on autocatalysis and the demonstration of bacterial involvement therefore requires careful controls. One type of control is the comparison with sterilized samples.

Sorokin (1972) used chloroform-killed controls in the Black Sea chemocline. Freshly collected samples were supplied with ^{35}S-labelled sulphide and the transformation of the label into oxidized sulphur pools was followed over several days (figure 3). The rates of H_2S oxidation were found to be similar in the fresh and in the killed samples, and Sorokin concluded that bacteria were not involved in the direct H_2S oxidation. Thiosulphate and sulphate in approximately equal amounts were the immediate products of the H_2S oxidation. In the live sample, the thiosulphate accumulated only transiently and it was further oxidized to sulphate. In the killed control, thiosulphate was a stable oxidation product.

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These results show that bacteria were responsible for the second oxidation step only, transforming thiosulphate into sulphate. The bacteria would thereby have only one-third of the reducing equivalents from the H_2S available:

$$\text{chemical: } 3H_2S + 4O_2 \longrightarrow S_2O_3^{2-} + SO_4^{2-} + 4H^+ + H_2O$$
$$\text{bacterial: } S_2O_3^{2-} + 2O_2 + H_2O \longrightarrow 2SO_4^{2-} + 2H^+$$
$$\text{total: } 3H_2S + 6O_2 \longrightarrow 3SO_4^{2-} + 6H^+.$$

Several other studies on sulphide oxidation in oxic water have confirmed that thiosulphate and sulphate are the main products of chemical oxidation, at least when sulphide or oxygen concentrations are low (see, for example, Cline & Richards 1969). If sulphide is introduced into oxic water in millimolar concentrations, elemental sulphur may become an important oxidation product.

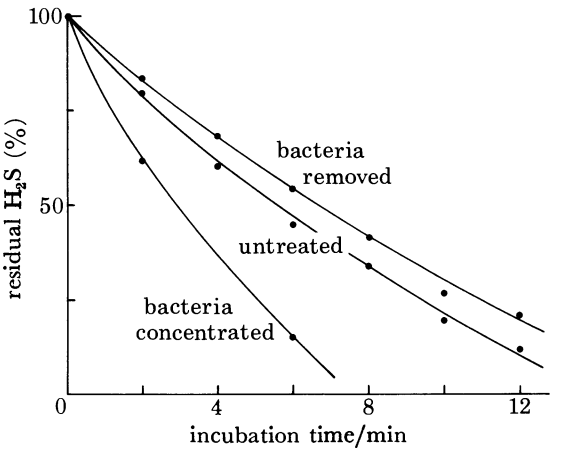

FIGURE 4. Disappearance of H_2S by oxidation in chemocline samples from the Solar Lake, Sinai. (Redrawn from Jørgensen *et al.* (1979).)

In contrast to the Black Sea, measurements of sulphide oxidation in the narrow chemocline of the Solar Lake indicated a significant contribution by bacteria to the direct H_2S oxidation (figure 4). The control experiments were here done by physically removing or concentrating the bacteria. Chemical inhibition of the bacteria was avoided because the introduction of poisons into the sample may possibly affect the autocatalytic pathway. When bacteria were removed from the chemocline sample by filtration, the rate of H_2S oxidation decreased. If the bacteria were concentrated by centrifuging the sample and resuspending the pellet in a smaller sample volume, the oxidation of H_2S was significantly enhanced. There was no stimulation if the pellet was first boiled to kill the bacteria (Jørgensen *et al.* 1979).

These results indicate that bacteria can in some chemocline environments compete with the spontaneous chemical oxidation. It is not yet understood what limits their competitive efficiency but a possible mechanism will be discussed below.

Sulphide oxidation at the O_2–H_2S *interface: three examples*

Most studies of the O_2–H_2S interface in stratified waters do not, unfortunately, have a sufficient depth resolution of the collected field data to determine the coexisting concentrations of the two compounds accurately. This is partly caused by sampling problems. The depth

interval of O_2–H_2S coexistence is usually very narrow relative to the total depth of the interface below the water surface and it is therefore difficult to find. The mere fact that waves tend to move the sampler up and down in the water may frequently make accurate depth sampling impossible and will lead to a mixing of different water layers from the chemocline. Furthermore, the measurement of low concentrations of coexisting oxygen and sulphide requires special precautions and analytical methods which in many studies have not been applied.

Three examples of O_2–H_2S interfaces in Nature are discussed here. The examples have been chosen mainly because the experimental work allows a direct evaluation of the contribution by bacteria to the process of sulphide oxidation. There are many other chemocline studies that show sulphide- or thiosulphate-stimulated bacterial activity through measurements of CO_2 fixation in the dark. The quantitative conversion of such data to sulphur metabolism involves, however, assumptions about autotrophy and heterotrophy of the organisms that still need to be proven.

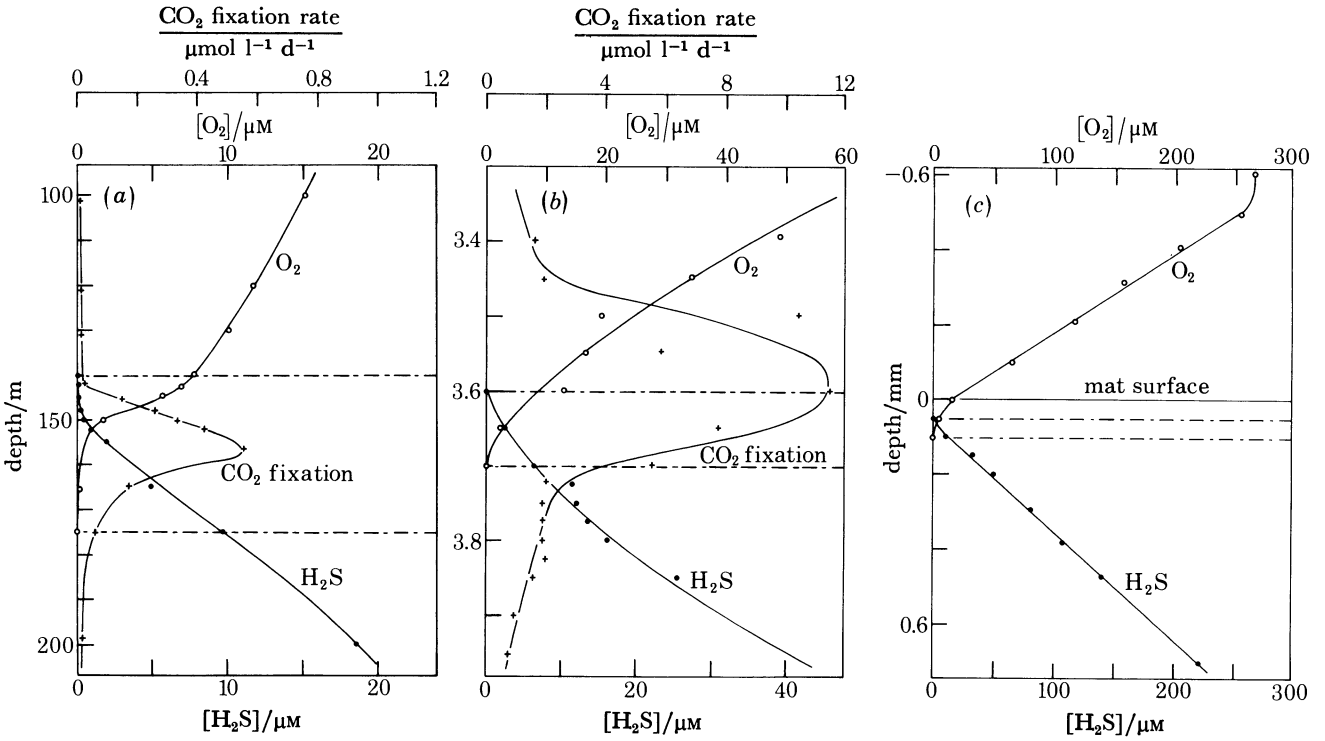

FIGURE 5. Three examples of the O_2–H_2S interface in stratified water bodies ((*a*) Black Sea; (*b*) Solar Lake) and in a *Beggiatoa* mat growing on a mud surface (*c*). Dark CO_2 fixation by bacteria shows a maximum at the interfaces. Broken lines indicate boundaries of O_2–H_2S coexistence. Note differences in scale. (Redrawn from Sorokin (1972), Jørgensen *et al.* (1979) and Jørgensen & Revsbech (unpublished).)

The three chemoclines are shown in figure 5. The data are from the permanently stratified Black Sea (Sorokin 1972, Station 1301), from the monomictic Solar Lake (Jørgensen *et al.* 1979), and from a *Beggiatoa* mat growing on the mud surface of a Danish bay (Jørgensen & Revsbech, unpublished).

The Black Sea has a total depth of about 2000 m. The water column is anoxic below 150–200 m and an O_2–H_2S interface of 35 m thickness was measured at that depth by Sorokin (1972) (figure 5). A maximum of CO_2 fixation in the dark was confined to the chemocline and

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was attributed to the activity of chemoautotrophic thiobacilli conserving energy from the oxidation of reduced sulphur compounds. Later investigators have, as previously mentioned, questioned the presence of significant numbers of true thiobacilli. The physiology of the CO_2-fixing organisms is therefore still not clear.

The Solar Lake has a total depth of only 5 m. Whereas the temperature of the Black Sea was 6 °C at the chemocline, the Solar Lake was hot, around 50 °C. The layer of O_2–H_2S coexistence at 3.6 m below the water surface was only 10 cm thick. Its exact position varied between day and night owing to the photosynthetic activity of planktonic cyanobacteria, which oxidized the sulphide and produced oxygen in the light. High rates of CO_2 fixation in the dark were found within the chemocline and above it where thiosulphate was present. The CO_2 fixation could be stimulated by H_2S, S^0 or $S_2O_3^{2-}$ and autotrophic thiobacilli could be enriched from O_2–H_2S interface samples. These studies indicated that reduced sulphur compounds could serve as energy sources for bacteria living in the chemocline (Jørgensen *et al.* 1979).

The dense *Beggiatoa* mat in figure 5 formed a thick white sheet 0.5 mm thick on the surface of the black mud in which an intensive sulphate reduction took place. The oxygen and sulphide gradients were measured at 0.05–0.1 mm depth intervals by the use of microelectrodes. The pO_2, pS^{2-} and pH electrodes were operated with a micromanipulator while observing the mat under a dissection microscope. This technique has a spatial resolution that makes it ideal for measurements in sediments and other microbial microenvironments. The electrode tips have diameters of 1–10 μm for pO_2 and 20–200 μm for pH and pS^{2-}. Oxygen and sulphide showed very steep gradients above and below the mat surface. In this extremely narrow and dynamic chemocline, CO_2 fixation rates have not been measured.

The difference in depth scale of the O_2–H_2S curves in figure 5 is striking and must be of significance for the bacterial contribution to sulphide oxidation. In table 1 the three systems are compared in more detail. There is almost a millionfold difference in the thickness of the O_2–H_2S interface in the Black Sea and in the *Beggiatoa* mat. The rates of H_2S oxidation in the mat must therefore be extremely high relative to the Black Sea.

The rates of $H_2{}^{35}S$ oxidation *in situ* in samples from the Black Sea as calculated from the data of Sorokin (1972) give a residence time of H_2S within the chemocline in the order of 5 days. This is a surprisingly long time as the purely chemical oxidation of H_2S in the presence of oxygen was generally found to be only a few hours. It does, however, show that the sulphide oxidation rates measured in the Black Sea can easily be explained as a purely chemical process. Similar measurements in the Solar Lake (see figure 4) gave residence times ranging from 10 to 20 min. The rapid oxidation is partly due to the high temperature. When recalculated to 20 °C on the basis of a Q_{10} of 2, the residence times are a few hours, just as in the chemical oxidation. The H_2S dynamics in the *Beggiatoa* mat were calculated from the diffusion gradients and from the pool sizes within the O_2–H_2S interface (Jørgensen & Revsbech, unpublished). The residence time was only 0.6 s. Thus the turnover of H_2S in the mat was almost a millionfold faster than in the Black Sea.

The possibilities of bacteria to compete favourably with the chemical sulphide oxidation vary greatly between the three systems. Sorokin (1972) found only chemical H_2S oxidation in the Black Sea and thus 0 % bacterial contribution (see figure 3). In a number of experiments in the Solar Lake, bacteria were responsible for 30–50 % of the H_2S oxidation (see figure 4). The very rapid sulphide oxidation in the *Beggiatoa* mat must be almost exclusively due to bacterial metabolism, i.e. 100 %. Even the very efficient chemical catalysts studied by Chen & Morris

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(1972) would reduce the normal half-life of H_2S in oxic water by only 10–100-fold. It would be highly unlikely that non-biological catalysts within the *Beggiatoa* mat could then speed up the process by 10^5—10^6-fold as observed in table 1.

It is an interesting but puzzling observation that O_2 and H_2S at equimolar values coexist in similar concentrations of a few micromoles per litre in all three systems. If the field data are accurate, this means that bacteria even in the Black Sea chemocline over a residence time of 5 days are unable to assimilate the H_2S efficiently and reduce the concentration below the micromolar level. In aerobic chemostat cultures of autotrophic thiobacilli growing on sulphide, the level of added sulphide is rapidly reduced below the detection limit of about 0.1 μM (J. G. Kuenen, personal communication). The apparently low efficiency of bacteria in the Black Sea may be a physiological characteristic. It could perhaps also be because the bacterial population is relatively scattered. It can be calculated from the data of Sorokin (1972) that, if a tenth of the bacteria directly counted at the O_2–H_2S equimolar depth (figure 5, 152 m depth) were to oxidize H_2S at the observed rate, each bacterium would have to clear 300 times its own volume for H_2S per second. This is significantly higher than the normal range of uptake efficiencies for organic substrates in, for example, *Escherichia coli* (Koch 1971).

TABLE 1. COMPARISON OF THREE O_2–H_2S INTERFACES: DIMENSIONS AND RATES

	Black Sea	Solar Lake	*Beggiatoa* mat
temperature/°C	6	50	20
O_2–H_2S interface depth	35 m	10 cm	50 μm
(proportion	700000	2000	1)
H_2S residence time	5 days	10–20 min	0.6 s
(proportion	700000	1500	1)
bacterial H_2S oxidation (%)	0	30–50	100
concentration at $C_{O_2} = C_{H_2S}$/μM	1–3	2–4	3–5
H_2S oxidation rate			
peak/(μmol l^{-1} d^{-1})	0.8	250	250000
areal/(mmol m^{-2} d^{-1})	10	20–30	12

Further studies are required to show what actually limits bacterial H_2S oxidation in natural chemoclines. At the present stage we can state that an important factor is the scale, both in depth and in time. On the small scale, bacteria are very efficient and decisive for both the dimensions of the O_2–H_2S interface and for the rates of turnover. On the large scale, these variables are determined by physical mixing and by chemical reaction. The total amount of H_2S that becomes available per unit area and time is of less importance, as shown in table 1. All three systems had similar areal oxidation rates. The peak rates of sulphide oxidation, however, varied over many orders of magnitude.

An important difference between the large-scale and small-scale chemoclines is also the intermediate products of sulphide oxidation. In the Black Sea, the Cariaco Trench, the Solar Lake and several other stratified water bodies, sulphate and thiosulphate are the immediate products of sulphide oxidation, and thiosulphate has a concentration maximum in the chemocline. In the small-scale mats of colourless sulphur bacteria, elemental sulphur is an important intermediate. The advantage for the bacteria in producing elemental sulphur in such a system seems obvious. Elemental sulphur is kept either intracellularly or it precipitates as largely non-diffusible granules around the cells. The sulphur is thus retained within the mat for further oxidation or reduction. If thiosulphate were produced as an extracellular intermediate it

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would diffuse away from the interface within a few seconds and the chemical energy or reducing power would be lost to the bacteria. Molecular diffusion is insignificant for the large-scale transport of sulphur within the Black Sea chemocline.

The H_2S *specialists*: *Thiovulum and Beggiatoa*

Colourless sulphur bacteria, which live in sheets or mats such as *Beggiatoa* and turn over the H_2S pool within less than a second, must be optimally adapted to a life with aerobic H_2S oxidation as a metabolic process. Although it is not yet clear to what extent the bacteria conserve energy from this oxidation process, it is obviously of great significance for their growth. It is

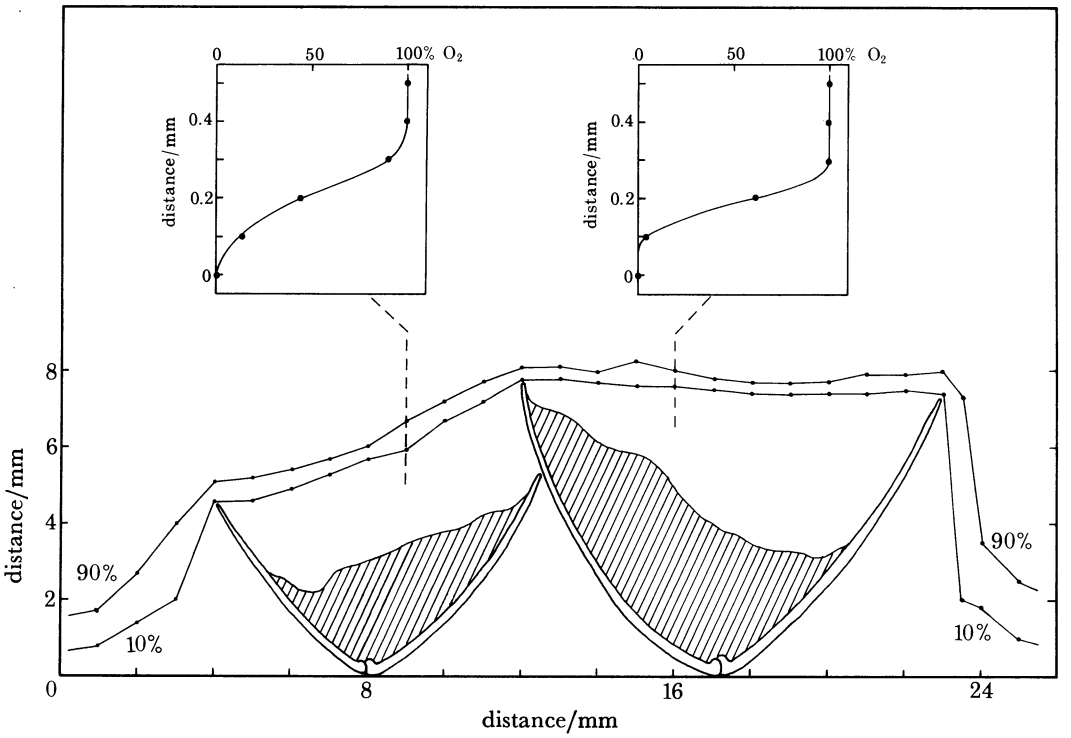

FIGURE 6. Oxygen distribution around decaying bivalves overgrown by a *Thiovulum* veil. The position of the veil is indicated by the isopleths of 90% and 10% air saturation of oxygen. (E. Chin, Jr & B. B. Jørgensen, unpublished.)

known that these gradient bacteria inhabit the surface layer of sulphide-rich sediments or cover decaying plant material or dead animals in both freshwater and marine exosystems. But the microenvironmental factors that determine the growth of the organisms are not well understood. It has mainly been the insufficient resolving power of the conventional chemical techniques that has impeded progress in this field. With the development of microelectrodes it is now possible to go below the millimetre scale and analyse the interactions between the sulphur bacteria and the chemical microenvironment.

A chemocline in the water column can be created not only by density stratification but, on the small scale, even by sulphur bacteria themselves. *Thiovulum* may form fragile slime webs or veils suspended over decaying animals and plants. Figure 6 shows the steep gradients of oxygen created by such a veil which grew over dead bivalves, *Solemya* sp. The position of the veil is detectable in the vertical section of figure 6 from the 90% and 10% O_2 saturation isopleths.

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The veil sharply separated the overflowing oxic sea water from the anoxic, sulphidic water below which surrounded the decaying bivalves. The oxygen gradient was only 200–400 μm thick.

The exact position of the *Thiovulum* cells relative to an oxygen microgradient is shown in figure 7. In this case a veil was established in a microaquarium and was observed under the microscope while the O_2 profile was measured. The cells flickered back and forth within the 100 μm thick veil and experienced only between 0 and 1% air saturation of oxygen. Similar studies have been made also on larger veils covering marine sediments, and the *Thiovulum* cells were always found to live under microaerobic conditions (Jørgensen & Revsbech, unpublished manuscript).

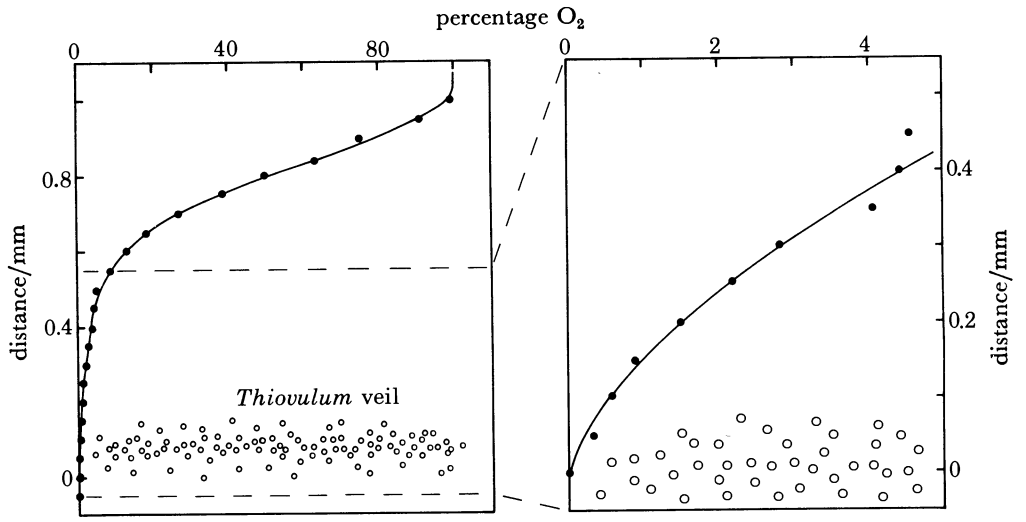

FIGURE 7. Oxygen gradient over a *Thiovulum* veil growing between the glass walls of a microaquarium 1 mm thick The cells were positioned exactly at the lower boundary of oxygen. The nonlinear gradient was caused by an irregular shape of the O_2-consuming veil. (E. Chin, Jr & B. B. Jørgensen, unpublished.)

The veil formation seems to serve two main purposes. One is to position the swarming cells exactly at the narrow O_2–H_2S interface. The other is to create and stabilize this interface and thereby to maximize the H_2S availability for the organisms. Just like any other solid surface in a liquid, a veil will create a stagnant boundary layer of water a few hundred micrometres thick. This water will not participate in the convective or turbulent mixing of the bulk water but will tend to stick to the veil by internal friction. Oxygen, sulphide and other dissolved substrates move rapidly through the stagnant layer by molecular diffusion along the steep gradients. Thus oxygen diffusion through a water film 300 μm thick takes only 10–20 s. The turnover time of oxygen within a veil was calculated to be only 0.1 s and the turnover time for sulphide must be similarly short. The obvious advantage in veil formation is thus that the oxic and sulphidic water masses are efficiently separated, which prevents any chemical sulphide oxidation. The two compounds only meet within the veil where the bacterial uptake is very rapid.

This picture is quite similar to that of the *Beggiatoa* mat in figure 5. The mat was covered by a stagnant boundary layer 500 μm thick. The *Beggiatoa* cells were also living under microaerobic conditions created by their own oxygen consumption. In the microaquarium they were found to avoid higher oxygen tensions actively but to gather near the oxic–anoxic interface. The mat was about 500 μm thick but the O_2–H_2S interface was only 50 μm thick. No more than 10%

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of the trichomes should therefore be able to oxidize H_2S to intracellular sulphur globules at any time. Cells above the interface may oxidize the sulphur further to sulphate while those below may possibly use the sulphur as an internal electron acceptor and reduce it back to sulphide (Nelson & Castenholz 1981). As the trichomes continuously glide around at a speed of about 150 μm min^{-1} (Crozier & Stier 1926) they may pass the different zones within a relatively short time. Their movement as well as the transport of elemental sulphur internally in their trichomes therefore gives a very flexible adaptation to the life in an O_2–H_2S gradient.

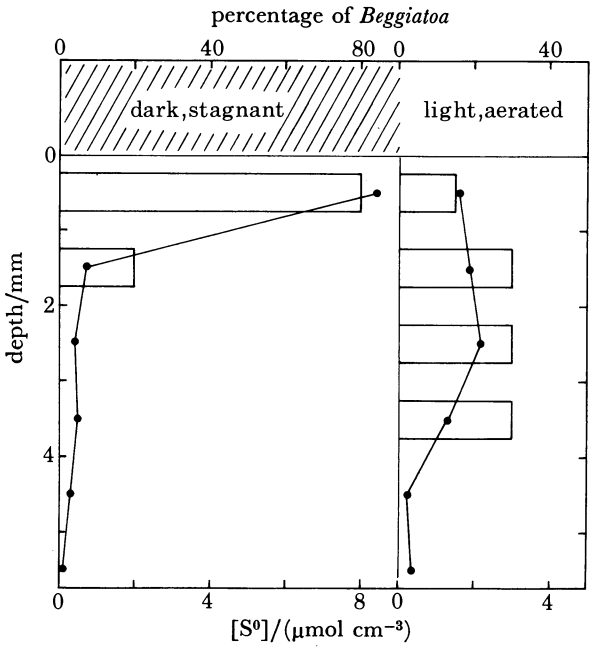

FIGURE 8. Vertical distribution of *Beggiatoa* (histograms) and of elemental sulphur (points) in an organic-rich marine sediment. The sediment was kept in the laboratory under alternating darkness, with stagnation of the overlying seawater, and light, under aerated flowing water. (Redrawn from Troelsen & Jørgensen (1982).)

A *Beggiatoa* mat also adjusts quite readily to movements of the oxic–anoxic interface as shown in figure 8. In the dark and under relatively stagnant conditions the trichomes as well as the intracellular sulphur were concentrated within the uppermost 1 mm of the sediment. Under strong aeration or in the light, where the benthic microflora produced O_2 in the uppermost 1–2 mm, *Beggiatoa* migrated a few millimetres downwards and so did the elemental sulphur; this showed that the sulphur in the mat was almost exclusively contained within the organisms.

The photic chemocline and the sulfuretum

The highest and most complex development of the sulphur cycle is reached in those ecosystems where light reaches into the sulphide zone. Such environments occur scattered along sheltered coasts and in some smaller lakes and are generally of very limited extension. Photosynthetic bacteria, which use H_2S as an electron donor in their anoxygenic photosynthesis, may here reach such high densities that they give the water or the sediment a distinct colouration. Depending mainly on the light intensity and the sulphide level, the dominant phototrophs may be purple or green sulphur bacteria. Also, cyanobacteria can grow phototrophically on H_2S and in some sulphide environments they may become dominant.

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The sulfuretum constitutes a gradual transition to the completely anaerobic ecosystem in which inorganic sulphur compounds serve as the main electron acceptors and donors for energy metabolism. Such an environment could in theory function as a completely closed ecosystem even today. In the modern world, however, the sulfuretum is in practice always bounded by oxic environments and will invariably lose reduced sulphur by oxidation with O_2. It therefore needs to be refuelled by an influx of reduced compounds in the form of organic matter. The O_2–H_2S interface of the sulfuretum is not only a habitat for the colourless sulphur bacteria but it is also strongly influenced by the photosynthetic organisms which tend to oxidize the environment in the light. The whole microbial community at the interface is therefore highly adapted to survive and exploit the varying oxidized and reduced conditions that they experience during the diurnal light and dark cycle. A few examples will be given to illustrate this.

During studies on the sulphur and carbon cycles in the Russian meromictic Lake Belovod, Sorokin (1970) observed a diurnal change in the H_2S level and in the distribution of phototrophic bacteria near the chemocline at 10 m depth. The H_2S was almost depleted from 10 to 13 m depth on sunny days but built up again during the night. The maximum of purple sulphur bacteria was reported to follow the retreating sulphide zone downwards by 2–3 m during the day. There was little change in the distribution of oxygen, probably due to the lack of oxygenic phototrophs in and below the O_2–H_2S interface.

Cyclic movements of the O_2–H_2S interface were observed in the Solar Lake (Jørgensen *et al.* 1979), where filamentous cyanobacteria (*Microcoleus* sp. and *Oscillatoria* sp.) were the main planktonic phototrophs. These organisms usually grow aerobically and produce O_2 in the light, like algae. At high H_2S concentrations they have the potential to switch to anoxygenic photosynthesis and oxidize H_2S to S^0, which is excreted from the filaments (Cohen *et al.* 1975). The cyanobacteria in the Solar Lake performed oxygenic photosynthesis in the oxic region just above the O_2–H_2S interface and anoxygenic photosynthesis just below it. The H_2S was gradually depleted at 0–20 cm below the interface during the day and at a certain stage a new peak of O_2 suddenly began to build up there. By the use of DCMU as a specific inhibitor of oxygenic photosynthesis it was shown that the cyanobacteria had switched the type of photosynthesis. These organisms are evidently able to grow like phototrophic, anoxygenic bacteria in the morning and like oxygenic algae in the afternoon. During the night, when O_2 is again depleted, they are even able to reassimilate external elemental sulphur and use it as an electron acceptor for their anaerobic respiration (Oren & Shilo 1979).

The elemental sulphur thus has an important role in this environment both as electron acceptor and as electron donor. In addition to the normal cycling between H_2S and SO_4^{2-}, the phototrophic bacteria created a cycle between H_2S and S^0. In the zone of O_2–H_2S coexistence $S_2O_3^{2-}$ was also produced, which could either be further oxidized or again reduced to H_2S. Chemical and radiotracer studies showed a very complex diurnal sulphur cycle in which the oxidative processes were dominating during the day and reductive processes during the night. There was no vertical migration of the organisms, which in the lower chemocline experienced alternating oxygen and sulphide, at peak concentrations above 20 μM.

The mass occurrence of photosynthetic bacteria on H_2S-rich sediments (sulfureta) has received much less attention than the photic sulphide zone of lakes and fjords. The same chemical and biological stratification recurs in the sediment, but the vertical scale is 1000-fold compressed relative to the water column. The aerobic zone, the chemocline and the photic sulphide zone all occur within a film of 1–3 mm thickness. Studies on the distribution of O_2 and H_2S as well as

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of microbenthic photosynthesis were recently initiated in sulphide-rich sediments by using the previously described microelectrode techniques. By rapid shifts from light to darkness it is possible to measure rates of photosynthesis within the microbial film at a spatial resolution of 100 μm (Revsbech *et al.* 1981; Revsbech & Jørgensen, unpublished manuscript). Two examples from an oxidized and a H_2S-rich coastal sediment, respectively, are shown in figure 9. The oxidized sediment had a dense population of diatoms in the uppermost 2–3 mm and no sulphur bacteria. Oxygenic photosynthesis took place down to 2 mm depth and produced a high O_2 peak that reached 3–5 mm depth. The sediment was thus oxic 1.5 mm below the photic zone of the algae and there was consequently not enough light in the anoxic zone for phototrophic

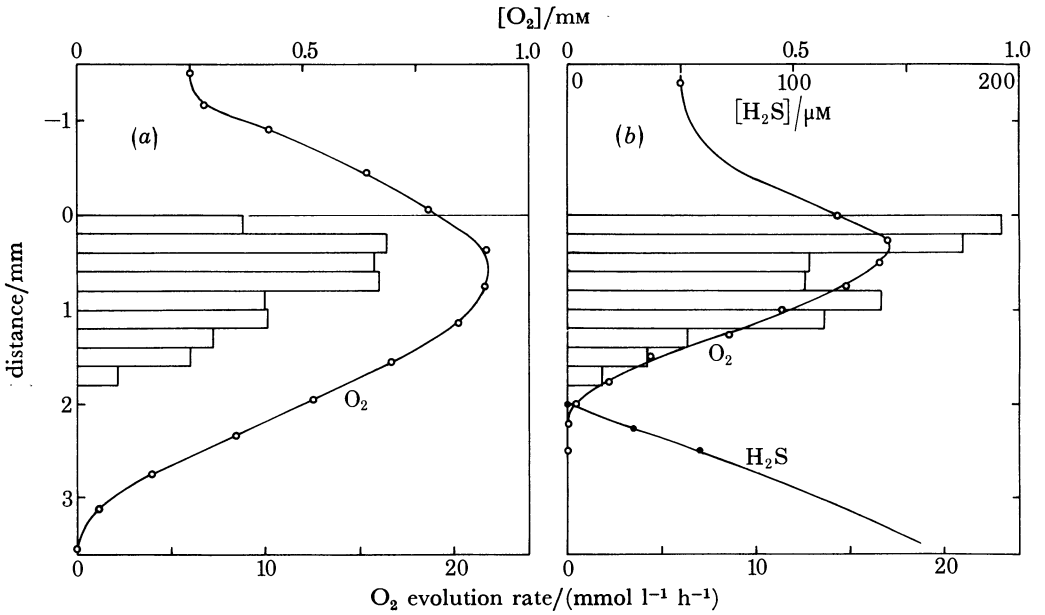

FIGURE 9. Vertical distribution of O_2, H_2S and oxygenic photosynthesis in two coastal sediments (Denmark). (*a*) Oxidized sediment without H_2S in the photic zone; (*b*) reduced sediment with phototrophic sulphur bacteria beneath the oxic zone. (N. P. Revsbech, B. B. Jørgensen & P. Wassman (unpublished).)

bacteria to grow. This is the normal situation in coastal sediments. In the H_2S-rich sediment, sulphide production was so high that oxygen was depleted immediately below the zone of oxygenic photosynthesis, owing to rapid reaction with H_2S. Sufficient light, outside the absorption maxima of chlorophyll, ought therefore to penetrate into the sulphide zone and permit the growth of phototrophic sulphur bacteria. In accordance with this view, a coloured band of purple sulphur bacteria was visible at 2–4 mm depth while diatoms were dominant at 0–2 mm. Techniques to quantify anoxygenic photosynthesis in these dimensions have not yet been developed.

In the fully developed sulfuretum, dense populations of diatoms, cyanobacteria, purple and green sulphur bacteria, and colourless sulphur bacteria such as *Beggiatoa* interact and compete to maximize their exploitation of light, oxygen and sulphide. As an important difference from the similar planktonic communities, the organisms in the sulfuretum are mostly motile. They constantly move around throughout the diurnal cycle and redistribute to where the environmental conditions are favourable for their growth. An example of this is shown in figure 10

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which is based on several studies from a sulfuretum at Kalø Vig near Aarhus, Denmark (Hansen *et al.* 1978; Ingvorsen & Jørgensen 1979; Jørgensen & Revsbech, unpublished; Troelsen & Jørgensen 1982).

The visibly dominant organisms were the filamentous cyanobacteria, *Oscillatoria* spp., and colourless sulphur bacteria, *Beggiatoa* spp., as well as small purple sulphur bacteria, *Chromatium* spp. Diatoms were largely mixed in between the cyanobacteria. During the sunny day (top frame in figure 10), the oxygenic phototrophs produced a sharp O_2 peak in the upper 1.5 mm.

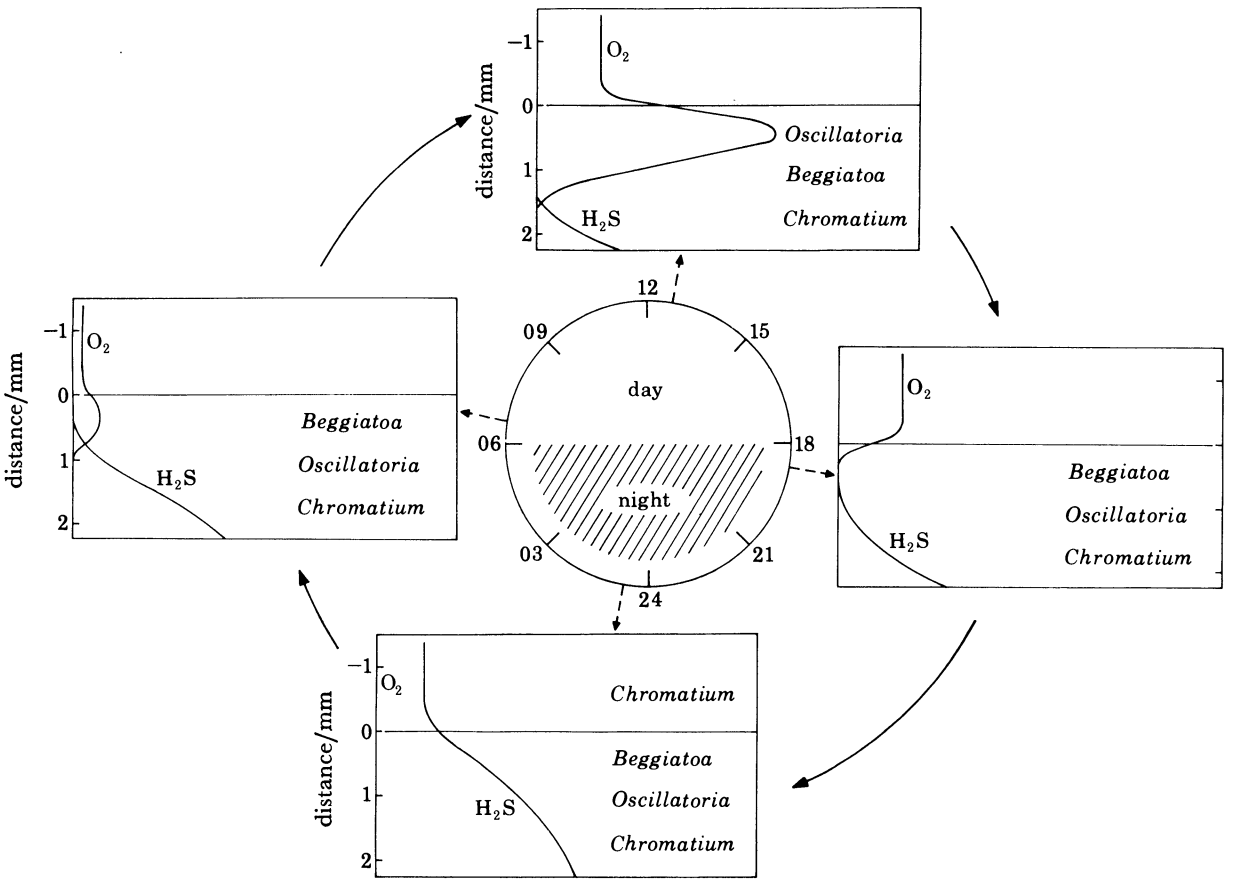

FIGURE 10. Diurnal cycle of oxygen and sulphide distribution and of microbial zonation in a marine sulfuretum.

Beggiatoa were concentrated at the O_2–H_2S interface and colonies of *Chromatium* were abundant in the upper H_2S zone. Soon after sunset, the O_2 peak was gone and the H_2S zone expanded upwards towards the surface, which changed in colour from dark green to white. Around midnight, the high oxygen uptake of the microbial community had depleted the oxygen from the overlying shallow water into which H_2S now diffused freely. The microbial film 2 mm thick on the sediment surface, which during the day constituted an impermeable barrier for the ascending H_2S, now had become completely leaky and permitted the emission of H_2S into the water and atmosphere (see Hansen *et al.* 1978). A part of the *Chromatium* population began to swarm up into the sulphidic water immediately above the sediment, which thereby changed in colour from white to purple. Soon after sunrise, however, oxygenic photosynthesis again blocked the H_2S release and O_2 built up in sediment and water. The clouds of swarming *Chromatium* cells

rapidly disappeared into the sediment to follow the retreating sulphide. During a short transition period, the sediment surface remained white from *Beggiatoa* but soon these had migrated down below the oxygenic phototrophs and the situation of the previous day was restored.

The diurnal migrations in figure 10 indicate how highly adapted the sulfuretum microorganisms are in their chemotactic and phototactic responses to their changing environment. Important information on their metabolism, tactic mechanisms and mutual competition under the actual conditions *in situ* are, however, still required to understand the details of their fascinating ecology and adaptation to a life at the oxic–anoxic interface.

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Discussion

J. R. Postgate, F.R.S. (*U.N.F., University of Sussex, Brighton, U.K.*). How confident can Professor Jørgensen be that his O_2 electrode measurements indicate absence of O_2? Are they sensitive to O_2 levels below the K_m of the oxygen uptake systems of organisms such as *Beggiatoa*?

B. B. Jørgensen. The detection limit of the O_2 microelectrodes, as they have been applied in *Beggiatoa* mats, is below 1 % air saturation. The electrodes showed a linear calibration between N_2, air and pure O_2. The transport of O_2 in the dimensions of the mat is primarily by diffusion. The diffusion gradients showed that O_2 was rapidly consumed within the uppermost 100 μm of the *Beggiatoa* mat. The transport and presence of undetected concentrations of O_2 in lower *Beggiatoa* layers would require that the oxygen here was used only very slowly by the bacteria. There was no significant bioturbation that could have brought O_2 down rapidly. I find it difficult to believe in such an inefficient bacterial uptake of O_2, even at trace levels. Anyhow, if there were traces of O_2 that were not taken up by the bacteria they would be of little significance for their metabolism.

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