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Ecology of the Colourless Sulphur Bacteria

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Introduction

In our treatment of the ecology of the colourless sulphur bacteria, we have been rather selective in our choice of illustrative material. We have attempted to find a median point between the ideals of seeking to familiarize the reader wholly unfamiliar with these organisms and of dealing in great depth with specific environments and organisms or with laboratory techniques such as mixed chemostat culture that have been used to test interactions (such as competition) between sulphur bacteria. The term "colourless sulphur bacteria" is the classic term used to describe one of the three main groups of "sulphur bacteria." The sulphur bacteria comprise the phototrophic bacteria (including some cyanobacteria), the sulphur- and sulphate-reducing bacteria, and the colourless sulphur bacteria. The interaction of these groups is essential to the sustained activity of the global sulphur cycle. The colourless sulphur bacteria are able (or in some cases, believed to be able) to generate metabolically useful energy from the oxidation of reduced inorganic sulphur compounds. In other words, the sulphur compounds act as an electron donor and a respiratory energy source for growth. Different species of colourless sulphur bacteria can use oxygen or oxidized nitrogen compounds (nitrate, nitrite, nitrous oxide) as electron acceptors for sulphur compound oxidation. The term "colourless" is sometimes rather a misnomer, as concentrated samples of bacteria such as the thiobacilli may be highly pigmented

with cytochromes. None of the colourless sulphur bacteria contain photosynthetic pigments. Some of the phototrophic bacteria are able to grow not only phototrophically, with sulphide as an electron donor for carbon dioxide fixation, but also in the dark using the oxidation of reduced inorganic sulphur compounds as the sole source of energy. Clearly, such organisms are not included as members of the colourless sulphur bacteria group. The difference between the sulphur- or sulphate-reducing bacteria and the colourless sulphur bacteria is, of course, that the former organisms use organic compounds as electron donors and the latter are used only in respiratory electron acceptors for energy conservative (Postgate, 1979; Peck and Le Gall, 1982; Pfennig and Widdel, 1982).

In general, we have taken the view that the best approach to understanding the ecology of these bacteria is through their physiology. Ecological interactions are generally competitive or cooperative interactions between physiological types. The type of biochemistry being expressed is not necessarily unique to a morphologically or taxonomically discrete group of organisms. In many cases, the study of microbial ecology is less the study of morphological identification and enumeration of specific types than the evaluation of chemical manifestations of the activity of those bacteria, possibly functioning in a cooperative or synergistic manner in a given environment.

It has become apparent in recent years that the microbiology of sulphur oxidation is rather complex: The thiobacilli and filamentous types such as *Beggiatoa* are part of a system containing many physiological types, several of very restricted ecological adaptability. We are fortunate that some aspects of the ecology of sulphur bacteria have recently been reviewed (Jørgensen, 1982), as have the microbiology and biochemistry of sulphur oxidation (Kelly, 1982; Kuenen and Beudeker, 1982), as well as the role of sulphur bacteria in sulphur geochemistry and the global sulphur cycle (Kuenen, 1975; Pfennig, 1975; Kelly, 1980; Trudinger, 1982). There have also been numerous comprehensive studies on special environments and the importance of metabolic flexibility as an ecological factor (Jørgensen *et al.*, 1979; Gottschal and Kuenen, 1981; Gottschal *et al.*, 1979, 1981a; Smith and Kelly, 1979; Beudeker *et al.*, 1982).

Physiological Flexibility Among the Colourless Sulphur Bacteria and the "Dark Oxidation" of Sulphur in the Natural Environment

The differences in physiological types among the nonphotosynthetic sulphur compound-oxidising bacteria are summarised in Table 1, which illustrates that the physiological types among these organisms can be grouped under four headings. The confusion sometimes experienced by newcomers to the terminology of these bacteria is avoidable, especially if the proliferation of descriptive subter-

Table 1. *Physiological types among the nonphototrophic sulphur compound-oxidising bacteria*[a]

Physiological type[b]	Synonyms or alternative name commonly used	Energy source (electron donor)		Carbon source	
		Inorganic sulphur compound oxidation	Organic compound oxidation	CO_2	Organic compound
A. Obligate chemolithoautotroph	Obligate chemolithotroph Obligate autotroph Obligate chemoautotroph Obligate lithotroph	+	−	+	−
B. Facultative chemolithoautotroph	Facultative chemolithotroph Facultative autotroph Facultative chemoautotroph Facultative lithotroph Mixotroph	+	+	+	+
C. "Symbiont"	—	+	?	+	?
D. Chemolithoheterotroph	Heterotroph able to obtain energy from oxidation of an inorganic sulphur compound	+	+	−	+
E. Chemoorganoheterotroph	Heterotroph able to oxidise sulphur compounds but unable to obtain energy	−	+	−	+

[a] By definition, the colourless sulphur bacteria belong to the first four groups.
[b] Chemo, metabolic energy obtained from chemical oxidation rather than from photosynthesis; litho, inorganic rather than organic (organo-) sources of energy; auto, carbon dioxide used as the carbon source (in contrast to heterotrophic use of organic carbon sources).

minology is avoided. Thus, most of the bacterial world is composed of "chemo-organotrophic heterotrophs" (normally simply called "heterotrophs"). Some chemolithotrophic autotrophs are also capable of growth as chemoorganotrophic heterotrophs (type B) and are frequently referred to as "facultative autotrophs," a term which defines precisely the easy choice of these two types of physiology in response to environmental conditions. As these organisms can grow on mixtures of organic and inorganic compounds, they may also be called "mixotrophs." Some chemolithotrophic autotrophs (e.g., *Thiobacillus neapolitanus* and *T. denitrificans;* see Table 2) are "obligate" (type A), that is, they are capable of growth only in this physiological mode, although they may have a limited heterotrophic capacity to assimilate some exogenous organic matter at the expense of energy from sulphur compound oxidation and to metabolise intracellular polyglucose for energy generation (Kelly, 1971, 1981; Rittenberg, 1972; Kuenen and Beudeker, 1982). The occurrence and biochemistry of these two types have been considered in detail (Kuenen and Tuovinen, 1981; Kelly, 1982; Kuenen and Beudeker, 1982). Those capable of true mixotrophy (type B) seem to have the ability to sustain regulated metabolism using organic and inorganic sources of carbon and energy, and to have a considerable survival and competitive capacity in the environment (Gottschal *et al.*, 1979; Smith *et al.*, 1980; Perez and Matin, 1980; Wood and Kelly, 1981; Kuenen and Beudeker, 1982).

Table 2. *Examples of sulphide-oxidising bacteria including the colourless sulphur bacteria, the phototrophic bacteria, and the inorganic sulphur compound-oxidising bacteria*

Physiological type	Example
Obligate chemolithoautotroph	*Thiobacillus neapolitanus* *T. denitrificans,* including marine strains *Thiomicrospira pelophila*
Facultative chemolithoautotroph	*T. novellus* *Thiobacillus* A2 *Sulfolobus acidocaldarius* *Beggiatoa* sp. *Chromatium* sp. *Thiocapsa* sp.
Symbiont able (at least) to generate energy from S^{2-} oxidation and to fix carbon dioxide	Symbionts of *Riftia pachyptila, Solemya,* and symbionts with other eukaryotes or prokaryotes?
Chemolithotrophic heterotroph	*Thiobacillus perometabolis* *Beggiatoa* sp.
Chemoorganotroph	*Pseudomonas aeruginosa* and many other soil and marine microorganisms

Among the facultative species, only *Thiobacillus* A2 and *T. novellus* have been shown capable of growth as chemoorganotrophic autotrophs. Thus, both grow on formate as an energy source but fix carbon dioxide by the Calvin cycle (Chandra and Shethna, 1977; Kelly *et al.*, 1979), and *Thiobacillus* A2 has been proved to oxidise methanol as an energy substrate while obtaining all of its carbon from carbon dioxide (Kelly and Wood, 1982). This organism also shows a physiological mixture of chemoorganotrophic heterotrophy and autotrophy when cultured on mixtures of formate and glucose (Wood and Kelly, 1981).

In Table 1, the third type of organism (C) comprises the symbiotic organisms of unknown physiological status, which may prove to be similar to that of type A or B. The type D organisms, which we shall discuss in some detail, are essentially chemoorganotrophic heterotrophs that are able to use sulphur compounds as (additional) energy sources for growth. Finally, a whole range of chemoorganotrophic heterotrophs is known to be able to oxidise reduced inorganic sulphur compounds, but they cannot obtain energy from such oxidations.

We have emphasised the physiological possibilities available to many of the colourless sulphur bacteria (including the less studied filamentous forms and a diversity of eubacterial forms that probably remain to be isolated or reisolated) because they are likely to experience mixed substrate environments in natural habitats, so that the regulatory processes and environmental pressures operating to determine their physiological behaviour are probably more complex than the relatively simple responses elicited by laboratory culture under single substrate-defined conditions.

Before proceeding to a detailed study of the types of bacteria, we have summarised in Table 2 the types of bacteria likely to affect the "dark oxidation" of inorganic sulphur in diverse environments. For completeness, the sulphur-oxidising photolithotrophs have been included because they are now known to carry out significant oxidation of sulphide in the dark under microaerophilic conditions. In quantitative terms, the greatest activity of all of these organisms is probably exhibited in environments where sulphide and oxygen (or nitrate) coexist. They are thus likely to be preponderant in oxic–anoxic interface habitats. Such environments are diverse in character, ranging from the broad sulphide-oxygen coexistence layers in stratified lake and ocean systems (Richards, 1965; Sorokin, 1972; Tuttle and Jannasch, 1973; Jørgensen, 1982; Indrebø *et al.*, 1979a,b; Jørgensen *et al.*, 1979; Kelly, 1980) to the narrower interface bands found in some sediments or in bacterial mats (Jørgensen, 1982). The general occurrence of the different metabolic types in soil and freshwater environments and marine (estuarine) ecosystems indicates that niches for the different types must be available in all of these habitats. Their ubiquity even in the open ocean further indicates their potential ability to scavenge both locally produced sulphide and (dilute) supplies of dissolved compounds such as thiosulphate or

locally available sources of particulate elemental sulphur of natural or agricultural origin.

Heterotrophic Sulphur-Oxidising Bacteria

The first reports of heterotrophic bacteria that oxidise inorganic sulphur compounds dealt with their ability to develop in media containing thiosulphate. The thiosulphate was often quantitatively converted into tetrathionate, and no sulphate was formed (Trudinger, 1967). In other cases, heavy precipitates of elemental sulphur were generated during growth. At the same time, organic compounds were required for growth. It was assumed that these organisms could obtain metabolically useful energy from this oxidation process, but evidence for this was inconclusive (Starkey, 1935, 1966). Over the years, numerous papers have reported the presence of high numbers of such organisms, particularly in soils (Swaby and Vitolins, 1969; Kelly, 1972) and later in marine environments (Tuttle and Jannasch, 1972, 1973; Tuttle *et al.*, 1974). The heterotrophic sulphur compound-oxidising bacteria described were usually unable to oxidise any compound other than thiosulphate. Aerobic media with sulphide as a sulphur source will inevitably contain thiosulphate (Kuenen, 1975), so that even if growth (or oxidation) on sulphide was reported, this may in fact have been growth on thiosulphate. The often raised questions were (1) whether these organisms were able to obtain energy from this oxidation step and (2) whether the oxidation of thiosulphate to tetrathionate has any ecological significance. To date, these questions have only partially been answered, and a great need exists for further details and quantitative analysis of the metabolism of these organisms. In the 1960s, Trudinger (1967) presented data to show that some thiosulphate-oxidising heterotrophs oxidised thiosulphate to tetrathionate, but the bacteria were apparently unable to generate energy from the oxidation. *Pseudomonas aeruginosa* was reported to oxidise thiosulphate and other sulphur compounds to sulphate via tetrathionate (Schook and Berk, 1979), but in further experiments with this strain in our laboratory (J. G. Kuenen, unpublished data), we were unable to demonstrate that this oxidation process produced metabolically useful energy.

The first well-described example of a chemolithotrophic heterotroph was *Thiobacillus perometabolis* (London and Rittenberg, 1967), an organism which grew best in mixtures of organic substrates and thiosulphate. The thiosulphate was converted into sulphuric acid, but autotrophic growth was not possible. However, Katayama-Fujimura *et al.* (1982) reported that this organism (ATCC 23370) could be grown autotrophically. Apparently, further work is necessary to prove that carbon is indeed obtained from CO_2 and not from traces of organic compounds.

In the 1970s, Tuttle (1980) and Tuttle and Jannasch (1977) studied large numbers of heterotrophic bacteria, isolated from the sulphide–oxygen interface of the Black Sea, that were able to oxidise thiosulphate to tetrathionate. In a series of papers, these authors presented evidence for the ability of some of their strains to obtain energy from this oxidation step. In many cases, experiments designed to demonstrate increases in growth yields on addition of thiosulphate to heterotrophic media showed marginal and pH-dependent increases. Later results indicated unrealistically high yields from the single step of oxidation of thiosulphate to sulphate (for a discussion, see Kuenen and Beudeker, 1982). On the other hand, in Tuttle's laboratory (Tuttle, 1980) and also in our own (J. G. Kuenen, unpublished), it has been clearly demonstrated that on addition of thiosulphate to starving cells of these heterotrophs, the ATP pools of the cells rapidly increase. In further experiments on one Black Sea isolate, it was shown that $^{14}CO_2$ fixation was clearly thiosulphate dependent (Tuttle and Jannasch, 1977). CO_2 incorporation was at a level of 30% of a control experiment with an obligately chemolithoautrophic *Thiobacillus* strain. Later work with the same strain (Tuttle, 1980) clearly demonstrated that the organism was a heterotroph that could use thiosulphate as an additional energy source for organic carbon assimilation. This finding seems to contradict the previous one unless this organism carried out very active heterotrophic CO_2 fixation, for example, by means of PEP carboxylase.

To avoid further confusion in this field of research, it seems extremely important that before claims are made, quantitative studies should be done on (1) yields obtained from the oxidation of sulphur compounds, preferably in continuous culture, and (2) levels of CO_2-fixing enzymes, not only those specific for autotrophic CO_2 fixation [notably ribulose 1,5-bisphosphate (RuBP) carboxylase and phosphoribulokinase] but also anaplerotic enzymes such as PEP and pyruvate carboxylase. Those strains showing substantial $^{14}CO_2$ fixation under some experimental conditions should obviously be used for such tests.

Gottschal and Kuenen (1980) developed a new method to isolate various metabolic types of sulphur-oxidizing bacteria from freshwater sources by enrichments in a continuous culture. The technique allows selection at will for obligate, facultative, or heterotrophic sulphur-oxidizing bacteria. Depending on the ratio of organic and inorganic sulphur compounds fed into the continuous culture (chemostat), the different physiological types will be selected according to Fig. 1. In the actual experiments, all metabolic types have been enriched for, with the possible exception of the heterotrophs that are able to oxidise sulphur compounds but not to generate energy. This latter type may have been present in the enrichments but was not identified. One organism was apparently a chemolithotrophic heterotroph. The organism could not be grown autotrophically but, in contrast to the organisms mentioned before, could oxidise thiosulphate completely to sulphate. This strain proved to be extremely sensitive to sulphite, which was easily

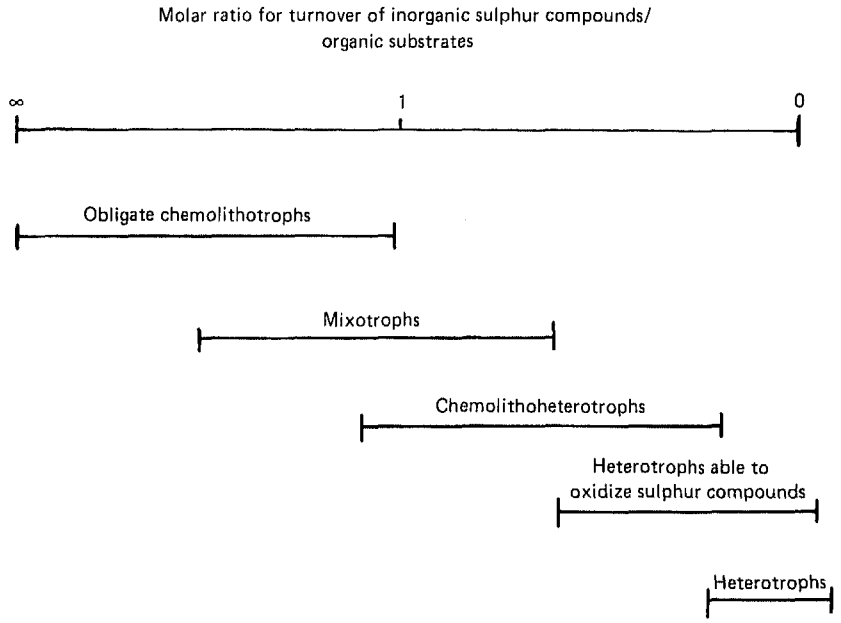

Fig. 1. Representation of the turnover ratio of inorganic compounds and the occurrence of different physiological types among sulphur-oxidising bacteria.

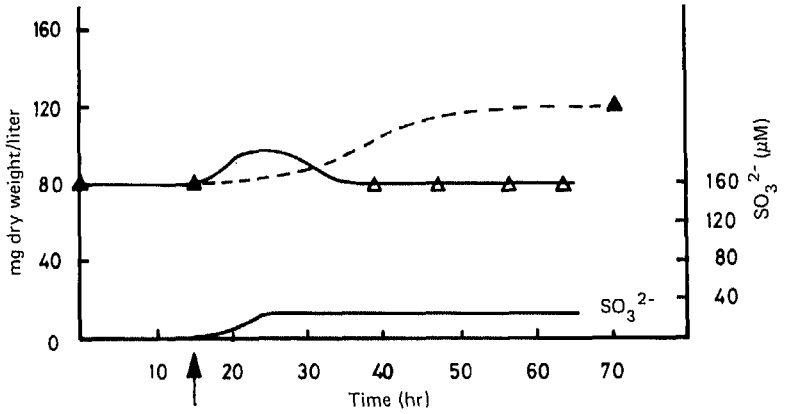

Fig. 2. Effect of thiosulphate on heterotrophic sulphur-oxidising bacteria in acetate-limited chemostat culture. ▲, $D = 0.02$; △, $D = 0.05$. Arrow indicates addition of 20 m*M* thiosulphate.

produced from incomplete oxidation of thiosulphate. Figure 2 shows the dry weight (equivalent to bacterial protein) as a function of time in a chemostat under acetate limitation. When thiosulphate was added to the inflowing medium while the dilution rate remained constant, an initial increase in cell density was observed. This was interpreted as evidence for the ability of the organism to obtain energy from the oxidation of the thiosulphate. However, the cell density returned

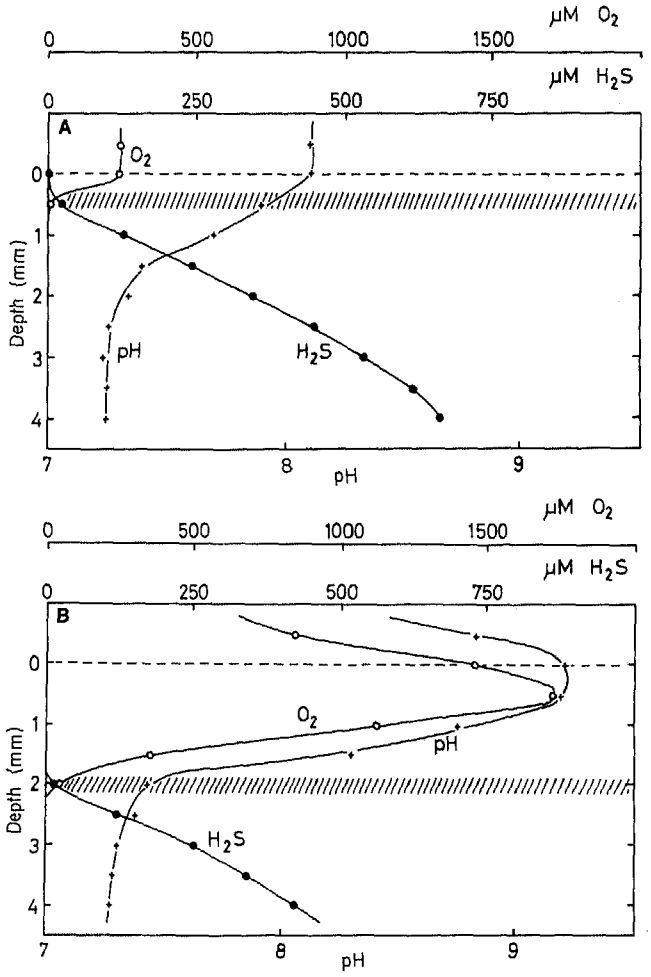

Fig. 3. Dark (A) and light (B) profiles of the O_2, H_2S, and pH of microbial mats from Solar Lake, Sinai. Dashed line indicates mat surface. Data were obtained with microelectrodes. In B, oxygenic photosynthesis creates a supersaturated oxygen concentration in the microbial mats. Note the extremely narrow zone of coexisting O_2 and H_2S both in the light and in the dark. With permission from Jørgensen (1983).

to its initial value after another 12 hr. After this period, thiosulphate remained untouched, whereas all acetate was still utilised. In a second experiment, the same sequence of substrate supply was followed, but on addition of the thiosulphate to the inflowing medium, the dilution rate was reduced in order to provide thiosulphate to the culture at a slower rate than in the first experiment. Figure 2 shows that in that case, the increase in yield persisted. The increase was of the same order of magnitude as that found for mixotrophic thiobacilli (Perez and Matin, 1980; Gottschal and Kuenen, 1980). It seems that if the thiosulphate concentration in the culture increased too rapidly, complete oxidation did not occur and sulphite accumulated in the culture, which subsequently irreversibly inhibited the thiosulphate oxidation. Small amounts of sulphite would continue to be produced, as a result of which the culture remained unable to oxidize thiosulphate. This interpretation could also explain why this organism, when grown in batch culture in the presence of excess thiosulphate, never showed substantial thiosulphate oxidation, since under such conditions sulphite would certainly have been produced.

In our laboratory we have observed that most, if not all, commercial thiosulphate preparations contain sulphite, which may inhibit the activity of heterotrophic sulphur-oxidising bacteria. This may explain why, in many cases, results of growth experiments in batch culture have been irreproducible. Finally, it should be realized that some heterotrophic sulphur-oxidising bacteria tend particularly to produce sulphite, whereas the specialist obligate chemolithotrophs can rapidly oxidise the sulphite to sulphate. This may also be of ecological importance. In mixtures of specialised obligate chemolithotrophic bacteria with heterotrophs, sulphite may not accumulate due to the activity of the specialists.

Curiously, in our hands, the continuous culture enrichment technique was not successful for the enrichment of facultative chemolithotrophs from the marine environment. Instead, large numbers of thiosulphate-oxidising heterotrophs mixed with specialists were obtained from enrichments with mixtures of organic substrates and inorganic sulphur compounds. However, Smith and Finazzo (1981) successfully isolated a marine strain of the facultatively chemolitho(auto)trophic *Thiobacillus intermedius* in a chemostat enrichment culture, showing that differences in inoculum material and perhaps also other experimental conditions may greatly influence the outcome of the experiments.

Fieldwork with Colourless Sulphur Bacteria

The colourless sulphur bacteria can be found almost anywhere in nature where inorganic sulphur compounds and oxygen or other suitable electron acceptors, such as nitrate, are available. Such is the case at the sulphide–oxygen interface of marine and freshwater sediments. Figure 3 shows an example of a cyanobac-

terial mat with such an interface. In sediments the oxygen–sulphide interface is not necessarily limited to a narrow horizontal zone, but can also reach deeper into sediment due to channels made by burrowing animals. Furthermore, isolated anaerobic pockets with active sulphate reduction can be found in otherwise oxic sediments (Jørgensen, 1977). Other well-known examples of habitats for the sulphur-oxidising bacteria are sulphur deposits, sulphur springs in areas with geothermal activity (Brock *et al.*, 1972), and waste treatment plants receiving sulphur compounds such as sulphide or thiocyanate (Woodard *et al.*, 1976).

Another important habitat of sulphur-oxidising bacteria is the oxygen–sulphide interface of stratified water bodies. Notable examples are the Black Sea, the Cariaco Trench, deep freshwater lakes throughout the world, and unusual environments such as hypersaline Solar Lake (Sinai). Figure 4 gives a typical example of such a layer of coexisting S^{2-} and O_2 where sulphur-oxidising bacteria may thrive. Since the literature on the role of colourless sulphur bacteria was reviewed by Kuenen (1975), relatively few advances have been made in field techniques to detect specifically the activity of colourless sulphur bacteria in

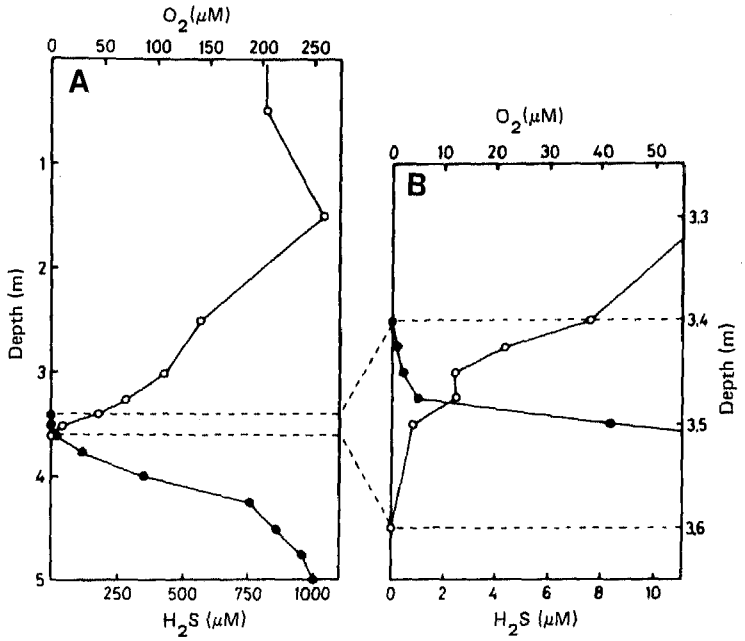

Fig. 4. Gradients in Solar Lake (Sinai). (A) Oxygen (○) and sulphide (●) gradients. (B) Oxygen–sulphide interface from (A) shown with expanded scales to demonstrate the zone of coexisting O_2 and H_2S (16 February). From Jørgensen *et al.* (1979).

the natural environment. Also, techniques to enumerate sulphur bacteria have not been improved significantly. The methods available in the literature are

1. Thiosulphate or elemental sulphur oxidation.
2. Decline in pH due to the production of sulphuric acid from sulphur, sulphide, or thiosulphate.
3. Rise in pH due to the production of tetrathionate from thiosulphate.
4. Production and/or turnover rates of intermediates from sulphide or thiosulphate labelled with ^{35}S.
5. Fixation of $^{14}CO_2$ in the dark.
6. Direct measurement of RuBP carboxylase in samples from permanently dark environments.
7. Most probable number (MPN) counts combined with (1), (2), or (3).
8. Direct count and measurements of colourless sulphur bacteria which can be directly seen and measured under the microscope.
9. Microelectrode measurements in sediments and aquatic environments.
10. Fluorescent antibody techniques.

The advantages and disadvantages of many of these methods have been discussed in detail by Kuenen (1975), with the exception of the last two methods. The microelectrode technique is a new and interesting field of research now being developed by Jørgensen's group.

The conclusion was, and still is, that in most cases no one of these methods will give a reliable estimate of the role of colourless sulphur bacteria in nature. A combination of these methods will certainly increase their meaningfulness, but even in that case, a quantitative estimate of microbial activity will be possible only in those (natural) environments where sulphide-oxidising bacteria dominate the total population. Such is the case, for example, in acid hot sulphur springs (Mosser *et al.*, 1973, 1974) and some highly acidic soils (Fliermans and Brock, 1972; Kuenen, 1975), and in environments dominated by the unusually large colourless sulphur bacteria such as *Beggiatoa* or *Thiovulum*, which can be recognized under the microscope (Jørgensen, 1982). A few examples will serve to make this point.

Beggiatoa

For a long time, *Beggiatoa* has been considered one of the most typical colourless sulphur bacteria in that it can generate energy from the oxidation of inorganic sulphur compounds and may therefore be able to grow autotrophically. Unchallenged is its ability to oxidise sulphur compounds, but the role of sulphur oxidation may be only indirectly linked to energy generation. Only one report (Kowallik and Pringsheim. 1966) has described autotrophic growth. Nelson and Castenholz (1980a,b) discovered that their strain of *Beggiatoa* was a heterotroph

which used its "intracellular" elemental sulphur as an electron acceptor for the oxidation of organic compounds under anaerobic conditions. This sulphur is located between the cellular membrane and the cell wall of *Beggiatoa* (Strohl and Larkin, 1978). During the oxidation of organic compounds, this sulphur was converted to sulphide. Subsequently, *Beggiatoa* used its high mobility to move in the sediment from the anaerobic environment to microaerophilic conditions, where the available sulphide was reoxidised to elemental sulphur. The *Beggiatoa* returned with stored sulphur to the anaerobic zone, where organic substrates such as acetate were available for the next round of oxidation. In this way, *Beggiatoa* shuttled between aerobic and anaerobic conditions to renew its internal electron acceptor. These observations, however, do not disprove the idea that *Beggiatoa* may also be able to oxidise elemental sulphur to sulphate, thereby obtaining chemolithotrophic energy.

In the same period, Strohl *et al.* (1981; Strohl and Larkin, 1978) reported on the heterotrophic abilities of *Beggiatoa* strains, which leaves little doubt that these strains are indeed heterotrophs. The same workers (Güde *et al.*, 1981) also reported "mixotrophic growth" of *Beggiatoa* on a mixture of sulphide and acetate. Under these conditions, sulphide may be oxidised, at least in part, to sulphate. Sulphide stimulated growth, but it was pointed out (Kuenen and Beudeker, 1982) that the increase in yield observed on addition of sulphide to acetate cultures may be too high to be explained by sulphur oxidation. CO_2 fixation in these organisms was demonstrated but was extremely small. Quantitative balances of input and output of organic compounds (oxidised, assimilated, etc.), CO_2, and/or sulphur metabolism will be needed to establish a good basis for further work on *Beggiatoa*. It should be emphasized here that it is most important for the initial physiological work to concentrate on a few strains, since it may very well be that, like the thiobacilli, the *Beggiatoa* comprise the complete spectrum of obligate chemolithotrophs, facultative chemolithotrophs, and chemolithotrophic heterotrophs.

As *Beggiatoa* are conspicuous organisms which can be recognized directly under the microscope, they can be studied, counted, and observed in samples taken from nature. Jørgensen (1977) reported large numbers of *Beggiatoa* in a coastal marine sediment. The organisms were hardly present in sandy sediments, but were abundant in muds aggregated around faecal pellets. It was postulated that *Beggiatoa* would be able to move more freely in the muddy than in the sandy sediment, and might therefore not be able to live in the sandy environment. Furthermore, the presence of sulphide-producing pockets (faecal pellets) in the softer, otherwise aerobic sediments would provide an ideal niche for these organisms. On the basis of the observed rate of oxidation of intracellular elemental sulphur in *Beggiatoa* (rate of disappearance of sulphur under aerobic conditions) and the biomass measured, an estimate was made of the potential rate of oxidation of sulphur in the sediments. The calculated value of 5–15 mmol S^{2-} m^{-2}

day $^{-1}$ was of the same order as the local sulphide production (sulphate reduction), which was approximately 10 mmol S^{2-} m^{-2} day^{-1}. This indicated that *Beggiatoa* could play an important role in the oxidation of sulphur compounds in the marine sediment. Microelectrodes were used to study the behaviour of populations of *Beggiatoa* and *Thiovulum* species in the microgradients in and above sulphide-containing sediment. Both populations located themselves very accurately at the interface between O_2 and S^{2-}, and maintained a steep gradient of sulphide diffusing upwards and oxygen diffusion downwards (Jørgensen, 1982). In the case of *Thiovulum*, the individual cells very actively moved in and out the gradient, until eventually the organisms arranged themselves in their veils of slime where the exact gradient could be maintained. As pointed out, these experiments show that the environments in which these gradient organisms live are extremely dynamic and obviously easy to disturb.

Competition Between Biological and Spontaneous ("Chemical") Oxidation of Sulphide with Oxygen

The H_2S formed in anoxic water bodies or sediments diffuses upwards towards the interface with oxygen, where it can react spontaneously with oxygen. Oxygen and sulphide coexist in salt environments at concentrations between 10^{-4} and $10^{-6}M$. The half-life of the spontaneous oxidation of sulphide ranges from a few minutes to an hour. Oxidation rates are dependent on a variety of environmental parameters such as temperature, salt concentration, presence of trace metals, and catalytic amounts of other sulphur compounds (Chen and Morris, 1972; Jørgensen *et al.*, 1979; Cline and Richards, 1969). The interesting question is whether bacteria would be able to compete successfully with the spontaneous reaction at low concentrations of O_2 and sulphide. Products of spontaneous oxidation are sulphur (10%), thiosulphate (30%), sulphite (30%) and sulphate (30%) (Chen and Morris, 1972). All of the available fieldwork seems to indicate that this is indeed so. For example, microelectrode measurements in *Beggiatoa* and *Thiovulum* veils show that these bacteria can oxidise sulphide at such a rate that neither sulphide nor oxygen is detectable, whereas without bacteria oxygen and sulphide coexist (Jørgensen, 1982).

Laboratory work with pure cultures of *T. neapolitanus* and *Thiobacillus* A2 also provides strong evidence that these organisms can efficiently and directly oxidise sulphide. This was shown by growing organisms under sulphide limitation in the chemostat. Table 3 shows the production of *T. neapolitanus* biomass during growth on sulphide and presents similar data for thiosulphate-limited growth. In the chemostat at pH 7.0, sulphide was below the level of detection, which is about 5×10^{-7} *M*. The thermodynamics of thiosulphate and sulphide oxidation to sulphate predict that the energy yield of both oxidation reactions should be equal (for further discussion, see Kelly 1982). Furthermore, the rates

Table 3. *Growth of* Thiobacillus neapolitanus *in an aerobic energy substrate-limited chemostat at a dilution rate of 0.05 hr^{-1} in a mineral medium with thiosulphate (40 mM) or sulphide (40 mM) as the growth-limiting nutrient*

	Growth-limiting substrate	
	$S_2O_3^{2-}$	S^{2-}
Biomass (mg dry wt l^{-1})	200	196
Residual concentration of $S_2O_3^{2-}$ or S^{2-} (*M*)	$<10^{-4}$	$<5 \times 10^{-7}$

[a] Data from Beudeker *et al.* (1982).

of oxidation of both sulphide and thiosulphate are identical under a variety of growth conditions. One would therefore predict that yields per mole of thiosulphate should be equal to the yield per mole of sulphide oxidised. Table 3 shows that this was indeed the case. If any substantial spontaneous oxidation had taken place, the yield on sulphide should have been lower.

Analogous experiments with *Thiobacillus* A2 gave identical results. Interestingly, this organism is unable to oxidise elemental sulphur. Thus, if substantial spontaneous oxidation had occurred, this would have led to accumulation in the medium of elemental sulphur, which was not the case (Beudeker *et al.*, 1982). These results indicate that thiobacilli can indeed compete very successfully with spontaneous oxidation. This is, however, not always true. A well-documented case was described by Sorokin (1972), who studied the oxidation of H_2S in the sulphur–oxygen interface in the Black Sea. The rate of H_2S disappearance was estimated by adding [^{35}S]sulphide to samples of water and incubating in the absence or presence of chloroform, which was added to inhibit biological oxidation. The rate of sulphide disappearance was the same in the presence of chloroform as in untreated samples. From this observation, it was concluded that the initial reaction of H_2S with oxygen was "chemical" and not "biological." Thiosulphate, a major product of spontaneous oxidation, was, however, oxidised to sulphate at a higher rate in the untreated samples than it was by the chloroform-killed controls. From this it was concluded that bacteria are involved primarily in the oxidation in the chemocline of thiosulphate rather than of sulphide. Although this interpretation may certainly be correct, it could be argued that even if chemical and biological rates of sulphide oxidation are very similar, the microorganisms present *in situ* might still be able to interfere with the chemical reaction by removing polysulphides, which have been shown to act autocatalytically on the chemical oxidation of sulphide (Kuenen, 1975). In the absence of these catalysts, the spontaneous oxidation would proceed at a lower rate and thus allow the organisms to oxidise a greater proportion of the sulphide

directly, thereby possibly gaining an enhanced supply of metabolically useful energy.

In this context, it should be mentioned that Tuttle and Jannasch (1972) and J. G. Kuenen (unpublished results), in contrast to Sorokin, were unable to isolate any obligate chemolithotrophic thiobacilli from the Black Sea. Instead, large numbers of sulphur- or thiosulphate-oxidising heterotrophs were isolated. Their properties have already been discussed in this chapter, but one interesting property was not mentioned. It was shown that a number of the thiosulphate-oxidising strains could also use this sulphur compound as electron acceptor for heterotrophic growth under anaerobic conditions (Tuttle and Jannasch, 1977). Such a property would render the organisms extremely suited for life at interfaces where oxygen is always available. It remains unclear, however, why true specialist thiobacilli are apparently not present at the interface. This becomes even more puzzling if it is realized that in the oxic zone thiosulphate is often available.

In the layer of coexisting sulphide and oxygen in the hypersaline Solar Lake (see also Fig. 4), the spontaneous oxidation rate may also be significant. Laboratory simulation experiments showed that the half-life of sulphide in filtered controls was 7.4 min, whereas half-lives of 5.1 and 2.7 min were exhibited by untreated and concentrated samples, respectively, showing that the biological oxidation was somewhat faster. As discussed in the previous paragraph, this may imply that in the presence of microorganisms spontaneous oxidation may be negligible. Indeed, an important role for biological oxidation has been established beyond any doubt. In the interface of Solar Lake, a major role is played by a bloom of sulphide-oxidising cyanobacteria which oxidise the sulphide to elemental sulphur in the light under anaerobic conditions. When the sulphide in the layer becomes depleted, the cyanobacterial layer switches to normal oxygenic photosynthesis.

In an extensive field experiment, Jørgensen *et al.* (1979) measured light and dark CO_2 fixation over a 36-hr period at various depths around the interface of Solar Lake. Variations of dissolved O_2 and S^{2-} concentrations in the diurnal cycle were also measured. The processes of sulphide-dependent or oxygenic photosynthesis (CO_2 fixation) can be discriminated experimentally by the addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (which inhibits photosystem II) to the samples, thereby inhibiting oxygenic- but not sulphide-dependent CO_2 fixation. Figure 5 shows, in the first row of panels, the changes in parameters with depth at 2300–0100 hr (nighttime). Two peaks of dark CO_2 fixation are evident. The lower peak may be due to sulphide-dependent chemolithotrophic CO_2 fixation. The upper peak may also be chemolithotrophic CO_2 fixation dependent on thiosulphate, which was shown to be present at low concentrations at this depth.

After sunrise (middle panels, 0600–0900 hr, Fig. 5), the lower peak of dark CO_2 fixation disappeared, concomitantly with a considerable decrease in the

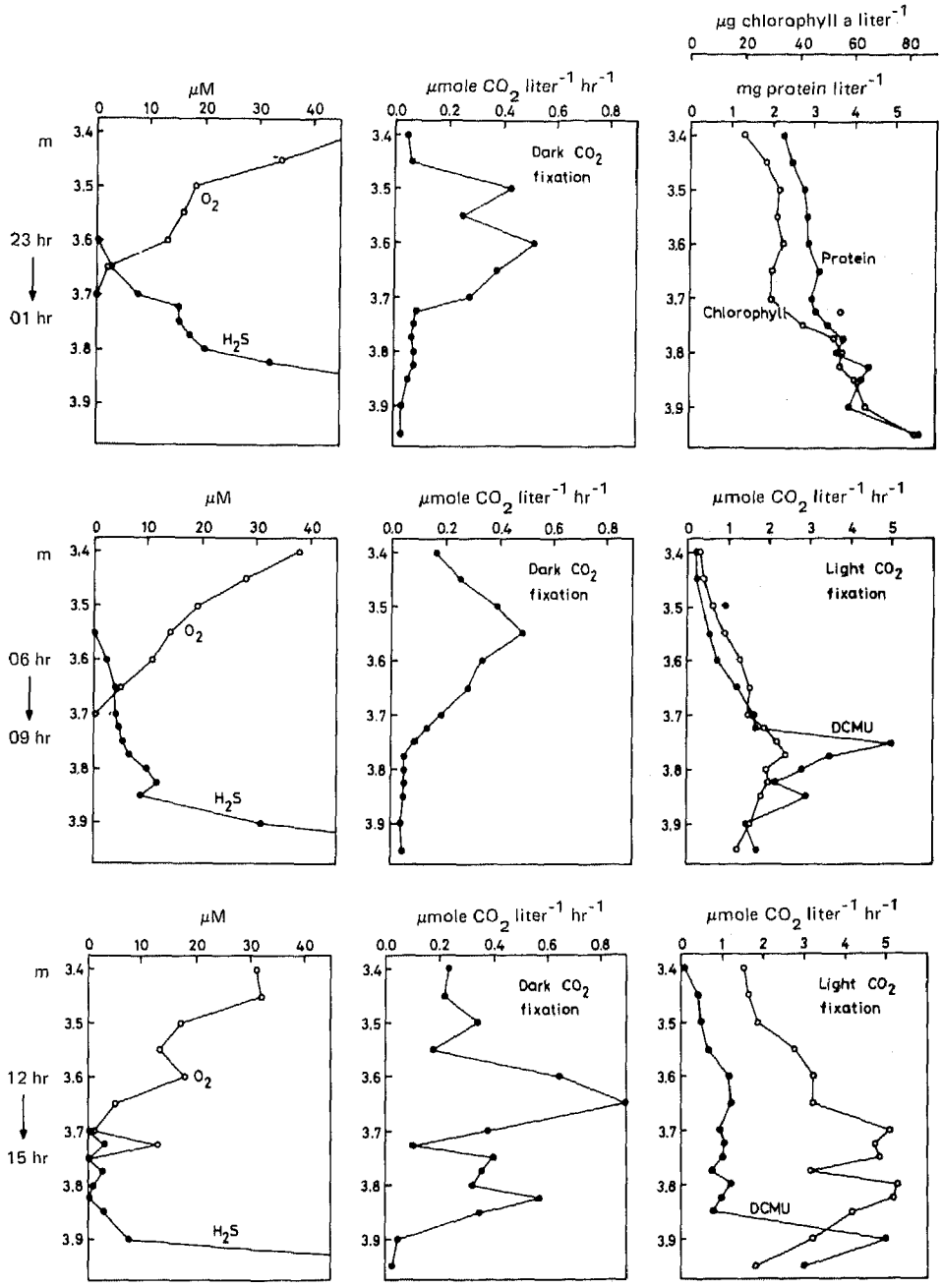

Fig. 5. Distribution in the chemocline of oxygen and sulphide; dark CO_2 fixation and light CO_2 fixation with or without DCMU. *In situ* incubations for 30 min at different times. Protein and chlorophyll a gradient also shown (28–29 March). From Jørgensen *et al.* (1979).

sulphide concentration. The disappearance of sulphide was clearly due to sulphide-dependent photosynthesis (in fact, DCMU stimulated photosynthesis, a phenomenon which remains unexplained).

The decrease in the lower dark CO_2 fixation at this time of the day indicates that the CO_2 fixation was indeed sulphide dependent. Separate experiments confirmed this contention since addition of sulphide to this layer increased dark CO_2 fixation. After midday (1200–1500 hr, Fig. 5), a very interesting phenomenon was observed. At that time, light-dependent CO_2 fixation increased to its maximum and clearly become strongly DCMU sensitive. This means that considerable oxygen production must have taken place at a depth of 3.5–3.9 m. However, at 3.75–3.9 m, no oxygen was detectable; at this depth, dark CO_2 fixation had again risen significantly. This can be taken as evidence for a very rapid, nonphotosynthetic biological oxidation of the sulphide, leading to CO_2 fixation. Particularly interesting is the small peak of oxygen at 3.725 m, which coincided with a low in the dark CO_2 fixation. This might mean that at this depth oxygen/sulphide-dependent dark CO_2 fixation does not occur, perhaps because of the absence of suitable organisms at this very dynamic sulphide–oxygen interface. Additional experiments showed that at 3.55 m the dark CO_2 fixation rate was generally electron donor (S^{2-}, $S_2O_3^{2-}$) limited, whereas at 3.75 m this rate was usually electron acceptor (CO_2) limited. Taken together, these data strongly indicate that an active chemolithoautotrophic population of sulphide-oxidising bacteria is present at the interface in different layers. Further circumstantial evidence for their presence comes from the fact that a chemostat enrichment culture prepared by inoculating interface sample water into a saline-mineral salts medium supplied with growth-limiting thiosulphate or sulphide (input concentration, 50 m*M*) resulted in a chemolithotrophic population with a yield of about 5 g dry weight (per mole of thiosulphate or sulphide oxidised). This is a typical yield for *Thiobacillus* and *Thiomicrospira* species (Kelly, 1982).

A practical aspect of the work on the activity of chemolithotrophic bacteria growing at the oxygen–sulphide interface is the sampling. The actual layer of coexistence in aqueous systems is often less than a few centimeters, making extremely accurate sampling essential. One method is the use of a pumping system with a specially designed inlet which ensures withdrawal of liquid from a horizontal circular segment of the column at 1- to 2-cm intervals.

The usefulness of this system was already apparent from the result of the Solar Lake work (for a description of the sampling device, see Jørgensen *et al.*, 1979). Later work showed that it can be used successfully not only in the very stable salt gradient of Solar Lake but also in a brackish environment where the density gradient is much less steep. For example, Saelenvaan Lake (near Bergen, Norway) on a still day with little or no wind showed a clear pattern of sulphide and oxygen concentration and of dark CO_2 fixation (Fig. 6). Coexistence of O_2 and S^{2-} was observed only over a few centimeters. At that level, a clear peak of dark

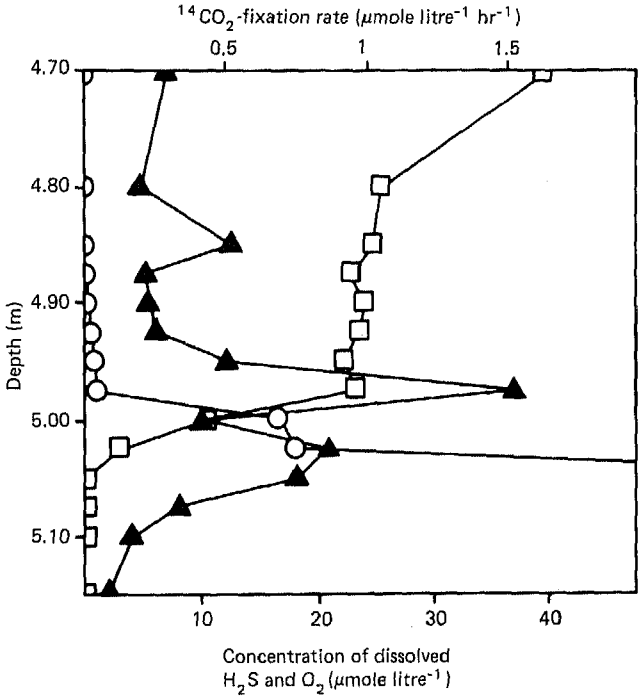

Fig. 6. Profiles of CO_2 fixation (▲, μmole CO_2 litre^{-1} hr^{-1}), dissolved oxygen (□), and dissolved H_2S (○) concentrations (μmole litre^{-1}) in Sælenvaan Lake, Norway, sampled at 0500 hr, 15 August 1978.

CO_2 fixation was also visible (Børsheim, 1979). The important point to be noted here is that sampling instruments with lower resolution, such as those commonly used in fieldwork, will not only fail to give accurate data but will also give a "smear" of dark CO_2 fixation over about 10–20 cm. It is obvious that had this been the case, the peak of dark CO_2 fixation at 4.975 m in the case of Fig. 6 would have disappeared into the background measurements. As a consequence, an erroneous conclusion would have been drawn concerning the possible presence of colourless sulphur bacteria.

An entirely new approach has been chosen with the use of microelectrodes for O_2, pH, and sulphide (Jørgensen *et al.*, 1979; Revsbech and Jørgensen, 1981; Jørgensen, 1982). The use of electrodes has proved very useful for the study of the static and dynamic properties of algal mats. The O_2 microelectrode has a sensitive tip of only 20–30 μm, which permits accurate measurements of oxygen profiles over less than 1 mm. Such profiles are essentially similar to those found at interfaces of water bodies, but on a very much more compressed scale. The algal mats can be removed from the natural environment without disturbing the

system, and can subsequently be studied under controlled laboratory or field conditions. By turning the light off and on, the dynamic behaviour of the oxygen profile can be studied, and initial rates of O_2 appearance and disappearance, in light and dark, respectively, can be measured. From such data, *in situ* rates of photosynthetic oxygen production can be calculated. Changes in sulphide content and pH can be measured in the same samples. pH is often very sensitive to changes in the bicarbonate concentration, which in turn is directly related to CO_2 fixation. Once a CO_2 microelectrode is available, this should provide a beautiful opportunity to study not only light but also sulphide/oxygen-dependent dark CO_2 fixation.

These examples have shown that the possibilities of achieving more than a general indication of the role of sulphide-oxidising bacteria are still very limited. This is also due to the fact that appropriate enumeration methods for the spectrum of sulphide-oxidising bacteria are still lacking. Consequently, in most cases, the presence of high numbers of sulphide-oxidising bacteria has almost never been correlated with the activities of these organisms. An interesting exception to this rule was reported by Mosser *et al.* (1974).

In the hot acid springs of Yellowstone Park, a dominant population of *Sulfolobus* species has been found. As sulphide and elemental sulphur are the predominant substrates in these ponds, direct measurements of turnover rates were possible. In these environments, it is likely that the greater part of the sulphide is spontaneously oxidized to sulphur, and that sulphur is then oxidized further to sulphuric acid by *Sulfolobus*.

A most interesting but further complicating phenomenon has been reported by Kondratieva *et al.* (1976) and Kämpf and Pfennig (1980). They found that phototrophic bacteria belonging to the *Chromatiaceae* can grow aerobically in the dark as chemolithotrophic autotrophs. Phototrophs that can also grow very well as chemolithoautotrophs may present a very serious challenge to the colourless sulphur bacteria. It may safely be assumed that the affinity $(\mu)/K_s$ of the phototrophs for sulphide is less than that of the colourless sulphur bacteria, but if a bloom of *Chromatiaceae* develops at the sulphide–oxygen interface, such organisms can outcompete the few thiobacilli in the dark by mere numbers. Therefore, at those interfaces which receive light, chemolithotrophic dark CO_2 fixation may not be due to thiobacilli and other colourless sulphur bacteria, but rather to blooms of the photosynthetic sulphur bacteria.

Colourless Sulphur Bacteria in the Cycles of Elements and in Food Chains

The role of colourless sulphur bacteria in the sulphur cycle hardly needs to be stressed. Their role in the carbon flow is also obvious since they act as primary producers in many ecosystems. For example, protozoa can often be seen in

Beggiatoa mats, their cells filled with ingested filamentous, sulphur-containing bacteria. In these environments, where 50% of the mineralization proceeds by sulphate reduction and subsequent sulphide oxidation by *Beggiatoa,* their contribution to the total energy and carbon flow may indeed be substantial. Principally, however, light remains the primary energy source for the food chains.

An entirely different situation exists in the ecosystems around the hydrothermal vents near the Galapagos Islands. From the vents, sulphide-rich water is forced into the ocean, mixes with oxic seawater (giving initial sulphide concentrations up to 150 μM), and thereby provides an ideal substrate for the growth of colourless sulphur bacteria. Around the vents, which are located at a depth of 2000–2500 m in complete darkness, large numbers of worms, clams, anemones, and even crabs can be found. Since light or imported organic compounds can be ruled out as sources of energy for this ecosystem, it was postulated that this system might live on sulphide as the *primary* energy source. Indeed, around thermal vents, high numbers of bacteria which could have been colourless sulphur bacteria were observed. From water samples taken from the vents, a large number of *Thiobacillus* and *Thiomicrospira* species were isolated. Typical of the *Thiomicrospira* was that it displayed the same high sulphide tolerance observed in the original isolate (Kuenen and Veldkamp, 1972; Ruby *et al.*, 1981; Ruby and Jannasch, 1982). Large numbers of *Hyphomicrobium*-like organisms were also observed (Jannasch and Wirsen, 1981). The genus *Hyphomicrobium* is known to harbour many methylotrophs (Harder and Atkinson, 1978), some of which can denitrify. As the gases in the vent water also contain methane (H. W. Jannasch, personal communication), these organisms may be involved in the metabolism of methane and other methylated compounds formed in the vents.

It is thus likely that in this specialized hydrothermal ecosystem, sulphide-oxidising bacteria could be the major primary producers, being consumed by animals such as clams and possibly providing dissolved organic matter for uptake by the indigenous marine worms.

Even more fascinating is the discovery that bacteria were living in symbiosis with eukaryotic organisms, such as the pogonophore worms, around the vent. One of these worms, the 2-m-long *Riftia pachyptila,* which lacks both mouth and gut, possesses an organ called the "trophosome," which fills most of the inside of the worm. Organisms were found in thin sections of the trophosome which were clearly prokaryotic (Cavanaugh *et al.*, 1981). At the same time, it was discovered that the tissues contained high concentrations of two key enzymes of the Calvin cycle, which occur only in prokaryotes and plants, and not in animals (Table 4). It is now postulated that the prokaryotes in the tissue could be sulphide-oxidising chemolithoautotrophic symbionts which are provided with substrates (S, S^{2-}, O_2, CO_2) by the worm through an elaborate network of blood vessels surrounding the trophosome. The blood has been shown to possess haemoglobin with a very high affinity for oxygen and to be insensitive to sulphide poisoning. Apart from the Calvin cycle enzymes, enzymes for sulphur

metabolism were also detected in the tissue, namely, rhodanese, APS reductase, and ATP sulphurylase. These may play an important role in the energy generation of the bacteria (Kelly, 1982; Table 4, this chapter). Subsequently, *Riftia* species and bivalve molluscs such as *Solemya,* living in sulphide-containing mud from other nonhydrothermal habitats, have been shown to contain putatively chemolithotrophic CO_2-fixing bacteria in their tissues (Felbeck *et al.,* 1981). Work on the comparative $^{13}C/^{12}C$ ratios of cellular and available carbon sources further indicates that bacterial CO_2 fixation rather than the assimilation of dissolved organic matter could be a significant factor in providing fixed carbon for the animals (Southward *et al.,* 1981). Studies on small species of Pogonophora from the Bay of Biscay and the Norwegian fjords (Southward and Southward, 1980; Southward *et al.,* 1979) showed that these were able to take up significant quantities of dissolved organic matter from their environment. In some cases at least, it was proposed that the bulk of the carbon nutrition could be provided in this way (Southward and Southward, 1981). Numerous examples of the small species have now been shown to contain intracellular bacteria (Southward, 1982). This observation, together with the fact that ^{13}C depletion values of -35 to $-45‰$ have been found for all the pogonophores so far examined (A. J. Southward and E. C. Southward, personal communication, 1983) and the occurrence of ribulose bisphosphate carboxylase, predominantly in the bacteria-filled tissues in two small species (P. Dando, A. J. Southward and E. C. Southward, personal communication, 1983), all indicate that all the pogonophores have internal bacterial symbionts that provide fixed organic compounds to the animals. The relative importance of sulphur compound oxidation and carbon dioxide fixation is uncertain, as oxidisable ammonia and methane could conceivably also be substrates for the symbionts. Clearly, this study is in its infancy. It is of course possible that diverse symbiotic relationships exist, depending on whether an environment is sulphide or methane rich. Indeed, morphological investigations have indicated that the bacterial symbionts are of several types, including examples with extensive or no internal membranes and of rod or coccoidal shape (Southward, 1982; Southward *et al.,* 1981; E. C. Southward, personal communication; H. W. Jannasch, personal communication). The nature of the symbiosis also needs much clarification. It is not known whether carbon is supplied to the animals by *secretion* of fixed carbon from the bacteria, or whether the bacteria are actually digested at a fixed rate, maintaining a constant population in the tissues. There is some visual evidence for actual digestion of the bacterial cells (Southward, 1982). Using known growth yields for sulphide-oxidising bacteria (e.g., Kelly, 1982) and estimating fixed carbon excretion rates (e.g., from the observed rate of excretion of glycollate by *T. neapolitanus;* Cohen *et al.,* 1979), one could determine what growth rates might be supported for worms growing in habitats in which a constant supply of dissolved sulphide was available. If it is assumed that the symbiotic bacterium in a pogonophore is a sulphide-oxidising

Table 4. *Enzymes of the Calvin cycle and sulphur metabolism in marine animals living at the sulphide–oxygen interface*[a]

Species and habitat	Enzyme activity (μmol min^{-1} per g wet mass of tissue)				
	RuBP carboxylase	Phosphoribulokinase	ATP sulphurylase	APS reductase	Rhodanese
Rift vents					
Riftia pachyptila (worm)	0.22	19.0	74.0	23.3	7.6
Riftia sp. (worm)	1.13	—	133.0	30.1	5.2
Sewage outfall					
Solemya panamensis (bivalve)	2.4	4.4	77.0	4.1	0.7

[a] Adapted from Felbeck *et al.* (1981).

chemolithotroph capable of a yield *in vivo* of 2.5 g fixed carbon per mole of H_2S oxidised, and that the pogonophore digests the whole bacterium with an ecological efficiency conversion factor (trophic level 1 to trophic level 2) of 20%, then the production of new worm tissue containing 0.5 g carbon would require the oxidation of 32 g sulphide ion. Given that the wet weight of an individual *Siboglinum fiordicum* ranges from about 0.43 to 1.21 mg (Southward *et al.*, 1979) and that of *S. ekmani* between 0.11 and 0.47 mg (Southward and Southward, 1980), and assuming a carbon content of 10% of the wet weight, one animal would require carbon needing the oxidation of 0.7–7.7 mg S^{2-} to produce the observed size range if all of the carbon were provided by the symbiont. The mean size of *S. fiordicum* at 0.8 mg and its maximum number at around 7000 m^{-2} (Southward *et al.*, 1979) would imply consumption of about 36 g H_2S m^{-2} for their production. This calculation is simplistic, as the habitats for *Siboglinum* contain considerable biomass of other marine animals (Brattegard, 1967; Southward and Southward, 1980) and may have considerable dissolved organic carbon input from external sources (Southward *et al.*, 1979). It does, however, show that the sulphide requirement, given also the slow growth rates of the pogonophores, is not impossibly unrealistic and indicates that sulphide-dependent chemolithoautotrophy could contribute significant amounts (>10%) of fixed carbon for pogonophore development in typical sediment environments in which sulphide production occurs.

It would thus seem that both free-living and symbiotic sulphide-oxidising bacteria may play a significant role as primary producers in food chains in marine environments, especially as the pogonophora are of global distribution (Southward, 1979, 1980). The existence of such symbiosis in freshwater animals or terrestrial environments with available sulphide remains to be established.

Some Concluding Remarks on Competition, Specialization, and Restricted Ecological Niches

We have not dwelt in detail on the topics mentioned in this heading, as they have either been considered extensively elsewhere or are as yet little investigated. The competitive advantage of potentially mixotrophic thiobacilli has been demonstrated by continuous culture methods both under steady state conditions and with fluctuating nutrient supplies (Smith and Kelly, 1979; Gottschal, 1980; Gottschal and Kuenen, 1981; Gottschal *et al.*, 1981b), which illustrates that habitats containing both organic nutrients and oxidisable sulphur are likely to be dominated by mixotrophs. This success is attributable, at least in part, to the enhancement of the growth rate by mixotrophic metabolism (Smith and Kelly, 1979; Wood and Kelly, 1981) and will consequently be influenced by other

factors that alter the growth rate, such as pH or the concentration of available nutrients. Thus, a mixotroph may be outcompeted by a specialist chemolithotroph (e.g., *Thiobacillus* A2 versus *T. neapolitanus;* Smith and Kelly, 1979) under autotrophic conditions at a pH most suitable for the specialist, but will dominate at that pH if an organic nutrient is also available.

The web of interactive effects of physical and chemical variables in any habitat explains the apparent occurrence of different organisms of similar physiology in apparent coexistence, when the ecological exclusion principle would predict that one only would survive the competition. In practice, the environments in which colourless sulphur bacteria are abundant contain great physicochemical diversity. These are characteristically gradients of oxygen, sulphide, dissolved sulphur compounds, dissolved carbon dioxide, and organic nutrients such as lactate, acetate, and possibly other organic acids and even sugars. Small differences in pH could influence the nature of the dominant organism in a microenvironment, just as could the absolute concentration of available oxygen or sulphide. Affinity for oxygen and tolerance of sulphide can both be crucial determinants in a stratified environment. Recent work has illustrated how particular types of physiology are found in organisms clearly adapted to fill each "niche" available in sulphide-generating habitats. Thus, the relatively sulphide-sensitive *Thiobacillus thioparus* and *T. neapolitanus,* which are also vigorous aerobes not noticeably stimulated by microaerophilic conditions, are likely to predominate in aerobic situations where subtoxic levels of sulphide occur. They may thus be found on the *surface* of sulphide-generating muds, where oxygen is high and sulphide low. There they may have to compete with mixotrophs such as *Thiobacillus* A2. Deeper in the mud are the more sulphide-tolerant and microaerophilic types such as *Thiomicrospira pelophila* (Kuenen and Veldkamp, 1972). Under anaerobic conditions when nitrate is available, the facultatively aerobic *Thiobacillus denitrificans* would be expected to succeed rather than *T. thioparus* or *Tms. pelophila,* but this expectation is now also complicated by the isolation of an *obligately* anaerobic nitrate reducer, *Thiomicrospira denitrificans* (Timmer-ten Hoor, 1975). The outcome of competition between this organism and *T. denitrificans* (both of which are obligate chemolithotrophs) would presumably be determined by relative sulphide tolerance, affinity for nitrate uptake, or reduction and possibly susceptibility to oxygen toxicity, as *Tms. denitrificans* is remarkably oxygen sensitive (Timmer-ten Hoor, 1975). Further competition for these organisms is indicated by the isolation of *Thiosphaera pantotropha* (Robertson and Kuenen, 1982), which is a *mixotrophic* facultative denitrifier, and is thus likely to have a competitive advantage in environments containing organic nutrients as well as sulphide and nitrate. Again, the dominating organism in any situation is determined by a multiplicity of factors, a particular combination of variants leading to ideal conditions for each organism.

The Future

It is clear that many habitats still need to be investigated (including the tissues of possible host animals—and plants?), and many habitats deserve much more intensive study. Little is known of the physiology of *thermophilic* autotrophic sulphur bacteria (other than *Sulfolobus*), even though the existence of "*Thiobacterium*" and "*Thiospirillum*" has been known for many years (Czurda, 1935, 1937). The approaches are many, including improvement of technique to study the dynamics *in situ* of sulphur transformations. The greater understanding of the microbiology and biochemical ecology of colourless sulphur bacteria will, however, come from selective enrichment culture and isolation (e.g., by continuous culture technique) of new organisms, followed by detailed study of their physiology in order to establish how they adapt to their habitat and the features that enable them to dominate particular environmental niches.

A combination of studies on the chemistry and kinetics of their habitats, with a full understanding of their individual properties will allow the interaction of the colourless sulphur bacteria to be understood. Much can be learnt from the isolated organism in pure culture, but we must heed the warning by Baas Becking and Wood (1955): "It seems, therefore, a hopeless task to reproduce the happenings at a mud surface by means of pure culture . . . in ecology their use is limited. . . . In order to reconstruct such a play as 'Macbeth' it seems unsatisfactory to study Banquo's part alone."

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