

CHAPTER 8

Interaction of Sulphur and Carbon Cycles in Microbial Mats

Y. COHEN, V.M. GORLENKO AND E.A. BONCH-OSMOLOVSKAYA

8.1 PHOTOSYNTHESIS IN CYANOBACTERIAL MATS AND ITS RELATION TO THE SULPHUR CYCLE

Cyanobacterial mats are organosedimentary structures composed primarily of benthic cyanobacteria together with diverse communities of microorganisms which trap, bind and precipitate sediment particles. Often cyanobacterial mats produce laminated sediments—biogenic stromatolites due to seasonal changes in the environment of deposition.

Cyanobacterial mats may serve as an ideal model for the study of basic mechanisms of the microbial sulphur cycle and the evolution of the sulphur cycle for several reasons:

- (a) Cyanobacterial mats develop under environmental conditions excluding, or at least limiting, eukaryotic organisms such as grazing metazoans. This allows the study of microbial interactions with limited bioturbation.
- (b) In most microbial mats the organic matter produced is autochthonous, allowing the study of *in situ* primary production and the coupled processes of mineralization of primary organic carbon of a known source. In contrast, in most other systems, part or all of the primary organic carbon is transported from other, often unknown, sources and is partially decomposed during transport under unknown conditions. This aspect is particularly important in the study of the decomposition and maturation of microbial mats as a model for oil shale formation (Aizenshtat *et al.*, 1984).
- (c) Cyanobacterial mats are the oldest known biogenic sedimentary structures found as stromatolites dating back to 3.5×10^9 years ago. As such

*Yehuda Cohen.

stromatolites are the only fossil record which can be found throughout the entire geological era, even though they were most abundant during the Precambrian (Shopf, 1983).

Presently, cyanobacterial mats are confined to restricted habitats including hypersaline coastal marine environments, hot springs and alkaline lakes. Major marine cyanobacterial mats studied include: Solar Lake, Gulf of Aquaba (Krumbein and Cohen, 1974, 1977; Krumbein *et al.*, 1977; Cohen *et al.*, 1980; Jørgensen *et al.*, 1979, 1983; Cohen 1984); Shark Bay, Western Australia (Logan *et al.*, 1970; Playford and Cockbain, 1969; Bauld *et al.*, 1979; Bauld, 1984); Spencer Gulf, South Australia (Bauld *et al.*, 1979; Bauld, 1984); Laguna Figuerea, Baja California, Mexico (Stolz, 1984); Guerrero Negro, Baja California, Mexico (Javor and Castenholz, 1984a, 1984b) and many other mats in Bermuda, Persian Gulf; Bonair, Caribbean Sea; the Bahamas, and the USSR (Gorlenko *et al.*, 1984). Hot spring cyanobacterial mats have been primarily studied in Yellowstone National Park, Wyoming, USA (Brock, 1978; Castenholz, 1984), Hunter's Spring, Oregon, USA (Castenholz, 1973) and other hot springs in the USA, New Zealand (Castenholz, 1976), Iceland (Schwabe, 1960; Castenholz, 1976), in the Far East Kamchatka (Gorlenko and Bonch-Osmolovskaya, 1986a) and in various hot springs in Europe. Cyanobacterial mats of alkaline lakes have been described in Ethiopia, USSR and various locations in the USA.

8.1.1 Oxygenic and anoxygenic photosynthesis

Cyanobacteria carry out oxygenic photosynthesis with two photosystems in series, in a manner identical to eukaryotic phototrophs. Water is the electron donor in oxygenic photosynthesis, and oxygen is the ultimate oxidation product. Cyanobacteria are similar to eukaryotic algae and plants in that they have chlorophyll *a*. In addition, the main light-harvesting pigments are phycobiliproteins, which are also found in eukaryotic red algae together with β -carotene and zeaxanthin as the most common carotenoids (Stanier, 1974).

The phototrophic green bacteria and the purple bacteria, in contrast, carry out anoxygenic photosynthesis using only one photosystem and then require electron donors of redox potential lower than that of water. The suitable donors are reduced sulphur compounds and CO_2 . The photosynthetic pigments are various bacteriochlorophylls—*a*, *b*, *c*, *d* or *e*, together with a great variety of accessory carotenoids. Though many phototrophic bacteria can grow aerobically in a chemoheterotrophic model of growth, their long-

*The term 'sulphide' will be used to designate the total dissolved sulphide including H_2S , HS^- , and S^{2-} . The population of these species is pH-dependent.

term photosynthesis is generally restricted to anaerobic reduced environments where a suitable electron donor can be found. The physiological and ecological consequences of the differences between oxygenic photosynthesis of the cyanobacteria and the anoxygenic photosynthesis of the other phototrophs are profound. However, cyanobacteria often share the same ecological niche together with other phototrophs.

8.1.2 The sulphide-rich environment and toxicity

Cyanobacteria are sometimes found in environments with continuous exposure to sulphide, such as hot springs with a constant sulphide supply or in sulfureta, where sulphide is produced biogenically and where fluctuations of anaerobic and aerobic conditions are common. These oscillations may occur seasonally (Kuznetsov, 1970), or diurnally (Ganf and Viner, 1973) or even during shorter time periods (Jørgensen *et al.*, 1979).

Life in sulphide-rich environments has both advantages and disadvantages. Sulphide is an effective reducing agent and provides the required low redox potential for growth under anaerobic conditions. Sulphide toxicity is quenched by chemical or biochemical combination with oxygen and oxidized compounds. This may reduce or even eliminate the oxygen effects of photoinhibition, photo-oxidation and photorespiration. Furthermore, sulphide serves as an electron donor to anoxygenic photosynthesis. It may also serve as an assimilatory sulphur source, especially in organisms lacking the capability of assimilating sulphate.

Sulphide is highly toxic for most microorganisms. It reacts with various cytochromes, haemoproteins and other compounds. It inhibits the electron transport chain, blocking respiration as well as oxygenic and anoxygenic photosynthesis. Sulphide toxicity is also apparent in organisms which are well adapted to sulphide-rich environments, but the sensitivity varies markedly among the different groups of microorganisms. The green phototrophic sulphur bacterium *Chlorobium* is the most tolerant. This organism may grow in the presence of as much as 8 mM sulphide (Pfennig, 1975). *Chloroflexus* is found in sulphide concentrations up to 1.5 mM (Castenholz, 1976). Purple sulphur bacteria (*Chromatium*, *Thiocystis*, and *Thiocapsa*) can tolerate 0.8–4 mM sulphide, whereas purple non-sulphur bacteria are less tolerant (0.4–2 mM).

8.1.3 Anoxygenic photosynthesis in *Oscillatoria limnetica* and other cyanobacteria

A flocculent mat from the bottom of the hypolimnion of Solar Lake, Sinai (Cohen *et al.*, 1977b) is composed mostly of *Oscillatoria limnetica* together with the green sulphur bacterium *Prosthecochloris* sp. and the purple sulphur

bacteria *Lamprocystis* sp. and *Chromatium* sp. Sulphide concentrations of up to 5 mM accumulate in this layer and persist for about ten months before holomixis occurs. Sulphide is oxidized during holomixis, and the sulphur-dependent phototrophic community disappears while *Oscillatoria limnetica* thrives under the oxic conditions which persist for two months before stratification sets in again (Cohen *et al.*, 1977a).

High primary production of up to 8 g (C) cm⁻²d⁻¹ was measured at Solar Lake under stratification (Cohen *et al.*, 1977b), of which about 60% may be attributed to the activity of *Oscillatoria limnetica* in the presence of sulphide.

This organism, though capable of oxygenic photosynthesis whenever sulphide is absent, switches off photosynthesis II (PS II) when sulphide is present and conducts anoxygenic photosynthesis (Cohen *et al.*, 1975a, 1975b). Very low concentrations (0.1–0.2 mM) of sulphide immediately inhibit the oxygenic system (Oren *et al.*, 1979). However, after two hours of exposure to light in the presence of high sulphide concentration (3 mM), photo-assimilation reappears and is insensitive to the photosystem II inhibitor DCMU 3(3,4,dichlorophenyl) 1,1 dimethylurea (Cohen *et al.*, 1975a). The possible participation of PS II in anoxygenic CO₂ photoassimilation has been further excluded (Cohen *et al.*, 1975a) by the results of experiments in which PS II was simply not activated (rather than being inhibited) by the use of infrared light. Oxygenic photosynthesis requiring the operation in both photosystems decreased drastically in the infrared (red drop), whereas anoxygenic photosynthesis with sulphide was fully operative under such conditions. Furthermore, if both photosystems could contribute to the reaction, the enhancement in quantum yield would be predicted with respect to that obtained with only PS I in operation. However, the 'enhancement phenomenon' was observed only with oxygenic photosynthesis (Oren *et al.*, 1977). The new photosynthesis type is therefore anoxygenic, independent of PS II and driven by PS I with sulphide as electron donor.

Two hours' preincubation in the presence of sulphide and light is required for anoxygenic photosynthesis, indicating that induction may be involved. Indeed, the protein synthesis inhibitor chloramphenicol inhibits the initiation of anoxygenic photosynthesis (Oren and Padan, 1978). In order to survive sulphide inhibition of photosystem II an adaptation which allows the use of sulphide with photosystem I is necessary. We suggest that low redox leads to a sulphide resistant modification which enables sulphide electrons to be used. Protein synthesis occurs and electron carriers may be reduced. Addition of sodium dithionate eliminates the lag period observed when *Oscillatoria limnetica* is transferred to sulphide, possibly by providing the low redox potential needed for the reduction and modification of electron carriers.

Sulphide is oxidized to elemental S° by *Oscillatoria limnetica* according to the following stoichiometric relationship (Cohen *et al.*, 1975a):



Elemental sulphur was observed as refractile granules either free in the medium or adhering to the cyanobacterial filament. Otherwise the appearance of the cells under the electron microscope was the same under both anoxygenic and oxygenic conditions.

If sulphide is removed from sulphide-adapted cells, *Oscillatoria limnetica* immediately returns to oxygenic photosynthesis (Oren and Padan, 1978). This capacity must therefore be present in the cells. The photosynthetic system of *Oscillatoria limnetica* thus operates facultatively both oxygenically and anoxygenically.

When CO_2 is eliminated from the reaction system in *Oscillatoria limnetica*, sulphide donates electrons to hydrogen evolution (Belkin and Padan, 1979). This reaction occurs only in cells originally adapted to sulphide in the presence of CO_2 . It is dependent on light and sulphide and is also insensitive to DCMU. Like the CO_2 photoassimilation reaction, it requires an induction period of two hours and is driven by PS I. Whereas CO_2 photoassimilation is fully induced after two hours of incubation in the presence of sulphide, the capacity of hydrogen evolution is very low. An additional 46 hours of incubation in the presence of sulphide is needed for a full induction of hydrogen evolution. Sulphide-dependent hydrogen evolution must require an additional step (S) to that required for the sulphide-dependent photoassimilation. Yet, addition of sodium dithionate eliminates the required incubation period, as in the case of anoxygenic photosynthesis (Belkin and Padan, 1984).

The concentration of light harvesting pigments (chlorophyll a, phycobilins and carotenoids) in the cell are identical under both oxygenic and anoxygenic photosynthesis (Oren *et al.*, 1977). Quantum yield spectra of the two photosynthetic modes show that, whereas only a narrow light band is efficiently utilized in the oxygen mode, the entire absorbed spectrum is used at high quantum efficiency in the anoxygenic mode. The drop in the quantum efficiency of oxygenic photosynthesis at both the blue and red ends of the visible spectrum is marked. This limited range of utilization of the visible light spectrum in oxygenic photosynthesis of *Oscillatoria limnetica* is similar to that of other cyanobacteria (Lemasson *et al.*, 1973). It is markedly different, however, from that of eukaryotic algae and plants which contain in addition chlorophyll b in their light harvesting system. In these organisms, almost the entire absorbed spectrum is utilized in oxygenic photosynthesis, with the exception of the far 'red drop' (Govindjee *et al.*, 1968).

A comparison of rates of anoxygenic photosynthesis as a function of sulphide concentration was made for 11 strains of cyanobacteria including the mat-forming *Lynogbia* 7104, *Aphanotheca halophilica* and *Oscillatoria limnetica* (Garlick *et al.*, 1977). In these organisms, the dependence on

sulphide concentration was similar, generating an optimum curve rather than a saturation curve. The drop in the photosynthetic rates at higher sulphide concentrations is caused by sulphide toxicity effects on photosystem I. The maximal rates of oxygenic and anoxygenic photosynthesis are similar in both *Osc. limnetica* ($1\text{--}2\ \mu\text{M}$ (C) $\text{mg protein}^{-1}\ \text{h}^{-1}$) and *Aphanotheca halophilica* ($0.5\text{--}1\ \mu\text{M}$ (C) $\text{mg protein}^{-1}\ \text{h}^{-1}$). While the dependencies on sulphide are similar, both the affinities to sulphide and tolerances are different. Each strain exhibits a different range of sulphide concentrations at which anoxygenic photosynthesis can be performed. The sulphide ranges at pH 6.8 are $0.1\text{--}0.3\ \text{mM}$ for *Lynogbia 7104*, $0.1\text{--}1.5\ \text{mM}$ for *Aphanotheca halophilica* and $0.7\text{--}9.5\ \text{mM}$ for *Osc. limnetica*. Furthermore, in each of these cases the range is markedly affected by the pH of the medium (Howsley and Pearson, 1979), which governs the dissociation of H_2S . Differences in pH-dependent sulphide ranges are known to constitute a determining factor in the ecology of photosynthetic sulphur bacteria (Baas-Becking and Wood, 1955; Pfennig, 1975).

The pattern of sulphide oxidation during anoxygenic photosynthesis of cyanobacteria seems to be thermodynamically less efficient than oxygenic photosynthesis. Photosynthetic bacteria oxidize sulphide to sulphate (Pfennig, 1975, 1977) gaining eight electrons for each H_2S molecule, while the cyanobacteria *Oscillatoria limnetica* and *Aphanotheca halophilica* remove only two electrons per molecule of sulphur. Elemental S^0 is the only end-product of anoxygenic photosynthesis in these cyanobacteria (Cohen *et al.*, 1975b). Elemental sulphur may also be intermediate in the sulphide oxidation process of several sulphur phototrophic bacteria (Trüper, 1973). Finally, other sulphur-containing electron donors which are utilized by photosynthetic bacteria do not seem to serve the photosynthetic system of sulphide-utilizing cyanobacteria (Gromet-Elhanan, 1977; Pfennig, 1975, 1977). *Anacystis nidulans*, however, can oxidize $\text{S}_2\text{O}_3^{2-}$ to SO_4^{2-} , yet $\text{S}_2\text{O}_3^{2-}$ cannot serve as the sole or even the major electron donor (Utkilen, 1976).

As in many photosynthetic bacteria and heterocystous cyanobacteria, H_2 has been shown to serve as an efficient electron donor for CO_2 photoassimilation in an anoxygenic reaction driven by photosystem I in both *Oscillatoria limnetica* and *Aphanotheca halophilica*. Hydrogen evolution dependent on sulphide oxidation has been demonstrated in both these strains (Belkin and Padan, 1979) and in heterocystous species (Bothe *et al.*, 1977; Tel-Or *et al.*, 1977; Weisman and Benemann, 1977).

In cyanobacteria, sulphide appears to be more toxic to oxygenic than to anoxygenic photosynthesis. In *Oscillatoria limnetica*, the former was inhibited by $0.1\ \text{mM}$ sulphide (Oren *et al.*, 1979) while the latter was partially inhibited by $4\ \text{mM}$ (at pH 6.8) (Garlick *et al.*, 1977). These differences have also been observed in other facultative anoxygenic strains. This difference in toxicity implies that at the higher range of sulphide concentrations,

oxygenic photosynthesis will make only a minor contribution, if any, to photoassimilation of CO_2 . However, at lower ranges, oxygenic photosynthesis may occur simultaneously with anoxygenic photosynthesis as demonstrated in *Oscillatoria amphigranulata* (Utkilen and Castenholz, 1979) and in the chemocline of Solar Lake (Jørgensen *et al.*, 1979). Low sulphide concentrations (0.2 mM) are toxic to eukaryotic phototrophs and cyanobacterial strains that do not carry out anoxygenic photosynthesis (Castenholz, 1976; Knobloch, 1969; Howsley and Pearson, 1979).

The greater sulphide tolerance of anoxygenic photosynthesis operative with PS I is a selective advantage in sulphide-rich habitats. Some cyanobacteria cannot grow continuously under strictly anaerobic conditions but they can temporarily maintain themselves in the presence of sulphide. Other cyanobacteria are capable of permanent growth under these conditions. *Oscillatoria limnetica* thrives at the hypolimnion of Solar Lake under a 3 cm column of sulphide-rich water and grew well in the laboratory with 3.5 mM sulphide and at pH 6.8 for a period of several months (Oren *et al.*, 1977). Facultative anoxygenic photosynthesis is clearly an advantage for life under prolonged periods of exposure to sulphide.

8.1.4 Sulphide-oxygen fluctuation in cyanobacterial mats

The *in situ* use of microelectrodes (O_2 , sulphide, and pH) in various microbial mats demonstrated the establishment of a sharp redoxcline of less than 1 mm as a result of oxygenic photosynthesis of cyanobacteria at the proximity of the sulphide-rich microzone. The first measurements of microgradients of O_2 , sulphide and Eh in microbial mats were carried out in the flat shallow cyanobacterial mats at Solar Lake, Sinai (Jørgensen *et al.*, 1979; Padan and Cohen 1982). This mat, dominated by the cosmopolitan mat-forming cyanobacterium *Microcoleus chthonoplastes*, is situated under 30 cm of oxic water. Extreme diurnal fluctuations were found, in the position of the O_2 maximum (0.5 mM at 1 to 2 mm depth at night). The O_2 - H_2S interface migrates diurnally from the mat surface at night to 3 mm below surface at noon. The photic zone extends down to 2.5 mm below the surface and H_2S peaked right below the photic zone. H_2S and O_2 were found to coexist at 2.5 mm depth over a depth interval of 0.2–1 mm, with a turnover rate of less than a minute.

A more detailed study of the microstructures of the various microbial mats in Solar Lake and their O_2 , H_2S and pH microprofiles was carried out (Jørgensen *et al.*, 1983). The dominant cyanobacterium in the flat and blister mats was *Microcoleus chthonoplastes* in association with filamentous flexibacteria, tentatively identified as *Chloroflexus*-like organisms. Similar associations were observed in cyanobacterial mats at Sabhat Gavish, southern Sinai (Gerdes and Krumbein, 1984), microbial mats in Laguna Figueroa,

Baja California, Mexico (Stolz, 1984), and in various microbial mats in Shark Bay, Western Australia, and Spencer Gulf, South Australia (Bauld, 1984). The flat mat from the most shallow parts of Solar Lake has a photic zone of merely 0.8 mm with a maximum photosynthetic activity of $50 \text{ mM O}_2 \text{ cm}^{-3} \text{ h}^{-1}$ at 0.3–0.4 mm depth. In deeper waters, the mats are less compacted and the photic zones extend from 2.5 to over 10 mm with increasing depth of overlying water. In all the microbial mats examined, a pH shift of up to two units developed at the maximal photosynthetically active zone causing rapid deposition of CaCO_3 in this microzone (Revsbech *et al.*, 1983).

In the hypolimnetic flocculent mat at Solar Lake, Sinai, where *Oscillatoria limnetica* is the dominant cyanobacterium at 3 mM sulphide (Krumbein and Cohen, 1977; Krumbein *et al.*, 1977), no O_2 could be detected at the photosynthetically active layer. Yet, this organism can produce O_2 well at higher light intensities, even at 3 mM sulphide (Cohen *et al.*, 1985).

All cyanobacterial mats examined so far exhibit sharp microgradients of sulphide, O_2 and pH which fluctuate diurnally, thus exposing both the cyanobacteria and sulphate-reducing bacteria to alternating conditions of highly oxygenated water and elevated sulphide concentrations.

8.1.5 Strategies of oxygenic and anoxygenic photosynthesis in mat-forming cyanobacterial isolates

Axenic cultures of cyanobacteria isolated from various biotopes exposed to varying sulphide concentrations were examined for their capacity to carry out oxygenic and/or anoxygenic photosynthesis under a range of sulphide concentrations. Four different strategies of photosynthetic life under varying degrees of sulphide exposure were detected.

1. Irreversible cessation of CO_2 photoassimilation upon brief exposure to sulphide

Anacystis nidulans isolated from planktonic blooms is highly sensitive to sulphide. Exposure to $100 \mu\text{M}$ sulphide causes 50% inhibition of CO_2 photoassimilation, and at $200 \mu\text{M}$ sulphide it is blocked completely. When sulphide was removed after 2 hours of incubation in the presence of sulphide in the light, no regenerated photoassimilation could be detected. A similar sensitivity to sulphide was shown by Schwabe (1960) for *Mastigocladus*, the thermophilic cyanobacterium found under very low sulphide concentrations (up to $2 \mu\text{M}$) in various hot springs in Iceland, New Zealand, and the USA (Schwabe, 1960; Castenholz, 1976, 1977; Brock, 1978).

2. Enhancement of oxygenic photosynthesis upon exposure to sulphide and inability to utilize sulphide as an electron donor for anoxygenic photosynthesis

This type of photosynthesis is represented by *Oscillatoria* sp. isolated from Wilbor Springs, California. This organism, which grows at 1 mM sulphide at neutral pH, shows $45 \times$ enhancement of oxygenic photosynthesis upon exposure to 800 μM sulphide at neutral pH. With increasing sulphide concentrations CO_2 photoassimilation was gradually inhibited, yet no anoxygenic, DCMU-insensitive photosynthesis could be detected. Similar activities were reported for *Phormidium* sp. isolated from a hot spring at Yellowstone National Park by Weller *et al.*, (1975).

3. *Enhancement of oxygenic photosynthesis at low sulphide concentrations, inhibition of photosystem II at higher sulphide concentrations and a concomitant induction of anoxygenic photosynthesis operating in concert with the partially inhibited oxygenic photosynthesis at higher sulphide concentration* *Microcoleus chthonoplastes*, representing this type of photosynthesis, is a cosmopolitan mat-forming cyanobacterium in hypersaline coastal lagoons. Isolates from Solar Lake and Sabhat Gavish, Sinai; Laguna Figueroa and Guerrero Negro salt pans, Baja California, Mexico; Spencer Gulf, South Australia; and Shark Bay, Western Australia all show virtually the same photosynthetic activity. The ultrastructures of the cyanobacterial mats dominated by *Microcoleus chthonoplastes* from Solar Lake (Cohen, 1984); Laguna Figueroa (Stolz, 1984), Shark Bay (Bauld *et al.*, 1979; Bauld, 1984) and the Persian Gulf (Stolz, personal communication) are all extremely similar. The same type of photosynthetic activity was described by Utkilen and Castenholz (1979) for *Oscillatoria amphigranulata* isolated from alkaline hot springs in New Zealand at 2.2 mM sulphide.

4. *Complete reversible inhibition of photosystem II at low sulphide concentrations and induction of efficient anoxygenic photosynthesis at higher sulphide levels.*

This type of photosynthesis was initially described in *Oscillatoria limnetica* from Solar Lake by Cohen *et al.* (1975a), and later for other cyanobacteria by Garlick *et al.* (1977). Oren *et al.* (1979) demonstrated that, unlike types 2 and 3, photosystem II is completely blocked at 100 μM sulphide. When the cyanobacterium is exposed to high sulphide levels an induction period of 2 hours is needed for anoxygenic, photosystem I-dependent photosynthesis to be fully induced.

Recently this type of activity was also found in a hot spring isolate, *Oscillatoria* sp., growing in Stinky Spring, Utah at 1.1 mM sulphide and pH of 6.3.

Cyanobacteria exhibit a great degree of variability in their photosynthesis as a function of varying exposures to sulphide. These four strategies represent increasing degrees of adaptation to photosynthetic life under sulphide. Prolonged exposures to increasing sulphide concentrations result in the

dominance of cyanobacterial strains which can better utilize sulphide, and hence one finds a gradual shift from type 1 to type 4.

Generally, type 1 are either not exposed at all to sulphide or exposed to negligibly low concentrations of it. Type 2 are found among cyanobacteria exposed to up to 1 mM sulphide at neutral or basic pH. Anoxygenic photosynthesis could not be demonstrated in these organisms, yet their oxygenic activity is enhanced at low redox potentials which allow protection from photoinhibition and increased efficiency of CO₂ photoassimilation. Types 3 and 4 develop at relatively high sulphide concentrations at neutral or acidic pH. Sulphide toxicity is pH-dependent; it is more toxic at lower pH. Presumably, H₂S penetrates passively through the cell membrane obeying diffusion laws, whereas the ionized forms, HS⁻ and S²⁻, need active transport mechanisms. Thus, *Oscillatoria amphigranulata* occurring at 2.2 mM sulphide at alkaline pH is a type 3 organism, whereas *Oscillatoria* sp. from Stinky Spring, Utah, developing under 1.1 mM sulphide but at a pH of 6.3, is a type 4 cyanobacterium.

Photosystem II of the various types of cyanobacteria show different sensitivities to sulphide inhibition. Exposure to 50 µM sulphide at pH 7.5 induces maximal variable fluorescence of PS II in both *Anacystis nidulans* (type 1) and *Oscillatoria limnetica* (type 4). The addition of 10⁻⁴ M DCMU does not further enhance PS II fluorescence. Yet, in *Oscillatoria* sp. from Wilbor Springs, California (type 2), PS II variable fluorescence is only partially affected even at 1 mM sulphide and pH 7.5. Further addition of 10⁻⁴ M DCMU induces the maximal variable fluorescence of PS II. Similar results were obtained in cultures of *Synechococcus lividus* from Yellowstone National Park, *Oscillatoria* sp. from Stinky Spring, Utah, and the various *Microcoleus chthonoplastes* isolates from Solar Lake, Sabhat Gavish, Baja California and Spencer Gulf.

The ability of various cyanobacterial mat communities to produce and accumulate oxygen under increasing sulphide concentrations as well as the efficiency of recovery of oxygenic photosynthesis upon gradual removal of sulphide was measured by the introduction of O₂, S²⁻ and pH microelectrodes into small blocks of various cyanobacterial mats suspended in sulphide-containing media. Given sufficiently high light intensities, all cyanobacteria of the types 2, 3 and 4 are capable of producing and accumulating oxygen under sulphide. Yet, the level of sulphide at which oxygen production is detected changes significantly from one type to another. *Oscillatoria limnetica*—type 4—produces O₂ at only low sulphide concentrations of 50 µM, whereas *Microcoleus chthonoplastes*—type 3—undergoes efficient oxygenic photosynthesis at higher sulphide levels of 250 µM at neutral pH, and *Oscillatoria* sp. from Wilbor Springs—type 2—produce O₂ at much higher sulphide (i.e. of several mM) concentrations (Jørgensen *et al.*, 1983).

Various types of photosynthesis are found both in hot sulphur springs and various sulfureta. The degree of exposure to sulphide in sulfureta depends

on the coupling of primary production and sulphate reduction—the only source of sulphide in these biotopes. The importance of sulphate reduction as the major process in the mineralization of the produced organic matter was demonstrated by Jørgensen and Cohen (1977) and by Skyring (1984).

8.1.6 Coupling of primary production and sulphate reduction in cyanobacterial mats

Since the coupling of the two processes in cyanobacterial mats must occur in close proximity to the photic zone, which is highly oxygenated during most of the day, the usual technique for the measurement of sulphate reduction cannot be accurately applied in these systems. Sulphide undergoes fast turnover due to efficient oxidation photosynthetically, chemolithotrophically, heterotrophically or even chemically in the presence of O_2 . Hence, H_2S produced by sulphate-reducing bacteria cannot be quantitatively measured by injecting $Na_2^{35}SO_4$ into sediment cores. A new method has been developed in order to assess the degree of coupling of sulphate reduction to primary production in these systems (Cohen, 1985a). Silver wires, 0.15 mm in diameter and 1 cm in length, are coated with $Na_2^{35}SO_4$ of high activity and are introduced into the mat by means of a micromanipulator alongside the microelectrodes for O_2 , sulphide and pH. The mat is then incubated either in the light or in the dark for periods of 10 minutes to 2 hours, after a period of preincubation under the same conditions. A series of silver wires are pulled out of the mat at different time intervals. They are autoradiographed for the activity of the $Ag^{35}S$, after washing to remove all remaining $^{35}SO_4^{2-}$. The vertical microprofiles of sulphate reduction are then compared to the microprofiles of O_2 , sulphide and pH as well as to the profiles of oxygenic primary production. The silver wires are later cut into 1 mm segments and each is counted in a scintillation counter. This new method has several major advantages, but also presents some problems compared to the conventional method for the determination of sulphate reduction. The new method allows the determination of SO_4^{2-} reduction rate in close proximity to oxic microzones since the sulphide produced binds to the silver wire at high affinity and resists oxidation. Although there may not be an overall sulphide accumulation, as it is immediately oxidized, it can still be measured using this technique while this was impossible to determine in the previous method. The other advantage is the understanding of the microenvironmental conditions of sulphate reduction and a better insight of its coupling to primary production. The disadvantage of this technique is the difficulties in the quantification of the activities since diffusion of the labile SO_4^{2-} causes a decrease of specific activity with time. Therefore, the time dependence of the sulphate reduction activity is necessary to quantify its activity.

The new technique reveals an extremely tight coupling of sulphate reduction and primary production in the Solar Lake cyanobacterial mat. Not only can sulphate reduction be detected under the highly oxygenated conditions at the photic microzone of the cyanobacterial mat, but this activity is enhanced in the light where O_2 concentrations may increase to $4.5 \times$ the saturation value at 1 atm O_2 . Furthermore, the light spectrum that is most efficient in oxygenic photosynthesis is responsible for the induction of sulphate reduction. Specifically, wavelengths of 590–660 nm which are absorbed by the major light-harvesting pigment, phycocyanine, induce both primary production and sulphate reduction. The sulphate reduction activity under these conditions is fuelled directly by photosynthates excreted by the cyanobacterial oxygenic photosynthesis under high O_2 concentrations and high light intensities. The nature of these excretions is currently being studied.

Many sulphate-reducing bacteria have been shown to be strict anaerobes and highly sensitive to O_2 toxicity (LeGall and Postgate, 1973; Pfennig *et al.*, 1981). Yet, in the Solar Lake cyanobacterial mats, and probably in many other mat systems, these organisms operate well under periodic exposures to high O_2 concentrations. The mechanisms allowing sulphate reduction under these conditions are not fully understood. Preliminary results indicate the involvement of H_2 in the coupling of primary production and sulphate reduction. Several mat-forming cyanobacteria have been shown to produce H_2 under CO_2 limitations. Temporal CO_2 limitations may well occur at the photic microzone of the cyanobacterial mats due to the high specific rate of CO_2 photoassimilation creating high pH values (>9.5) and precipitation of $CaCO_3$ in this Ca-rich system.

8.1.7 Fe^{2+} -dependent photosynthesis in benthic cyanobacteria

The diurnal migration of the redoxcline through the photosynthetic layer assures the release of Fe^{2+} from the pool of monosulphide. High concentrations of dissolved Fe^{2+} were observed in the interstitial water in the upper 2 mm of the Solar Lake cyanobacterial mat in the morning hours. The ferrous ion is a good potential electron donor for photosynthesis in cyanobacteria and may thermodynamically operate well in the range of redox potential values of -50 mV to $+50$ mV, which is typical for the photic microzone during the diurnal transitions from fully reducing conditions at night to the high O_2 concentrations in the daytime. Banded microlayers of iron oxides are not very common in recent cyanobacterial mats, yet they are found in several mats in Spencer Gulf, South Australia and in Shark Bay, Western Australia as well as in several coastal lagoons such as the Sippewissett Marsh at Cape Cod, USA. Iron-dependent photosynthesis of mat-forming cyanobacteria has long been speculated to be responsible, at

least in part, for the extended deposition of Banded Iron Formations during the Precambrian (Knoll, 1979; Cloud and Gibor, 1983). This type of photosynthesis was speculated to be an important step in the evolution of oxygenic photosynthesis (Hartman, 1983).

Several cyanobacteria were examined for their capacity to use Fe^{2+} as an electron donor in photosynthesis. *Oscillatoria* sp. from Wiebor Springs and Stinky Hot Spring as well as all *Microcoleus chthonoplastes* isolates showed Fe^{2+} -dependent CO_2 photoassimilation (Cohen and Gack, 1985). Ferrous ion donates electrons primarily to photosystem II and this activity is thus DCMU sensitive. Ferrous ion initially blocks photosynthesis as indicated by a sharp temporal decrease in the PS II variable fluorescence in the presence of 10^{-5} M DCMU. Yet, after a short incubation in the presence of Fe^{2+} , the fluorescence reappears and with it an efficient rate of CO_2 photoassimilation and iron oxidation. Iron is oxidized through a ferritine intermediate. The end-product of the oxidation, iron oxide, is excreted outside the cell, similar to the excretion of S^0 in sulphide-dependent anoxygenic photosynthesis.

Preliminary results of the diurnal fluctuation of Fe^{2+} and $\delta^{13}\text{C}$ values for ΣCO_2 in the interstitial water in the upper 10 mm of the sediment core from the San Francisco Marsh, showed loss of Fe^{2+} from the photic zone during the day. There were two peaks of ^{13}C enrichment, one at the deepest part of the photic zone at 4–6 mm, and one at the surface. This indicated two microzones of autotrophic activities. The upper zone is clearly the result of oxygenic photosynthesis, whereas at the lower layer a Fe^{2+} -dependent CO_2 photoassimilation is inferred.

The importance of Fe^{2+} -dependent photosynthesis as an intermediate between sulphide-dependent activity and oxygenic photosynthesis is not yet understood.

8.1.8 Regulation of the sulphur cycle in recent cyanobacterial mats

Over 99% of CO_2 photoassimilation and SO_4^{2-} reduction as well as all sulphide oxidation processes in cyanobacterial mats take place in the upper 5–10 mm of the sediment. Any attempt to estimate the rates of processes in this system must be carried out on a microscale. Analysis of bulk samples must be cautiously treated.

In these microdimensions, very sharp gradients of O_2 , sulphide, pH, Eh, Fe^{3+} and other parameters are established. These sharp gradients within a few millimetres are the result of highly specific photosynthetic activities, sulphate reduction and sulphide oxidation and heterotrophic microbial activity. The chemical microgradients fluctuate drastically daily and expose the microbial communities to 1 mM O_2 , pH of 9–10, Eh of +200 mV, and over 10 mM Fe^{3+} at noon, in contrast to 5 mM sulphide, pH 6.5–7.0, and

Eh of -100 mV at night. Microorganisms must adapt to these drastic microenvironmental changes by developing elaborate regulatory mechanisms.

The regulation of photosynthetic mat-forming cyanobacteria has been studied in detail since 1974. Anoxygenic, sulphide-dependent photosynthesis and oxygenic photosynthesis under sulphide both play a major role in sulphide oxidation processes in these environments. Using our present knowledge we can predict which type of cyanobacteria will dominate a given biotope by measuring the diurnal microgradients of oxygen and sulphide (Cohen *et al.*, in press).

Since iron serves as a major trap of sulphide, the regulation of Fe^{2+} , Fe^{3+} in sediments is highly interrelated to the sulphur cycle. The information on Fe^{2+} photosynthesis in cyanobacteria is presently inadequate to properly assess its ecological importance.

8.1.9 Precambrian cyanobacteria and stromatolites

Cyanobacterial mats are an extremely ancient phenomenon, documented in the oldest fossils known, dating 3.5×10^9 years ago. For the remaining period of the Archaean and throughout the Proterozoic era, up to 0.57×10^9 years ago, stromatolitic communities of cyanobacterial mats are the most abundant fossils. In the Palaeozoic era, the fossil record of stromatolites is limited and restricted to intertidal and supratidal hypersaline marine environments, thermal springs and alkaline lakes (Awramik, 1984), where they were protected from the newly evolved grazing activity.

Another major sedimentary record of the Proterozoic is widely spread deposition of finely laminated ferro-ferric oxides known as the Banded Iron Formations. The understanding of microbial activities in recent cyanobacterial mats may throw light on our understanding of evolution processes in the Precambrian. The recent mat-forming cyanobacteria present a protocyanobacterial group which differs markedly from modern planktonic cyanobacteria.

8.2 MICROORGANISMS OF THE SULPHUR CYCLE AND THEIR ACTIVITY IN MICROBIAL MATS OF HOT SPRINGS

8.2.1 Introduction

Certain aquatic microorganisms find a favourable ecological niche at solid-liquid interfaces where particular physicochemical conditions have been established (Marshall, 1980; Gorlenko *et al.*, 1983). In waters lacking nutrients, such as springs, inorganic ions and organic ions may be concentrated

*V.M. Gorlenko and E.A. Bonch-Osmolovskaya.

on the sediment surface. This promotes the colonization of the spring bed by microorganisms which form benthic associations referred to as microbial mats. The composition of microbial mats depends upon the characteristics of the spring.

Microbial mats often contain a phototrophic component, mainly cyanobacteria, which are primary producers of organic matter (Bauld, 1981a; Krumbein, 1983). However, in some cases, phototrophic microorganisms are not found in microbial mats. Examples are sulphur mats of submarine vents (see Chapter 7) and regions near extremely hot sulphur spring orifices. In these systems, the primary producers are chemolithotrophic sulphur bacteria (Brock, 1978; Caldwell *et al.*, 1976; Gorlenko *et al.*, 1988).

According to Bauld (1981b) microbial mats are associations of microorganisms which colonize benthic surfaces and form adhesive, extended and often layered structures. The terms cyanobacterial and algal bacterial mats should be used carefully, since they characterize only certain types of mats.

Emphasis has been placed on benthic microbial associations inhabiting shallow marine systems (Krumbein *et al.*, 1979; Cohen *et al.*, 1984), but information about mats in thermal springs is rather scanty (Brock, 1978; Cohen *et al.*, 1984). These environments (dominant sulphide, CH_4 , CO_2 and N_2) are of particular interest because they are believed to be similar to Precambrian systems (Awramic, 1984; Walter, 1976; Zavarzin, 1984).

Due to the diversity of types, springs are convenient natural model systems. It is possible to study individual factors which determine populations of species and regulate microbial processes, in different springs.

Hot springs are particularly interesting because high temperatures restrict the number of species (Brock, 1978; Gorlenko *et al.*, 1985). Downstream, at lower temperatures more diverse microbial associations develop. Hence, one spring can provide considerable information about the structure of microbial communities.

The present paper considers microbial mats in thermal springs with high concentrations of sulphur compounds. The sulphide concentration determines species and regulates carbon and sulphur metabolism in these systems (Brock, 1978; Castenholz, 1984a).

8.2.2 Types of hot springs

The mineral composition of a given spring depends on the composition of the rocks through which the hydrothermal solution migrates. The total mineralization of springs varies over the range of 10^{-2} to $10^2 \text{ g(salt) l}^{-1}$. Therefore both fresh water and halophilic microorganisms can develop in different springs. In some mineral springs, the cation concentration sequence is $\text{Na}^+ > \text{Ca}^{2+} > \text{Mg}^{2+}$ (Miyaki, 1965), and the anion sequence may be $\text{SO}_4^{2-} > \text{Cl}^- > \text{HCO}_3^-$; $\text{Cl}^- > \text{SO}_4^{2-} > \text{HCO}_3^-$; or $\text{Cl}^- > \text{HCO}_3^- > \text{SO}_4^{2-}$. The

second anion sequence resembles seawater in composition. The water of some hot springs contains high concentrations of heavy metals and toxic compounds such as Sb and As salts. These substances might influence the microbial population.

Hot springs can be divided into two main groups: alkaline to neutral and acid. Acid springs have unique microflora which have adapted to extreme conditions: pH (1–5), high temperature and H_2S , the latter being especially toxic at low pH values. Acid springs are found in zones of solfataric and fumarole activity. Here, the water may be acidified by bacterial oxidation of fumarole elemental sulphur to sulphate (Zavarzin, 1984).

Boiling acid sulphur springs are especially interesting since they are inhabited by archaebacteria including the sulphur oxidizers Sulphobales and the elemental sulphur reducers, Thermoproteales. In acid hydrothermal springs, neither mats nor prokaryotic phototrophs have been found. However, the eukaryotic alga *Cyanidium acidocaldarium* may be found at 45–55°C (Brock, 1978). In contrast to acid springs, microbial mats may develop to a considerable thickness near the orifices of neutral or alkaline hot springs.

Hydrotherms are subdivided according to their gas composition (Zavarzin, 1984). Nitrogen- CO_2 thermal springs have highest temperatures and are formed by mantle emanations. Examples that have been studied microbiologically include springs in Iceland, North America (Yellowstone National Park), Kamchatka (caldera of the Uzon volcano) and Kunashir Island (the southernmost Kuril island). Nitrogen-rich hydrothermal springs are formed outside the regions of active volcanism and have lower temperatures. There are also CO_2 springs which are relatively cold. Rather rare hydrogen springs are found in Iceland.

Hydrogen sulphide, usually a product of mantle exhalations, is found in many types of springs: high concentrations are present in association with CO_2 in thermal springs in craters and calderas of volcanoes. Secondary biogenic H_2S is generated in springs flowing through sedimentary sulphate rocks with organic inclusions. Exogenic gases from deep crustal or upper mantle sources may serve as energy and carbon sources for microflora. Potential hydrogen donors are H_2 , CH_4 and H_2S whereas CO_2 is an electron acceptor. Carbon dioxide (bicarbonate) and dissolved organic substances may be assimilated by microorganisms. Mantle gases usually contain no more than 0.05% oxygen, but downstream water may derive O_2 from the atmosphere and photosynthesis by cyanobacteria.

The microbial communities are relatively stable in neutral-alkaline springs because of the steady water inflow. Seasonal fluctuations of temperature, mineralization and other physicochemical factors are minimal (Castenholz, 1984a). Photo- and chemolithotrophic microorganisms dominate these waters because the small quantities of nutrients, especially organic matter, do not meet the requirements of most heterotroph species (Campbell, 1983).

However, excreted organic matter and decaying cells create conditions suitable for the development of some heterotrophic microorganisms (Ward *et al.*, 1984). Therefore, hot springs are characterized by a great diversity of microorganisms dependent upon factors such as concentrations of H_2S , pH and salinity.

8.2.3 Microorganisms of the sulphur cycle

8.2.3.1 Cyanobacteria

Cyanobacteria are primary producers which form the basis of most hot spring microbial mats at temperatures below $74^{\circ}C$ and pH higher than 5.5 (Castenholz, 1984a). The unicellular bacterium *Synechococcus lividus* was found at $74^{\circ}C$ in alkaline springs of Yellowstone National Park. One of the isolated strains was cultured at 63 – $67^{\circ}C$ (Meeks and Castenholz, 1971). In nature, other species of *Synechococcus* are found at lower temperatures such as *S. elongatus* (66 – $70^{\circ}C$) and the widely spread *S. minervae* ($60^{\circ}C$) which prefers hot springs with low sulphide content (Castenholz, 1969; Gerasimenko *et al.*, 1983). Usually these species develop jointly with the filamentous *Chloroflexus* which imparts rigidity to the mat. Other species of cyanobacteria colonize low-temperature (45 – $55^{\circ}C$) zones of thermal springs. Filamentous cyanobacteria of the genera *Oscillatoria*, *Spirulina* and *Phormidium* are also widespread. Some species tolerate temperatures as high as $60^{\circ}C$, e.g. *Oscillatoria okenii*, *Spirulina* sp., *Phormidium laminosus*. Highly differentiated cyanobacteria which are capable of N_2 fixation (e.g. *Mastigocladus laminosus*) occur in many low sulphide springs of the USA, New Zealand, Iceland and the USSR (Castenholz, 1969).

In 1975, it was shown for the first time that *Oscillatoria limnetica* may completely switch to anoxic photosynthesis consuming dissolved sulphide as a hydrogen donor (Cohen *et al.*, 1975a, 1975b) and oxidizing it to extracellular S^0 . It was further shown that cyanobacteria can be divided into four groups, on the basis of their tolerance to sulphide and ability to use sulphide as an H-donor (Castenholz, 1973, 1976, 1977; Garlick *et al.*, 1977; Howsley and Pearson, 1979; Cohen *et al.*, 1984). Various cyanobacteria from hot spring environments are listed in their respective groups in Table 8.1. It is seen that a large variety of bacteria inhabiting hot sulphur springs participate actively in the sulphur cycle. In some species, sulphide oxidation occurs by anoxic photosynthesis similar to bacterial photosynthesis, whereas in others it occurs with the help of O_2 released by O_2 photosynthesis.

Cyanobacteria performing anoxic photosynthesis are able to compete for H-donors with green and purple bacteria which also inhabit microbial mats of sulphur springs. The role of thiophilic cyanobacteria in the sulphur cycle is not confined to oxidative processes. Some, belonging to the fourth group,

Table 8.1 Cyanobacteria-sulphide relation in hot springs

Species	[HS ⁻] in nature	Response* to HS ⁻	Reference
<i>Mastigocladus laminosus</i>	2 µM	I	Castenholz, 1976; 1977; Gerasimenko <i>et al.</i> , 1983
<i>Synechococcus lividas</i>	trace	II	Castenholz, 1977; Brock, 1978
<i>Oscillatoria</i> sp.	6 µM	II	Cohen (this volume, Section 8.1)
<i>Phormidium</i> sp.	—	II	Weller <i>et al.</i> , 1975
<i>Oscillatoria amphigranulata</i>	2.2 µM	III	Cohen (this volume, Section 8.1)
<i>Phormidium amphigranulata</i>	0.1 µM	III	Bildushkinov and Gerasimenko, 1985
<i>Oscillatoria</i> sp.	1.1 µM	IV	Cohen (this volume, Section 8.1)

* I—HS⁻ is not used as H-donor by cyanobacteria: they are highly sensitive to HS⁻.

II—HS⁻ is not used as H-donor but it stimulates oxygenic photosynthesis.

III—HS⁻ is used as H-donor; photosystem II is tolerant to [HS⁻].

IV—HS⁻ is used as H-donor; photosystem II is sensitive to low [HS⁻].

are able to consume S⁰ as an electron acceptor for oxidation of endogenic substrates under dark anaerobic conditions (Oren and Shilo, 1979). As a result of 'sulphur respiration', elemental sulphur is reduced to sulphide. Sulphur respiration has also been found in *Chloroflexus* and in purple sulphur bacteria (Van Gemerden, 1968).

Organisms in microbial mats can also participate in the sulphur cycle by assimilatory reactions. Like other prokaryotes, cyanobacteria are able to assimilate sulphate. Some of them prefer to utilize more reduced products for assimilatory purposes. It has been shown that *Oscillatoria amphigranulata* can utilize S⁰ as well as sulphate during phototrophic growth (Castenholz and Utkilen, 1984). Sulphur accumulated by such microorganisms undergoes further transformation during the decomposition of dead cells and diagenesis.

8.2.3.2 Anoxic phototrophs

The green filamentous bacterium *Chloroflexus* is a common component of microbial mats in hot springs with low mineralization (Pierson and Castenholz, 1974; Bauld and Brock, 1973; Castenholz, 1984a, 1984b; Gorlenko *et al.*, 1985). This organism is capable of phototrophic growth when consuming sulphide or organic compounds as electron donors and chemotrophic growth in the dark (Madigan and Brock, 1973, 1975; Madigan *et al.*, 1974). The most rapid growth for all isolated strains occurs photoheterotrophically

(Castenholz, 1984a, 1984b). *Chloroflexus*, considered to be the most active consumer, uses both excretion products of oxygenic phototrophs, i.e. cyanobacteria (Bauld and Brock, 1974) and decomposition products of dead microorganisms (Ward *et al.*, 1984). Bildushkinov and Gerasimenko (1985) are of the opinion that *Chloroflexus* plays an important role in the oxidation of organic matter with O₂, since it inhabits the interface between the anaerobic and aerobic zones. *Chloroflexus* occurs both in high sulphide springs and in hydrothermal springs containing trace sulphide. Evidently the role of *Chloroflexus* in different mats varies depending upon redox conditions, availability of sulphide, and light intensity (Doemel and Brock, 1977). At high sulphide concentrations in mats which contain *Chloroflexus* as the sole phototrophic component (Castenholz, 1976, 1984b) sulphide inhibits many species of cyanobacteria. Therefore, in a number of sulphur springs, only *Chloroflexus* develops, and its upper temperature limit (65–66°C) is lower than that for low sulphide springs (72°C). Anaerobic mats are common in springs of Iceland, New Zealand and some regions of North America. Primary production in anaerobic mats is maintained exclusively at the expense of anoxic phototrophic green bacteria. Elemental sulphur produced by photo-oxidation serves as terminal electron acceptor for anaerobic destructors, such as sulphur-reducing bacteria. Since S⁰ does not accumulate in macrobial mats, one can assume that sulphur reduction is a basic removal process.

Anaerobic sulphur mats of hot springs are characterized by relatively simple interdependent cycles of carbon and sulphur. They can be regarded as the most probable analogues of ancient mats or stromatolites which existed before the appearance of O₂ photosynthesis (Awramic, 1984).

It is noteworthy that strains isolated from the upper part of anaerobic mats cannot develop under anaerobic conditions in the dark (Castenholz, 1984b). It is still unknown whether these strains are ecotypes of *Chloroflexus aurantiacus* or an independent species.

In warm sulphur springs (28–40°C), there are filamentous green bacteria, e.g. *Oscillochloris trichoides*, containing gas vacuoles (Gorlenko and Korotkov, 1979). This green bacterium occurs in springs of Kamchatka, Dagestan, and Lenkoran (Gorlenko *et al.*, 1985) and has been studied in a monoculture (Gorlenko and Korotkov, 1979). It has been shown that the physiology of *O. trichoides* is similar to *Chloroflexus*. However, *C. aurantiacus* and *O. trichoides* have ecologically different temperature regimes. *Oscillochloris* tends towards anaerobiosis and photoautotrophy and exhibits a greater tolerance to sulphide (204 mM). During photosynthesis, sulphide is oxidized by *Oscillochloris* to S⁰. Field studies show that *O. trichoides* inhabits low temperature sulphur mats often beneath purple sulphur bacteria. From these observations one can assume that photosynthesis by *O. trichoides* depends upon sulphide ascending from lower layers of the mat.

Purple sulphur bacteria have often been observed in hot springs (Miyoshi, 1897; Castenholz, 1969, 1977). Madigan (1984) isolated the thermophilic strain *Chromatius* strain MC from a reddish bacterial mat embedded in carbonate sinter of a sulphide thermal spring (45°C) in the Upper Terrace area of Mammoth hot springs, Yellowstone National Park, Wyoming, USA. The optimal temperature range of this organism in laboratory cultures was found to be 48–50°C and the upper limit, 58°C. *Chromatium* strain MC morphologically resembles the smaller species *C. vinosum*. It differs from the other mesophilic species by its inability to grow on organic substrates in the absence of sulphide and CO₂. The thermophilic culture also differs from *C. vinosum* in carotenoid composition, its basic carotenoid being rhodovibrin.

Other thermophilic strains of *Chromatium* were obtained from thermal springs in New Mexico (47–48°C) (Madigan, 1984). Evidently, purple sulphur bacteria are widely spread in springs enriched in sulphide at temperatures of 40–58°C. Mesophilic *Chromatium* are often found in mats of hot springs in the temperature range 28–45°C (Gorlenko *et al.*, 1985).

Hot springs of high salinity in Dagestan in the Caucasus (Baftugai springs; mineralization, 70–100 g salt l⁻¹; pH, 6.5–7.0; sulphide, 30–40 mg l⁻¹; 45–52°C) contain sulphur mats with a red-brown layer consisting of *Ectothiorhodospira* sp. (authors, unpublished data). Some species of this genus (*E. halophila* and *E. abdelmalekii*) develop at elevated temperatures (45–47°C) in shallow saline water basins and soda lakes (Raymond and Sistrom, 1969; Imhoff *et al.*, 1978).

Purple sulphur bacteria in microbial mats utilize dissolved sulphide during anoxic photosynthesis. Since they occupy the upper boundary of the anaerobic zone, it is evident that they primarily consume biogenic sulphide arriving from lower layers of the mat. It is well known that many small forms of Chromatiaceae can grow aerobically in the dark (Bogorov, 1974; Gorlenko, 1974; Kämpf and Pfennig, 1980). Therefore, one can assume that during the dark and perhaps at low light intensities during the day, purple bacteria oxidize sulphide by chemosynthesis. Thus they perform the same function as colourless chemosynthetic bacteria in the community. In summary, the role of purple bacteria as producers of organic matter is very important since they function both photosynthetically and chemosynthetically.

Non-sulphur purple bacteria are widely spread in thermal springs at moderate temperatures (28–55°C) (Gorlenko *et al.*, 1985). *Rhodopseudomonas palustris*, *Rhodobacter capsulatus*, and *Rhodocyclus gelatinosus* are frequently encountered (Table 8.2). The first two are able to consume sulphide at low concentrations both in light and the dark (Gorlenko, 1981).

Hence, some species of non-sulphur purple bacteria may participate in sulphur metabolism in microbial mats, performing the function of sulphur bacteria. The physiology of non-sulphur purple bacteria resembles that of

Table 8.2 Temperature limits for anoxic phototrophic bacteria in some springs

Species	Temperature (°C)						
	10	20	30	40	50	60	70
<i>Phodopseudomonas viridis</i>		*	*	*			
<i>Phodopseudomonas palustris</i>	*	*	*	*	*	*	
<i>Rhodobacter capsulatus</i>			*	*	*	*	
<i>Rhodocyclus gelatinosus</i>		*	*	*	*	*	
<i>Thiocapsa roseopersicina</i>	*	*	*				
<i>Chromatium vinosum</i>		*	*	*			
<i>Chromatium minutissimus</i>			*	*			
<i>Chlorobium limicola</i>			*	*			
<i>Chloroflexus aurantiacus</i>			*	*	*	*	*
<i>Oscillochloris trichoides</i>	*	*	*	*			
<i>Rhodospirillum mediosalinum</i>				*	*		
<i>Rhodobacter</i> sp.			*	*			
<i>Ectothiorhodospira</i> sp.			*	*	*		
<i>Prosthecochloris</i> sp.			*	*	*		

Chloroflexus aurantiacus. The temperature ranges for *Chloroflexus* and non-sulphur purple bacteria barely overlap. So it is reasonable to conclude that as the temperature decreases downstream, *Chloroflexus* is replaced functionally by mesophilic species of non-sulphur purple bacteria (Gorlenko *et al.*, 1985).

In the Dagestan saline springs, the sulphide-tolerant halophilic non-sulphur bacterium *Rhodobacter* sp. (similar to *R. euruhaline*) coexists with *Ectothiorhodospira* sp. (Kompantseva, 1985). *Rhodobacter* sp. easily switches from photoheterotrophic to photoautotrophic metabolism, consuming sulphide as an electron donor in the latter state. Extracellular S^0 is the main oxidation product. This purple bacterium functions like *Chloroflexus* which has never been found in saline springs.

All known species of green sulphur bacteria (Chlorobiaceae) develop only in stable anaerobic ecosystems (Pfenning, 1967; Gorlenko *et al.*, 1984). These obligate photolithoautotrophic bacteria utilize sulphide as H-donor. They can only use certain organic acids together with CO_2 , and in the presence of sulphide they utilize H_2 . These highly specialized organisms are restricted ecologically. They must compete for sulphide with the more universal purple bacteria and some non-sulphur purple bacteria. However, their chlorosomes, containing particularly bacteriochlorophylls c, d and e which absorb light with wave lengths from 730 to 760 nm, allowing them to occupy an independent niche. Green sulphur bacteria are widely distributed in cold, high sulphide springs. A high sulphide tolerance facilitates their

development at sulphide concentrations of 200–300 mg l⁻¹ without extra specific competition (van Neil, 1931; Pfennig, 1967, 1975, 1978). Such sulphide concentrations at pH 6.5–7.0 inhibit the growth of other phototrophs including purple bacteria. It is also remarkable that green bacteria can develop in association with sulphate- and sulphur-reducing bacteria at very low sulphide concentrations (Pfennig, 1978).

Gorlenko *et al.*, (1985) isolated *Chlorobium* at temperatures below 45°C in thermal sulphide springs at Lenkoran, Dagestan, and Kamchatka in line with Kaplan (1956) who concluded that they grew above 42°C. In the hot saline springs of Dagestan, the temperature range for green sulphur bacteria is wider, the upper limit being 52–55°C. Here only the species *Prosthecochloris* sp. has been found. It is morphologically identical to *P. aestuarii*, which is widespread in seas and shallow saline basins. Green sulphur bacteria may occupy a narrow microzone adjacent to anaerobic destructors in highly developed multi-component microbial mats in warm springs.

8.2.3.3 Non-photosynthetic aerobic bacteria of the sulphur cycle

In microbial mats of sulphide-containing hot springs, aeration and O₂ photosynthesis may create aerobic conditions, especially during the day (Revsbech and Ward, 1984). The availability of both sulphide and O₂ is favourable for the development of chemolithotrophic sulphur bacteria.

The thermophilic facultative autotrophic thiobacterium *Thiobacillus thermophila* was isolated from a hot spring in Yellowstone National Park (Golovacheva, 1984). The growth of this bacterium occurs at 35–55°C, the optimum being 50°C (at pH 5.6). Of particular interest are sulphur bacteria such as *Thermothrix thiopara* which can develop at temperatures above 55°C (Caldwell *et al.*, 1976). In nature, *Thermothrix* exists at 72°C, pH 7.0 and a sulphide concentration of 17.4 µM (0.57 mg l⁻¹). In the laboratory, it grows between 67 and 77°C, 70–73°C being the optimum at pH 7.0. This facultative autotroph, neutrophile and facultative anaerobe capable of denitrification utilizes sulphide or thiosulphate substrates. In addition, it may develop heterotrophically on amino acids and simple media aerobically or anaerobically, reducing nitrate (Brannan and Caldwell, 1980). *Thermothrix thiopara* exhibits polymorphism. In hot springs, it grows as slimy interwoven filaments containing sulphur particles, *Thermothrix* communities may consist of several morphologically different bacteria: filamentous, small rods and cocci (Balashova, 1986).

Some authors believe that *Thermothrix thiopara* was described earlier as *Thiospirillum pistiense*. These microorganisms were observed in thermal sulphide springs of Japan, and in the Ozerkovo and Yuzhno-Koshelevo hot springs in Kamchatka (Kuznetsov, 1955).

Microorganisms similar to *Thermothrix* are found in hot sulphur springs

of caldera of the Uzon volcano in Kamchatka (Gerasimenko *et al.*, 1983; Gorlenko *et al.*, 1987a). In the Thermophilny spring, sulphur bacteria dominate and develop as interwoven filaments covered by amorphous sulphur (66–70°C; pH, 6.3–6.7; sulphide, 10–16 mg l⁻¹; trace O₂). There are also abundant large curved bacteria belonging to the genus *Macromonas*. Thermophilic (65°C) microorganisms morphologically similar to *Thermothrix thiopara* and a thermophilic variant of *Thiobacterium bovista* were isolated from thermal sulphur mats. Small rod-shaped cells of these sulphur bacteria were submerged in globular or dentritic gel-like masses. Cells grew on a mineral medium containing 1% Na₂S₂O₃ at 65°C and a pH of 4.5 (Balashova 1985, 1986).

8.2.3.4 Anaerobic microorganisms of the sulphur cycle

Thermodesulfobacterium commune is at present the only sulphate-reducing organism isolated from very hot springs (Zeikus *et al.*, 1983). It was found in different springs (50–72°C) and in cyanobacterial mats at 59°C. *Th. commune* is a small gram-negative rod able to grow on lactate and pyruvate (more slowly on acetate and hydrogen) in the presence of sulphate. Its temperature optimum is around 70°C. However, it grows between 45 and 85°C. *Th. commune* is not an archaeobacterium but it possesses some unique properties, e.g. lipids in this organism are branched diesters of glycerol. It contains cytochrome C₃, no desulphoviridine, and differs from other sulphate-reducing bacteria by its low content of G+C nucleotide pairs in DNA (34.4 mol %).

Microbial mats are probable sites for sulphur-reducing bacteria but information about these organisms is scanty. An organism identified as a thermophilic variant of *Pseudomonas mendocina* (Balashova, 1986) was isolated from a *Thermothrix* association. This facultative aerobe can utilize different inorganic compounds, including S⁰ as an electron acceptor. In springs with a high colloidal sulphur content near the Uzon volcano, there is a predominance of obligate anaerobic microflora in cyanobacterial mats (Bonch-Osmolovskaya, 1986). The number of bacteria reducing sulphur during caseine hydrolysate oxidation was found to be very high (10¹⁰ cell cm⁻³). The dominant forms are small obligate anaerobic rods. They reduce sulphur and are resistant to streptomycin.

Organisms utilizing other organic substrates to reduce sulphur in thermophilic cyanobacterial associations have not been found as yet. Undoubtedly, they would be of much interest since the known sulphur-reducing bacteria are either mesophiles (Pfennig and Biebl, 1976) or extreme thermophiles (optimal temperatures for growth >80°C) (Stetter, 1985). The latter are Thermoproteales belonging to archaeobacteria with their distinct structural and molecular organization as Thermoproteales (Zillig *et al.*, 1981a). Extreme

thermophilic sulphur-reducing archaeobacteria belonging to the genera *Thermoproteus* (Zillig *et al.*, 1981b), *Thermofilum* (Zillig *et al.*, 1983) and *Desulfurococcus* (Zillig *et al.*, 1982) were isolated from thermal springs in Iceland. The first two are obligate sulphur reducers. The latter can grow by sulphur respiration and anaerobic decomposition of organic matter in the absence of sulphur. Thermoproteales are capable of both lithoautotrophic growth by hydrogen oxidation (Fisher *et al.*, 1983) and organotrophic growth. The range of organic substances consumed is very wide (Zillig *et al.*, 1981).

A new extreme thermophilic archaeobacteria was isolated from Kamchatka hot springs—*Desulfurococcus amylolyticus*—with a temperature optimum at about 90°C (Bonch-Osmolovskaya *et al.*, 1988). The lower temperature limit for Thermoproteales is 70°C, but this temperature is too high for their participation in cyanobacterial associations. Perhaps Thermoproteales associates with *Thermothrix thiopara* but this has not been demonstrated. A high number of extreme thermophilic bacteria (10^5 – 10^7 cells cm⁻³) capable of utilizing casein hydrolysate for sulphur reduction were found in the caldera of the Uzon volcano (Bonch-Osmolovskaya and Svetlichny, 1987). Evidently their substrate is dissolved organic matter arriving from low temperature zones by thermal convection. Therefore, extreme thermophilic sulphur reducers can interact indirectly with cyanobacterial associations and couple the anaerobic cycles of carbon and sulphur in very high temperature ecosystems.

8.2.4 Extreme factors in the formation of microbial mats in hot sulphur springs

In the following sections we discuss how the structure and function of sulphur microbial mats in hot springs are controlled by extremes of temperatures, sulphide concentrations, pH and salinity.

8.2.4.1 Temperature

Temperature greatly affects the diversity of microbial associations in hot springs (Brock, 1978). In alkaline and neutral low mineralized springs (temperature over 72°C), phototrophic microorganisms are absent. In high temperature sulphur springs, sulphur oxidizers and reducers develop. The primary producers of organic matter in sulphur mats are colourless sulphur bacteria which develop in microaerophilic conditions. At extremely high temperatures, the most abundant sulphur bacteria are *Thermothrix thiopara*, *Thiobacterium bovista* and some thionic bacteria (Balashova, 1985; Brock, 1978).

Under natural conditions (e.g. Thermophilny spring), sulphur bacteria associate with sulphur-reducing microorganisms. The conditions for the

development of strictly anaerobic bacteria are created inside slimy structures during degradation of sulphur bacterial biomass (Gorlenko *et al.*, 1987a).

Therefore, in high temperature regimes of hot sulphur springs, unique mats develop which consist of microorganisms highly dependent on sulphur compounds. Biomass accumulates due to chemosynthesis by sulphur bacteria. Sulphur, and probably sulphate-reducers, are the dominant destructors. Oxygen plays an important role as an electron acceptor during bacterial oxidation of sulphur compounds. However, in the Thermophilny spring *Thermothrix* grows abundantly at an extremely low O₂ content (less than 1 mg l⁻¹). There are always phototrophic microorganisms in microbial mats in sulphur springs at temperatures below 72–74°C. These include unicellular and filamentous thermophilic cyanobacteria, as well as the green filamentous *Chloroflexus aurantiacus*.

As mentioned previously, the distribution of some species and groups of cyanobacteria is regulated by their temperature tolerance and strategy in relation to sulphide (Table 8.1). Among anoxic phototrophs, the most thermophilic is *Chloroflexus aurantiacus* (Table 8.2). This microorganism dominates in all springs with low mineralization.

Purple non-sulphur and sulphur bacteria as well as green sulphur bacteria are found in microbial mats below 60–65°C. Their density can be very high (10²–10⁷ cells l⁻¹) (Gorlenko *et al.*, 1985). The diversity of phototrophs increases gradually as the temperature decreases to 45°C (Figure 8.1, Gorlenko *et al.*, 1985). If the index of diversity (Odum, 1983) is zero at temperatures higher than 60°C, then it gradually decreases from 0.27 to 0.5 over the range of 60 to 45°C. At lower temperatures, the variability index

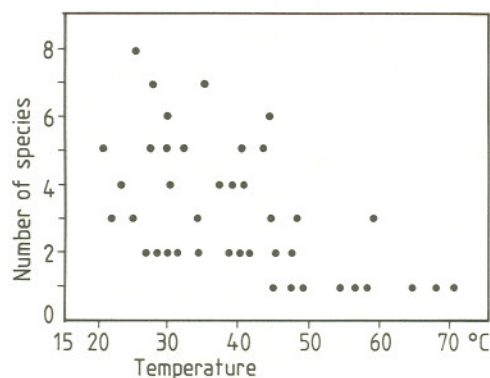


Figure 8.1 Number of species of phototrophic bacteria in 41 different microbial mats at different temperatures

fluctuates irregularly from 0.52 to 0.83. In the temperature range of 20 to 45°C, the phototrophic community is most diverse.

Gorlenko *et al.* (1985) identified a number of species of phototrophic bacteria in hot springs of the USSR (Table 8.2). It is noteworthy that at elevated temperatures (45–60°C), non-sulphur purple bacteria were found (*Rhodopseudomonas palustris*, *Rhodobacter capsulatus*, *Rhodocyclus gelatinosus*, *Chromatium vinosum*, *Rhodospirillum mediosalinum*). Below 45°C, the purple *Rhodopseudomonas viridis*, a number of species of purple and green sulphur bacteria, and the green filamentous *Oscillochloris trichoides* were found.

Thus, temperature exerts a significant effect on the composition of microflora in hot spring microbial mats. It is evident, however, that below 45°C, its influence is minimal and the structure of microbial associations is determined by other parameters.

8.2.4.2 Sulphide concentration

Even trace concentrations of sulphide completely exclude certain cyanobacteria in microbial mats (Castenholz, 1973). The effect of increasing sulphide concentrations on other cyanobacteria is highly species-dependent (see Section 8.1). *Chloroflexus aurantiacus* exhibits rather a low tolerance of sulphide (1–2 mM) which is comparable to that of some non-sulphur purple bacteria (Hansen and Van Gemerden, 1972). Since sulphide concentrations are relatively low in hot springs, conditions are favourable for *Chloroflexus aurantiacus*. In contrast, extremely high sulphide contents may be observed in warm and cold springs. For instance, in Talgi spring (Dagestan, Caucasus) (28°C; pH, 6.3), the sulphide concentration may be as high as 218 mg l⁻¹ (authors, unpublished data). Sulphide in this spring is the main factor which determines the phototrophic population.

Near the spring orifices, there are only green sulphur bacteria *Chlorobium limicola* which form thin films on stones. At decreasing sulphide concentrations (less than 190 mg l⁻¹) and pH greater than 6.7, a sulphur mat consists of purple *Thiospirillum* sp. and *Chromatium* sp. forms on the spring bed. Green bacteria are also abundant in this association. Of interest is the presence of the non-sulphur purple *Rhodobacter* sp. in high sulphide systems. Mechanical rigidity of the microbial mat in Talgi spring is provided by the filamentous cyanobacterium *Oscillatoria* sp. At lower sulphide concentrations (50 mg l⁻¹) downstream, *Phormidium* sp. and the green filamentous bacterium *Oscillochloris trichoides* provide rigidity. The total mat accumulates by anoxic photosynthesis (see Section 8.1). Sulphide inhibits photosystem II in cyanobacteria. Therefore, high sulphide concentrations result in the formation of completely anaerobic mats in sulphur springs.

8.2.4.3 pH

It appears that pH variations from 5.7 to 10 do not significantly affect the species composition of photosynthetic bacteria in springs (Brook, 1978; Gorlenko *et al.*, 1985). Mildly acidic conditions and high sulphide concentrations are unfavourable for purple bacteria (Pfennig, 1967), which cannot compete with the more sulphide-tolerant green sulphur bacteria. The composition of cyanobacteria in association with *Chloroflexus aurantiacus* changes depending on pH and sulphide concentration. In alkaline low sulphide springs, it is *Synechococcus* sp.; in neutral springs lacking sulphide it is *Mastigocladus laminosus* (Nikitina and Gerasimenko, 1983); in mildly acidic and neutral springs with moderate sulphide concentrations—*Phormidium* sp. and *Oscillatoria* sp.

8.2.4.4 Combined effect of salinity and temperature

Salinity is a very important ecological factor. In mesophilic saline benthic sulfureta, the phototrophic species composition differs greatly from those of freshwater basins (Gorlenko *et al.*, 1984). Some hot sulphur springs with highly mineralized water are interesting for studying the combined effect of salinity and temperature. An example is the microbial mat in springs at Berikei, Dagestan (authors, unpublished data). These are NaCl springs with mineralization of 60–97 g l⁻¹; pH, 5.7–6.7 and sulphide, 30–40 mg l⁻¹.

Microbial mats begin to form at 54°C and lower whereas at higher temperatures, there is no visible development of microorganisms. Therefore, the upper temperature limit for cyanobacteria and anoxic phototrophic bacteria is significantly lower in saline springs than in others (authors, unpublished data).

The microbial mat of Berikei springs consists of species unique to saline water systems. The number of cyanobacterial species is limited, and filamentous *Phormidium* sp. predominate. There are also small quantities of unicellular *Synechocystis* sp. During the day, the mat is covered by a sulphur film indicating microbial sulphide oxidation. It was shown that cyanobacteria carry out O₂ photosynthesis in the presence of sulphide (>20 mg l⁻¹). These cyanobacteria can be assigned to the second or third group by their relation to sulphide (Table 8.1).

The microbial mat of Berikei saline springs is multilayered. The purple bacteria *Ectothiorhodospira* sp. and *Rhodobacter* sp. and green bacterium *Prostecochloris* sp. coexist in the upper layer. These purple and green bacteria were found for the first time in hot sulphur springs (50–54°C). Thus, the combined action of high salinity and high temperature promotes specific halophilic forms of cyanobacteria, and purple and green bacteria which are unable to develop in fresh water. In spite of a favourable

temperature regime, *Chloroflexus aurantiacus* is absent from saline sulphur springs.

8.2.5 Horizontal and vertical zonality in microbial associations of hot sulphur springs

A zonal distribution and variation of microbial associations downstream in springs were observed by many investigators (Streczewski, 1913; Brock, 1967, 1969, 1978; Castenholz, 1984a, 1984b; Gorlenko *et al.*, 1985). This results from gradual changes in physicochemical parameters of the medium. The temperature, pH and sulphide concentrations decrease, and the O₂ concentration increases, i.e. the conditions change gradually from anaerobic to aerobic in the horizontal direction. In CO₂ springs, the bicarbonate content decreases downstream. An example is the change in microbial associations observed in the Thermophilny spring (Gorlenko *et al.*, 1987a). At 78°C, sulphide concentration of 16–19 mg l⁻¹, pH of 6.3, in the absence of O₂, the dominant processes are anaerobic sulphate reduction and methanogenesis. Downstream, at 67–76°C and O₂ concentration of 0.1–1.0 mg l⁻¹, one can observe an intensive development of the chemolithoautotrophic microorganisms *Thermothrix thiopara*, *Macromonas* sp., and *Thiobacterium bovista* (Figure 8.2). This zone is characterized by abundant S⁰. In lower temperature zones (54–62°C) and sulphide concentrations of 7–10 mg l⁻¹, photosynthetic microorganisms form olive or orange mats (Figure 8.7). The latter are found at higher O₂ concentrations (1–3 mg l⁻¹). The basic components of the olive and orange zones are the green bacterium *Chloroflexus aurantiacus*, cyanobacteria *Phormidium* sp., and *Synechococcus* sp. Further downstream, the temperature drops below 50°C, the sulphide concentration decreases to 1 mg l⁻¹, and the O₂ content increases to 5–7 mg l⁻¹. Over the temperature range 28–50°C, a large multi-component green mat forms consisting of several species of cyanobacteria (Figure 8.7), *C. aurantiacus*, and sulphur and non-sulphur bacteria. The different biological regimes are presented on Eh versus pH and rH₂ versus temperature diagrams in Figures 8.2 and 8.3. The systematic change in microbial associations in response to a gradual decrease of temperature and an increase in redox potential is very evident in the diagrams.

For the development of photosynthetic and chemotrophic sulphur bacteria, the availability of sulphide as H-donor (in discharge water and generated during microbial destruction) is important.

Sulphur bacteria can develop in low-sulphide springs if the sulphate concentration is high.

The thickness of microbial mats in different springs and different zones of the same spring vary from 1 to more than 100 mm (Castenholz, 1984a). Photosynthesis proceeds actively in the upper 1–3 mm. Below the

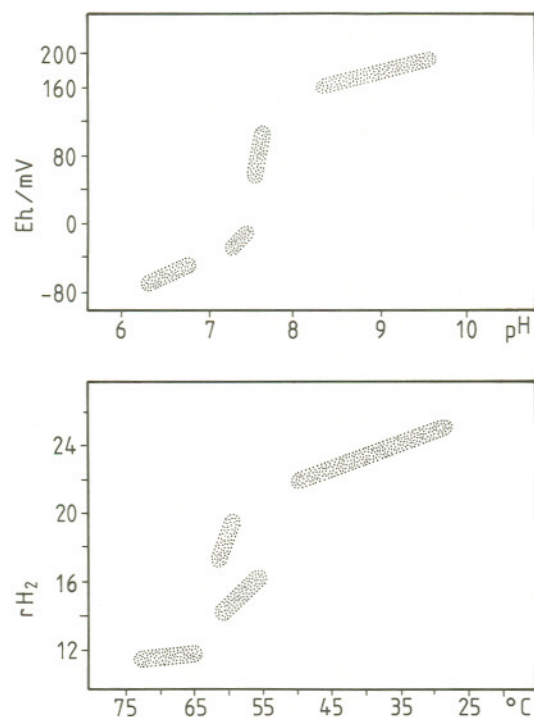


Figure 8.2 Location of different biological zones in Thermophilny spring versus physicochemical conditions: Eh-pH; rH_2 - $t^{\circ}C$. 1, 2, 3, 4 are white, olive, orange and green mats, respectively

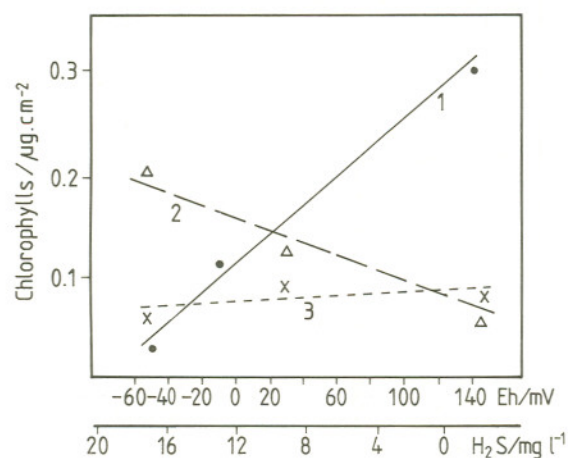


Figure 8.3 Redistribution of chlorophylls in different types of photosynthetic communities in Thermophilny spring versus physicochemical conditions. 1—chlorophyll-a; 2—bacteriochlorophyll-c; 3—bacteriochlorophyll-a

photosynthetic zone, anaerobic destruction proceeds with the participation of methanogenic, sulphate-reducing and sulphur-reducing microorganisms (Doemel and Brock, 1976). This causes an abrupt change in the redox potential in the uppermost layer of the mat. The Eh distribution in mats of the Thermophilny spring is shown in Figure 8.5. The slope of the Eh gradient determines the spatial distribution of microbial groups within the benthic association. A green low-temperature (40–50°C) loose mat, 1–2 cm thick, has the greatest potential for the development of physiologically different groups. Figure 8.4 shows the vertical distribution in green mats. Five species of cyanobacteria can develop in different microzones. *Chloroflexus aurantiacus* is found in close association with these producers (Figure 8.4). The highest number of filamentous green bacteria is observed during the day, 3–5 mm below the surface. The optimal sulphate reduction and methanogenesis are found at 15 and 20 mm depth respectively (Figure 8.6).

8.2.6 Productivity of microbial associations in different types of springs

The productivity of phototrophic associations in different springs correlates with the total chlorophyll pigment content. However, when making calculations, one should take into account that a significant part of the pigments is in the form of pheophytin (Gorlenko *et al.*, 1987a).

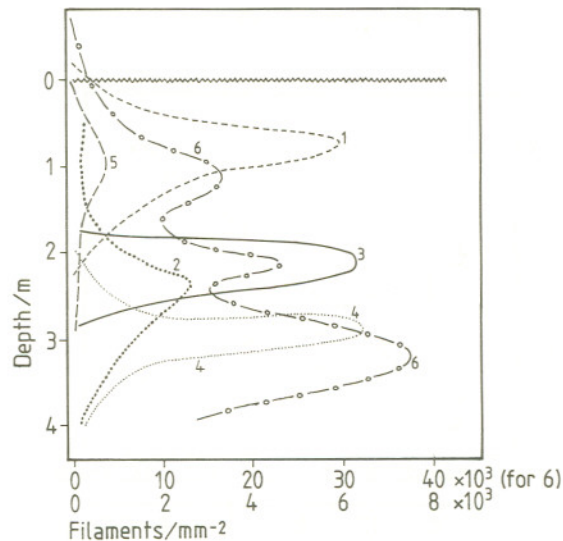


Figure 8.4 Vertical distribution of different morphological forms of cyanobacteria (1–5) and *Chloroflexus aurantiacus* (6) in the body of a green mat in Thermophilny spring

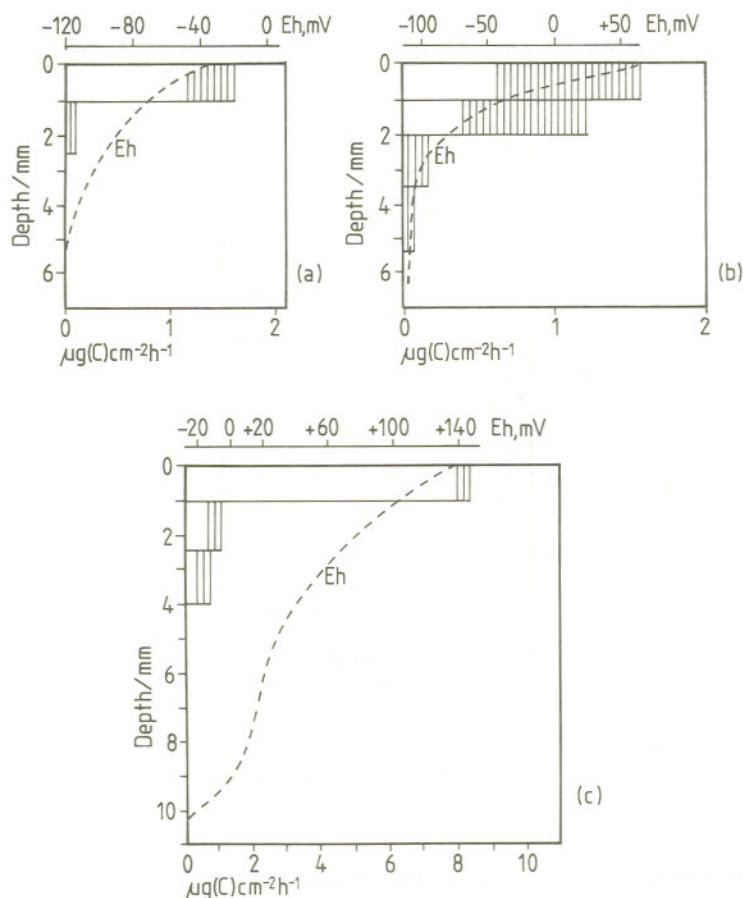


Figure 8.5 Rate of photosynthesis and redox potential in microbial mats of Thermophilny spring. □—oxygenic photosynthesis; ▨—anoxygenic photosynthesis; (a) olive mat; (b) orange mat; (c) green mat

Primary production changes along the stream gradient (Table 8.3), while the level of photosynthesis is generally high in all types of springs. For instance, in the hot saline Berikei springs, the photosynthetic production ranges from 4.7 to 53.8 g (C) $\text{cm}^{-2}\text{h}^{-1}$ (which means about 0.37–4.3 g (C) m^{-2} for a typical 8 hour daylight period) at different sites. The maximum value of photosynthesis approaches the highest productivity of the cyanobacterial mat in Solar Lake (Cohen *et al.*, 1977b).

Anoxic photosynthesis varies depending on the type of spring (Table 8.3), and is mainly performed by purple and green bacteria and to a lesser extent by unicellular and filamentous cyanobacteria.

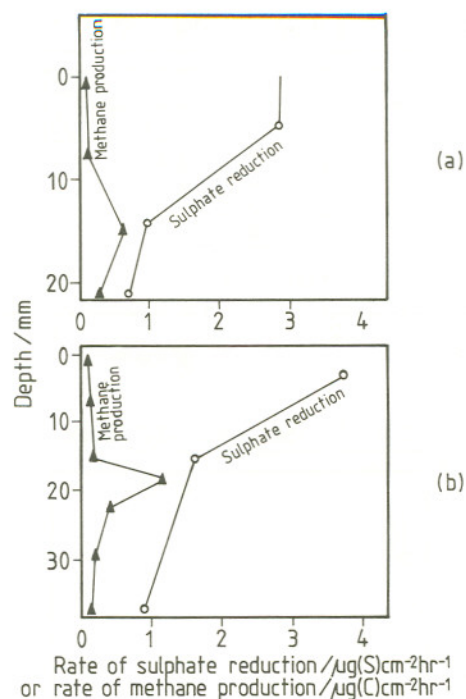


Figure 8.6 Rates of sulphate reduction and methanogenesis in orange (a) and green (b) mats in Thermophilny spring

In the springs studied, extracellular production makes up 53% of the total fixed carbon (Bauld and Brock, 1974; Gorlenko *et al.*, 1987b). Therefore excretions from photosynthetic microorganisms play an important role in trophic chains of ecosystems. Specifically, they create favourable conditions for the development of photoheterotrophic microorganisms such as *Chloroflexus* and non-sulphur purple bacteria.

The biomass accumulated during photosynthesis in microbial mats undergoes partial or complete destruction. Research into the degradation of mat components should indicate the pathways of burial and lithification of organic matter as well as the formation of some economic minerals.

8.2.7 Anaerobic destruction of organic matter in thermophilic mats

The bulk of the biomass of primary producers undergoes anaerobic destruction since the lower parts of mats are usually under reduced conditions (Table 8.5).

Table 8.3 Photosynthetic and dark production in microbial mats of Thermophilny spring

Type of mat, depth, (mm)	P-photo- synthesis ($\mu\text{g}(\text{C})$ $\text{cm}^{-2} \text{h}^{-1}$)	P-extra cellular (%)	P-anoxic (%)	P-dark ($\mu\text{g}(\text{C})$ $\text{cm}^{-2} \text{h}^{-1}$)	Chl-a BChl-a
White	—	20.0	—	0.074	—
Olive					
(0–1)	1.57	18.8	35.3	0.23	0.07
(1–2.5)	0.04	46.5	40.0		0.63
Orange					
(0–1)	1.62	2.9	59.3		1.1
(0–2)	1.40	7.4	62.0	0.68	1.2
(2–3.5)	0.13	24.0	76.0		—
(3.5–5.5)	0.07	—	—		—
Green					
(0–1)	7.63	22.2	1.0		6.0
(1–2.5)	0.77	8.5	10.1	1.21	4.35
(2.5–4)	0.54	3.8	2.9		—

Not much is known about the microbiota which hydrolyse biopolymers in thermophilic microbial mats. To date, the only hydrolytic organism isolated from a cyanobacterial mat is *Clostridium thermosulfurogenes* which can degrade pectin (Schink and Zeikus, 1983). It has the unique ability to form S^0 from thiosulphate. However, it is quite probable that this organism is not important in the destruction of cyanobacterial biomass because of its low population ($10^3 \text{ cells cm}^{-3}$). Sugar-consuming bacteria are more abundant (10^8 – $10^9 \text{ cells cm}^{-3}$) (Zeikus *et al.*, 1980). Among the saccharolytic organisms is the earlier known species *Cl. thermohydrosulfuricum* (Zeikus *et al.*, 1980). In addition, three saccharolytic anaerobic organisms were isolated and assigned to new genera: *Thermoanaerobium brockii* (Zeikus, *et al.*, 1979), *Thermobacterioides acetoethylicus* (Ben Bassat and Zeikus, 1981) and *Thermoanaerobacter ethanolicus* (Wiegel and Ljungdahl, 1981). They ferment hydrolysis products of algal polysaccharides into acetate, lactate, ethanol, H_2 and CO_2 . However, the abundance of proteolytic bacteria in the thermophilic mat (45°C) of Thermophilny spring is $10^9 \text{ cells cm}^{-3}$, i.e. an order higher than the number of saccharolytic bacteria (Bonch-Osmolovskaya *et al.*, 1988). Consequently, one can infer that protein is the basic substrate for destruction processes whereas polysaccharide is decomposed more slowly. Intermediate products (acids, alcohols, hydrogen) from hydrolysis and fermentation serve as energy substrates for bacteria carrying out the final

step of anaerobic destruction. Depending on the type of the spring temperature zone, and vertical position within the mat, the terminal processes may be sulphate reduction, sulphur reduction or methanogenesis.

The intensity of sulphate reduction in cyanobacterial mats is determined primarily by the sulphate content (Sandbeck and Ward, 1981). For example, at a concentration of 718 mg l^{-1} , sulphate reduction is the main terminal process of destruction, and H_2S is the principal reduced product (Ward and Olson, 1980). These authors found that sulphate reduction was the most intensive in the uppermost 5 mm of the mat. In Thermophilny spring, sulphate concentration was much lower (36 mg l^{-1}). The rate of sulphate reduction, determined by means of $^{35}\text{SO}_4^{2-}$ *in situ*, reached a maximum of $6 \text{ mg (S) cm}^{-2} \text{ h}^{-1}$ in the most productive green mat (Bonch-Osmolovskaya *et al.*, 1988; Tables 8.4 and 8.5). In the dark, sulphate reduction is most intensive in the uppermost (0–1 mm) layer of the mat, below which it decreases rapidly (Figure 8.6). The residence time of sulphate in cyanobacterial mats is very short: 2 to 3.7 hours (Table 8.4). In the depth interval, 1.5–2 cm, methanogenesis is most intensive and sulphate reduction is probably limited by a slow sulphate diffusion.

At lower SO_4^{2-} concentrations (16.6 mg l^{-1} , Octopus spring in Yellowstone National Park), sulphate reduction is evidently a minor process and does not inhibit methanogenesis even in the upper layer (Ward, 1978).

In model systems, sulphate reduction usually inhibits methanogenesis (Bonch-Osmolovskaya *et al.*, 1978). This is usually explained by the high energy yield of sulphate reduction:



compared to the reaction of methanogenesis:



where ΔG^0 is the free energy change associated with these reactions (Thauer *et al.*, 1977).

However, in nature, methanogenesis and sulphate reduction often occur simultaneously (Oremland and Polcin, 1982). This is explained by the difference in affinities of methanogens and sulphate reducers to the substrate, which allows them to prevail in different ecological situations (Lovley *et al.*, 1982). A number of substrates (methanol, methylamines) are non-competitive since they are utilized only by methanogens. Thus methanogens can develop even in biotopes rich in sulphate (Oremland and Polcin, 1982).

In thermophilic cyanobacterial mats, methanogenesis does not occur at high SO_4^{2-} concentrations (Ward and Olson, 1980). During incubation of

Table 8.4 Rates of terminal reduction processes in different mats of Thermophilny spring

Type of mat	H ₂ S (mg cm ⁻³)	S ^O (mg cm ⁻³)	Sulphate reduction rate (μg cm ⁻² h ⁻¹)	Sulphur reduction rate (μg cm ⁻¹)	CH ₄ production rate (μg cm ⁻² h ⁻¹)	Sulphate residence time (h)
White	1.3	3.1	0.16	0.4	trace	75
Olive	0.39	8.0	3.3	—	0.12	3.6
Orange	0.41	9.3	3.2	—	0.58	3.7
Green	0.47	9.6	6.0	—	1.77	2.0

Table 8.5 Sulphate reduction and methanogenesis in destruction processes in microbial mats of Thermophilny spring

Type of mat	Production (μg (C) cm ⁻² l ⁻¹)	Destruction, μg (C) cm ⁻² h ⁻¹ , due to:			$\frac{D(SO_4^{2-})}{D(CH_4)}$	P-D*
		sulphate reduction	sulphur reduction	methanogenesis		
White	0.074	0.09	0.28	trace	900	-0.31
Olive	1.87	2.48	—	0.24	10.30	-0.85
Orange	3.90	2.40	—	1.16	2.00	+0.34
Green	10.80	4.50	—	3.54	1.27	+2.18

*P—production, D—destruction

samples from such a spring, methanogenesis started only after 200 hours, and according to these authors, it is exclusively a laboratory process which does not occur in nature. As mentioned above, at low concentrations (16.6 mg l⁻¹), sulphate reduction does not occur, and methanogenesis is the main terminal anaerobic process (Ward, 1978).

Both destructive processes occur at varying rates in Thermophilny spring, having a sulphate concentration of 36 mg l⁻¹ (Table 8.4). The contribution of methanogenesis to the total destruction increases gradually, as the temperature decreases, and reaches a maximum in the green mat zone. Methanogenesis increases with depth and is a maximum immediately under the sulphate reduction zone (Figure 8.6).

A study of methanogenesis with different substrates showed that the main process in thermophilic mats is CO₂ reduction by hydrogen. This pathway in Thermophilny spring accounts for 89% of the methane produced (Bonch-Osmolovskaya *et al.*, 1988). This is consistent with the high number (10⁸

cells cm^{-3}) of the methanogen consuming H_2 , which was identified as *methanobacterium thermoautotrophicum*.

Acetate did not appear to be consumed in hot spring mats of Yellowstone National Park during methanogenesis (Ward, 1978) and does not stimulate sulphate reduction (Ward and Olson, 1980). It has been shown that the bulk of the acetate in mats of Octopus spring, Yellowstone National Park, is assimilated by *Chloroflexus* (Ward *et al.*, 1984). In contrast to this a thermophilic organism which produces CH_4 from acetate was isolated from Thermophilny spring (Nozhevnikova and Yagodina, 1982). Radioisotopic study *in situ* showed that up to 11% of methane in Thermophilny spring is formed from acetate (Bonch-Osmolovskaya *et al.*, 1987).

Usually methane can be formed intensively from C_1 -compounds by representatives of the genus *Methanosarcina*. However, microorganisms of this genus are not found in thermophilic cyanobacterial mats. A rod-like methanogenic microflora similar to *Methanobacillus kuzneceovii* (Pantshava and Pcholkina, 1969) was isolated using a methanol medium (Zhilina *et al.*, 1983). It was shown that such a culture is in a symbiotic association with *Clostridium thermoautotrophicum* producing H_2 from methanol, and *Methanobacterium thermoautotrophicum* consuming this hydrogen to reduce CO_2 to CH_4 (Ilarionov and Bonch-Osmolovskaya, 1986). Thus in anaerobic destruction of thermophilic cyanobacterial mats, hydrogen is the main substrate for competition between methanogenic and sulphate-reducing bacteria.

Sulphur reduction in microbial mats has been studied less. However, this process may also play an important role in the anaerobic destruction of thermophilic systems. Sulphur may be an electron acceptor during anaerobic destruction of organic matter, H_2S being the reduction product. Elemental sulphur in mats may have different origins.

For instance, in the white mat of Thermophilny spring, S^0 is the product of H_2S oxidation by *Thermothrix thiopara* (Figure 8.7a). Organic matter synthesized by this reaction undergoes decomposition in anaerobic conditions, and the terminal electron acceptor is again S^0 . It was shown that *Thermothrix thiopara* exists in association with facultative anaerobic thermophilic bacteria including those able to reduce S^0 (Balashova, 1986).

We have established that under 1 cm^2 of the white mat, $0.4 \mu\text{g}$ (S^0) is reduced per hour. This is 2.3 times higher than the rate of sulphate reduction in the same zone. Therefore sulphur reduction dominates in the destruction of organic matter synthesized by *Thermothrix thiopara*.

Cyanobacterial mats of Thermophilny spring contain significant quantities of S^0 ($8\text{--}9.6 \text{ mg cm}^{-2}$). The largest amount accumulates in the upper layers of green and orange mats, which correspond to the photosynthetic zone and the transition between aerobic and anaerobic zones (Figure 8.7b; Jørgensen, 1982). The source of S^0 may be anaerobic oxidation of H_2S by phototrophic

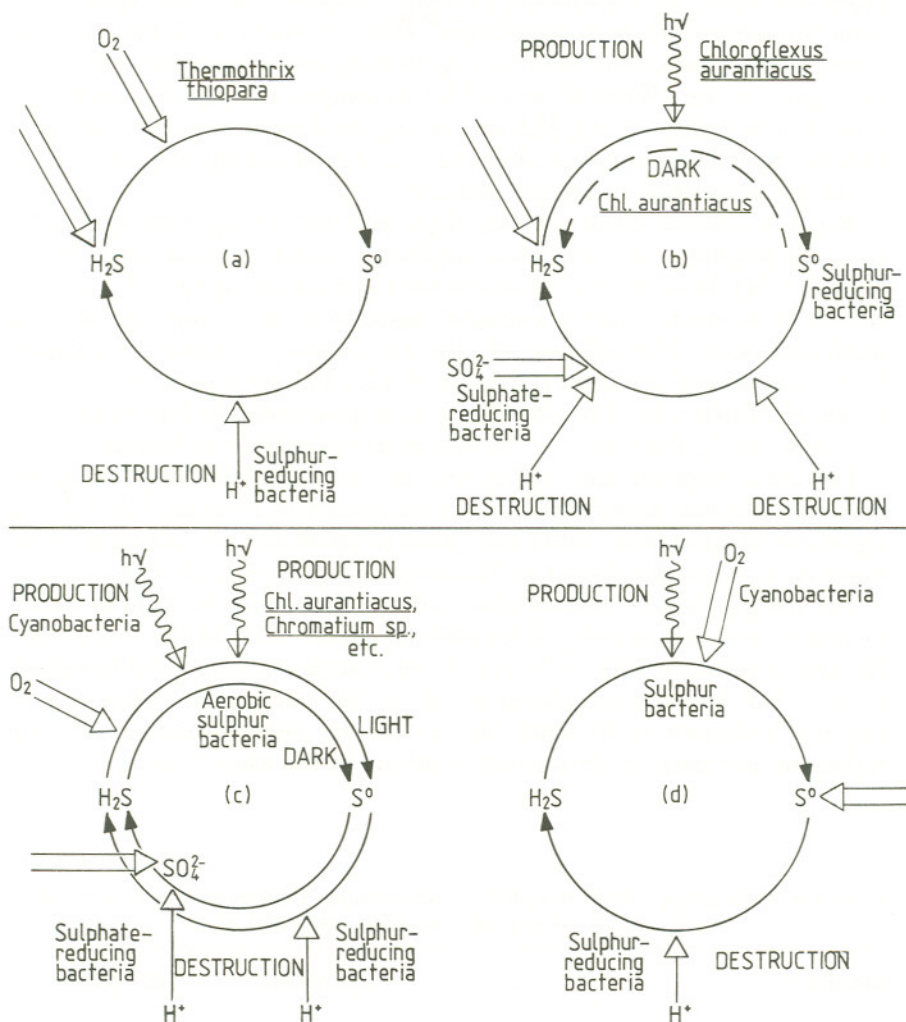


Figure 8.7 Hypothetical schemes of the sulphur cycle in thermophilic microbial mats of different types.

(a) Microbial mat associated with *Thermotrix thiopara* (72°C)

(b) Microbial mat with *Chloroflexus aurantiacus* as main producent (65°C)

(c) Multi-component cyanobacterial mat (45–55°C)

(d) Cyanobacterial mat with exogenic source of sulphur (50–55°C)

⇒ influx of compounds from outside;

→ the sulphur cycle inside the mat;

H⁺ → formation of a reducer during destruction

bacteria, aerobic oxidation of H_2S by sulphur bacteria, or the activity of organisms similar to *Clostridium thermosulfurogenes* which can produce S^0 from thiosulphate (Schink and Zeikus, 1983). It would seem that in these zones, sulphur reduction contributes significantly to the anaerobic destruction of organic matter. When S^0 was added to samples of cyanobacterial mats from Thermophilny spring, H_2S was evolved and methanogenesis inhibited (Table 8.6). This points to the ability of cyanobacterial associations to reduce S^0 during destruction of organic matter.

When a cyanobacterial mat develops in a hot spring with suspended colloidal sulphur, the exogenous sulphur is found between algal layers (Figure 8.7a). In such a mat (found in the Uzon caldera at 50–57°C) sulphur reduction dominates during anaerobic destruction and completely inhibits methanogenesis. The addition of different organic substrates to samples from such a mat increases the yield of H_2S but methanogenesis is still inhibited (Table 8.6). The abundance of sulphur-reducing bacteria (up to 10^{10} cells cm^{-3}) also points to the dominance of sulphur reduction.

Therefore, sulphate- and sulphur-reducing bacteria of thermophilic cyanobacterial associations participate in the destruction of biomass. The balance equations of Krumbein (1983) were used to calculate their contribution to destruction of organic matter at Thermophilny spring (Table 8.5).

The participation of methanogenesis in the destruction of organic matter increases gradually in going from white to green mats. In the green mat, the contributions of SO_4^{2-} reducers and methanogens to the decomposition of microbial biomass are almost equal. In the olive mat, destruction by sulphate reduction is 10 times higher than by methanogenesis. Sulphur reduction dominates in destruction occurring in the white mat.

Table 8.6 Reduction products in anaerobic destruction of different organic substrates with samples of sulphur-containing microbial mats

Substrate*	CH_4 (mM)	HS^- (mM)
Pectin	0	12.5
Yeast extract	0	7.1
Casein hydrolysate	0	12.8
Lactate	0.97	17.6
Formate	0.87	19.2
Acetate	0	23.5
Methanol	0	22.3
Ethanol	0	21.5
Glycerol	0	21.3

*Samples of mat (1 cm^3) were incubated anaerobically for 14 days in 50 ml vials with mineral medium and organic substrate (5 mg ml^{-1}) (Pfennig, 1965).

Visual examination showed that organic remnants do not accumulate and are not buried in white and olive mats. This means that production is balanced by destruction. Calculations confirm this conclusion. Organic matter accumulates in the orange and especially green mats (3 and 15 cm thick, respectively). The calculated production is higher than the anaerobic destruction by methanogenesis and sulphate reduction.

8.2.8 Conclusion

Microbial associations of hot sulphur springs have a local character of development and do not appreciably affect terrestrial geochemical processes which occur nowadays. Nonetheless their study gives insight into ideas about the evolutionary pattern of the atmosphere and lithosphere, and indicate which microorganisms could play a leading part in biogeochemical processes at raised temperatures and O₂ deficiency. The species composition of microbial mats depends upon a set of physicochemical parameters and is different in various sources.

In thermophilic microbial mats the sulphur cycle is coupled with the cycle of carbon, i.e. the primary production and destruction of organic matter. In all cases studied, the production leads, directly or indirectly, to elemental sulphur formation.

In the *Thermothrix* community, the aerobic oxidation of H₂S yields elemental sulphur as the main catabolic product (Figure 8.7a). In a mat formed by *Chloroflexus aurantiacus*, sulphur is produced in the course of photosynthesis as a result of H₂S oxidation in the light (Figure 8.7b). In multi-component mats consisting mainly of cyanobacteria, elemental sulphur is the product of chemical or biological H₂S oxidation with O₂ brought about by phototrophs (Figure 8.7c).

Reduced compounds are formed during biomass destruction; H₂S is produced with elemental sulphur as terminal electron acceptor.

Thus we see that in thermophilic microbial mats of sulphur springs a short sulphur cycle $\text{H}_2\text{S} \rightarrow \text{S}^0$ is taking place. This supports the idea of an analogy between 'oxygenic' and 'sulphur' life (Wolfe and Pfennig, 1977). But, unlike the closed cycles in symbiotic associations, described by Pfennig *et al.* (Wolfe and Pfennig, 1977; Biebl and Pfennig, 1978), open sulphur cycles take place in thermal springs. In the *Thermothrix* community, exhalations of juvenile gases contribute to the H₂S pool in the system (Figure 8.7a). Elemental sulphur, when reaching high concentrations in the spring, also enters the cycle (Figure 8.7d). Sulphate can be of exogenous origin, or produced endogenously in the course of aerobic sulphur bacteria activity.

Thus, microbial communities in thermal sulphur springs can be regarded as natural model systems. Further investigations will contribute to a better understanding of processes that took place in the geological past of the Earth.

REFERENCES

- Aizenshtat, Z., Lipiner, G., and Cohen, Y. (1984). Biogeochemistry of carbon and sulphur cycle in the microbial mats of Solar Lake (Sinai). In: Cohen, Y., Castenholz, R.W., and Halvorson, H.O. (Eds.) *Microbial Mats: Stromatolites*. Alan R. Liss, New York, pp. 281–312.
- Awramik, S.M. (1984). Ancient stromatolites and microbial mats. In: Cohen, Y., Castenholz, R.W., and Halvorson, H.O. (Eds.) *Microbial Mats: Stromatolites*. Alan R. Liss, New York, pp. 1–22.
- Baas-Becking, L.G.M., and Wood, E.J.F. (1955). Biological processes in the estuarine environment: 1–11. Ecology of the sulphur cycle. *Proc. K. Med. Akad. Wet. Serct.*, **B58**, 160–81.
- Balashova, V.V. (1985). Use of molecular sulphur as H_2 oxidiser by facultative anaerobic pseudomonade. *Mikrobiologia*, **54**, 324–6 (in Russian).
- Balashova, V.V. (1986). Thermophilic sulphureta modelling. In: Imshenetsky, A.A. (Ed.) *Biology of Thermophilic Microorganisms*, Nauka, Moscow, pp. 113–16 (in Russian).
- Bauld, J. (1981a). Geobiological role of cyanobacterial mats in sedimentary environments: production and preservation of organic matter. *BMR J. Aust. Geol. Geophys.*, **6**, 307–17.
- Bauld, J. (1981b). Occurrence of benthic microbial mats in saline lakes. *Hydrobiologia*, **81**, 87–111.
- Bauld, J. (1984). Microbial mat in marginal marine environments: Shark Bay, Western Australia and Spencer Gulf, South Australia. In: Cohen, Y., Castenholz, R.W., and Halvorson, H.O. (Eds.) *Microbial Mats: Stromatolites*, Alan R. Liss, New York, pp. 39–58.
- Bauld, J., and Brock, T.D. (1973). Ecological studies of *Chloroflexus*, a gliding photosynthetic bacterium. *Arch. Microbiol.*, **92**, 267–84.
- Bauld, J., and Brock, T.D. (1974). Algal excretion and bacterial assimilation in hot spring algal mats. *J. Physiol.*, **10**, 101–6.
- Bauld, J., Chambers, L.A., and Skyring, G.W. (1979). Primary productivity, sulphate reduction and sulphur isotope fractionation in algal mats and sediments of Hamelin Pool, Chark Bay, W.A. *Aust. J. Mar. Freshwater Res.*, **30**, 753–64.
- Belkin, S. (1983). Hydrogen metabolism in the facultative anoxygenic cyanobacteria. Ph.D. Thesis, Hebrew University, Jerusalem.
- Belkin, S., and Padan, E. (1979). Hydrogen metabolism in the facultative anoxygenic cyanobacteria (blue green algae) *Oscillatoria limnetica* and *Aphanotheca halophila*. *Arch. Microbiol.*, **116**, 109–11.
- Ben-Bassat, A., and Zeikus, J.G. (1981). *Thermobacteroides acetoethylicus* Gen.nov. and spec.nov., a new chemoorganotrophic, anaerobic thermophilic bacterium. *Arch. Microbiol.*, **128**, 365–70.
- Biebl, H., and Pfennig, N. (1978). Growth yields of green sulphur bacteria in mixed cultures with sulphur and sulphate reducing bacteria. *Arch. Microbiol.*, **117**, 9–16.
- Bildushkinov, S.S., and Gerasimenko, L.M. (1985). Thermophilic cyanobacteria from cyanobacterial community. *Mikrobiologia*, **54**, 490–3 (in Russian).
- Bonch-Osmolovskaya, E.A. (1986). Reduction of elemental sulphur in thermophilic algal bacterial communities of Uzon caldera (Kamchatka). In: Imshenetsky, A.A., (Ed.) *Biology of Thermophilic Microorganisms*, Nauka, Moscow, pp. 112–13 (in Russian).
- Bonch-Osmolovskaya, E.A., and Svetlichny, V.A. (1989). Extremely thermophilic sulphur-reducing archaeobacteria. *Archaeobacteria*, Puschino, USSR (in Russian).

- Bonch-Osmolovskaya, E.A., Vedenina, I.Ya., and Balashova, V.V. (1978). The influence of inorganic electron acceptors on methane formation during anaerobic cellulose destruction. *Mikrobiologia*, **47**, 611–16 (in Russian).
- Bonch-Osmolovskaya, E.A., Gorlenko, V.M., Karpov, G.A., and Starynin, D.A. (1987). Anaerobic destruction of organic matter in cyanobacterial mats of Thermophilny spring (Uzon, Kamchatka). *Mikrobiologia*, **56**, 1022–8 (in Russian).
- Bonch-Osmolovskaya, E.A., Slesarev, A.I., Miroshnichenko, M.L., Svetlichnaya, T.P., and Alekseev, V.A. (1988). *Desulfurococcus amylolytuus* n.sp.—a new extremely thermophilic archaeobacterium from Kamchatka and Kunashire hot springs. *Mikrobiologia*, **57**, 94–101 (in Russian).
- Bogorov, L.V. (1974). On capacities of *Thiocapsa roseopersicina* strain BBS isolated from the White Sea estuaries. *Mikrobiologia*, **43**, 326–31 (in Russian).
- Bothe, H., Tennigkeit, Y., and Eisbrinner, G. (1977). The utilisation of molecular hydrogen by the blue green alga *Anabaena cylindrica*. *Arch. Microbiol.*, **114**, 43–9.
- Brannan, D.K., and Caldwell, D.E. (1980). *Thermotrix thiopara*: growth and metabolism of a newly isolated thermophile capable of oxidising sulphur and sulphur compounds. *Appl. Environ. Microbiol.*, **40**, 211–16.
- Brock, T.D. (1967). Relationship between standing crop and primary productivity along a hot spring thermal gradient. *Ecology*, **48**, 566–71.
- Brock, T.D. (1969). Vertical zonation in hot spring algal mats. *Phycologia*, **8**, 201–5.
- Brock, T.D. (1978). *Thermophilic Microorganisms and life at High Temperatures*, Springer-Verlag, New York.
- Cairns-Smith, A.G. (1978). Precambrian solution photochemistry, inverse segregation and banded iron-formations. *Nature*, **276**, 807–8.
- Caldwell, D.E., Caldwell, S.J., and Laycock, J.P. (1976). *Thermotrix thioparus* gen. et sp.n., a facultatively anaerobic facultative chemolithotroph living at neutral pH and high temperature. *Can. J. Microbiol.*, **22**, 1509–17.
- Campbell, R. (1983). Microbial ecol. In: Wilkinson, J.F. (ed.) 2 edn. Blackwell Scientific Publications, Oxford.
- Castenholz, R.W. (1969). Thermophilic blue-green algae and the thermal environment. *Bact. Rev.*, **33**, 476–504.
- Castenholz, R.W. (1973). The possible photosynthetic use of sulphide by the filamentous phototrophic bacteria of hot springs. *Limnol. Oceanogr.*, **18**, 863–76.
- Castenholz, R.W. (1976). The effect of sulphide on blue green algae of hot springs. I. New Zealand and Iceland. *J. Phycol.*, **12**, 57–68.
- Castenholz, R.W. (1977). The effect of sulphide on blue green algae of hot springs. II. Yellowstone National Park. *Microb. Ecol.*, **3**, 79–68.
- Castenholz, R.W. (1984a). Composition of hot spring microbial mats: A summary. In: Cohen, Y., Castenholz, R.W., and Halvorson, H.O. (Eds.) *Microbial Mats: Stromatolites*, Alan R. Liss, New York, pp. 101–20.
- Castenholz, R.W. (1984b). Habitats of *Chloroflexus* and related organisms. In: King, M.J., and Reddy, C.A. (Eds.) *Current Perspectives of Microbial Ecology*, ASM, Washington, DC.
- Castenholz, R.W., and Utkilen, H.C. (1984). Physiology of sulphide tolerance in a thermophilic *Oscillatoria*. *Arch. Microbiol.*, **138**, 299–305.
- Cloud, P. and Gibor, A. (1970). The oxygen cycle. *Scientific American* (Sept.).
- Cohen, Y. (1985a). Microtechnique for *in situ* sulphate reduction measurement in proximity to oxygen. *Arch. Microbiol.* (in press).
- Cohen, Y. (1985b). Sulphate reduction under oxygen in cyanobacterial mats and its coupling to primary production. *Limnol. Oceanogr.* (in press).

- Cohen, Y. and Gack, E. (1985). Fe^{++} -dependent photosynthesis in cyanobacterial. *Nature* (in press).
- Cohen, Y., Castenholz, R.W., and Halvorson, H.O. (Eds.) (1984). *Microbial Mats: Stromatolites*, MBL Lectures in Biology, Vol. 3. Alan R. Liss, New York, pp. 1–498.
- Cohen, Y., Padan, E., and Shilo, M. (1975a). Facultative anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. *J. Bacteriol.*, **12**, 855–61.
- Cohen, Y., Jørgensen, B.B., Padan, E., and Shilo, M. (1975b). Sulphide-dependent anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. *Nature*, **257**, 489–91.
- Cohen, Y., Goldberg, M., Krumbein, W.E., and Shilo, M. (1977a). Solar Lake (Sinai). I. Physical and chemical limnology. *Limnol. Oceanogr.*, **22**, 597–607.
- Cohen, Y., Krumbein, W.E., and Shilo, M. (1977b). Solar Lake (Sinai). 2. Distribution of photosynthetic microorganisms and primary production. *Limnol. Oceanogr.*, **22**, 609–10.
- Cohen, Y., Aisenshtat, Z., Stoler, A., and Jørgensen, B.B. (1980). Microbial geochemistry of Solar Lake Sinai. In: Trudinger, P.A., and Walter, M.R. (Eds.) *Biogeochemistry of Ancient and Modern Environments*, Australian Academy of Science, Canberra, pp. 167–77.
- Doemel, W.N., and Brock, T.D. (1976). Vertical distribution of sulphur species in benthic algal mats. *Limnol. Oceanogr.*, **21**, 237–44.
- Doemel, W.N., and Brock, T.D. (1977). Structure, growth and decomposition of laminated algal-bacterial mats in alkaline hot springs. *Appl. Environ. Microbiol.*, **34**, 433–52.
- Fisher, F., Zillig, W., Stetter, K.O., and Schreiber, G. (1983). Chemolithoautotrophic metabolism of anaerobic extremely thermophilic archaebacteria. *Nature*, **301**, 511–13.
- Ganf, G.G., and Viner, A.B. (1973). Ecological stability in a shallow equatorial lake (Lake George, Uganda). *Proc. R. Soc. Lond. (Biol.)*, **184**, 321–46.
- Garlick, S., Oren, A., and Padan, E. (1977). Occurrence of facultative anoxygenic photosynthesis among filamentous and unicellular cyanobacteria. *J. Bacteriol.*, **129**, 623–9.
- Gerasimenko, L.M., Karpov, G.A., Orleansky, V.K., and Zavarzin, G.A. (1983). The role of cyanobacterial filter in gas component of hydrotherms transformation with caldera Uzon in Kamchatka as an example. *Zhurnal obshchei biologii*, **44**, 842–51 (in Russian).
- Gerdes, G., and Krumbein, W.E. (1984). Animal communities in recent potential stromatolites of hypersaline origin. In: Cohen, Y., Castenholz, R.W., and Halvorson, H.O. (Eds.) *Microbial Mats: Stromatolites*. Alan R. Liss, New York, pp. 59–84.
- Golovacheva, R.S. (1984). Aerobic thermophile chemolithotrophic bacteria participating in the sulphur cycle. *Uspehi Mikrobiologii*, **19**, 166–202 (in Russian).
- Gorlenko, V.M. (1974). Dark thiosulphate oxidation by *Amoebobacter roseus* in microaerophilic conditions. *Mikrobiologia*, **43**, 729–31 (in Russian).
- Gorlenko, V.M. (1981). Purple and green bacteria and their role in the carbon and sulphur cycles. Thesis, INMI Acad. Sci. USSR, p. 51 (in Russian).
- Gorlenko, V.M., Dubinina, G.A., and Kuznetsov, S.I. (1983). The ecology of aquatic microorganisms. *Die Binnengewässer*, Volume XVIII, Stuttgart, E. Schweizerbartsche Verlagsbuchhandlung (Nägele und Obermiller), 252 pp.
- Gorlenko, V.M., Kompantseva, E.I., and Puchkova, N.N. (1985). Influence of temperature on phototrophic bacteria distribution in thermal springs. *Mikrobiologia*, **54**, 848–53 (in Russian).

- Gorlenko, V.M., and Korotkov, S.A. (1979). Morphological and physiological features of the new filamentous gliding green bacteria *Oscillochloris trichoides* nov.comb. *Izv. Akad. Nauk SSSR. Ser. Biol.*, **5**, 848–57 (in Russian).
- Gorlenko, V.M., Kompantseva, E.I., Korotkov, S.A., Puchkova, N.N., and Savvichev, A.S. (1984). Growth conditions and diversity of phototrophic bacteria species in saline shallow waters of Crimea. *Izv. Akad. Nauk SSSR, Ser. Biol.*, **3**, 362–73 (in Russian).
- Gorlenko, V.M., Bonch-Osmolovskaya, E.A., Kompantseva, E.I., and Starynin, D.A. (1987). Differentiation of microbial communities in connection with changes in physico-chemical parameters in Thermophilny spring. *Mikrobiologia*, **56**, 314–22 (in Russian).
- Gorlenko, V.M., Starynin, D.A., Bonch-Osmolovskaya, E.A., and Kachalkin, V.J. (1987b). Primary production processes in microbial mats of Thermophilny spring. *Mikrobiologia* (in Russian).
- Govindjee, R., Rabinovitch, E., and Govindjee, (1968). Maximum quantum yield and action spectrum of photosynthesis and fluorescence in *Chlorella*. *Biochem. Biophys. Acta.*, **162**, 539–44.
- Gromet-Elhanan, Z. (1977). Electron transport and photophosphorylation in photosynthetic bacteria. In: Trebt, A., and Avron, M. (Eds.) *Encyclopedia of Plant Physiology*, Springer-Verlag, Berlin, pp.637–62.
- Hansen, T.A., and Van Gemerden, H. (1972). Sulphide utilization by purple bacteria. *Arch. Microbiol.*, **86**, 49–56.
- Hartman, H. (1983). The evolution of photosynthesis and microbial mats: A speculation on the Banded Iron Formation. In: Cohen, Y., Castenholz, R.W., and Halvorson, H.O. (Eds.) *Microbial Mats: Stromatolites*, Alan R. Liss, New York, pp. 441–54.
- Howsley, R., and Pearson, H.W. (1979). pH dependent sulphide toxicity to oxygenic photosynthesis in cyanobacteria. *FEMS Lett.*, **6**, 287–92.
- Ilarionov, S.A., and Bonch-Osmolovskaya, E.A., (1986). Methane production from methanol by bacterial associations. *Mikrobiologia*, **55**, 282–8 (in Russian).
- Imhoff, J.F., Hashwa, F., and Truper, H.G. (1978). Isolation of extremely halatrophic bacteria from the alkaline Wadi Natrun, Egypt. *Arch. Hydrobiol.*, **84**, 381–8.
- Javor, B.J., and Castenholz, R.W. (1984a). Invertebrate grazers of microbial mats, Lagoon Guerrero Negro, Mexico. In: Cohen, Y., Castenholz, R.W., and Halvorson, H.O. (Eds.) *Microbial Mats: Stromatolites*, Alan R. Liss, New York, pp. 85–94.
- Javor, B.J., and Castenholz, R.W. (1984b). Productivity studies of microbial mats, Lagoon Guerrero Negro, Mexico. In: Cohen, Y., Castenholz, R.W., and Halvorson, H.O. (Eds.) *Microbial Mats: Stromatolites*, Alan R. Liss, New York, pp. 149–70.
- Jørgensen, B.B. (1982). Ecology of the bacteria of the sulphur cycle with special reference to anoxic–oxic interface environments. *Phil. Trans. R. Soc. Lond. B.*, **298**, 543–61.
- Jørgensen, B.B., and Cohen, Y. (1977). Solar Lake (Sinai). 5. The Sulphur cycle of benthic cyanobacterial mats. *Limnol. Oceanogr.*, **22**, 657–66.
- Jørgensen, B.B., Revsbech, N.P., and Cohen, Y. (1983). Photosynthesis and structure of benthic microbial mats: microelectrode and SEM studies of four cyanobacterial communities. *Limnol. Oceanogr.*, **28**, 1075–93.
- Jørgensen, B.B., Revsbech, N.P., Blackburn, T.H., and Cohen, Y. (1979). Diurnal cycle of oxygen and sulphide microgradients and microbial photosynthesis in a cyanobacterial mat sediment. *Appl. Environ. Microbiol.*, **38**, 46–58.
- Kämpf, C., and Pfennig, N. (1980). Capacity of Chromatiaceae for chemotrophic

- growth. Specific respiration rates of *Thiocytis violaceae* and *Chromatium vinosum*. *Arch. Microbiol.*, **127**, 125–35.
- Kaplan, I.R. (1956). Evidence of microbiological activity in some of the geothermal regions of New Zealand. *N.Z. J. Sci. Technol.*, **37**, 639–48.
- Knobloch, K. (1969). Sulphide oxidation via photosynthesis in green alga. In: Muntzer, H. (Ed.) *Progress in Photosynthesis Research*, Vol. II, International Union of Biological Sciences, Tubingen, pp. 1032–4.
- Knoll, A.H. (1979). Archaeal photoautotrophy: some alternatives and limits. *Origins of Life*, **9**, 313–27.
- Kompantseva, E.I. (1985). New halophilic purple bacteria *Rhodobacter euryhalinus* sp. nov. *Mikrobiologia*, **55**, 974–82 (in Russian).
- Krumbein, W.E. (Ed.) (1983). *Microbial Geochemistry*, Blackwell Scientific Publications, Oxford, 330 pp.
- Krumbein, W.E., and Cohen, Y. (1974). Klastische und evaporitische Sedimentation in einem mesothermen monomiktischen ufernahen See (Golf von Aqaba, Sinai). *Geol. Rundschau*, **63**, 1035–65.
- Krumbein, W.E., and Cohen, Y. (1977). Primary production, mat formation and lithification contribution of oxygenic and facultative anoxygenic cyanobacteria. In: Flugel, E. (Ed.) *Fossil Algae*, Springer-Verlag, New York, pp. 37–56.
- Krumbein, W.E., Cohen, Y., and Shilo, M. (1977). Solar Lake (Sinai). 4. Stromatolitic cyanobacterial mats. *Limnol. Oceanogr.*, **22**, 635–56.
- Krumbein, W.E., Buchholz, H., Franke, P., Giani, D., Giele, C., and Wonneberger, C. (1979). O₂ and H₂S coexistence in stromatolites. A model for the origin of mineralogical lamination in stromatolites and banded iron formations. *Naturwissenschaften*, **66**, 381–9.
- Kuznetsov, S.I. (1955). Microorganisms of Kamchatka thermal springs. *Trudy Inst. Mikrobiologii*, **4**, 130–154 (in Russian).
- Kuznetsov, S.I. (1970). The Microflora of Lakes and its Geochemical Activity. University of Texas Press, Austin, 424 pp.
- Lauterborn, R. (1915). Sapropelische Lebewelt. *Verh. Dtsch. Naturhist. Med. Ver. Heidelberg, N.F.*, **13**, 395–481. (in German).
- LeGall, J., and Postgate, J.R. (1973). The physiology of sulphate reducing bacteria. In: Rose, H.A., and Tempest, D.W. (Eds.) *Methods in Microbiology*, Vol. 3A, Academic Press, London, New York, pp. 81–133.
- Lemasson, C., Tandeau de Marsac, N., and Cohen-Basier, G. (1973). Role of allophycocyanin as a light harvesting pigment in cyanobacteria. *Proc. Natl Acad. Sci. USA* **70**, 3130–3.
- Logan, B.W., Davies, G.R., Read, J.F., and Cebulski, D.E. (1970). Carbonate sedimentation and environments, Shark Bay, Western Australia. *Tulsa Am. Assoc. Petrol. Geol. Mem.*, **13**, 223.
- Lovley, D.R., Dwyer, D.F., and Klug, M.J. (1982). Kinetic analysis of competition between sulphate reducers and methanogens for hydrogen in sediments. *Appl. Environ. Microbiol.*, **43**, 1373–9.
- Madigan, M.T. (1984). A novel photosynthetic bacterium isolated from a Yellowstone hot spring. *Science*, **225**, 313–15.
- Madigan, M.T., and Brock, T.D. (1973). CO₂ fixation in photosynthetically growing *Chloroflexus aurantiacus*. *FEMS Microbiol. Lett.*, **1**, 301–4.
- Madigan, M.T., and Brock, T.D. (1975). Photosynthetic sulphide oxidation by *Chloroflexus aurantiacus*, a filamentous photosynthetic gliding bacterium. *J. Bacteriol.*, **122**, 782–4.
- Madigan, M.T., Petersen, S.R., and Brock, T.D. (1974). Nutritional studies on

- Chloroflexus*, a filamentous photosynthetic, gliding bacterium. *Arch. Microbiol.*, **100**, 97–103.
- Margulis, L., Ashendorf, S., Banerjee, S., Francis, S., Giovannoni, S., Stolz, J., Barghoorn, E.S., and Chase, O. (1980). The microbial community in the layered sediments at Laguna Figueroa, Baja California, Mexico: does it have Precambrian analogues? *Precambrian Res.*, **11**, 93–123.
- Marshall, K.C. (1980). Reactions of microorganisms, ions and macromolecules at interfaces. In: Ellwood, D.C., Hedger, J.N., Latham, M.J., Lineh, J.M., and Slater, J.H. (Eds.) *Contemporary Microbial Ecology*, Academic Press, London, New York, pp. 93–106.
- Meeks, J.C., and Castenholz, R.W. (1971). Growth and photosynthesis in an extreme thermophile *Synechococcus lividus* (Cyanophyta). *Arch. Microbiol.*, **78**, 25–41.
- Miyake, Ya. (1965). *Elements of Geochemistry*, Marazen Company Ltd, Tokyo, 326 pp.
- Miyoshi, M. (1897). Studien über die Schwefelbakterien der Thermen von Yumoto bei Nikko. *J. College Sci. Imp, Univ. Tokyo*, X. Pt. 2. 143, Referat: *Zbl. Bakteriol. Parasitenkunde, Infektionskrankh. und. Hyg.*, **11**, Abt., B.3, 526.
- van Niel, C.N. (1931). On the morphology and physiology of the purple and green sulphur bacteria. *Arch. Microbiol.*, **3**, 1–112.
- Nikitina, V.N., and Gerasimenko, L.M. (1983). An unusual thermophilic form of *Mastigocladus laminosus*. *Mikrobiologia*, **52**, 477–82 (in Russian).
- Nozhevnikova, A.N., and Yagodina, T.G. (1982). A thermophilic acetate-consuming methane-producing bacteria. *Mikrobiologia*, **51**, 642–7 (in Russian).
- Odum, E. (1983). *Basic ecology*, CBS College Publ., Philadelphia, USA.
- Oremland, R.S., and Polcin, S. (1982). Methanogenesis and sulphate reduction: competitive and noncompetitive substrates in estuarine sediments. *Appl. Environ. Microbiol.*, **44**, 1270–6.
- Oren, A. and Padan, E. (1978). Induction of anaerobic photoautotrophic growth in the cyanobacterium *Oscillatoria limnetica*. *J. Bacteriol.*, **133**, 558–63.
- Oren, A., Padan, E., and Avron, M. (1977). Quantum yields for oxygenic and anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. *Proc. Natl Acad. Sci. USA*, **73**, 2152–6.
- Oren, A., Padan, E., and Malkin, S. (1979). Sulphide inhibition of photosystem 2 in cyanobacteria (blue green algae) and tobacco chloroplast. *Biophys. Acta*, **546**, 270–9.
- Oren, A., and Shilo, M. (1979). Anaerobic heterotrophic dark metabolism in the cyanobacterium *Oscillatoria limnetica*: sulphur respiration and lactate fermentation. *Arch. Microbiol.*, **122**, 77–84.
- Padan, E., and Cohen, Y. (1982). Anoxygenic photosynthesis. In: Carr, N.C., and Whitton, B.A. (Eds.) *The Biology of Cyanobacteria*, Blackwell Scientific, Oxford, pp. 215–35.
- Pantshava, E.S., and Pchyolkina, V.V. (1969). Methane fermentation of methyl alcohol by the culture of *Methanobacillus kuzneceovii*. *Prikl. biochim. Mikrobiologia*, **5**, 416–20 (in Russian).
- Pfennig, N. (1965). Anreicherungskulturen für rote und grüne schwefelbakterium. *Zbl. Bakter. I Abt, Suppl.*, (1), p.179 (in German).
- Pfennig, N. (1967). Photosynthetic bacteria. *Ann. Rev. Microbiol.*, **21**, 285–324.
- Pfennig, N. (1975). Phototrophic bacteria and their role in the sulphur cycle. *Plant Soil*, **43**, 1–16.
- Pfennig, N. (1977). Phototrophic green and purple bacteria: a comparative systematic survey. *Ann. Rev. Microbiol.*, **31**, 275–90.

- Pfennig, N. (1978). General physiology and ecology of photosynthetic bacteria. In: Clayton, R.K., and Sistron, W.R. (Eds.) *The Photosynthetic Bacteria*, Plenum Press, New York, London, pp. 3–18.
- Pfennig, N., and Biebl, H. (1976). *Desulfuromonas acetooxidans* gen.nov. and sp.nov, a new anaerobic sulphur-reducing, acetate-oxidising bacterium. *Arch. Microbiol.*, **110**, 3–12.
- Pfennig, N., Widdel, F., and Truper, H.G. (1981). The dissimilatory sulphate-reducing bacteria. In: Starr, M.P., Stop, H., Truper, H.G., Balows, A., and Schlegel, H.C. (Eds.) *The Prokaryotes*, Springer, Berlin, pp. 926–40.
- Pierson, B.K., and Castenholz, R.W. (1974). A phototrophic gliding filamentous bacterium of hot springs. *Chloroflexus aurantiacus* gen. and sp.nov. *Arch. Microbiol.*, **100**, 5–24.
- Playford, P.E., and Cockbain, A.E. (1969). Algal stromatolites: deep water forms in the Devonian of Western Australia. *Science*, **1965**, 1008–10.
- Raymond, J.C., and Sistron, W.R. (1969). *Ectothiorhodospira halophila*. A new species of the genus *Ectothiorhodospira*. *Arch. Microbiol.*, **69**, 121–6.
- Revsbech, N.P., and Ward, D.M. (1984). Microelectrode studies of interstitial water chemistry and photosynthetic activity in a hot spring microbial mat. *Appl. Environ. Microbiol.*, **48**, 270–5.
- Revsbech, N.P., Jørgensen, B.B., Blackburn, T.H., and Cohen, Y. (1983). Microelectrode studies of the photosynthetic and O₂, and H₂S and pH profiles of a microbial mat. *Limnol. Oceanogr.*, **28**, 1062–74.
- Sandbeck, K.A., and Ward, D.M. (1981). Fate of immediate methane precursors in low-sulphate hot spring algal-bacterial mats. *Appl. Environ. Microbiol.*, **41**, 775–82.
- Schink, B. and Zeikus, J.G. (1983). *Clostridium thermosulfurogenes* sp.nov., a new thermophile that produces elemental sulphur from thiosulphate. *J. Gen. Microbiol.*, **129**, 1149–58.
- Schopf, W.H. (Ed.) (1983). *Earth's Earliest Atmosphere, its Origin and Evolution*, Princeton University Press, Princeton, NJ., 543 pp.
- Schwabe, G.H. (1960). Über den thermobioten Kosmopoliten *Mastigocladus laminosus*. Cohn. Blaugen und lebensraum. V. Schweeig. *J. Hidrol.*, **22**, 757–92.
- Skyring, F.W. (1984). Sulphate reduction in marine sediments associated with cyanobacterial mats, Australia. In: Cohen, Y., Castenholz, R.W., and Halvorson, H.O. (Eds.) *Microbial Mats: Stromatolites*, Alan R. Liss, New York, pp. 265–76.
- Stanier, R.Y. (1974). The origin of photosynthesis in eukaryotes. *Soc. Gen. Microbiol. Symp.*, **24**, 219–40.
- Stanier, R.Y. (1977). The position of the cyanobacteria in the world of phototrophs. *Carlsberg Res. Commun.*, **42**, 77–98.
- Stetter, K.O. (1985). Extremthermophile Bakterien. *Naturwissensch.*, **72**, 281–301.
- Steward, W.D.P., and Pearson, W.H. (1970). Effects of aerobic and anaerobic conditions on growth and metabolism of blue-green algae. *Proc. R. Soc. London, Ser. B*, **175**, 293–311.
- Stolz, J.F. (1984). Fine structure of the stratified microbial community at Laguna Figueroa, Baja California, Mexico. II. Transmission electron microscopy as a diagnostic tool in studying microbial communities *in situ*. In: Cohen, Y., Castenholz, R.W., and Halvorson, H.O. (Eds.) *Microbial Mats: Stromatolites*, Alan R. Liss, New York, pp. 23–38.
- Streczowski, B. (1913). Beitrag zur Kenntnis der Schwefelflora in der Umgebung von Krakau. *Bull. Acad. Sci. Cracovie, Ser. B*, pp. 309–12.
- Tel-Or, E., Luijk, C.W., and Packer, L. (1977). As inducible hydrogenase in cyanobacteria enhances NH₂ fixation. *PEBS Lett.*, **78**, 49–52.

- Thauer, R.K., Jungermann, K., and Decker, K. (1977). Energy conservation in chemotrophic anaerobic bacteria. *Bact. Rev.*, **41**, 100–80.
- Trüper, H.G. 1973. The present state of knowledge of sulphur metabolism in phototrophic bacteria. *Abstr. Symp. Prokaryotic. Photosyn. Organisms, Freiburg*, pp. 160–6.
- Utkilen, H.C. (1976). Thiosulphate as electron donor in the blue-green alga *Anacystis nidulance*. *J. Gen. Microbiol.*, **95**, 177–80.
- Utkilen, H.C., and Castenholz, R.W. (1979). Physiological aspects of adaptation to sulphide in a thermophilic *Oscillatoria*. In: Nichols, J.M. (Ed.) *Abstract of Third International Symposium on Photosynthetic Prokaryotes*, Oxford, Liverpool, England, p. 12.
- Van Gernerden, H. (1968). Utilisation of reducing power in growing cultures of Chromatium. *Arch. Microbiol.*, **64**, 111–17.
- Walter, M.R. (Ed.) (1976). *Developments in Sedimentology 20, Stromatolites*, Elsevier, Amsterdam.
- Ward, D.M. (1978). Thermophilic methanogenesis in a hot spring algal-bacterial mat (71–30°C). *Appl. Environ. Microbiol.*, **35**, 1019–26.
- Ward, D.M., and Olson, G.I. (1980). Terminal processes in the anaerobic degradation of an algal-bacterial mat in a high-sulphate hot spring. *Appl. Environ. Microbiol.*, **40**, 67–74.
- Ward, D.M., Beck, E., Revsbech, N.R., Sandbeck, K.A., and Winfrey, M.R. (1984). Decomposition of hot spring microbial mats. In: Cohen, Y., Castenholz, R.W., and Halvorson, H.O. (Eds.) *Microbial Mats: Stromatolites*, Alan R. Liss, New York, pp.191–214.
- Weisman, J.C., and Benemann, J.R. (1977). Hydrogenase production by nitrogen starved culture of *Anabena cylindrica*. *Appl. Environ. Microbiol.*, **35**, 123–31.
- Weller, D., Doemel, W., and Brock, T.D. (1975). Requirement of low oxidation-reduction potential for photosynthesis in blue green alga (*Phormidium* sp.). *Arch. Microbiol.*, **104**, 7–13.
- Wiegel, J., and Ljungdahl, L.G. (1981). *Thermoanaerobacter ethanolicus* gen. nov., sp.nov., a new extreme thermophilic anaerobic bacterium. *Arch. Microbiol.*, **128**, 343–8.
- Wolfe, R.S., and Pfennig, N. (1977). Reduction of sulphur by *Spirillum 5175* and syntrophism with *Chlorobium*. *Appl. Environ. Microbiol.*, **33**, 427–33.
- Zavarzin, G.A. (1984). *Bacteria and the Atmosphere Composition*, Nauka, Moscow, 192pp. (in Russian).
- Zeikus, J.G., Hegge, P.W. and Anderson, P.W. (1979). *Thermoanaerobium brockii* gen.nov. and sp.nov., a new chemoorganotrophic caldoactive anaerobic bacterium. *Arch. Microbiol.*, **122**, 41–8.
- Zeikus, J.G., Ben-Bassat, A., and Hegge, P.W. (1980). Microbiology of methanogenesis in thermal volcanic environments. *J. Bacteriol.*, **143**, 432–40.
- Zeikus, J.G., Dawson, M.A., Thompson, T.E., Ingoorsen, K., and Hatchikian, E. (1983). Microbial ecology of volcanic sulfidogenesis: isolation and characterisation of *Thermodesulfobacterium commune* gen.nov. and sp.nov. *J. Gen. Microbiol.*, **129**, 1159–69.
- Zhilina, T.N., Chudina, V.I., Ilarionov, S.A., and Bonch-Osmolovskaya, E.A. (1983). Thermophilic methane-producing bacteria from *Methanobacillus kuzneceovii* methylotrophic associations. *Mikrobiologia*, **52**, 328–34 (in Russian).
- Zillig, W., Stetter, K.O., Prangishvilli, D., Schäfer, W., Wunderl, S., Janeovic, D., Holz, I., and Palm, P. (1982). Desulfurococcaceae, the second family of the extremely thermophilic anaerobic sulphur-respiring Thermoproteales. *Zbl. Bakt. Hyg., Abt I Orig. C3*, pp. 304–17.

- Zillig, W., Gierl, A., Schreiber, G., Wunderl, S., Jankovic, D., Stetter, K., and Klenk, H.P. (1983). The archaeobacterium *Thermophilum pendens*, a novel genus of the thermophilic anaerobic sulphur-respiring Thermoproteales. *System. Appl. Microbiol.*, **4**, 79–87.