Cyanobacterial Mats and Stromatolites

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Lucas J. Stal

Contents

L.J. Stal (✉)
Department of Marine Microbiology, Royal Netherlands Institute of Sea Research (NIOZ),
P. O. Box 140, NL-4400 AC Yerseke, The Netherlands
e-mail: lucas.stal@nioz.nl

Summary

Cyanobacteria are often the key organisms comprising microbial mats. They form dense micrometer-scale communities in which the full plethora of microbial metabolism can be present. Such mats are therefore excellent model systems and because of their analogy with Precambrian stromatolites they are also attractive subjects for evolutionary studies. Growth and metabolism of the oxygenic phototrophic cyanobacteria enrich the sediment with organic matter. However, in mature mats net growth of cyanobacteria appears to be of less importance. Most of the organic matter produced from photosynthetic CO_2 fixation is liberated in the sediment by one of the following: fermentation, photorespiration, pouring out of solutes or secretion of mucus although grazing may also be important. This organic matter is degraded by chemotrophic microorganisms, among which sulphate-reducing bacteria are particularly prominent. The combined activities of the cyanobacteria and sulphate-reducing bacteria result in steep and fluctuating gradients of sulphide and oxygen. Cyanobacteria therefore have to cope with

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high concentrations of sulphide, oxygen supersaturated – and anoxic conditions. These physicochemical gradients force different functional groups of microorganisms to particular vertical stratified positions in the mat. This, and the fact that accretion of sediment fluctuates, gives rise to one of the most conspicuous properties of microbial mats namely their laminated structure. Modern microbial mats have this laminated structure in common with Precambrian stromatolites. Most modern mats do not lithify but this may also have been the case for Archean microbial mats. Only a few examples of modern calcifying stromatolithic microbial mats are known. A hypothesis has been developed which conceives a role for extracellular polysaccharides in calcification. Extracellular polysaccharides in cyanobacterial mats are often produced as the result of unbalanced growth caused by nitrogen deficiency. The mat organisms are embedded in the extensive polysaccharide matrix that inhibits calcification. All cyanobacterial mats can fix atmospheric dinitrogen, which covers part of their nitrogen demand, but the fluctuating physicochemical gradients limits the efficiency of this process.

4.1 Introduction

The term microbial mat is used for multilayered microbial communities growing on sediments in diverse habitats such as tidal sand flats, hypersaline ponds, hot springs and other. Microbial mats are generally formed by filamentous, entangled organisms that produce a macroscopic 'mat-like' structure. In some cases such mats can indeed be peeled off from the sediment as a large coherent piece. However, benthic microbial communities of unicellular organisms, that usually do not form such coherent structures, are also called mats. Microbial mats exhibit great variety in morphology and composition, and they may include mats of diatoms and other biofilms of immobilized microorganisms (Bauld 1984). Nevertheless, eukarya are few or excluded altogether from environments in which extreme conditions prevail but in some cases meiofauna and other grazers are active in the habitats in which microbial mats are formed. One reason for the exclusion of eukarya is the wide spectrum of metabolic capabilities of bacteria and archaea and the great capacity these 'prokaryotes' display to adapt to changes and fluctuations in environmental conditions. Purple and sometimes green sulphur bacteria are normal components of most cyanobacterial mats (Nicholson et al. 1987; Pierson et al. 1987). This review focuses on mats formed by cyanobacteria.

The reason why cyanobacteria are typically the most successful mat-building organisms may be found in the combination of a number of the characteristic properties of this unique group of microorganisms. Cyanobacteria are the only oxygenic phototrophic bacteria and this metabolism is absent in archaea. As their predominant metabolism is oxygenic photosynthesis, cyanobacteria use light as an energy source, water as an electron donor and CO_2 as a carbon source. These are the major requirements for growth and are abundant in the environments where most microbial mats are found. Another important property of many cyanobacteria, which is not shared by eukarya (and hence not by algae), is their ability to fix atmospheric N_2, allowing them to grow independent of a source of combined nitrogen. Photosynthesis in cyanobacteria saturates at low light intensity, cyanobacteria have a high affinity for light, and maintenance requirements are extremely low (Van Liere and Mur 1979). These properties allow photosynthesis even under extremely low light conditions. Moreover, several species are capable of sulphide-dependent anoxygenic photosynthesis (Garlick et al. 1977). Mat-forming cyanobacteria are well-adapted for life under anoxic conditions. In addition to the normal aerobic dark respiration, virtually all species of cyanobacteria in microbial mats are capable of fermentation (Stal and Moezelaar 1997). These properties of cyanobacteria are essential for life in microbial mats in which environmental conditions strongly fluctuate.

A typical property of microbial mats is their laminated structure in which different functional groups of microorganisms occur in vertically stratified layers (Stal et al. 1985). In addition to biological stratification biomineralogical stratification can be distinguished (Monty 1976). This type of lamination can be attributed to different growth periods, seasonal events, periodical events (e.g. tides) or episodic or erratic events (e.g. storms). Often, this laminated pattern is restricted, since most of the organic matter of the mat is degraded. When conditions allow, mats precipitate minerals, mainly calcite (Golubić 1973; Monty 1976; Krumbein 1979). This calcification is strongly associated with microbial metabolism and it may therefore give rise to the formation of distinct laminae and eventually to consolidated rock. Laminated rocks dating from the Precambrian and later eras are known as stromatolites (Krumbein 1983). Modern microbial mats built by cyanobacteria show remarkable similarities to fossil stromatolites. Stromatolites date back to 3.5 billion years (Mason and Von Brunn 1977; Lowe 1980; Walter et al. 1980; Orpen and Wilson 1981; Chap. 2). In some of these stromatolites well-preserved microfossils have been found that in some cases showed a remarkable resemblance to modern cyanobacteria (Schopf and Walter 1982; Awramik 1984; Chap. 2). It is therefore attractive to consider modern microbial mats as analogues of Precambrian stromatolites; however, structural differences do not always seem to justify this comparison. A major problem is the fact that the great majority of present day microbial mats does not form consolidated rock.

This review will discuss the metabolic activities of cyanobacteria that allow them to form microbial mats and stromatolites. This is a revised, updated and extended version of the chapter with the same title that appeared in the first edition of The Ecology of Cyanobacteria (Stal 2000).

4.2 Microbial Mats, Stromatolites and Their Environments

4.2.1 What Are Microbial Mats and Stromatolites? Some Definitions

Krumbein (1983), referring to the work of Kalkowsky (1908), proposed the following definition: "*Stromatolites are laminated rocks, the origin of which can clearly be related to the activity of microbial communities, which by their morphology, physiology, and arrangement in space and time interact with the physical and chemical environment to produce a laminated pattern which is retained in the final rock structure*". This definition includes fossil as well as recent formations. Modern stromatolites that fit this definition are rare. Awramik and Margulis (in Walter 1976) defined stromatolites as: "*Organosedimentary structures produced by sediment trapping, binding and/or precipitation as a result of the growth and metabolic activity of microorganisms, principally cyanophytes*". This definition includes fossil and recent consolidated stromatolites as well as unconsolidated microbial mats. Both definitions, however, emphasize the role of microbial mats and their microflora in the formation of stromatolites. Walter (1976) formulated the following conditions necessary to form a microbial mat:

(i) environmental conditions must allow growth of the mat-building microorganisms; growth rate of the mat-building organisms must be faster than consumption by grazers;
(ii) sedimentation rates should not be exceedingly high to allow stabilized colonization of the surface by the mat-building organisms;
(iii) destructive forces from burrowing organisms and mechanical and chemical erosion must be absent or at least not prevent accretion of organisms.

In order to produce a stromatolite, preservation of the structure must occur. In modern day environments unconsolidated microbial mats are formed, i.e. systems that do not have the potential to preserve its structure, defined by Krumbein (1983) as: "*Unconsolidated laminated systems, clearly related to the activity of microbial communities, often called recent stromatolites or living stromatolites are defined as potential stromatolites*". Indeed, stromatolites *sensu* Krumbein are still being formed today. Excellent examples of consolidated, well-laminated stromatolites formed by the growth and metabolic activity of a microbial mat are found in the Exuma Cays, Bahamas (Reid and Browne 1991; Pinckney et al. 1995). Stromatolites are just one form of calcified microbial mats that are jointly termed microbialites, a term that includes thrombolites, characterized by a cohesive macrofabric, and leiolites, which are without defined structure (Dupraz et al. 2009).

Also, non-consolidated, non-lithified microbial mats may leave traces of microbially induced sedimentary structures especially in siliciclastic deposits in shallow coastal environments (abbreviated as MISS) (Noffke et al. 2006). These structures have in some cases been preserved in the fossil record going back to the early Archean, emphasizing that not all Archean microbial mats were microbialites.

Since their discovery in 1961 in Shark Bay, Western Australia, poorly-laminated consolidated calcareous stromatolites (thrombolites) have been presented as strikingly similar to Precambrian stromatolites (Logan 1961). Contemporary calcareous stromatolites are also formed in Polynesian atolls (Kopara) (Défarge et al. 1994a, b) and other lacustrine and perimarine settings (Kempe et al. 1991; Kempe and Kazmierczak 1993). Such stromatolites can be called 'modern stromatolites' to distinguish them from fossil formations. It is not necessary to name unconsolidated microbial mats 'potential stromatolites', since they are not stromatolites (*sensu* Krumbein) and most of these microbial mats will never become such. Nevertheless, unconsolidated mats may keep in themselves the capacity for consolidation. This was shown by a transplantation experiment in which a non-lithifying microbial mat was placed in an environment with lithifying microbial mats. This mat calcified, demonstrating its potential to become a microbialite. Calcification clearly depends on the prevailing local environmental conditions (Dupraz et al. 2009).

Calcification is required for consolidation and preservation of microbial mats. In many microbial mats, calcification does not occur and the reasons for this are discussed later on in this chapter. Many consolidated rocks without a clear lamination are formed by microbial communities. It may be that laminations were lost during the process of diagenesis or that neither vertical stratified communities of microorganisms nor clear seasonal variations were involved in growth and metabolic activity. Another proposed mechanism is that carbonate sand accretes through trapping and binding in the microbial mat without *in situ* calcification. Such cohesive but poorly laminated microbialites are known as thrombolites (Kennard and James 1986). The stromatolites of the Exuma Cays, Bahamas, in contrast with other recent formations, possess a fine laminated structure. There, in addition to trapping and binding of carbonate sand, *in situ* precipitation of calcium carbonate produces distinct layers of cement (Reid and Browne 1991).

4.2.2 Microbial Mats and Stromatolites: The Geological Evidence

The Hadean era from the origin of the Earth 4.5×10^9 years before present to 3.9×10^9 is the period of which no rock record exists. The oldest rock known from the early Archean

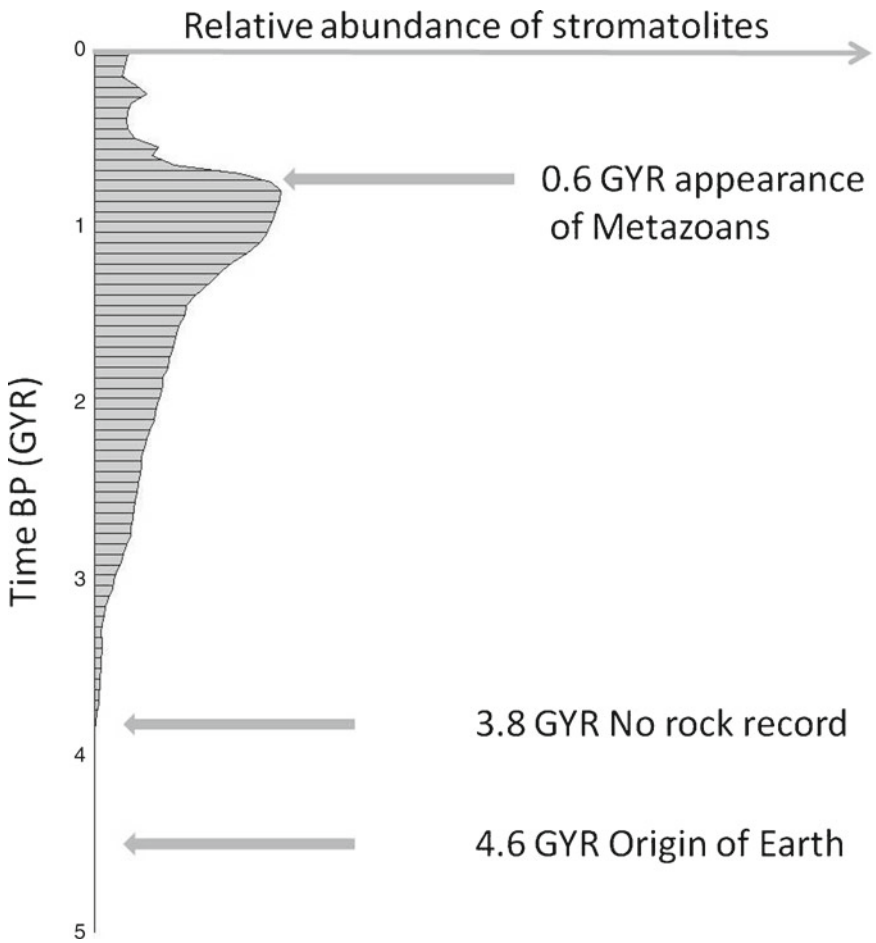

Fig. 4.1 Relative abundance of stromatolites plotted against time (After Awramik 1984)

may not be of biogenic origin. The oldest stromatolites date back in the Archean about 3.5×10^9 years ago. Only a few examples are known from this era, but they were undoubtedly biologically produced. Microfossils have been found in these stromatolites, but it is premature to identify them as cyanobacteria. From measurements of carbon isotope compositions in these rocks it was deduced that autotrophic microorganisms must have been active at that time. However, it could have been chemoautotrophs that fixed the CO_2, rather than photoautotrophs. The morphology of the microfossils from these oldest stromatolites also does not give an unequivocal clue about the identity of the organisms. Cyanobacteria are a group of oxygenic phototrophic organisms and it is well established that the Archean atmosphere did not contain oxygen.

During the Proterozoic, which started about 2.5×10^9 years ago, stromatolites became abundant (Fig. 4.1) and occur in a wide variety of facies. They occupied every major ecological niche, marine and lacustrine, shallow and deep water. Most of limestones, dolomites and magnesites as well as many phosphorites and iron formations of the Proterozoic contain stromatolites. Like modern microbial mats, it seems certain that the Proterozoic stromatolites were produced by growth and metabolic activity of cyanobacteria. The Proterozoic stromatolites contain a wealth of very well preserved microfossils that show striking similarity to present day cyanobacteria. Over 1,100 microfossils have been described from 190 stromatolite formations (Walter et al. 1992). Many of these fossils are preserved in the cherts of stromatolites. The best preservation occurred following early silicification of the stromatolites. Silica precipitation occurred possibly spontaneously because of its supposed high concentration in the seawater. Diatoms with their silicate frustules had not evolved yet and no other sink for silica is known.

Oxygen was present in the atmosphere at 2.3×10^9 years before present. It is now well accepted that the oxygenation of the atmosphere was the result of oxygenic photosynthesis. It might have taken considerable time after the origin of oxygenic photosynthesis until the atmosphere became oxygenated, since a large amount of reduced compounds had to be oxidized. Banded iron formations (BIFs) are known from 2.5×10^9 years before present. These are huge formations consisting of oxidized iron and they have been taken as evidence for the presence of oxygenic photosynthesis. However, iron oxidation could also have taken place by the activity of anoxygenic phototrophic bacteria under anaerobic conditions

(Widdel et al. 1993; Ehrenreich and Widdel 1994) or perhaps even by iron-dependent anoxygenic photosynthesis by cyanobacteria (Cohen 1989). Oxygenic photosynthesis most likely evolved at the beginning of the Proterozoic. Evidence for this is the presence of molecular markers such as the methylhopanes that are supposed to be specific for cyanobacteria 2.7×10^9 years before present (Brocks et al. 1999). Also, phylogenetic analysis date the origin of cyanobacteria at 2.6×10^9 (Hedges et al. 2001). However, many cyanobacteria in present day microbial mats are capable of anoxygenic photosynthesis and it seems likely that cyanobacteria were anoxygenic phototrophs before they evolved oxygenic photosynthesis (Olson 2006).

The morphology of the stromatolites of the 3.1×10^9 old Insuzi group of South Africa hints at the involvement in their formation of tactic filamentous organisms (Mason and Von Brunn 1977). Although it is tempting to suspect phototaxis and hence potentially photosynthetic organisms, a chemotactic response would also explain the structure of this formation (Schopf and Walter 1982).

Of the many different morphological forms of microfossils, some can be traced back to present day cyanobacteria such as *Oscillatoria* and *Lyngbya* (Schopf and Walter 1982). These organisms are common in modern microbial mats where they may be involved in N_2 fixation. It is difficult to determine whether these ancient mats were diazotrophic, although the signature of the stable isotope ^{15}N might give some hints in the direction of diazotrophic (N_2-fixing) cyanobacteria (Bauersachs et al. 2009). Microfossils resembling cyanobacteria of the heterocystous genera *Nostoc* and *Scytonema* were abundant in Archean stromatolites (Schopf and Walter 1982), and this is taken as evidence that N_2 fixation might have been important. No remnants of heterocysts are known, probably because these cells did not fossilize well. Fossil remnants of akinetes which are survival stages of cells that are known only from heterocystous cyanobacteria are known dating back 1.5×10^9 years (Srivastava 2005). However, they may not have originated from microbial mats and it is unknown whether they were already associated with heterocystous cyanobacteria. To date, heterocystous cyanobacteria are uncommon in most microbial mats.

Proterozoic stromatolites reached maxima in numbers and diversity towards the end of this era, after which it showed a rapid decline (Walter and Heys 1985) (Fig. 4.1). It has been postulated that metazoa, which then appeared on Earth, were responsible for this decline (Walter and Heys 1985). The grazing activity of these animals would prevent the accumulation of the benthic mat-forming organisms and destroyed the fabric of microbial mats by bioturbation. After the appearance of metazoa, microbial mats would be much more limited in their distribution and developed in environments in which these grazers are largely excluded (so-called extreme environments). Nevertheless, based on evidence from a modern hypersaline lagoon in Venezuela, Gingras et al. (2011) have suggested that the early evolution of mobile complex animals may have been in cyanobacterial dominated mats during the Ediacaran period (635–542 million years ago).

The appearance of algae that competed successfully for light and nutrients in many environments could help explain the eventual pushing back of cyanobacterial mats to extreme environments. Also, sea level changes, caused by changes of climate and by tectonic processes, could explain the sudden decrease in stromatolite abundance (Gebelein 1976). And finally, the seawater in the Proterozoic might have been greatly oversaturated with respect to calcium carbonate (alkaline soda ocean) facilitating the calcification and preservation of stromatolites, which is less the case in the modern moderately alkaline ocean (Kempe and Kazmierczak 1990a).

Proterozoic stromatolites probably formed through one or more of the following (Walter et al. 1992):

(i) *in situ* precipitation as cement;
(ii) *in situ* precipitation as micrite either accreted passively from suspension or through trapping and binding of the grains by the mat microorganisms;
(iii) precipitation of micrite imported from adjacent environments.

The fine and distinct lamination of Proterozoic stromatolites hints at *in situ* precipitation. Most Phanerozoic stromatolites are probably produced by trapping and binding of carbonate and sand grains and therefore show poor or no lamination (Cloud and Semikhatov 1969).

4.2.3 Stratification and Structure of Microbial Mats and Stromatolites

Microbial mats are characterized by the vertical stratification of different functional groups of microorganisms. This structure is the result of the physicochemical gradients that are present in mats and in fact produced by the metabolic activity of the mat organisms themselves (Jørgensen et al. 1983). The typical structure of a microbial mat build by cyanobacteria is schematically depicted in Fig. 4.2.

Cyanobacteria evidently form the top layer of microbial mats although they are sometimes overlain by a film of diatoms. These organisms need to harvest light for photosynthesis and are essentially aerobic organisms. The cyanobacteria may further be covered by a layer of sand or sediment of varying thickness or be covered by an organic-rich mucilaginous layer which may contain photoprotective pigments such as scytonemin, which is produced by cyanobacteria. It occurs predominantly in the extracellular polysaccharide sheaths. Scytonemin is highly recalcitrant remaining in the empty sheaths that are left behind by the cyanobacteria. Scytonemin protects the underlying community from damage

caused by UV irradiation (sunglass effect) (Garcia-Pichel and Castenholz 1991; Chap. 19). The organic matter introduced in the sediment through the photosynthetic activity of the cyanobacteria is decomposed by a variety of chemotrophic microorganisms. The degradation of organic matter and the accompanying demand of oxygen result in permanent anoxic conditions below the layer of cyanobacteria (Fig. 4.3). Obligate anaerobic sulphate-reducing bacteria play a major role in the decomposition of organic material in marine cyanobacterial mats and other sulphide dominated environments. These bacteria produce sulphide, which is used by anoxygenic phototrophic bacteria.

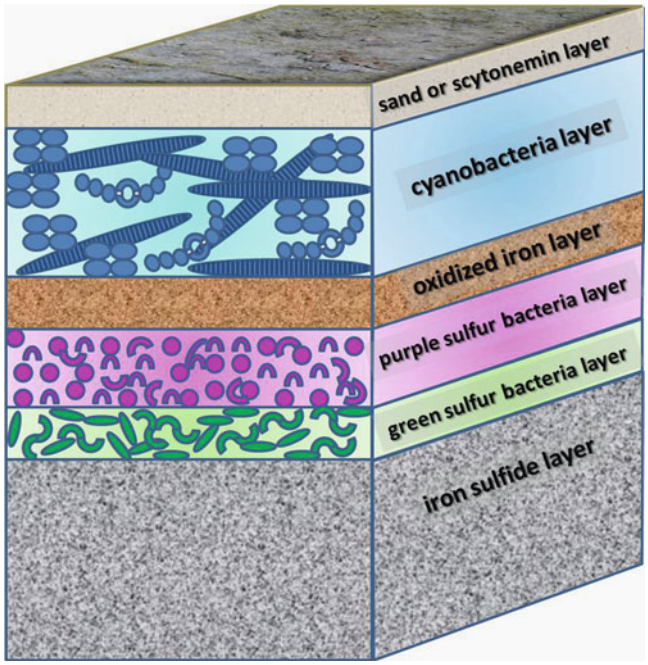

Fig. 4.2 Schematic drawing of a building of a typical microbial mat formed by cyanobacteria. The layer of green sulphur bacteria has been observed in only a few occasions

Purple sulphur bacteria are very common in microbial mats and are often seen as a pink layer below the cyanobacteria. Purple sulphur bacteria are essentially anaerobic bacteria, but species that occur in microbial mats are usually metabolically versatile (van Gemerden 1993). Anoxygenic photosynthesis in purple sulphur bacteria saturates at even much lower light intensities than photosynthesis in cyanobacteria. In addition, these organisms use a different part of the electromagnetic spectrum, not used by cyanobacteria (Pierson et al. 1987). This far red light is also least attenuated by the sediment (Fig. 4.4) (Stal et al. 1985; Jørgensen and Des Marais 1988). The biological stratification is thus the result of gradients of light, oxygen and sulphide and is found in virtually all cyanobacterial mats (Fig. 4.3). In some rare cases a layer of green anoxygenic phototrophic bacteria can be found underneath the purple bacteria (Nicholson et al. 1987).

A distinct layer of oxidized iron may be present between the cyanobacteria and the purple sulphur bacteria (Stal 1994). It is not clear how this layer is formed. It may be formed by chemical oxidation by the oxygen produced during photosynthesis. An alternative explanation is the anaerobic oxidation of iron by anoxygenic photosynthesis in a specific group of purple bacteria (Widdel et al. 1993; Ehrenreich and Widdel 1994). Aerobic oxidation of iron by chemotrophic bacteria seems unlikely at the alkaline pH that are usually encountered

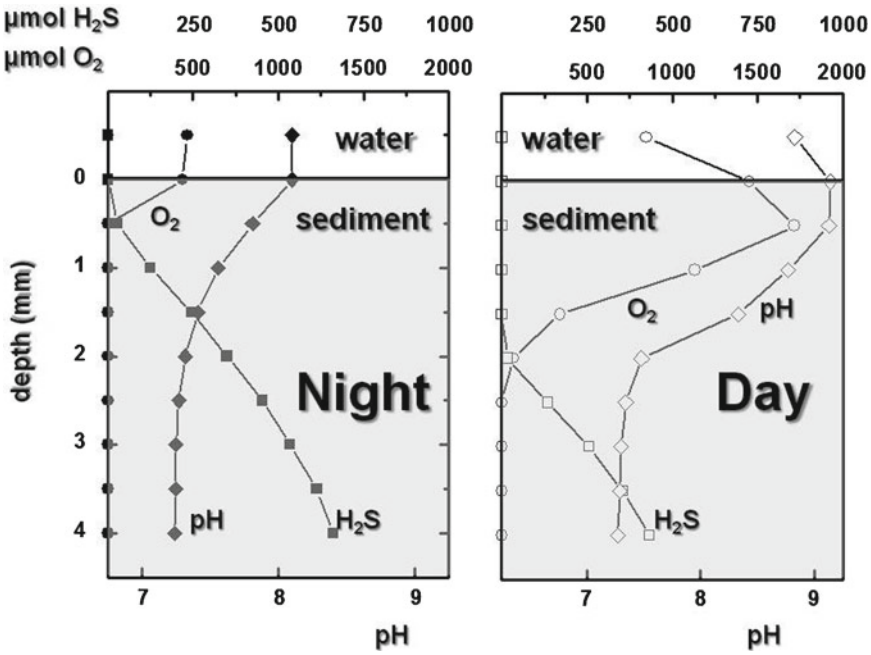

Fig. 4.3 Vertical profiles of oxygen, sulphide and pH at night (*left panel*) and during the day (*right panel*) in a mat of *Microcoleus chthonoplastes* from Solar Lake, Sinai (Redrawn from Revsbech et al. 1983)

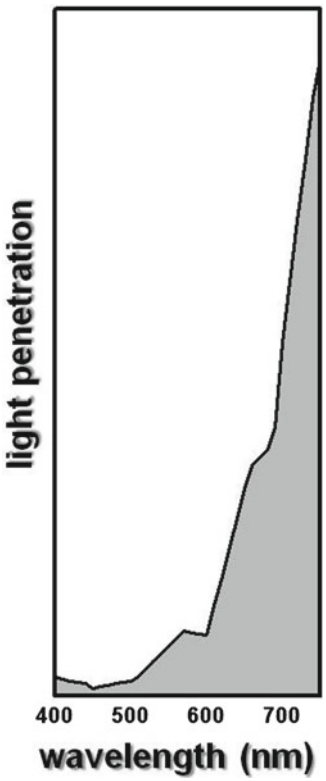

Fig. 4.4 Spectral light penetration through a 1.5-mm mat of *Microcoleus chthonoplastes* of the North Sea island of Mellum (Germany). Far-red light is least attenuated by the mat. The light absorption by chlorophyll *a* and phycobiliproteins at respectively 680 and 600 nm is clearly visible

in microbial mats (Fig. 4.3) although it should also not be excluded as a possibility as shown by Emerson and Revsbech (1994a, b). Other forms of anoxygenic photosynthesis that could potentially be important are those using nitrite (Griffin et al. 2007) and arsenate (Budinoff and Hollibaugh 2008; Kulp et al. 2008), although expected only in special cases.

The deeper layer of the mat is often black or gray, indicating the presence of iron sulphide (FeS) or pyrite (FeS_2). This layer has often been referred to as the layer of the sulphate-reducing bacteria but it has become clear that these bacteria in fact do not form a distinct layer and occur throughout the sediment (Visscher et al. 1992; Stal 1993). They are both abundant and highly active in the top layers of microbial mats. At first sight this distribution of sulphate-reducing bacteria is unexpected and odd. However, their substrates are mainly produced by the cyanobacteria and it is certainly beneficial to the organisms to be close to the site of production. At night when photosynthesis ceases, the mat turns anoxic (Fig. 4.3), providing the appropriate environment for sulphate-reducing bacteria. Sulphate reducing bacteria appear to be quite tolerant to oxygen and some are even capable of low rates of aerobic respiration although they are unable to grow aerobically (Dilling and Cypionka 1990; Marschall et al. 1993). It has also been shown that sulphate reduction in a microbial mat can occur in the presence of oxygen (Canfield and Des Marais 1991; Fründ and Cohen 1992).

Chemotropic bacteria can also oxidize sulphide and represent an important group of organisms in microbial mats. As many as 2×10^9 cm^{-3} sediment of colourless sulphur bacteria has been detected in the top layer of microbial mats (Visscher et al. 1992). These bacteria are quantitatively important in microbial mats. Colourless sulphur bacteria are essentially aerobic and gain energy from the aerobic oxidation of sulphide. They are autotrophic organisms and fix CO_2 through the reductive pentose phosphate pathway (Calvin-Benson-Bassham cycle). Colourless sulphur bacteria have high affinities for their substrates and their presence cause highly dynamic oxygen and sulphide gradients, thereby overruling the chemical oxidation of sulphide. Since the sulphide-oxygen interface is highly dynamic and not fixed at a certain depth in the sediment (Fig. 4.3), these bacteria also do not form a distinct layer, although they are clearly most abundant in the top layer (Visscher et al. 1992). The joint metabolic activity of microorganisms in microbial mats results in steep physicochemical gradients of e.g. light, oxygen, sulphide, carbon dioxide and pH; these gradients shift markedly during a 24-h cycle (Fig. 4.3) (Jørgensen et al. 1979; Revsbech et al. 1983) and also respond to fluctuations of incident light. All microorganisms in microbial mats must therefore be highly versatile and flexible in order to respond to the continuous changes in environmental conditions.

The biological stratification in microbial mats may however be far more complex than described above. Cyanobacteria may be sandwiched between layers of anoxygenic phototrophic bacteria and even perform oxygenic photosynthesis there. Microbial mats may be also just 'inverted', with cyanobacteria occurring underneath the layer of anoxygenic phototrophic bacteria (Van Gemerden et al. 1989). This type of microbial mats can develop when much organic matter is deposited on the sediment and its degradation results in very high rates of sulphide production.

The construction of extensive clone libraries has shed new light on the structure and composition of the microbial mat community. While microscopic examination of the mat suggests that the cyanobacteria are the dominant component, clone libraries of the 16S rRNA gene tell a different story. Ley et al. (2006) found cyanobacteria only important in the top most layer of a microbial mat but most of the 16S rRNA sequences belonged to other bacteria. These authors found 752 species in 42 bacterial phyla and *Chloroflexi* were identified as the dominant organism both in terms of biomass and in numbers of 16S rRNA genes and were present throughout the mat. Most cyanobacteria are large microorganisms. They have only few copies of the 16S rRNA gene and their DNA is often difficult to extract from natural environments and to amplify by PCR. Even so, the importance of the diversity

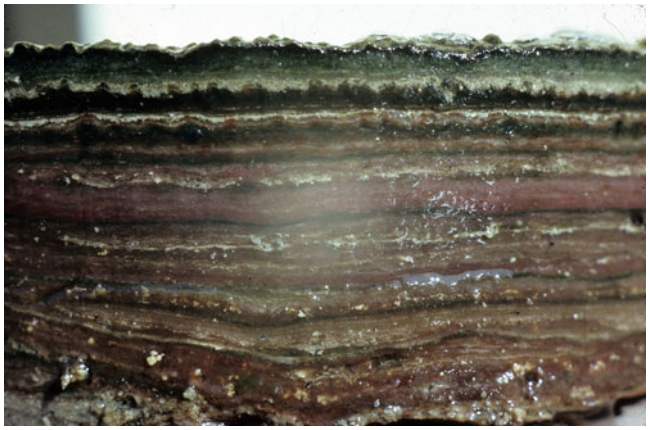

Fig. 4.5 The mat of *Microcoleus chthonoplastes* of hypersaline pond 5 of the saltern of Exportadora de Sal, S.A., Guerrero Negro, Baja California Sur, Mexico, showing the typical multilaminated structure

and biomass of other bacteria in microbial mats is probably severely underestimated. For instance, the clone libraries of the microbial mats of Hamelin Pool, Shark Bay, Western Australia, contained only less than 5% cyanobacteria. Ninety percent were bacteria and ten percent belonged to archaea. These mats did not reveal sequences belonging to eukarya (Papineau et al. 2005). The average sequence identity was only 92% emphasizing the high diversity of these microbial mats.

The organic matter produced by photosynthesis is actively cycled in mats. In mats net growth is often close to nil (Nold and Ward 1996). When growth occurs seasonally, a new mat may grow on top of the old one, resulting in a 'historical' lamination (Monty 1976). In many coastal environments the organic matter is fully degraded and a historical lamination is then absent. However, in other mats, particularly those growing in hypersaline environments, degradation may be incomplete. Examples of such mats are the well-investigated hypersaline mats of 'pond 5' in Guerrero Negro, Baja California, Mexico and those of Solar Lake, Sinai, Egypt (D'Antoni D'Amelio et al. 1989). The Guerrero Negro 'pond 5' mats are about 5–6 cm thick and are well-laminated (Fig. 4.5). This mat grows at a rate of about 1 cm year^{-1} but there is no net accretion, so that the thickness remains about the same. This means that the microbial decomposition of the mat also must proceed at a rate of approximately 1 cm year^{-1}. This mat therefore seems to be in steady state (Canfield and Des Marais 1994). The mineralization of the mat of Solar Lake is incomplete, although up to 99% of the primary production is immediately recycled in the mat, leaving only 1% for net accretion (Jørgensen and Cohen 1977; Krumbein et al. 1977). The Solar Lake microbial mat is about 1 m thick and the lamination goes back almost 2,000 years.

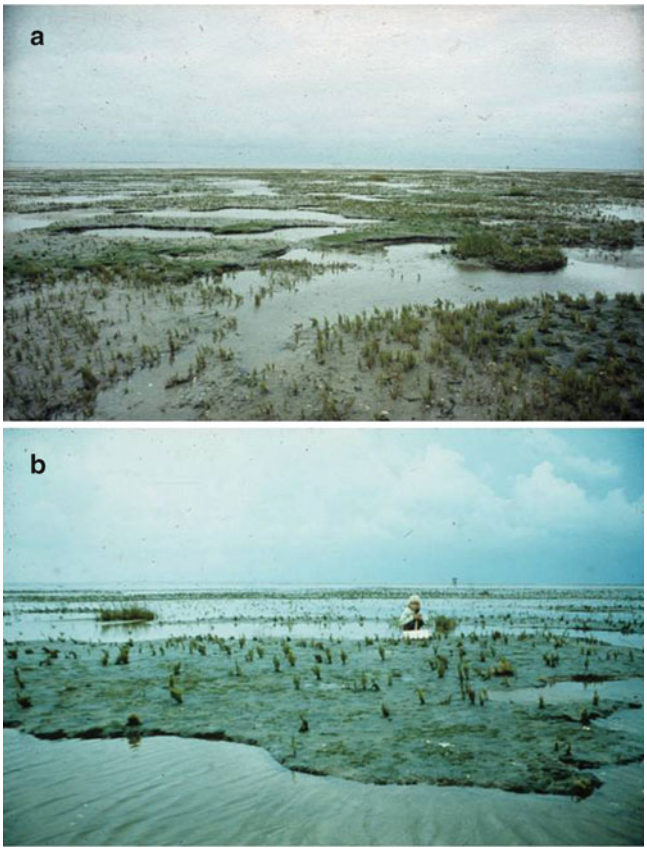

Fig. 4.6 (**a**) Extended tidal sand flat of the island of Mellum (Southern North Sea, Germany) at low tide covered with microbial mats. (**b**) Mature mats of *Microcoleus chthonoplastes* of the island of Mellum have accreted and fixed much sediment so that they are often not submersed at high tide. This decreases the grazing pressure

4.2.4 Environments Supporting Cyanobacterial Mats

4.2.4.1 Coastal Microbial Mats

Coastal tidal sand flats often are excellent environments for microbial mats to develop, particularly when the flats extend over a large area and when the slope of the flat is low (Fig. 4.6). Large areas will be covered by water for only a short period during the tide and often the sediment is not inundated for several days during neap tides. Such sediments often experience large fluctuations in water content, salinity and temperature, resulting in extreme conditions that limit the range of organisms able to inhabit this environment. The near absence of grazing organisms allows mat-building cyanobacteria and diatoms to accumulate. Coastal sand flats are usually nutrient-poor, but the phototrophic cyanobacteria have low nutrient demands and they can fix N_2. Moreover, most cyanobacteria resist long periods of drought, tolerating large fluctuations of salinity and temperature.

Often these coastal microbial mats are composed of filamentous cyanobacteria that form a dense entangled mass which traps and binds sediment particles. Such mats are clearly visible to the naked eye as massive structures that to a large extent resist erosion (Fig. 4.6b). Their sediment stabilizing effect is of great importance for coastal morphogenesis. Typical examples are found in tidal sand flats of islands of the southern North Sea (e.g. Mellum, Germany), along the east coast of North America (e.g. Great Sippewisset Marsh, Cape Cod, Massachusetts; Bird Shoal, North Carolina), Pacific Coast (e.g. Guerrero Negro, Mexico) (Fig. 4.7), Shark Bay and Spencer Gulf in, respectively, Western and South Australia. A more complete list is given by Pierson (1992). Most such coastal microbial mats are not stromatolites, but examples of stromatolites in coastal sediments can be found in El Hamira Bay, Sinai, where stromatolitic beachrock is formed (Krumbein 1979) and along the Exuma Cays of the Bahamas intertidal and subtidal stromatolites are formed (Reid and Browne 1991) (Fig. 4.8). *Schizothrix* sp. can settle there in spite of the high wave energy to which the Atlantic coast of the Bahamas is exposed. Calcification of the Exuma Cays microbial mats renders stability to the system, which is necessary to cope with the strong wave energy.

Other coastal mats are present in protected lagoons and are semi-permanently inundated. Examples of such coastal lagoons in which benthic microbial mats develop are found in many countries. Mats develop in the shallow parts of coastal lagoons, where large fluctuations of temperature and salinity may occur (Stal et al. 1996; Villbrandt and Stal 1996).

4.2.4.2 Hypersaline Microbial Mats

Hypersaline environments can be found in shallow and sheltered coastal lagoons and tidal channels with high rates of evaporation and low precipitation. In the Mediterranean, hypersaline lagoons may form when they have narrow connections to the open sea and exchange of water is limited because a tide is virtually absent. Alternatively they can be totally disconnected from the sea and are fed by sea water through a sand bar as is the case in Solar Lake on the Red Sea coast of Sinai, Egypt. When virtually all water in such coastal lagoon environments evaporates, a natural salt pond develops, forming structures known in the Sinai desert as Sabkhas. In those geographical regions were the combination of sun and wind result in sufficient evaporation, artificial salt ponds have been constructed. Other hypersaline environments are inland seas which can be found at many different locations on the globe (Oren 1988) and in shallow lagoons of many of these lakes cyanobacterial mats develop (e.g. Zavarzin et al. 1993).

Cyanobacterial mats develop under these hypersaline conditions thanks to the potential of certain cyanobacteria to accumulate compatible solutes such as betaine that allow osmoregulation up to high salinities (up to 25%). Depending on the salinity cyanobacteria with different osmoprotectants prevail. Cyanobacteria are not found under saturating salinities or when the salt composition differs strongly from that of seawater. In general, hypersaline environments are also strongly alkaline. Cyanobacteria tolerate high pH thanks to their capacity of taking up bicarbonate and accumulating inorganic carbon. As a result of the fixation of CO_2 cyanobacteria generate alkaline conditions themselves. The best studied hypersaline microbial mats are from the salt ponds in Guerrero Negro, Baja California Sur, Mexico and Solar Lake, Sinai, Egypt (D'Antoni D'Amelio et al. 1989; Des Marais et al. 1992). Salts and brines are discussed more fully by Oren in Chap. 15.

4.2.4.3 Hot Spring Mats of Cyanobacteria

Thermal hot springs are environments in which the high temperature in combination with H_2S or acidic conditions decreases biodiversity enormously. Cyanobacterial mats are most common in hot springs at near neutral or alkaline conditions and are described more fully in Chap. 3. Cyanobacteria are generally alkaliphilic organisms. Acidic hot springs are more likely to inhabit eukaryotic microalgae. Thermal springs that contain sulphide may limit the formation of mats since thermophilic cyanobacteria do not tolerate the combination of high temperature and high levels of sulphide (Castenholz 1976, 1977). At moderate concentrations of sulphide, mats of *Oscillatoria* spp. have been shown to lower the sulphide concentration by anoxygenic photosynthesis by the cyanobacteria (Cohen et al. 1986; Ward et al. 1989). Another strategy is found in the so-called inverted mats (Castenholz 1976). Here, mats of the anoxygenic phototroph *Chloroflexus* overlay the cyanobacterial mat. Anoxygenic photosynthesis scavenges the sulphide and protects the underlying mat of the oxygenic heterocystous cyanobacterium *Chlorogloeopsis* sp. (Jørgensen and Nelson 1988). The maximum temperature at which photosynthesis can take place is slightly above 70°C.

4.2.4.4 Terrestrial Cyanobacterial Mats

Terrestrial cyanobacterial mats can be found in a variety of different environments. De Winder et al. (1989a, b) described a cyanobacterial-algal crust in coastal dunes. Sand dunes have a poor capacity of retaining water and are therefore extremely dry environments that are characterized by a low biodiversity. Under certain conditions there develops a mat of *Crinalium epipsammum*, a unique band-shaped filamentous cyanobacterium (Fig. 4.9); its unusual cell envelope is exceptionally well-adapted to desiccation (De Winder et al. 1990). This species is important in the Netherlands in stabilizing and protecting dune sand from wind erosion. Once this organism has established a matrix the community is taken over by the green alga *Klebsormidium flaccidum*.

Desiccation and life under low water potential are also the controlling factors for the development of cyanobacterial mats

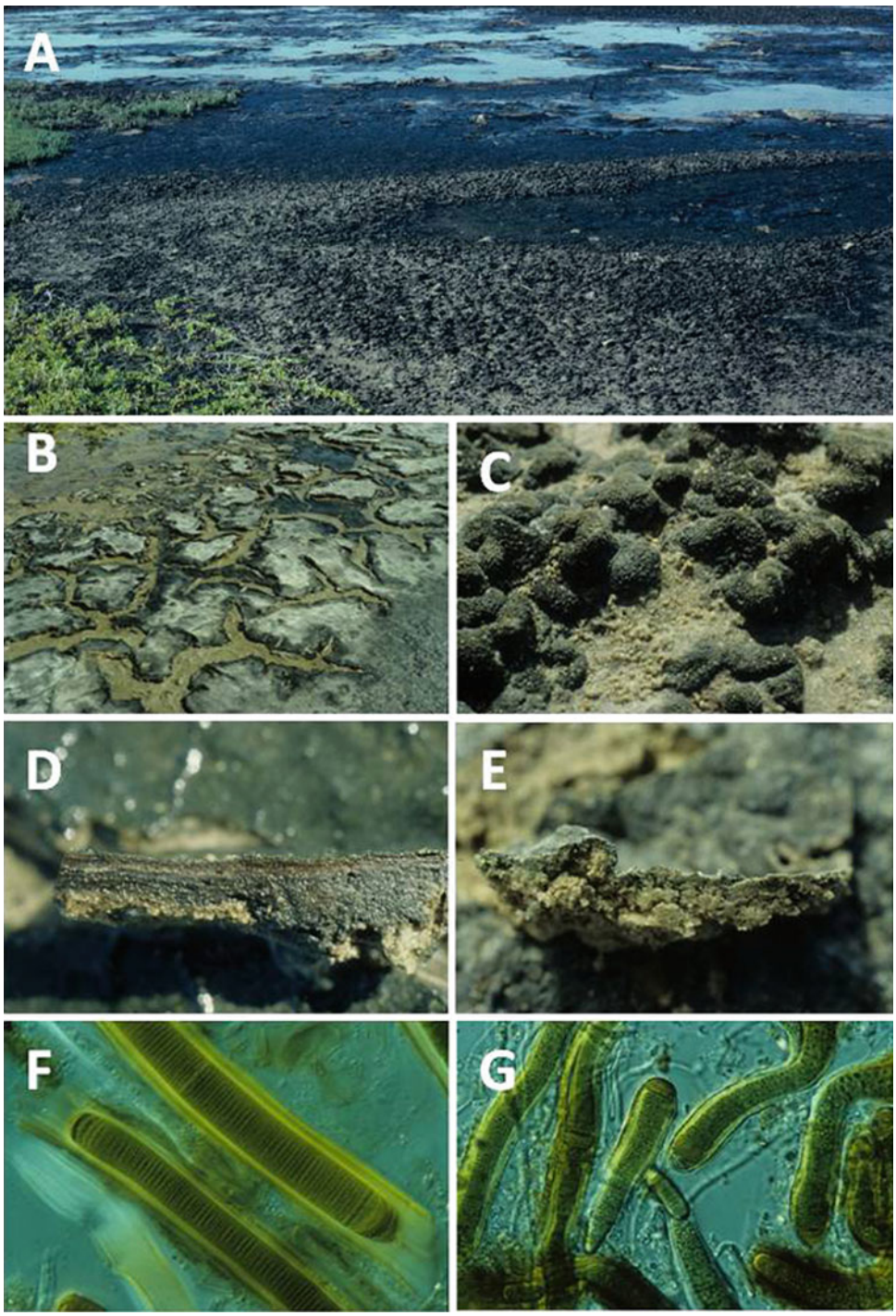

Fig. 4.7 Microbial mats on intertidal flats of the Pacific coast of Guerrero Negro, Baja California Mexico. (**a**) Two types of mat develop close to each other, with smooth mat (shown as *dark areas*) in the lower intertidal. (**b**) Smooth mat, showing cracks caused by desiccation at low tide. (**c**) Pustular mat. (**d**) Differences between the mats shown in cross-section: smooth mat has laminated structure typical of microbial mats – with a thin and dense layer of cyanobacteria on *top*, next a layer of anoxygenic purple sulphur bacteria, then a black layer of FeS, indicating that the mat is permanently anoxic below the layer of cyanobacteria. (**e**) Pustular mat showing a much looser mat of cyanobacteria on top, while layers of purple sulphur bacteria and FeS are absent, indicating that the sediment below the cyanobacteria is predominantly aerobic. (**f**) Smooth mat is composed of the non-heterocystous (but N_2-fixing) *Lyngbya aestuarii*, the trichomes of which are surrounded by a thick polysaccharide sheath and the organisms are embedded in a dense matrix of mucilage. (**g**) Pustular mat composed of *Calothrix*

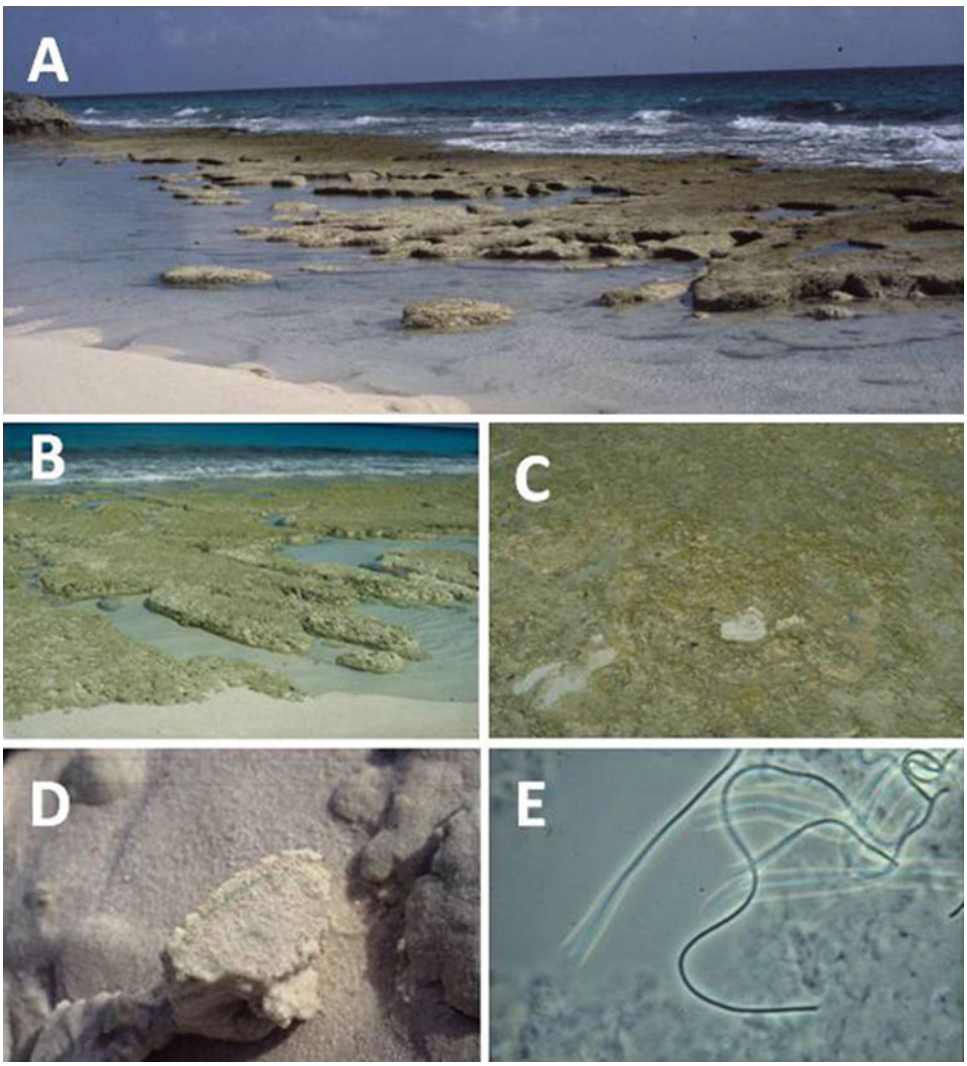

Fig. 4.8 (**a**) Stromatolites formed by cyanobacteria in the intertidal of Exuma Cays, Bahamas: these structures are considered as modern examples of known fossil stromatolites. (**b**) A closer look at these lithified sedimentary structures, which consist of trapped carbonate sediment cemented by micritic (microcrystalline) carbonate. (**c**) Surface of stromatolites is covered by a cyanobacterial-algal mat, which is thought to be involved in formation of the micritic horizons. (**d**) Cross-section of the *top* part of the stromatolite shows a distinct green layer of cyanobacteria. (**e**) *Schizothrix* is the dominant cyanobacterium in these modern stromatolites

and stromatolites in the hot desert. *Microcoleus chthonoplastes*, which occurs in some desert crusts (Brock 1975), has a polysaccharide sheath which plays an important role in protection from desiccation. After re-wetting the sheath absorbs water and the cyanobacterium resumes activity immediately (Campbell 1979). The sheaths of the unicellular desert *Chroococcus* sp. and *Chroococcidiopsis* sp. also have this function and these species are found hypolithically on rocks in the Negev desert (Potts and Friedman 1981; Potts et al. 1983; Caiola et al. 1993, 1996). Cyanobacterial mats are particularly well investigated in the Negev desert in Israel (Friedman et al. 1967; Berner and Jensen 1982). Krumbein and Giele (1979) found a calcifying mat of a unicellular cyanobacterium producing stromatolitic structures in the desert. Cyanobacterial mats also seem to be involved in the formation of rock varnish in the desert. Desert rock varnish is composed of iron and manganese oxides that are precipitated by the metabolic activity of mat microorganisms. The cyanobacterial mat is usually present underneath this hard brownish layer where they are protected from direct sunlight and are capable of retention of some water (Krumbein and Jens 1981).

Carbonate caves are other terrestrial environments that support the formation of microbial mats of the unicellular N_2-fixing *Gloeothece* (*Gloeocapsa*) and of the heterocystous *Nostoc* on walls that receive some daylight (or when artificial illumination is present) (Cox et al. 1981; Griffiths et al. 1987). Another example of such a low-light terrestrial environment is the mats of *Leptolyngbya* sp. described by Albertano and Kovacik (1996) on the walls of Casa Aureum

in Rome. Terrestrial mats of *Nostoc* have been reported from a variety of desert environments, including the cold desert in Antarctica (Davey 1983; Davey and Marchant 1983). Cyanobacterial mats from cold deserts have been described by Davey and Clarke (1992) and Vincent et al. (1993a, b).

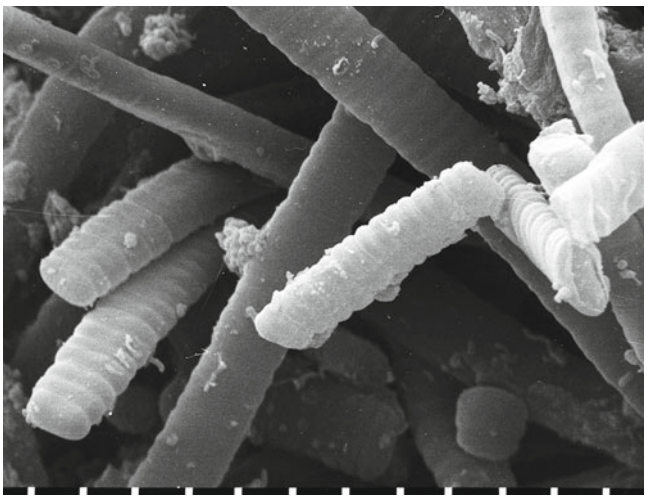

Fig. 4.9 Scanning electron micrograph of *Crinalium epipsammum*, a cyanobacterium forming phototrophic microbial crusts on coastal dunes. This organism is unusual because of the flat trichomes and the fact that the cell wall contains cellulose. Scale bar = 3 μm

4.3 The Organisms: Cyanobacteria That Build Microbial Mats

Cyanobacteria that build microbial mats include a variety of filamentous and unicellular species. The filamentous non-heterocystous *Microcoleus chthonoplastes* dominates marine intertidal microbial mats all over the world (Stal et al. 1985), hypersaline environments (Garcia-Pichel et al. 1996) and in the hot desert (Campbell 1979).

A notable characteristic of *M. chthonoplastes* is its occurrence in bundles containing many trichomes, often twisted like a rope. The bundles are enclosed in a common polysaccharide sheath (Fig. 4.10) which may be partitioned in different compartments (Fig. 4.10b). The rope morphology has been suggested to be an adaptation evolved to colonize unstable substrates (Garcia-Pichel and Wojciechowski 2009). Garcia-Pichel et al. (1996) investigated and compared cultures isolated from a variety of mats from geographically distant locations, both marine and hypersaline. Based on morphological and genetic characteristics, the authors concluded that all these isolates were closely related and belonged at least to the same genus and probably the same species.

The analysis of the 16S rRNA gene and morphological characteristics of a large number of strains that were assigned

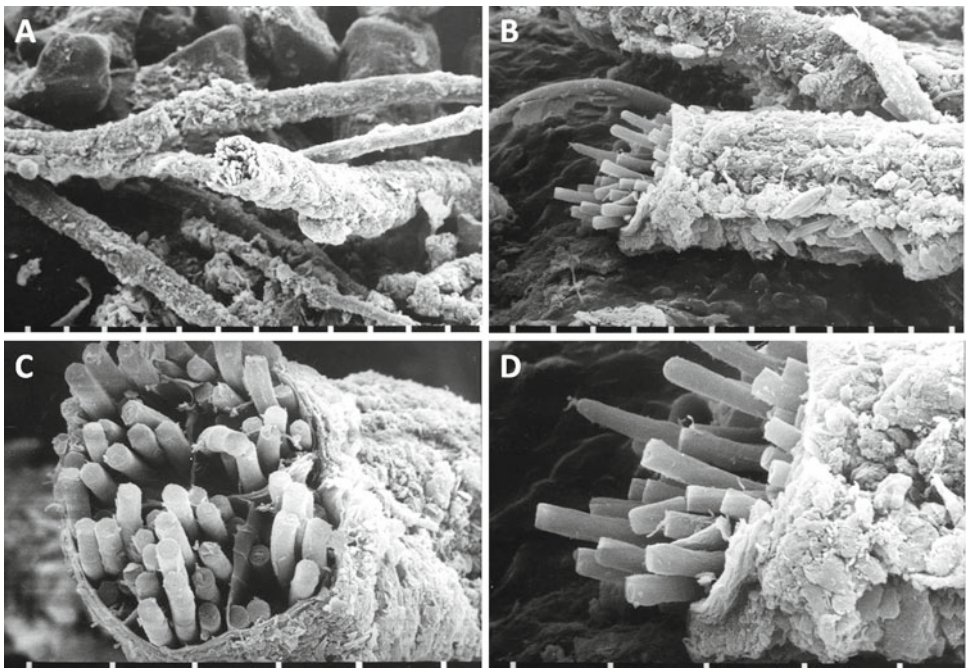

Fig. 4.10 Scanning electron micrographs of a mat of *Microcoleus chthonoplastes* of the island of Mellum, Germany. (**a**) Overview of the mat showing the large polysaccharide ensheathed bundles of trichomes. Scale bar = 30 μm. (**b**) Detail showing one end of a bundle of *M. chthonoplastes*. The *inner room* of the bundle is composed of different compartments separated by polysaccharide walls. This bundle contains a large number of trichomes. Scale bar = 10 μm. (**c**) A *side view* of a bundle of *M. chthonoplastes*. The *outside* is colonized by other microorganisms, including diatoms. Scale bar = 10 μm. (**d**) Detail of the end of the bundle with the individual trichomes sticking out. The trichomes can move freely in and out of the bundle. Scale bar = 10 μm

to *Microcoleus* led Siegesmund et al. (2008) to conclude that this genus belongs in fact to two families: the Oscillatoriaceae and the Phormidiaceae. The marine and hypersaline mat-forming *M. chthonoplastes* belongs to the latter and formed a coherent group that was proposed a new genus name, *Coleofasciculus* (only species so far *C. chthonoplastes*), in order to separate them from the freshwater and terrestrial *Microcoleus* (type strain *M. vaginatus*). However, in this Chap. 1 will refer to the better known name *M. chthonoplastes*.

More than 20 morphotypes of cyanobacteria were isolated from the mats of the intertidal sediments of the German North Sea island Mellum (Stal and Krumbein 1985) and a similar number with the same range of morphotypes were later isolated from a similar mat of the Dutch North Sea barrier island Schiermonnikoog. These also included several heterocystous species that were rarely observed in the field except in supratidal mats that received more freshwater than those in the intertidal regions. The cyanobacterial community composition of these mats varied considerably during the course of a year but also between different years. Sometimes the mats consisted virtually exclusively of *Spirulina* or *Merismopedia* (Palinska et al. 1996). Often these mats were composed of mixtures of different species (Fig. 4.11).

An important species that was always present and was frequently dominant was originally assigned to *Oscillatoria limosa* strain 23 (Stal and Krumbein 1985), which was shown to be the diazotrophic component of these mats. This strain was capable of aerobic N_2 fixation in culture (Stal and Krumbein 1981). Based on the 16S rRNA gene sequence analysis *O. limosa* was found to be related to *Lyngbya aestuarii*. This morphotype is observed frequently in microbial mats all over the world and as far as known all of these mats are diazotrophic. The trichomes have a thick polysaccharide sheath which is often pigmented (Fig. 4.7). Mats of *Lyngbya*/*Oscillatoria* can be found in geographically distant locations and are characterized by high rates of N_2 fixation.

Although many if not all microbial mats are capable of diazotrophy, the specialized heterocystous forms are only rarely the dominant component. That in some cases they can be isolated proves their presence, but obviously the prevailing conditions in the mat prevent their proliferation. Nevertheless, a few exceptions are known. In parts of the tidal flat in Guerrero Negro (Baja California Sur, Mexico) extensive mats of the heterocystous *Calothrix* sp. are present (Stal 1995) (Fig. 4.7). In a coastal lagoon near Bordeaux, France, mats of *Anabaena* sometimes develop (Villbrandt and Stal 1996). Mangroves often support extensive mats of the heterocystous *Scytonema* sp. (Potts 1979). *Calothrix* sp. is also known to form mats on rocks in the spray zone at the seashore (Whitton and Potts 1982). The development of these heterocystous diazotrophic mats is discussed further in Sect. 4.7.

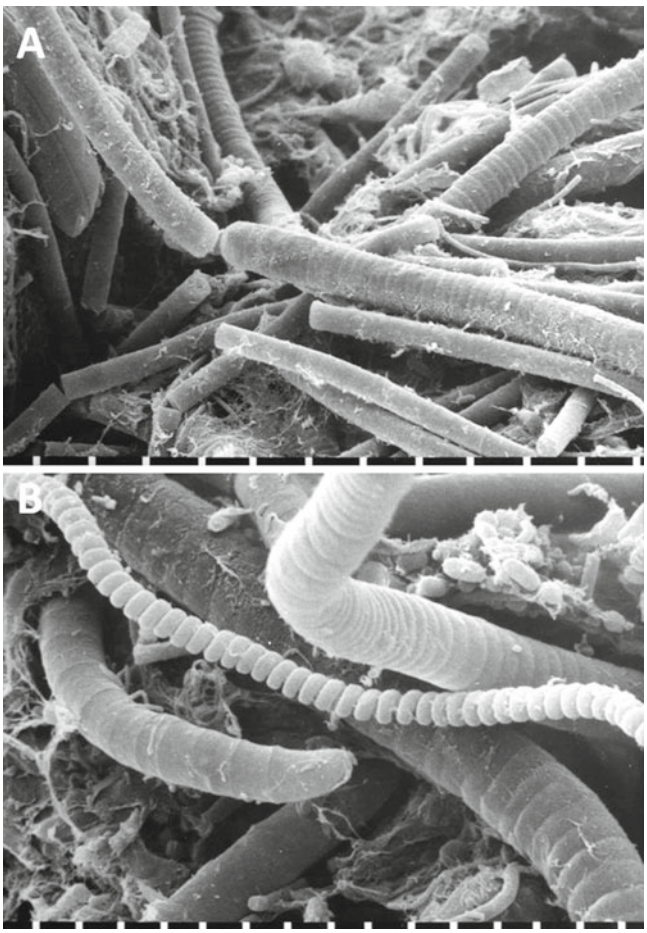

Fig. 4.11 (**a**) Scanning electron micrograph (SEM) of a N_2-fixing mat of *Oscillatoria* spp. and other cyanobacteria. (**b**) SEM photo of a detail of a mat of *Lyngbya* sp. with the typical coiled filament of *Spirulina subsalsa*, which is a typical component of these mats and a single trichome of *Microcoleus chthonoplastes*

Hot spring microbial mats such as Octopus Spring in the Lower Geyser Basin of Yellowstone National Park in the USA and similar microbial mats are dominated by the rod-shaped unicellular cyanobacterium *Synechococcus lividus* (Brock 1978). For a long time, strain *Synechococcus* sp. Y-7c-s was the only cultivated species. This strain was isolated from the 50–55°C Octopus Spring mat and considered to be representative for all thermophilic *Synechococcus* species since they possessed DNA with almost identical G+C ratios (Waterbury and Rippka 1989). In fact, at least seven different strains, all with identical morphology, are present (Ward et al. 1994). Y-7c-s was only detected in Clearwater Spring, which is slightly acidic (Ruff-Roberts et al. 1994; Ferris et al. 1996) and from which this strain may have been originally isolated. Hybridization probes have shown that *Synechoccoccus* sp. strain Y-7c-s was present in the Octopus Spring mats in extremely low frequency. Ferris et al. (1996) demonstrated that enrichment cultures selected for this strain.

Although morphological indistinguishable, the populations of *Synechococcus* sp. in Octopus hot spring microbial mats belong to a phylogenetic diverse group. Analysis of the 16S rRNA gene sequences of Octopus Spring revealed a high diversity of *Synechococcus* distantly related to *S. lividus*. These thermophilic *Synechococcus* are an ecologically diverse group of cyanobacteria that are distributed horizontally along a temperature gradient and vertically along light and oxygen gradients (Allewalt et al. 2006). However, by diluting the inocula prior to enrichment new strains of *Synechococcus* were obtained in axenic cultures. The different strains of hot-spring *Synechococcus* sp. have growth optima at different temperatures (Ward et al. 1994) and it was shown that their temperature ranges and optima were consistent with their distributions in the mats. Other adaptations may include those to pH and light.

In the hypersaline mats of Pond 5 of the Guerrero Negro saltern and the shallow flat mat of Solar Lake *M. chthonoplastes* is the dominant species and forms gelatinous organic mats (Fig. 4.5). Other cyanobacteria that may occur in these hypersaline mats are *Oscillatoria* sp. and *Spirulina* sp. Unicellular cyanobacteria may also be present. The Pond 5 mat of Guerrero Negro grows at salinities from 60‰ to 95‰. The salt content of the shallow flat mat of Solar Lake ranges from 45‰ to 180‰. At salinities that are permanently above 100‰ *M. chthonoplastes* does not proliferate well but *Spirulina subsalsa* may be found up to 150‰. At higher salinities up to 200‰, the unicellular *Aphanothece halophytica* usually dominates the mats (Dor and Paz 1989). The salinity tolerances for cyanobacteria seem to be higher in mats of the Sabkha, where *A. halophytica* occurs at 250‰, which is close to saturation, while *S. subsalsa* is present up to ~200‰ (Dor and Paz 1989). The reason for these differences is unclear. Salinity tolerance may be influenced by temperature. Although the sediment surface of the Sabkha may become hot from the solar radiation, the submersed mats in solar ponds may also be exposed to high temperatures. Therefore, halophilic cyanobacteria may also be moderately thermophilic such as was shown for a newly discovered organism with very thin trichomes of 1 μm *Halomicronema excentricum* that grows in the range of 3.2–12% salt and 28–50°C (Abed et al. 2002).

Lithified microbial mats found in the Exuma Cays, Bahamas, are built by the filamentous cyanobacterium *Schizothrix* (Pinckney et al. 1995). This forms thin trichomes about 1 μm wide, with cells 2–5 times as long as wide. The trichomes may be enclosed by a thick sheath. Communities of *Schizothrix* may form dense and tough mats that are often associated with calcium carbonate precipitation. The lithified microbial mats of Exuma Cays, Bahamas, result in the formation of stromatolites, a process which still goes on (Reid and Browne 1991; Pinckney et al. 1995; Reid et al. 2000).

4.4 Motility, Chemo- and Phototaxis of Cyanobacteria in Microbial Mats

Microbial mats are characterized by steep and fluctuating physicochemical gradients. In order to experience optimum conditions at all times, cyanobacteria must position themselves continuously in the mat. Microbial mats also often occur in environments with high sedimentation rates. This demands a light-oriented motility, in order to prevent permanent burial.

Cyanobacteria possess essentially three different ways in which they respond to light: phototaxis, photokinesis and photophobic response (Häder 1987a, b). Phototaxis is a movement, which is oriented along the direction of light. Phototaxis can be either positive or negative. Positive phototaxis is towards the direction of light whereas negative phototaxis is the movement away from the light. Both positive and negative phototaxis are important for cyanobacteria in microbial mats. Most cyanobacteria are adapted to growth at low light intensities. Excessive light may result in photooxidative stress and can cause damage. The combination of positive and negative phototaxis will allow the organism to obtain an optimum position in the mat. Most of the research on light responses of cyanobacteria has been carried out on *Phormidium* and *Anabaena* and more recently also on the unicellular *Synechocystis*. Little work has been carried out on light responses in cultures of mat-forming cyanobacteria.

Photokinesis is the term used for the phenomenon where speed of movement increases with light intensity. This is because of the greater supply of energy. Only positive photokinesis is known (negative photokinesis would be the decrease of speed at higher light intensities). The photophobic response is the reversal of the direction of movement as a result of a sudden change in light intensity. This response is very important for cyanobacteria. Both step-down and step-up responses are known (Häder 1987a). A step-down response causes the accumulation of the organisms in the light. At very high light intensities a step-up response may result in the accumulation of the organisms in a shaded area. Photophobic responses are clearly related to photosynthesis as could be concluded from action spectra (Häder 1988).

The light required for phototaxis might be extremely low (0.001 μmol photon m^{-2} s^{-1}) and also saturates at very low photon irradiance (1 μmol m^{-2} s^{-1}) (Ng et al. 2003). In most cases the action spectrum of phototaxis follows the photopigments of the cyanobacteria, the phycobiliproteins and chlorophyll *a* (Bhaya 2004). The low threshold for phototaxis is important for cyanobacteria in microbial mats to direct them to the surface after a large deposition event. The low light intensity and the complex action spectrum suggest that it is not required for providing the energy for locomotion,

which is confirmed by the ineffectivity of inhibitors of photosynthesis (Choi et al. 1999).

Motility is an extremely important property for most mat-forming cyanobacteria and occurs by gliding, which can be defined as a self-propulsion along a surface. This surface can also be the interior of the polysaccharide sheath. Trichomes may move forwards and backwards in their sheaths and may move out of it, leaving an empty sheath behind. Trichomes may also glide along each other. The hypotheses to explain gliding motility that have received most attention are:

(i) secretion of mucilage
(ii) contractile structures that cause surface undulations.

Some motile cyanobacteria possess junctional pore complexes, organelles that penetrate the cell wall and through which it is assumed that mucilage is secreted (Hoiczyk 2000). According to this hypothesis the mucilage adheres to the substrate and flows in tight contact with the trichome, thereby producing the propulsive force. The reversal of the movement would be obtained by using junctional pore complexes at the other end of the cell that are directed opposite. The rotation along the long axis in some Oscillatoriaceae could be produced through the presence of helically arranged fibrils. The arrangement of these fibrils determines indeed the left or right rotation, which is species specific. However, the highly motile *Phormidium uncinatum* does not possess junctional pore complexes, although it secretes polysaccharide (Häder 1987b).

Halfen and Castenholz (1971) and Castenholz (1973) suggested that the helically arranged microfibrils which they found in the external layers of the cyanobacterium *Oscillatoria princeps* can contract producing a surface wave that contacts the surface producing the force needed for the gliding movement.

Gliding movement is not restricted to filamentous cyanobacteria. The unicellular *Synechocystis* exhibits a motility that has been described as twitching and depends on type IV pili which moves the organism by pilus extension, adhesion and retraction (Bhaya 2004). Hence, gliding may well be a collection of different types of locomotion, each with their own specific mechanisms. One pelagic marine unicellular *Synechococcus* is capable of swimming, even if it lacks flagella (Waterbury et al. 1985). Swimming depends on swmA, a cell surface glycoprotein of *Synechococcus* and which is needed for the generation of thrust (McCarren et al. 2005). This mode of locomotion does not seem practical in the dense matrix of the microbial mat.

It is not certain how important the three different responses to light are in microbial mats. Ramsing and Prufert-Bebout (1994) concluded from light measurements in mats made by fiber-optic micro light sensors that light fields in microbial mats are uniform. This is caused by scattering of light, and it means that there is in fact no direction of light. Moreover, light intensity will not be subject to sudden changes in microbial mats. These authors therefore conceived that phototactic and photophobic responses would not be especially beneficial for mat-forming cyanobacteria. Studies with *M. chthonoplastes* indicated that the strategy of this organism is to minimize movement when conditions are favourable. Instead of varying the speed of movement (photokinesis) it moves less frequently. *M. chthonoplastes* also reverses its movement frequently. This is not a photophobic response because this would imply a step-down or step-up change in light intensity which is not the case. Ramsing and Prufert-Bebout (1994) further observed that *M. chthonoplastes* bends more frequently at optimum light intensity. In the long-term this could lead to curling of trichomes into bundles. Motility in such bundles is likely to be restricted. Such cyanobacteria are likely to be confined to a fixed position in the mat. In the hypersaline Guerrero Negro mats *M. chthonoplastes* was present throughout the mat and did apparently not migrate, whereas other bacteria, including other cyanobacteria, migrated through the mat and occupied different positions during the daily cycle (Dillon et al. 2009).

Garcia-Pichel et al. (1994) demonstrated that mat-forming cyanobacteria *Oscillatoria* sp. and *Spirulina subsalsa* migrated up and down in the mat in a daily manner (Fig. 4.12). At sunset these cyanobacteria moved towards the mat surface and stayed there throughout the night. At sunrise they migrated downwards. The depth to which they migrated appeared to be related to the light intensity, reaching the maximum depth in the mat at mid-day when the light intensity was highest. Interesting was also that *Oscillatoria* sp. and *S. subsalsa* contained unusually high amounts of chlorophyll *a* (3.9% d. wt). A unicellular cyanobacterium in this mat was non-motile and contained only 0.3% chlorophyll *a* (Garcia-Pichel et al. 1994). It was calculated that if *Oscillatoria* and *S. subsalsa* did not migrate they would be photoinhibited for most of the time, whereas the daily movement guaranteed optimum photosynthesis throughout the light period. Many cyanobacteria move deeper into the sediment at high light intensities (Pentecost 1984; Whale and Walsby 1984; Richardson and Castenholz 1987a) (Fig. 4.12).

Other researchers have also noticed that cyanobacteria migrate to the surface during the night or when the mat is shaded. Migration occurs also during the dark and Whale and Walsby (1984) therefore concluded that this upward movement was not controlled by light. Since these authors could not find any evidence for geotactic or magnetotactic responses, they assumed that migration of cyanobacteria was directed through chemotaxis in a chemical gradient. On the other hand, not all cyanobacteria are capable of moving in the dark. Malin and Walsby (1985) observed that motility of *Oscillatoria* sp. was strictly dependent on light and gliding stopped in the dark after a short period, presumably because energy reserves were exhausted. These authors demonstrated responses of *Oscillatoria* sp. to oxygen (aerotaxis) and CO_2 and bicarbonate. A light-dependent response to CO_2 would

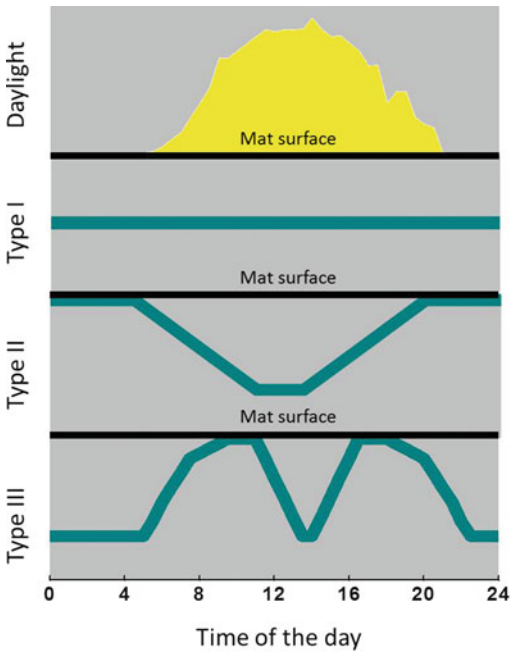

Fig. 4.12 Movements of cyanobacterial layer in mats during a 24-h period. *Upper panel* shows an example of the daily sinusoidal light curve. Example Type I is a mat which does not displace itself during a day-night cycle. This is either the case with unicellular cyanobacteria that are not motile and grow at optimal light intensity or by species that minimize movement when conditions are favorable, which may be the case in some populations of *Microcoleus chthonoplastes*. Example Type II is a mat that moves toward the surface at sunset and moves into the sediment during the day. *Upwards* movement may be controlled by chemical factors such as oxygen or sulphide. *Downwards* movement is in most cases attributed to negative phototaxis. Mats of *Oscillatoria* often show this type of displacement during a 24-h cycle. Example Type III is exhibited by the hot-spring *O. terebriformis*. In the dark the organism moves randomly, but motility is inhibited by sulphide, which eventually results in the accumulation of the population in the sulphide-rich layer deep in the sediment. Positive phototaxis occurs at low light and negative phototaxis at high light. This forces the organism to move deeper into the sediment during the middle of the day

be advantageous. Photosynthetic activity in microbial mats causes depletion of CO_2 and the high pH usually encountered in these environments as a result of photosynthetic activity and CO_2 fixation will shift the carbonate equilibrium resulting in even lower concentrations of CO_2. A light-dependent positive response to oxygen seems to be less advantageous. High concentrations of oxygen in the light may cause photo-oxidative reactions (Eloff et al. 1976) and photorespiration with therefore less efficient CO_2 fixation (Lorimer 1981; Reinhold et al. 1991).

Aerotaxis would be a useful strategy for dark migration. This would allow aerobic degradation of endogenous storage carbohydrate. The migration of *M. chthonoplastes* (Whale and Walsby 1984), *Oscillatoria* sp. and *S. subsalsa* (Garcia-Pichel et al. 1994) to the mat surface during the dark can be explained by a positive aerotaxis (Fig. 4.12). Alternatively, migration to the surface during the dark can be explained by a negative response to sulphide which is very toxic. In the dark the concentration of sulphide will increase because anoxygenic photosynthesis is absent and no oxygen is available for biological or chemical oxidation (Fig. 4.3). Castenholz (1982) therefore conceived a chemophobic response towards sulphide in cyanobacteria that migrate to the mat surface during the dark.

Chemotaxis in chemotrophic bacteria has received much attention but cyanobacteria have hardly been investigated for such migratory behaviour. Several cyanobacteria are capable of assimilating organic compounds such as glucose and fructose in the light (photoheterotrophy) and some even display a fully chemoorganotrophic metabolism (Smith 1982). *Oscillatoria terebriformis* is capable of fermenting extracellular compounds such as fructose and glucose (Richardson and Castenholz 1987b). Chemotactic responses of cyanobacteria to organic compounds are largely unknown. Fechner (1915) reported a negative chemotactic response to organic acids and Richardson and Castenholz (1989) observed inhibition of gliding of *O. terebriformis* by fructose. This effect was similar to that observed for sulphide. Glucose, the other substrate for this organism, did not have an effect, nor did lactate which is one of the fermentation products produced by *O. terebriformis*.

Cyanobacteria that form symbioses with plants were attracted by plant extracts, by certain sugars and particularly by mucilage (Nilsson et al. 2006). Higher temperature and darkness decreased chemotaxis, although this may be explained that light provides the energy for motility, rather than that it controls chemotaxis itself. Chemotaxis may be much more widespread in cyanobacteria than known until now, since operons for this process have been found in their genomes (Wuichet and Zhulin 2003) and the advantages for life in microbial mats with their steep and fluctuating chemical gradients are obvious.

An interesting behaviour has been encountered in *O. terebriformis*, which occurs in hot spring microbial mats and has a light-oriented motility. In the dark, this organism continues to move, albeit randomly. It may thus happen that it moves down into the sediment reaching the sulphide layer. Sulphide inhibits motility in *O. terebriformis* and therefore the organism is trapped in this layer (Richardson and Castenholz 1987a) (Fig. 4.12). Under laboratory conditions, 0.7 mM sulphide completely inhibited its gliding motility. Sulphide inhibited motility only in the dark or in the light when photosystem II was blocked by 3,-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). This inhibition was reversible and was abolished in the light. Since every individual organism has a high probability to become trapped in the sulphide layer, virtually the whole population will end up there. During the day the sulphide horizon will move down into the sediment relieving the inhibition of motility and at the same time the organism will move towards the light at the mat surface. At mid-day,

when light intensity is high, *O. terebriformis* shows negative phototaxis and moves deeper into the sediment in order to prevent photo-oxidative damage (Fig. 4.12). The majority of motile mat-forming cyanobacteria will prefer low light intensities and move deeper into the sediment during the day. The trapping of *O. terebriformis* in the sulphide layer during the dark is unusual, but essential for this organism to survive the dark period. In the presence of oxygen dark respiration will cause a rapid depletion of the endogenous storage carbohydrate which will result in the death of the organism in a matter of hours. The sulphide layer is of course anoxic. *O. terebriformis* is capable of fermentation and this process is slow, allowing for an extended period of energy generation. Indeed, the amount of energy that can be generated is small, but sufficient to cover its maintenance requirements (Stal and Moezelaar 1997). Most cyanobacteria, including mat-forming species, have low rates of dark respiration, allowing them to overcome long periods in the dark in the presence of oxygen. In the dark many microbial mats are virtually anoxic up to the surface, and fermentation is probably the only metabolism possible for the majority of cyanobacteria in the mat, with the exception of those that are exposed to the air. When the mat is submersed, oxygen decreases to zero within the diffusive boundary layer and no oxygen will be available to the mat (Fig. 4.3).

An unusual and new form of taxis is directed to gradients of water or water potential and has been termed hydrotaxis (Garcia-Pichel and Pringault 2001; Pringault and Garcia-Pichel 2004). Hydrotaxis has been found in a desert crust cyanobacterium related to a marine *Oscillatoria*. It was shown that migration depended on energy and probably occurred by gliding movement which is the mode of motility in this genus. When the surface of the crust dried out, the cyanobacteria migrated deeper into the crust where the water potential is higher. After a rain shower, the cyanobacteria moved quickly back to the surface. It is not known what exactly the cyanobacteria senses but it was speculated that it might be water potential or hydrophobicity (Pringault and Garcia-Pichel 2004). Migration towards water makes sense in microbial mats or crusts in desert environments but is probably unlikely in microbial mats in aquatic environments, even if coastal intertidal mats may become desiccated for prolonged periods as well. Hydrotaxis has hitherto only been described for this one occasion.

Mats of diatoms on intertidal mudflats in estuaries and bays have been reported to migrate into the sediments on a high tide (Serôdio et al. 1997). This migration might be under the control of an endogenous rhythm and was maintained for a certain period of time even when the trigger of the tidal cycle was taken away experimentally. For these diatoms it is important to migrate into the sediments when the tide comes in, in order to avoid grazing, even when this greatly limits their window for photosynthesis. In cyanobacterial mats such migration triggered by the tidal cycle has not been reported. Cyanobacterial mats are usually found in the higher reaches of the tidal flats and are not or for shorter periods inundated with water and therefore less subject to the tidal cycle. It is probably the lack of the tide-triggered migration that cyanobacteria can not escape grazing and therefore microbial mats do not occur there where diatom mats are found. In contrast to what had been assumed, it was demonstrated by Garcia-Pichel and Bebout (1996) that ultraviolet radiation penetrates well in microbial mats. The amount of penetration varies with the type of sediment on which microbial mats developed. Silty mud absorbed UV light most and quartz sand the least. Mats that are mainly organic take an intermediate position. UV light was absorbed in these mats more or less exponentially, in a similar way to visible light. There were two important aspects of the penetration of UV light in microbial mats, regardless of their sedimentological characteristics. In some mats the intensity of UV-B at the surface is considerably higher than the incident intensity; this is caused by scattering. Secondly, the total amount of UV-B in the euphotic zone of the mat ranged from 15% to 33% of incident irradiance which is high, particularly when compared with aquatic systems, where this number varies from 3% to 9%. These measurements carried out by Garcia-Pichel and Bebout (1996) were the first to demonstrate unequivocally that cyanobacterial mats develop under UV stress. Garcia-Pichel and Castenholz (1994) and Bebout and Garcia-Pichel (1995) provided also strong evidence that vertical migrations are partly under control of UV light. Garcia-Pichel and Castenholz (1994) reported that only 1.3 $W\,m^{-2}$ of UV-A (315–400 nm) was sufficient to keep the cyanobacteria *Oscillatoria* sp. and *Spirulina subsalsa* deep in the sediment. This intensity is only 3–4% of the level that these organisms would experience at mid-day. These cyanobacteria responded by negative phototaxis. In another study of microbial mats in Solar Lake (Sinai) it was shown that *M. chthonoplastes* responds clearly to UV-B light (310 nm). Exposure of the mat to 0.35–0.79 $W\,m^{-2}$ was sufficient to cause a downwards migration of the cyanobacteria. The effect of UV-B was about two orders of magnitude stronger than normal visible light. Also UV-A had this effect but was about five times less efficient than UV-B (Bebout and Garcia-Pichel 1995). It was concluded from these experiments that *M. chthonoplastes* is capable of sensing UV light, particularly UV-B.

There is no doubt that UV light causes serious damage to oxygenic phototrophic organisms (Cullen and Neale 1994) and has therefore negative effects on primary productivity (Smith et al. 1992). A mat-forming cyanobacterium will therefore benefit from the capability of sensing low levels of UV radiation and combining it with negative taxis. This will nevertheless result in a negative effect on total gross photosynthesis and productivity during exposure to UV light, but it is largely reversible (Bebout and Garcia-Pichel 1995).

Due to the downwards migration of the cyanobacterium, the biomass at the surface, and thus gross photosynthesis, decreases. In addition, surface photosynthesis may be partly inhibited by UV irradiation. Because in the deeper layers more biomass accumulates gross photosynthesis is even higher but due to the low level of photosynthetic active radiation (PAR), biomass specific photosynthesis is low. Not all cyanobacteria exhibit negative phototaxis with respect to UV light. Donkor and Häder (1991) and Donkor et al. (1993) showed that motility in the cyanobacteria they investigated was rather impaired by UV-B (280–315 nm). This may also have been the case in a mat of *M. chthonoplastes* of the temperate southern North Sea, where photosynthesis was strongly inhibited by UV-B radiation and did not recover during the subsequent 3 h when UV was excluded (Garcia-Pichel and Castenholz 1994).

Instead of migrating up- and down in a microbial mat, cyanobacteria have other possibilities to control the amount of light that they must absorb. Pierson and Parenteau (2000) observed for instance that cyanobacteria in the top layer oriented themselves vertically in the mat. This orientation may also have important consequences for the morphology of the microbial mat and may explain some of the morphologies of fossil stromatolites. *Merismopedia* is a unicellular cyanobacterium that occurs frequently in coastal microbial mats. It is characterized by its occurrence in plates in which the cells are well ordered. I have observed frequently that these plates may change its orientation from the large surface towards the light so that it receives maximum light to the side (one cell layer thick) to receive a minimum of light. The same was observed for the flat band-shaped filamentous cyanobacterium *Crinalium epipsammum* (de Winder et al. 1990).

4.5 Carbon Metabolism

4.5.1 Introduction

Cyanobacteria are the principal primary producers in the majority of microbial mats, although in some cases diatoms contribute as well. Oxygenic photosynthesis and sometimes anoxygenic photosynthesis and even chemosynthesis drives CO_2 fixation. Cyanobacteria enrich the microbial mat with organic matter. CO_2 fixation results in the formation of structural biomass of the cyanobacteria. This organic matter may become available to other organisms in the mat by the death and subsequent lysis of the cyanobacteria. However, it appears that, in spite of the high rates of photosynthesis usually observed, net growth of the cyanobacteria is often low in mature mats (Nold and Ward 1996). Hence, other processes must be involved in order to divert photosynthate to the mat community.

The benthic microbial mat community of the hypersaline lake, 'La Salada de Chiprana', northeastern Spain, produced dissolved organic carbon during the day and the night (Jonkers et al. 2003). These authors estimated that 14% and 49% of the mat gross and net photosynthetic production, respectively, diffused out of the mat in the form of low molecular weight fatty acids, although these compounds made up only 2% of the total dissolved organic carbon pool. The high flux of the dissolved organic carbon was generated by nutrient deficiency of the cyanobacteria. Photoheterotrophic *Chloroflexus*-like bacteria grew on top of the cyanobacterial mat at the expense of these phototrophic exudates. Also, large numbers of sulphate-reducing bacteria were found in the fully oxygenated surface layers. Another process that degrades dissolved organic carbon in microbial mats is by exposure of UV-B radiation (e.g. Häder et al. 1998). As will be discussed below, the flow of organic carbon from the cyanobacteria to the heterotrophic mat community may include the excretion of glycolate during photorespiration, the excretion of compatible solutes after an osmotic down shock, the excretion of fermentation products during dark anoxic conditions and the secretion of extracellular polymeric substances (EPS).

4.5.2 Oxygenic Photosynthesis

Oxygenic photosynthesis requires the presence of two photosystems (PS I and II). Cyanobacteria contain chlorophyll *a* in the reaction centers of both PS I and II, but the former contains about 2–3 times as many molecules of chlorophyll *a*. This chlorophyll may also contribute to the light harvesting, but the phycobiliproteins are far more important pigments as light-harvesting antennae. Jørgensen et al. (1987) demonstrated by recording photosynthetic action spectra in cyanobacterial mats that chlorophyll *a* contributed hardly to these action spectra even when additional 600 nm light was given to excite PS II.

Light is strongly attenuated in microbial mats, both by the sediment and by absorption by the dense phototrophic community. Sediments are transparent to light of long wavelengths (Stal et al. 1985). Dry sediments consisting of fine sandy quartz attenuate light much stronger than the same sediment saturated with water (Stal et al. 1985). Through the upper 1 mm of the latter more than 10% of surface irradiance penetrated, while this was only 2.5% of the dry sediment. The photic depth of the bare wet fine sandy sediment was about 4 mm. Through a 1.5 mm mat of cyanobacteria (0.5 g chlorophyll *a* m^{-2}), 0.45% of photosynthetically active radiation (PAR) penetrated. However, due to the specific absorption of the mat, wavelengths that would support oxygenic photosynthesis are specifically attenuated and oxygenic photosynthesis would not be possible below the cyanobacterial mat (Stal et al. 1985; Jørgensen et al. 1987; Pierson et al. 1987, 1990; Jørgensen and Des Marais 1988) (Fig. 4.4). Sediment and cyanobacterial mats are relatively transparent to light of

wavelengths above 700 nm, which explains the occurrence of communities of bacteriochlorophyll *a*-containing anoxygenic phototrophic purple sulphur bacteria (Pierson et al. 1987, 1990). These findings were confirmed by using fiber-optic microprobes connected to diode array detector (Kühl and Jørgensen 1992) and by a scalar irradiance microsensor which allowed spectral light measurements in sediments at the scale of the phototrophic microorganisms (Lassen et al. 1992a). More than 50% of the incident irradiance of 800 nm light penetrated a 1-mm thick microbial mat (Ploug et al. 1993). Lassen et al. (1992b) used this technique to study photosynthesis and photosynthetic efficiency in a microbial mat in Limfjorden, Denmark. This mat consisted of a top layer of diatoms and a cyanobacterial layer (*Oscillatoria* spp.) underneath. Using an oxygen micro-sensor, two peaks of oxygenic photosynthesis were found, corresponding to the diatom biofilm and the second deeper maximum corresponded with the layer of cyanobacteria. This latter maximum at 1 mm depth occurred at a light intensity of only 12 µmol photon m^{-2} s^{-1} i.e. 1.5% of incident light intensity. However, photosynthetic efficiency (rate of photosynthesis at a specific depth divided by the scalar irradiance at that depth) appeared to be tenfold higher in the cyanobacterial mat compared to the diatom film. This increased photosynthetic efficiency at low light intensity was the result of both the content of cyanobacteria at the depth of the second maximum of photosynthesis as well as a likely increased efficiency with which the available light was absorbed by the organisms (Lassen et al. 1992b). The report (Chen et al. 2010) that a cyanobacterium from a Shark Bay stromatolite can form a newly discovered chlorophyll, chl *f,* with the ability to absorb light in the infrared as well as red part of the spectrum, suggests the possibility that this pigment may also contribute to efficient use of light deeper in the stromatolite.

The dense biomass of cyanobacteria in the upper photic zone of microbial mats results in high rates of photosynthesis, and on a surface basis it compares to the productivity of rain forests, which are usually considered as the most productive ecosystems on Earth (Guerrero and Mas 1989) (Table 4.1). Revsbech et al. (1983) measured a total daily photosynthesis in a cyanobacterial mat in Solar Lake (Sinai) of 156 mmol O_2 m^{-2} and similar rates were found by Villbrandt et al. (1990) for a cyanobacterial mat in a temperate region (North Sea). The daily rates of photosynthesis measured by Revsbech et al. (1983) in Solar Lake microbial mats followed the light intensity during the day. The efficiency of photosynthesis was highest at low light intensity (up to 120 µmol photon m^{-2} s^{-1}). Photosynthesis was not inhibited at high light intensity. The same was found by Villbrandt (1992) when diurnal photosynthesis data on several days were plotted against light intensity (Fig. 4.13a). Photosynthesis increased in a linear way with light intensity. This photosynthesis versus light intensity curve of a microbial mat is completely different from isolated cultures of cyanobacteria (Fig. 4.13b). At low light intensity the photosynthesis curve is steep, depending on photosynthetic efficiency of the organism. At certain, usually moderate, light intensity photosynthesis saturates (P_{max}) and decreases again at higher intensity as a result of photoinhibition. The different *P* versus *I* curve of a mat is explained by the fact that all light is absorbed and used for photosynthesis. At higher light intensity cyanobacteria in the deeper parts of the mat will exhibit higher rates of photosynthesis. The diurnal variation of photosynthesis on a bright, cloudless day in July in a mature mat of *M. chthonoplastes* on the island of Mellum, North Sea (chlorophyll *a* content typically 0.3 g m^{-2}: Stal et al. 1985), showed a different pattern as the one measured by Revsbech et al. (1983). The rates of photosynthesis were highest during the morning hours and showed a sharp drop after mid-day (Villbrandt et al. 1990) (Fig. 4.14b). This mid-day drop in photosynthesis has been observed by other workers both in microphytobenthos as well as in phytoplankton (Paerl et al. 1989; Storch et al. 1990). An explanation for this observation may be that in the morning hours the concentration of dissolved inorganic carbon in the pore water of the mat is high as a result of decomposition of organic matter during the preceding night. After some hours of photosynthetic CO_2 fixation the pore water becomes depleted of dissolved inorganic carbon, which could explain the much lower rates of photosynthesis measured in the afternoon. A similar pattern as the one measured by Revsbech et al. (1983) was measured at another site of tidal flat on the island of Mellum. This site was characterized by freshly colonized sediment with *Oscillatoria limosa* (*Lyngbya aestuarii*) as the dominant species and with only 1/10 of the biomass compared to the mature mat (chlorophyll content typically 0.03 g m^{-2}, Stal et al. 1985) (Villbrandt 1992) (Fig. 4.14). Photosynthesis on a surface basis was much lower which is of course due to the much lower biomass and therefore the supply of dissolved inorganic carbon may have been sufficient throughout

Table 4.1 Comparison of primary productivity in microbial mats with other ecosystems (After Stal 1993)

Ecosystem	Primary productivity (mg C m^{-2} d^{-1})
Microbial mat	
Mellum (North Sea)	6,200
Solar Lake (Sinai)	5,000
Sea and ocean	
Mediterranean	60–500
Coastal upwellings	1,000–4,000
Ocean	<100
Lakes	
Oligotrophic lakes	40–80
Eutrophic lakes	300–3,000
Hypertrophic lakes	2,000–5,000
Mangrove forests	5,600
Rain forest	6,000

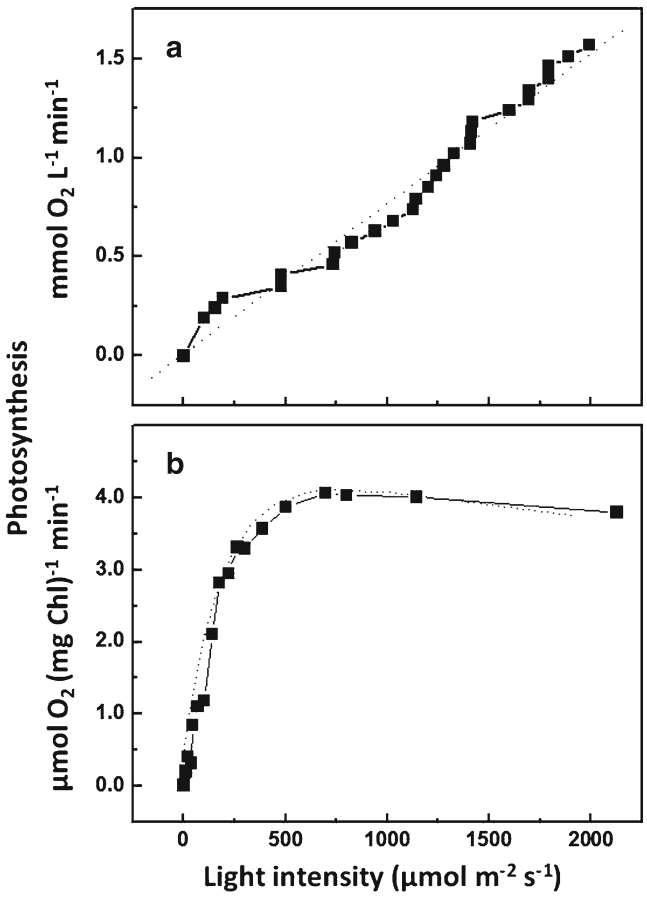

Fig. 4.13 (**a**) Photosynthesis versus light curve in a microbial mat of the North Sea island of Mellum (Germany) (Data from Villbrandt 1992). A large number of depth integrated measurements of photosynthesis recorded at different days and during different times of the day at the same location in the mat were used to plot in this *curve*. The curve was fitted by linear regression: P (mmol O_2 l^{-1} min^{-1}) = 0.01062 + (7.58 10^{-4}) I (R = 0.99 ± 0.08; N = 27 P < 0.0001). (**b**) Typical photosynthesis versus light intensity curve of a cyanobacterial culture

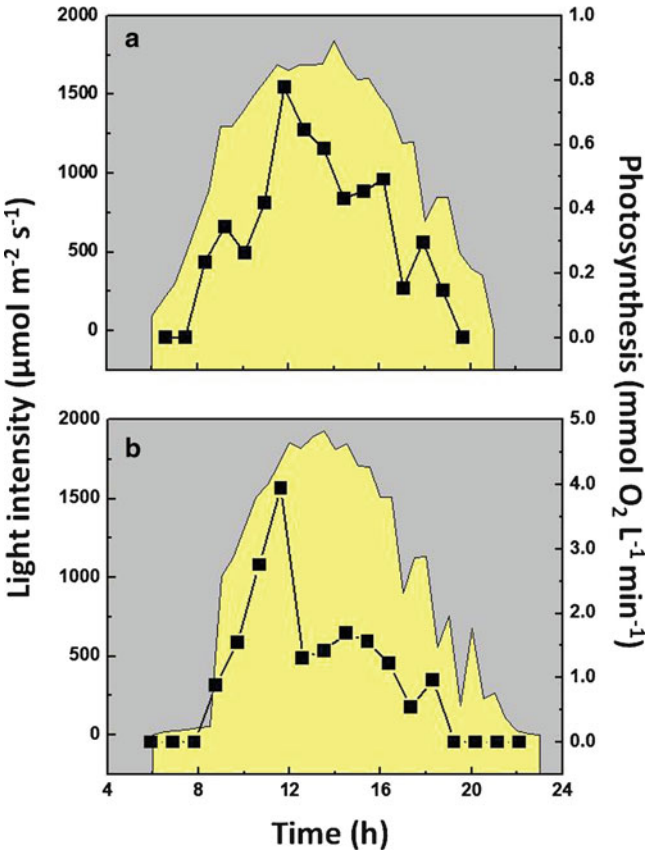

Fig. 4.14 Daily light curve (*shaded area*) and depth integrated photosynthesis of: (**a**) freshly colonized sediment with *Lyngbya* sp. as the dominant cyanobacterium; (**b**) mature mat of *Microcoleus chthonoplastes*. Both mats were located on tidal sand flats on the North Sea island of Mellum, Germany (Data from Villbrandt et al. 1990)

the day. Photosynthesis on a biomass basis was about twice as high as in the mature mat with comparable surface incident light intensity. This is attributed to the much smaller attenuation of light when biomass is low, i.e. the individual cyanobacteria receive much more light in the freshly colonized sediment compared to the mature mat.

The high rates of photosynthesis often observed in microbial mats may cause supersaturated oxygen conditions. The dense organic matrices represent a diffusion barrier that limits gas exchange. Oxygen bubbles that eventually develop may also be trapped in this matrix, causing the mats to become buoyant and lift off the sediments. Erosion of microbial mats as a result of this phenomenon can be regularly observed. Pieces of mat may be carried to vegetated areas and become desiccated when the tide has gone. Such desiccated pieces may be transported by wind over long distances. This phenomenon was first reported in 1686, becoming known as "Meteorpapier" because of the belief that it came from space. In his publication "Über das im Jahre 1686 in Curland vom Himmel gefallene Meteorpapier und über dessen Zusammensetzung aus Conferven und Infusorien" Ehrenberg (1838) identified this meteor paper as desiccated microbial mats.

4.5.3 Anoxygenic Photosynthesis

Anoxygenic photosynthesis in microbial mats is not the exclusive trait of purple- or green sulphur bacteria. Some species of cyanobacteria are capable of anoxygenic photosynthesis in which only photosystem I is involved. As with phototrophic sulphur bacteria, anoxygenic photosynthesis in cyanobacteria depends on sulphide as the electron donor. Roughly two categories of cyanobacteria can be distinguished with respect to the capacity of anoxygenic photosynthesis. In one group oxygenic photosynthesis is inhibited at low concentrations of sulphide and anoxygenic photosynthesis is induced. Inhibition of oxygenic photosynthesis by sulphide is

probably at the level of the manganese-containing, water-splitting enzyme (Oren et al. 1977). Both types of photosynthesis are mutually exclusive in these organisms. In the other group anoxygenic and oxygenic photosynthesis occur concurrently. At low sulphide concentrations oxygenic photosynthesis is more important and with increasing sulphide concentrations anoxygenic photosynthesis gradually takes over.

In both types of cyanobacteria, anoxygenic photosynthesis must be induced, a process which depends on a certain threshold of sulphide concentration and on light. Induction of anoxygenic photosynthesis in some organisms may take several hours. Therefore, cyanobacteria that possess the capability of carrying out oxygenic and anoxygenic photosynthesis concurrently have an ecological advantage in environments in which the sulphide concentration fluctuates, as is the case for instance in many marine and hypersaline microbial mats. Cyanobacteria that can carry out only one type of photosynthesis at a time are typical of environments with a constant supply of sulphide, as in certain hot spring microbial mats with an indigenous supply of sulphide. Moreover, these cyanobacteria tolerate higher concentrations of sulphide.

In cyanobacteria, anoxygenic photosynthesis is defined as energy generation through cyclic electron flow driven by photons absorbed by the reaction center of photosystem I and the fixation of CO_2 using sulphide as the electron donor. This means that this process also takes place when photosystem II is inhibited by the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) or when illuminated by far red light (>700 nm) which cannot be used by photosystem II. Cyanobacteria that perform anoxygenic photosynthesis oxidize sulphide to elemental sulphur according to the following stoichiometric equation:

$$12H_2S + 6\,CO_2 \rightarrow C_6H_{12}O_6 + 12S^o + 6H_2O$$

The elemental sulphur that is produced is deposited outside the cell but is often found attached to the outer sheath of the cyanobacterium as finely dispersed particles. The elemental sulphur is not further oxidized, as is the case in purple sulphur bacteria. In the mat-forming *Microcoleus chthonoplastes* (this particular strain is now assigned to *Geitlerinema* and is therefore closely related to the anoxygenic Solar Lake cyanobacterium '*Oscillatoria limnetica*', now also reassigned to *Geitlerinema*) thiosulphate was found to be the product of sulphide oxidation (De Wit and Van Gemerden 1987):

$$6H_2S + 3H_2O + 6\,CO_2 \rightarrow C_6H_{12}O_6 + 3H_2S_2O_3$$

While the oxidation of sulphide to sulphur yields only two electrons, the oxidation to thiosulphate yields four electrons per sulphide oxidized. Thus, the oxidation of sulphide to thiosulphate in *M. chthonoplastes* (*Geitlerinema*) seems to be twice as efficient as in other cyanobacteria in which zero-valent sulphur is the product. Rabenstein et al. (1995) reported that sulphite was the intermediate in those cyanobacteria that oxidized sulphide to thiosulphate. It is possible that thiosulphate is formed in a chemical reaction of sulphite with sulphide.

It is not clear why most anoxygenic cyanobacteria oxidize sulphide only to elemental sulphur. Phototrophic sulphur bacteria usually oxidize sulphide to sulphate, which yields eight electrons. However, in these bacteria the oxidation to elemental sulphur is usually much faster as the further oxidation to sulphate. Depending on the species this elemental sulphur is accumulated intra- or extracellularly. It can be hypothesized that the rapid oxidation of sulphide to elemental sulphur would be advantageous since it assures a rapid removal of the toxic sulphide. Moreover, in anoxygenic phototrophic bacteria, no matter whether they store the elemental sulphur intra- or extracellularly, it is not available for other organisms (Van Gemerden 1987). In that way, when sulphide is depleted these organisms continue anoxygenic photosynthesis at the expense of stored sulphur without competition with other organisms. In cyanobacteria the rapid consumption of sulphide most likely serves for detoxification since they can immediately switch to oxygenic photosynthesis when it has been completely oxidized. In addition, cyanobacteria can also use elemental sulphur as electron acceptor in anaerobic dark metabolism. *O. amphigranulata* is capable of using elemental sulphur for assimilatory purposes (Castenholz and Utkilen 1984). In the unicellular *Anacystis nidulans* low rates of CO_2 fixation are supported by the oxidation of thiosulphate (Utkilen 1976; Peschek 1978).

M. chthonoplastes (*Geitlerinema*) belongs to the group that performs oxygenic and anoxygenic photosynthesis concurrently. In this organism the growth rate decreased exponentially with increasing concentrations of sulphide. At a concentration of 1 mM sulphide (pH 8.0) growth was completely inhibited. Oxygenic photosynthesis was gradually inhibited with increasing concentrations of sulphide. The relative contribution of anoxygenic photosynthesis to total photosynthesis was >95% at concentrations of sulphide exceeding 0.35 mM (pH 8.0). The inhibition of growth at 1 mM of sulphide is probably caused by the complete inhibition of oxygenic photosynthesis, rather than anoxygenic photosynthesis. It was shown that *M. chthonoplastes* (*Geitlerinema*) requires oxygen for growth. When oxygenic photosynthesis was inhibited by DCMU, *M. chthonoplastes* (*Geitlerinema*) was not capable of sulphide-dependent anoxygenic phototrophic growth unless some oxygen was present. Therefore, a small contribution of oxygenic photosynthesis may be necessary in order to provide the essential oxygen. Oxygen may be required for the oxidation of fatty acids. *Anacystis halophytica*, for instance, possesses an oxygen-dependent

desaturation mechanism (Padan and Cohen 1982). Several cyanobacteria contain polyunsaturated fatty acids (Kenyon et al. 1972) and Padan and Cohen (1982) suggested that such cyanobacteria may be incapable of anaerobic growth. *O. limnetica* (*Geitlerinema*) does not contain polyunsaturated fatty acids which may explain its capacity for anaerobic growth (Padan and Cohen 1982). *M. chthonoplastes* (*Geitlerinema*) SAG 3192 contains considerable amounts of linoleate (18:2), linolenate (18:3) and tetradecadienate (14:2), regardless whether the culture was grown in the presence or absence of sulphide (De Wit et al. 1988).

The affinity of *M. chthonoplastes* (*Geitlerinema*) for sulphide is extremely low. The K_m for sulphide has been calculated as 974 μM, approximately the concentration at which growth of *M. chthonoplastes* (*Geitlerinema*) ceased (De Wit and Van Gemerden 1987). These authors analyzed the data of Jørgensen et al. (1986), who investigated the transition of anoxygenic photosynthesis to oxygenic photosynthesis in a mat of *M. chthonoplastes*. A K_m of 710 μM was calculated for sulphide oxidation in this mat. This affinity is close the one calculated in culture by De Wit and Van Gemerden (1987). These affinities for sulphide are extremely low when compared to the value of 5 μM for an anoxygenic phototrophic purple sulphur bacterium as *Thiocapsa roseopersicina,* which is frequently present in microbial mats (De Wit and Van Gemerden 1988). The similarity of the K_m of sulphide oxidation estimated in a culture to that estimated in a natural microbial mat of *M. chthonoplastes* indicates that this organism was probably responsible for the sulphide oxidation in the microbial mat. This was also suggested by Jørgensen et al. (1986) although they concluded this from the fact that purple sulphur bacteria constituted only a minor fraction in that mat.

The results on anoxygenic photosynthesis in a culture of *M. chthonoplastes* (*Geitlerinema*) obtained by De Wit and van Gemerden confirmed those for a natural mat by Jørgensen et al. (1986). These authors found that oxygenic and anoxygenic photosynthesis occurred concurrently. However, at higher concentrations of sulphide oxygenic photosynthesis was insignificant. Oxygenic photosynthesis in this mat started when the sulphide concentration decreased to about 0.3 mM, which is in agreement with the results obtained by De Wit and van Gemerden. The experiments of Jørgensen and co-workers showed that oxygenic photosynthesis could occur even when the microbial mat was exposed to 5–6 mM of sulphide in the overlying water. An oxygen peak was sandwiched between layers of sulphide. This sandwiching of cyanobacteria in microbial mats is often observed. Apparently, *M. chthonoplastes* is capable of resisting high concentrations of sulphide and recovers oxygenic photosynthesis. It is also capable of oxidizing this high concentration of sulphide in the light but this does not mean that the organism is indeed growing or even fixing CO_2.

Taking together the extremely low affinity of *M. chthonoplastes* for sulphide, the low growth rate with anoxygenic photosynthesis and the fact that this organism cannot grow in the absence of oxygen, the major function seems to be the detoxification of sulphide.

4.5.4 CO_2 Fixation

Carbon dioxide is the most important source of carbon for cyanobacteria and it is therefore crucial for the functioning of microbial mats. Cyanobacteria use the energy and low potential reductant (NADPH) produced during photosynthesis to fix CO_2 through the reductive pentose phosphate pathway (Calvin-Benson-Bassham cycle). The same pathway in the opposite direction, the oxidative pentose pathway, is used for the oxidation of storage carbohydrate during the dark in combination with aerobic respiration (Smith 1982; Schmetterer 1994).

High rates of photosynthesis will deplete the sediment of CO_2 and raise the pH. The pH may even reach values of over 9.5 (Revsbech et al. 1983) (Fig. 4.3) and any dissolved inorganic carbon will be present as bicarbonate or carbonate. Cyanobacteria are capable of adapting to growth at extremely low concentrations of dissolved inorganic carbon. Both CO_2 and bicarbonate are taken up by cyanobacteria. However, CO_2 is the substrate for RubisCO, the key enzyme of the Calvin-Benson-Bassham cycle and responsible for the fixation of CO_2. As is the case in other autotrophic organisms, this enzyme has also a low affinity for CO_2 in cyanobacteria, which means that both a high concentration of CO_2 and of RubisCO are prerequisites for the efficient fixation of carbon dioxide. Cyanobacteria possess an inorganic carbon-concentrating mechanism (CCM) which may result in up to 1,000-fold accumulation of inorganic carbon in the cell. A tentative model of this CCM in cyanobacteria proposes that either bicarbonate or CO_2 is taken up but that the former is the predominant species of inorganic carbon in the cytoplasm (Kaplan et al. 1994). Bicarbonate enters the carboxysome, a cell inclusion in autotrophic bacteria also known as polyhedral bodies. Carboxysomes contain virtually all RubisCO in organisms that possess these inclusions. The importance of carboxysomes for the CCM is also shown by the observations of Turpin et al. (1984) and McKay et al. (1992) that the number of these inclusions increases during adaptation of cyanobacteria to low CO_2 concentrations.

The fixation of CO_2 in microbial mats can be investigated by measuring the $^{12}C/^{13}C$ carbon isotope ratio in the organic matter. RubisCO discriminates between carbon isotopes with a slight preference for the lighter isotope ^{12}C. This fractionation factor α equals 1.029 (Roeske and O'Leary 1984), which means that organic matter may become 29‰ depleted in the heavy isotope ^{13}C when its origin is from RubisCO

mediated CO_2 fixation. This isotopic discrimination is only achieved when the CO_2 concentration is sufficiently high. This is generally not the case in microbial mats. Moreover, the measured value may differ from expected one because other organisms that were responsible for RubisCO independent CO_2 fixation may have been present in the system. Also cyanobacteria may fix significant amounts of CO_2 via alternative pathways such as PEP carboxylase or carbamylphosphate. Furthermore, the dissolved inorganic carbon produced from the decomposition of organic matter may be recycled and give rise to different net isotope discrimination. At the low concentrations of CO_2 that usually occur in cyanobacterial mats active transport of HCO_3^- becomes important (Badger and Andrews 1982) which results in a much smaller isotope discrimination than in the case of CO_2 uptake (Des Marais and Canfield 1994). Microbial mats are usually not much depleted in ^{13}C ($\delta^{13}C_{mat}$ is not very negative) because the pool of dissolved inorganic carbon is small compared to the rate of CO_2 fixation. This minimizes the isotope discrimination. The most negative values of $\delta^{13}C_p$ (photosynthate) are expected when CO_2 does not become depleted from the medium and when exchange between the medium and the site of fixation is rapid.

Des Marais and Canfield (1994) investigated the carbon isotope discrimination in two microbial mats in Guerrero Negro, Baja California, Mexico. The $\delta^{13}C_{mat}$ in these mats was slightly negative (−70‰). In the mat of *Lyngbya aestuarii* this value corresponded with the fractionation factor 1.007. This low value was evidently attributed to the closed reservoir behaviour of the system. The dissolved inorganic carbon that was produced by the mat during the night possessed the same negative value and therefore no changes in the $\delta^{13}C_{mat}$ were expected in the *L. aestuari* mat. In the mat of *M. chthonoplastes* photosynthesis did not discriminate between the lighter and heavier isotopes. At present a conclusive explanation for the negative value of $\delta^{13}C_{mat}$ in the *M. chthonoplastes* mat is not available (Des Marais and Canfield 1994). Processes such as excretion, fermentation and respiration do not change isotopic discrimination. Diagenesis of organic matter does not alter its isotopic composition (Des Marais et al. 1992) and the $\delta^{13}C_{DIC}$ is similar as $\delta^{13}C_{mat}$ (Bauer et al. 1991). It is known that the 'pond 5' mats of *M. chthonoplastes* are more or less in 'steady state', i.e. most of the organic matter that is produced by photosynthesis is mineralized in the mat. It is likely that photosynthesis scavenges very efficiently the dissolved inorganic carbon that is produced in the mat, thereby limiting net isotope fractionation. In fossil Proterozoic stromatolites $\delta^{13}C$ is much more negative than in present day microbial mats and stromatolites. This may reflect the higher levels of dissolved inorganic carbon in the Precambrian compared to today's concentrations (Kempe and Kazmierczak 1990a) or a more negative $\delta^{13}C_{DIC}$.

4.5.5 Photorespiration and Glycolate Excretion

During daylight, the dense phototrophic biomass in the cyanobacterial mat depletes CO_2 and accumulates oxygen, which sometimes may reach high supersaturation. It is assumed that such conditions will support photorespiration. Besides carboxylation, RubisCO also possesses oxygenase activity and can oxidize ribulose-1,5-bisphosphate to one molecule of each 2-phosphoglycolate (2PG) and 3-phosphoglycerate (3PGA) instead of two molecules of the latter during the carboxylation reaction (Lorimer et al. 1973; Lorimer 1981; Miziorko and Lorimer 1983). In fact, RubisCO has a much better affinity for oxygen as substrate than for carbon dioxide (Pierce 1988) and it has been suggested that the original function of the enzyme was an oxygenase rather than a carboxylase (Tabita 1988). Warburg (1920) discovered that O_2 inhibited CO_2 fixation in algae. Schau et al. (1950) showed that glycolate was a product of CO_2 fixation and Warburg and Krippahl (1960) demonstrated that its synthesis could be stimulated by oxygen. Glycolate is produced from 2PG by phosphoglycolate phosphatase and is metabolized via the glycine-serine (C2) pathway (Renstrom-Kellner and Bergman 1990), resulting in the production of 3PGA, CO_2 and NH_3. This light-dependent oxygen uptake and CO_2 evolution is called photorespiration. Photorespiration may represent a loss of fixed carbon which may be as high as 15–50% of net photosynthesis (Artus et al. 1986; Gerbaud and Andre 1987). What function of photorespiration is so important to justify this loss of fixed C?

In plants, the C2 pathway that metabolizes the toxic intermediate of photorespiration 2PG is essential for photosynthesis. Mutations in the C2 pathway result in plants with a high CO_2-requiring phenotype (high CO_2 would minimize photorespiration). In cyanobacteria the oxygenase reaction has been considered as irrelevant because these organisms possess a carbon concentration mechanism (CCM) which would allow sufficient high CO_2 concentration at the site of RubisCO. Cyanobacteria were known to convert 2PG to glycolate. However, it has now become clear that 2PG metabolism is probably present in all cyanobacteria, even in those with the smallest genomes, such as the marine picocyanobacteria *Prochlorococcus* and *Synechococcus*. The model strain *Synechocystis* PCC 6803 possesses three routes to metabolize 2PG: the plant-type C2 route, the bacterial glycerate pathway and the conversion of glyoxylate via oxalate to formate and subsequently to CO_2 (Eisenhut et al. 2008). It was also shown that 2PG metabolism was obligatory and that it could not be compensated by the CCM of this organism. Eisenhut et al. (2008) postulated that 2PG metabolism evolved simultaneously with oxygenic photosynthesis in cyanobacteria to allow them to cope with the toxic products generated by photorespiration that could occur because of the oxygen that accumulated in the cell and in the microbial mats, while

the CCM evolved only recently (Raven et al. 2008). Hence, the C2 pathway in plants was probably inherited from the cyanobacteria as well (Eisenhut et al. 2008).

Although it seems reasonable to assume that the metabolism of 2PG serves the elimination of toxic intermediates produced by the oxygenase reaction of RubisCO, there may be other benefits from photorespiration. 3PGA is regenerated for the Calvin-Benson-Bassham cyclus and other metabolic intermediates may be produced (Husic et al. 1987). The CO_2 produced may be re-fixed and the NH_3 may be re-assimilated even if this at the expense of ATP in both cases. In microbial mats photorespiration may help to prevent photooxidative damage by the lowering of O_2.

It is questionable whether in microbial mats the CCM would be sufficient to prevent photorespiration, because of the depletion in CO_2 during the daytime. If all cyanobacteria are capable of 2PG metabolism as is the case in *Synechocystis* PCC 6803, then it seems unlikely that glycolate is excreted, and this compound would therefore not be important as a substrate in microbial mats.

Many microbial mats are characterized by oxygen supersaturation in the light and CO_2 depletion and by very high pH (sometimes above 10). They are also subject to high light intensities and are chronically nitrogen depleted. All these factors will force the cyanobacteria to maximum rates of photorespiration. Glycolate metabolism, and therefore photorespiration, is closely associated with nitrogen metabolism. Renstrom-Kellner and Bergman (1989) demonstrated that the excretion of glycolate by *Anabaena cylindrica* decreased drastically in the presence of a source of nitrogen such as NH_4Cl or glutamate. As was shown by these authors, N_2-fixing cyanobacteria could lose up to 60% of photosynthetic fixed CO_2 as glycolate. Heterocystous cyanobacteria can access nitrogen through N_2 fixation. However, most mat-forming cyanobacteria are non-heterocystous and probably grow under severe nitrogen limitation. This suggests that these organisms may even lose the greater part of net photosynthesis. Bateson and Ward (1988) showed the importance of glycolate as a substrate for the microbial community in microbial mats. Glycolate may be utilized by sulphate-reducing bacteria, even in the presence of oxygen (Fründ and Cohen 1992). Glycolate-oxidizing sulphate-reducing bacteria have indeed been isolated from marine sediments (Friedrich and Schink 1993, 1995), but these organisms were strictly anaerobic.

4.5.6 Organic Compatible Solutes

Cyanobacteria exposed to high salinity or drought, accumulate osmoprotectors and extrude sodium ions through the activation or adaptation processes. These include (1) the uptake or biosynthesis of compatible solutes, (2) the active extrusion of sodium ions through the enhancement of ATPase activity, (3) modifications of the membrane lipid composition and (4) increase the energetic capacity through cyclic electron transport through photosystem I and through respiration.

In marine and hypersaline environments, micro-organisms accumulate solutes in order to obtain a sufficient turgor pressure necessary to allow cell division and growth (Taiz 1984). The cytoplasmic membrane is permeable to water and an organism that is exposed to an elevated salt concentration in the surrounding medium would tend to lose water. In order to retain water inside itself the cell can either take up ions until an osmotic equilibrium with the environment is obtained or accumulate low molecular weight organic solutes. High concentrations of inorganic ions are not compatible with the metabolism of cyanobacteria and cause inhibition of enzyme activity (Warr et al. 1984).

Cyanobacteria can be subdivided into three groups with respect to the type of organic solute they accumulate in response to osmotic stress (Reed et al. 1986a). Halotolerant freshwater cyanobacteria accumulate disaccharides (either sucrose or trehalose). Marine cyanobacteria accumulate the heteroside glucosylglycerol (2-O-α-D-glucopyranosylglycerol) and the very halotolerant hypersaline cyanobacteria accumulate quaternary ammonium compounds (glycine betaine and in one case glutamate betaine) (Mackay et al. 1984). There is no clear link between the type of solute and the taxonomic group of cyanobacteria, although all strains of *Anabaena* that were screened accumulated sucrose in response to osmotic stress. A habitat relation is suggested among species of the unicellular *Synechococcus.* Of the 33 strains investigated, all originating from freshwater environments accumulated sucrose, those isolated from marine systems accumulated glucosylglycerol and those from hypersaline habitats without exception betaine (Reed et al. 1986a). Stal and Reed (1987) screened 25 strains of cyanobacteria isolated from a microbial mat in the North Sea and found glycosylglycerol as well as trehalose and sucrose as osmolytes, suggesting no habitat relationship with the type of solute. Glucosylglycerol was nevertheless typically the dominant osmolyte in this marine ecosystem. The two dominant cyanobacteria in this mat, *M. chthonoplastes* (*Geitlerinema*) and *O. limosa* (*Lyngbya*) accumulated glucosylglycerol and trehalose, respectively. This property has been used to estimate the respective biomass of both species in these microbial mats (Stal and Reed 1987). Karsten (1996) measured the compatible solutes of a variety of strains of *M. chthonoplastes* isolated from various geographic locations and found that they contained glucosylglycerol as well as trehalose. However, the latter prevailed under sub-optimal salinities, while it appeared that the glucosylglycerol served as the only osmolyte. Betaine seems not to be limited to cyanobacteria from hypersaline environments since it has been identified in marine picocyanobacteria *Synechococcus* (Lu et al. 2006).

Although cyanobacteria normally accumulate a single low-molecular weight organic compound in response to osmotic stress, many species may produce a secondary compound. The synthesis of disaccharide is much faster than glucosylglycerol. Within 8 h of an osmotic upshock the disaccharide pool has reached 90% of its maximum, while with glucosylglycerol this is only the case after 24–48 h (Reed and Stewart 1988). Therefore the synthesis of disaccharide as secondary osmolyte may help for a quicker response to salt stress. Thus cyanobacteria that thrive under relative constant salinities may prefer glucosylglycerol while those that are exposed to fluctuating salinities may be better off with trehalose for example. This difference could explain why the pioneer in microbial mats, *Oscillatoria* (*Lyngbya*) sp., contains trehalose while the typical organism in established microbial mats, *M. chthonoplastes* contains glucosylglycerol. For the same reason hypersaline species contain sucrose in addition to betaine. Since betaine is a nitrogen-containing compound, nitrogen deficiency may also lead to the accumulation of sucrose as secondary osmolyte (Trüper and Galinski 1989). It has been shown that only glycine betaine provided a significant protection of enzyme activity against Na^+ ions, suggesting that sugars and polyols protect by a different mechanism (Warr et al. 1988).

Osmotic down shock exerted on betaine-containing *Aphanothece halophytica* resulted in the release of this osmolyte into the environment (Reed and Stewart 1988). This may have important consequences for an ecosystem such as a microbial mat because it may allow chemotrophic bacteria that cannot synthesize betaine to take it up from the environment (Reed and Stewart 1988). Moreover, betaine may serve as substrate for sulphate-reducing bacteria and the product of its metabolism, trimethylamine (TMA) is known as a so-called non-competitive substrate for methanogenic bacteria (Heijthuijsen and Hansen 1989).

Osmotic downshock in *Rivularia atra* resulted in a corresponding decrease of the osmoticum trehalose but only 10% was recovered from the medium and the rest was apparently metabolized or converted to glycogen (Reed and Stewart 1983). The glucosylglycerol accumulating strain *Synechocystis* PCC6714 and the sucrose-containing *Synechococcus* PCC6311 released 50% of their carbohydrates and over 70% of their amino acids after experiencing hypo-osmotic shock (Reed et al. 1986b). However, in some other cyanobacteria there is no evidence for the release of low molecular weight compounds upon hypo-osmotic shock (Reed and Stewart 1988). The release by cyanobacteria of low molecular weight compounds into a microbial mat would have a great impact on the ecosystem. The cellular concentration of these osmolytes is considerable and at full seawater salinity it may amount to as much 270 mM. These carbohydrates are easy accessible substrates for chemotrophic bacteria in the mat. Except in the case of a hypo-osmotic shock, which may occur in exposed microbial mats after a rain shower for instance, osmotica will also be liberated after death and lysis of the organism.

Microbial mats have often been found to evolve dimethylsulphide (DMS), a sulphur-containing organic volatile compound. It is known that DMS can be produced from dimethylsulphoniopropionate (DMSP) by microbial activity or by chemical decomposition at high pH (Kiene and Visscher 1987). DMSP occurs in a number of algae where its most likely function is that it serves as osmoprotectant (Turner et al. 1988). Vogt et al. (1998) suggested that some cyanobacteria might perhaps contain minor amounts of DMSP and Visscher and Van Gemerden (1991) suggested that *M. chthonoplastes* (*Geitlerinema*) may produce DMSP as a secondary osmolyte and could be the source of DMS in microbial mats. However, Van Bergeijk and Stal (1996) found a correlation between the number of diatoms in these mats and the amount of DMS that evolved from it. Some benthic diatoms accumulate large amounts of DMSP as osmoticum. It is possible that cyanobacteria take up DMSP from the environment (Vila-Costa et al. 2006).

4.5.7 Fermentation

When in microbial mats photosynthesis ceases, they may rapidly, sometimes even within minutes, turn anoxic. Cyanobacteria are essentially aerobic organisms that during the dark normally have a respiratory metabolism in which the endogenous storage carbohydrate glycogen is degraded (Smith 1982). When oxygen is absent, aerobic respiration is evidently not an option. Many cyanobacteria die and lysis occurs within 2–3 h after transfer to dark anoxic conditions. However, mat-forming cyanobacteria survive dark anoxic conditions for much longer time, often for several days. A number of these cyanobacteria were investigated in more detail and it was discovered that they were capable of fermenting glycogen. Fermentation as a constitutive property would have a number of advantages. Primarily, it would greatly increase the reactivity of the organism. Microbial mats are generally environments in which steep gradients of light and oxygen occur and these factors fluctuate strongly. If oxygen disappears rapidly, fermentation can immediately provide energy for maintenance, allowing the organism to survive. The excretion of fermentation products by cyanobacteria is an important process in microbial mats because it supplies other microorganisms, notably the sulphate-reducing bacteria, with substrate.

There is a great diversity of fermentation pathways in cyanobacteria. In some cases the pathways have been elucidated by the demonstration of the enzymes involved. In *O. limosa* (*Lyngbya*) the presence of the key enzymes of the homoacetate pathway has been demonstrated as well (Heyer et al. 1989).

Table 4.2 Cyanobacteria capable of fermentation (After Stal and Moezelaar 1997)

Organism	Strain, origin	Fermentation pathway	Products[a]
Anabaena azollae AaL	Symbiont from *Azolla caroliniana*	Homoacetate	Acetate (lactate, CO_2, H_2)
Anabaena azollae AaN	Symbiont from *Azolla caroliniana*	Homoacetate	Acetate (lactate, CO_2, H_2)
Anabaena azollae AaS	Symbiont from *Azolla filiculoides*	Homoacetate	Acetate (lactate, CO_2, H_2)
Anabaena siamensis As1	Paddy field	Homoacetate	Acetate (CO_2, H_2)
Cyanothece	PCC 7822 (Inst. Pasteur)	Mixed acid	H_2, ethanol, lactate, formate, acetate
Microcoleus chthonoplastes	Microbial mat	Mixed acid	H_2, ethanol, lactate, formate, acetate
Microcystis aeruginosa	PCC 7806 (Inst. Pasteur)	Mixed acid	H_2, ethanol, acetate
Nostoc sp. Cc	Symbiont from *Cycas circinalis*	Homoacetate	Acetate (lactate, CO_2, H_2)
Nostoc sp. Al2	Symbiont from *Anthoceros laevis*	Homoacetate	Acetate (lactate, CO_2, H_2)
Nostoc sp. Ef1	Symbiont from *Encephalartos ferox*	Homoacetate	Acetate (lactate, CO_2, H_2)
Nostoc sp. MAC	Symbiont from *Macrozamia lucida*	Homoacetate	Acetate (lactate, CO_2, H_2)
Nostoc sp. Mm1	Symbiont from *Macrozamia moorei*	Homoacetate	Acetate (lactate, CO_2, H_2)
Nostoc sp. M1	Symbiont from *Macrozamia* sp.	Homoacetate	Acetate (CO_2, H_2)
Nostoc sp. Gm	Symbiont from *Gunnera manicata*	Homoacetate	Acetate (lactate)
Nostoc sp. T1	Paddy field	Homoacetate	Acetate (formate, CO_2, H_2)
Nostoc sp. Bali	Paddy field	Homoacetate	Acetate (CO_2, H_2)
Oscillatoria limnetica	Hypolimnion Solar Lake	Homolactate	Lactate
Oscillatoria limosa	Microbial mat	Heterolactate Homoacetate	Lactate, ethanol, acetate
Oscillatoria sp.	Microbial mat	Not known	Lactate, ethanol, acetate, formate
Oscillatoria terebriformis	Hot spring microbial mat	Homolactate?	?
Spirulina (*Arthrospira*) *platensis*	Not known	Mixed acid	H_2, ethanol, acetate, formate, lactate
Spirulina sp.	Not known	Not known	Lactate, acetate

[a]Compounds in brackets are produced in minor quantities (From Stal and Moezelaar 1997)
?: uncertain

In the majority of pathways the Embden-Meyerhof-Parnas pathway (glycolysis) was involved in fermentation. Only the heterolactate fermentation makes use of parts of the oxidative pentose phosphate pathway. The oxidative pentose phosphate pathway is used by cyanobacteria during aerobic dark respiration and it is essentially the reverse of the reductive pentose pathway, which serves CO_2 fixation in the light (Smith 1982). In all cyanobacteria capable of fermentation the capacity for fermentation appears to be constitutive (Stal and Moezelaar 1997).

Stal and Moezelaar (1997) reviewed fermentation in cyanobacteria. Table 4.2 lists cyanobacteria capable of fermentation. The phenomenon was first discovered in *O. limnetica* (*Geitlerinema*) (Oren and Shilo 1979), which occurs in the sulphide-rich hypolimnion of Solar Lake, Sinai, and is typically adapted to anaerobic growth. In the dark this organism ferments glycogen to lactate. Since no other fermentation product was found, it was assumed that the homolactic acid pathway was used in this organism. In the non-heterocystous diazotrophic mat-building *O. limosa* (*Lyngbya*), heterolactic acid fermentation was found (Heyer et al. 1989). This organism produced equimolar amounts of lactate and ethanol from glycogen. In addition, it is capable of homoacetic fermentation, for which its osmoprotectant trehalose was used as substrate. Trehalose was degraded to 5–6 acetate and some hydrogen and CO_2. The occurrence of homoacetate fermentation in cyanobacteria is remarkable, since it further only occurs in a group of specialized anaerobic bacteria, the acetogenic bacteria. Homoacetate fermentation is energetically efficient. The occurrence of homoacetate fermentation has been proposed in a number of other cyanobacteria (De Philippis et al. 1996). The degradation of the osmoprotectant in *O. limosa* (*Lyngbya*) was another unexpected phenomenon. Trehalose represents a large amount of energy, which may be important for the organism to use under a situation of severe starvation. The question of how the organism compensates for the loss of compatible solute has not been answered, but it has been suggested that this may be through a temporary accumulation of inorganic ions such as K^+ (Stal and Moezelaar 1997). Also, the mat building cyanobacterium *M. chthonoplastes* (*Geitlerinema*) has been shown to ferment part of its osmoprotectant (Moezelaar et al. 1996). *M. chthonoplastes* (*Geitlerinema*) accumulates the heteroside glucosyl glycerol which is only degraded in cultures that contain low amounts of glycogen. Unlike in *O. limosa* (*Lyngbya*), *M. chthonoplastes* (*Geitlerinema*) possesses just one fermentation pathway. Glycogen and the glucose part of glucosyl glycerol are fermented via a mixed acid fermentation, resulting in the formation of formate, acetate, ethanol, lactate, H_2 and some CO_2. The presence of the homoacetogenic pathway allows

O. limosa (*Lyngbya*) acetogenesis from CO_2 and H_2 (Stal, unpublished observations). Acetogenesis from CO_2 has been observed in anoxic sediment (Hoehler et al. 1999).

Hydrogen is often a product of fermentation in cyanobacteria. Hydrogenases in cyanobacteria have been extensively reviewed by Tamagnini et al. (2002, 2007). Cyanobacteria possess different hydrogenases. N_2-fixing cyanobacteria produce hydrogen as a by-product of nitrogenase. Because nitrogenase obligatory produces hydrogen during N_2 fixation, aerobic N_2-fixing cyanobacteria usually possess an uptake hydrogenase. This enzyme carries out an oxy-hydrogen reaction. The third type of hydrogenase in cyanobacteria is reversible hydrogenase. This enzyme is frequently found in obligate anaerobic bacteria. Depending on the conditions it catalyses either the uptake or the production of hydrogen at approximately equal rates. Although its function in cyanobacteria has been debated for some time, reversible hydrogenase plays an important role in fermentation (Stal and Moezelaar 1997). Hydrogen concentrations are kept low in microbial mats because sulphate reducing bacteria, acetogenic bacteria, methanogenic bacteria and anoxygenic phototrophic bacteria use it as energy source and/or as electron donor (Hoehler et al. 2002). The escape of reduced gases from microbial mats into the atmosphere may have contributed to the oxygenation of the oceans on early earth (Hoehler et al. 2001).

Elemental sulphur may serve as electron acceptor in cyanobacteria. Many cyanobacteria have been shown to be able to reduce elemental sulphur to sulphide. It has been suggested that this process in *O. limnetica* (*Geitlerinema*) might represent a form of anaerobic respiration (Oren and Shilo 1979). However, in other cyanobacteria the advantage of the reduction of sulphide is probably that it serves as electron sink, allowing the formation of more oxidized product (acetate) which results in a higher amount of substrate phosphorylation.

Stal and Moezelaar (1997) have discussed the bioenergetics of fermentation in a number of different cyanobacteria for which sufficient information is available. Although evidently the amount of energy that is generated during fermentation is low, calculations showed that it usually exceeded the minimum amount required for maintenance. This remaining energy could potentially drive metabolic processes. For instance, *O. limosa* (*Lyngbya*) is even capable of maintaining a considerable rate of N_2 fixation under anaerobic conditions in the dark (Stal and Heyer 1987).

Hot spring microbial mats consisting of *Synechococcus* switched on a variety of genes involved in fermentation when in the dark and anaerobic conditions although the fermentation products were not identified (Steunou et al. 2006). This fermentation supported the fixation of N_2 that occurred in the dark in these mats. Anderson et al. (1987) investigated the fate of representative fermentation products (acetate, propionate, butyrate, lactate, and ethanol) in hot spring cyanobacterial mats. Fermentation occurred mainly in the top 4 mm of the mat. In the light, filamentous bacteria resembling *Chloroflexus aurantiacus* photoassimilated the fermentation products. In the dark under anaerobic conditions, only lactate was oxidized and also the extended incubation under these conditions did not enhance the metabolism of acetate, propionate, or ethanol. Acetogenic bacteria converted butyrate into acetate. In mats occurring at temperatures ranging from 50°C to 70°C acetate and propionate accumulated under dark anaerobic conditions.

4.5.8 Extracellular Polymeric Substances (EPS)

Extracellular polymeric substances are important components of microbial mats. They are involved in the attachment of cyanobacteria to the substrate and are essential structuring molecules by producing a matrix in which the organisms are embedded (Decho 2000). This polymeric matrix fulfils a number of other important functions in microbial mats, which will be discussed below.

Cyanobacterial exopolysaccharides are complex molecules composed of 6 or more different monosaccharides out of a suite of at least 12 sugars (De Philippis and Vincenzini 1998). The variety of linkages that is possible gives a broad range of possible structures. Cyanobacteria produce polysaccharides which can be roughly categorized in three groups: (i) endogenous polysaccharides that serve as storage compounds; (ii) cell envelope polysaccharides; (iii) extracellular polysaccharides. The endogenous polysaccharides in cyanobacteria can be found in the so-called α-granules, which are composed of a branched glycogen-like polymer. This polymer consists of α(1–4) and α(1–6) linked glucose molecules. The cell envelope consists of the cell wall polysaccharides and the external layers (glycocalyx). The glycocalyx can be subdivided in (i) the well-structured polysaccharide sheath, (ii) a polysaccharide capsule which extends outside the sheath but is clearly associated with the organism and is less structured and (iii) mucilage polysaccharide. The latter is not or very loosely associated with the organism. In fact, the polysaccharides that form the glycocalyx should all be considered as extracellular polysaccharides or exopolysaccharides (Fig. 4.16). Exopolysaccharides that are released into the surrounding environment may be the colloidal suspended molecules originating from any of the glycocalyx components. The different fractions are often poorly defined and mostly based on the different extraction procedures. Relatively little is known about cyanobacterial exopolysaccharides and their biosynthetic pathways are complex and not well known (Pereira et al. 2009).

Microbial exopolymers, including those produced by cyanobacteria, are high molecular weight mucous secretions that often have a complex structure. The molecular weight is

often more than 100,000 Da. Although polysaccharides are quantitatively the most important part of these exopolymers, other components are present as well. Proteins make up a significant part of the exopolymers (Decho 1990). These polymers are also known as extracellular polymeric substances (EPS). The composition and structure of EPS vary widely among different microorganisms (Tago and Aida 1977; Bertocchi et al. 1990; Decho 1994; Stal 1994) and even one single strain may produce more than one type EPS simultaneously or at different stages of growth (Christensen et al. 1985). Most of the polysaccharides in EPS are heteropolysaccharides that are composed of a variety of different monosaccharides, arranged in repeating units. EPS often contain uronic acids such as D-glucuronic acid, D-galacturonic acid and D-mannuronic acid. These are important functional groups because they contain carboxyl groups that are responsible for interactions with other EPS molecules or the binding of metals. However, other types of EPS are composed of neutral sugars. Extracellular polymeric substances may be hydrophilic or hydrophobic. Many are hydrophilic and may contain over 95% water by weight (Decho 1994). Depending on the chemical composition and the functional groups present, the tertiary structure of EPS is determined. The tertiary structure of EPS determines whether it is a cohesive gel or in a colloidal form. An intermediate form could be described as nonconsolidated mucilage (Decho 1994). The tertiary structure of EPS not only depends on the chemical composition but also strongly on temperature. Microbial mats and intertidal mudflats during emersion are subject to large variations in temperature and this will thus affect the cohesiveness and rheological properties of the sediment.

A large number of functions have been ascribed to EPS (Decho 1990). These include adhesion and immobilisation of the organism, protection against desiccation, protection from grazing, protection from toxic substances, scavenging of trace metals, and (anti-) calcification. Some of these functions will be discussed below as far as they are relevant to microbial mats.

Organisms in microbial mats are often subject to desiccation. EPS may retain large amounts of water and form a gel that stabilizes the macromolecular components and the cell structure of the cyanobacteria and organisms that produce it may overcome long periods of drought by forming hydrogen bonds with proteins, membrane lipids and DNA, thereby replacing the water shell surrounding these cell constituents (Caiola et al. 1996; Potts 1994). Some cyanobacterial EPS may be hydrophobic due to the presence acetyl-groups, peptide moieties or desoxysugars which determine the emulsifying properties and the rheological properties (Neu 1992). Particularly, EPS containing uronic acids or hydrophobic proteins may be important for micro-organisms, including cyanobacteria and diatoms, enabling these to attach to surfaces (Robins et al. 1986). For benthic organisms, it is important to stay on surfaces when conditions are optimal for growth. Some cyanobacteria are capable of modifying EPS from hydrophobic to hydrophilic and they may thus detach from a surface when conditions become inappropriate (Bar-Or et al. 1985). Benthic communities of diatoms may attach to the surface of intertidal mudflats by the production of hydrated and hydrophilic exopolymers during periods of emersion. During immersion, these polymers go into solution releasing the diatoms into the water column (Talbot et al. 1990). Benthic cyanobacteria may secrete flocculants, exopolymers that produce flocs with detritus and other material in the overlying water. These flocs eventually sediment, thereby clearing the overlying water and hence improving the conditions for these benthic phototrophs (Bar-Or and Shilo 1987, 1988).

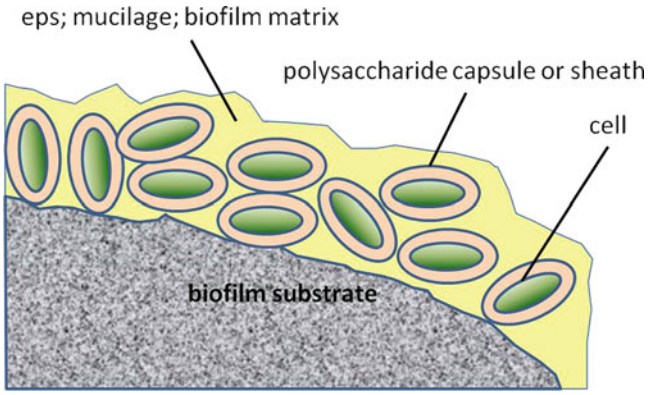

Fig. 4.15 Capsular and slime extrapolymeric substances (*EPS*) and the formation of a microbial mat

Mat-forming cyanobacteria that excrete EPS produce a matrix that stabilizes the sediment (Fig. 4.15). This is also the case with benthic films of diatoms that grow on intertidal mudflats (Paterson 1989; Stal 1994; Yallop et al. 1994). In the desert, hydrophobic EPS of microbial crusts cause the run-off of water preventing erosion (Mazor et al. 1996; Kidron et al. 1999).

Uronic acids are important components of EPS because these charged groups interact with sediment particles. Thus, EPS with a large content of uronic acids are more efficient in the stabilization of sediments (Martin 1971; Stal 1994). Sulphated groups and uronic acids contribute to the anionic nature of exopolysaccharides which determines the sticky properties of these molecules. EPS may also contain sulphated sugars. As the uronic acids, sulphate groups are also important for the tertiary structure of the polysaccharide and influence the stability of the microbial mat matrix (Decho 1990). Uronic acids as well as sulphate groups interact with a variety of metals. This may either result in the immobilization of toxic metals or scavenge trace metals that are important nutrients. The uronic acid groups of polysaccharides may be involved in the regulation of calcification. Sulphated polysaccharides are often encountered in algae but rarely in archaea and bacteria,

including cyanobacteria (Bertocchi et al. 1990). Nevertheless, sulphated polysaccharides have been found in cyanobacteria (Tease et al. 1991; Ortega-Calvo and Stal 1994) and a more thorough investigation of mat-forming cyanobacteria may reveal that such polysaccharides are more common in this group of organisms as previously thought (Pereira et al. 2009).

The polysaccharide produced by the mat-forming cyanobacteria fulfils an important function as a matrix for exoenzymes, plasmids and DNA (Decho 1990). Extracellular DNA is protected from DNases in the sediments (Romanowski et al. 1991), and may give rise to natural transformation in these ecosystems (Lorenz and Wackernagel 1990, 1994). Hence, in microbial mats, gene exchange may take place.

A considerable number of functions can be attributed to EPS but it is not clear what controls the formation of this polysaccharide and how this relates to one or more of the possible functions. It may very well be that the production of mucilage by cyanobacteria is the result of unbalanced growth caused by nutrient deficits (Lange 1976). Particularly a shortage or deficiency of nitrogen and sulphur results in the stagnation of protein synthesis while the full photosynthetic capacity remains. Under such conditions cyanobacteria accumulate large amounts of glycogen (Allen and Smith 1969; Lehmann and Wöber 1976). The capacity of the cell to store glycogen is limited and any additional polysaccharide may be excreted as mucilage. Old starved cultures often become viscous as a result of excess mucilage production. In the modern marine stromatolites of Highborne Cay, Bahamas, the maximum EPS production represented 7% of the total CO_2 fixation while most the fixed carbon was released as low-molecular weight dissolved organic carbon.

Little is known about the fate of EPS in mats. Some polysaccharides appear to be recalcitrant to microbiological degradation, whereas others are not. EPS that is newly formed in a microbial mat may be transformed rapidly (within 12 h) through the degradation by heterotrophic organisms, particularly by sulphate reducing bacteria (Decho et al. 2005). This is interesting because sulphate reducing bacteria are thought to degrade preferentially low-molecular weight organic compounds such as acetate, lactate and ethanol. The degradation of EPS was incomplete causing the accumulation of a more-refractory remnant polymer that was enriched in nitrogen. Net production of EPS in the Highborne Cay stromatolites was less than 2% of the total inorganic carbon uptake (Decho et al. 2005). A similar model of the origin of different fractions of EPS has been proposed for diatom biofilms (Stal 2010). In this model diatoms were supposed to produce one type of EPS which was enriched in glucose. Degradation and the preferential utilization of the glucose component left an EPS that was relatively depleted in glucose and enriched in uronic acids which was refractory and accumulated in the biofilm.

4.6 Calcification in Mats and Stromatolites

The biological control over calcium carbonate precipitation in the ocean leads to overproduction. It is estimated that 5 Gt calcium carbonate is annually produced in the ocean of which 3 Gt is removed from the system by incorporation and accumulation in sediments, while the other 2 Gt is dissolved (Milliman 1993). The weathering of rock on the continents causes a continuous runoff of calcium and carbonate into the sea. Therefore the oceans tend to be supersaturated with calcium carbonate. In order to maintain a steady state, the amount of calcium carbonate removed from the oceans must be the same as that entering. However, it is estimated that twice as much calcium is removed from the ocean by calcium carbonate precipitation than is brought in (Milliman 1993). This means that the ocean is not in equilibrium or that, sources and sinks are respectively under- or overestimated. The equilibrium of calcium carbonate in the oceans could be maintained by the dissolution of the excess calcium carbonate. Part of this dissolution is biologically controlled because it acts as a pH buffer for respiratory and fermentative processes. Another part dissolves in the deep sea, which is under saturated with calcium carbonate. Some calcium carbonate leaves the system by sinking as fecal pellets to the ocean floor or by fast burial. Although the surface waters of the ocean are supersaturated with calcium carbonate spontaneous precipitation does not normally occur.

Calcification is responsible for the lithification of microbial mats and is the basis of the formation of stromatolites. In most cases calcification seems under a stringent biological control, but the mechanisms by which living organisms influence the precipitation of calcium carbonate are poorly understood. Whereas the function of calcium carbonate precipitation in many organisms is obvious (e.g. shell or skeleton formation) this is not the case in microorganisms, including algae and cyanobacteria. Calcification in bloom-forming algae such as the coccolithophore *Emiliania huxleyi* would predominantly serve the production of CO_2 for subsequent photosynthetic fixation:

$$2HCO_3^- + Ca^{2+} \leftrightarrow CaCO_3 + CO_2 + H_2O$$

The so-called 'whitings', clouds of aragonite needles, that often occur in tropical lagoons and that have been considered as inorganic precipitates may be produced by the photosynthetic activity and CO_2 fixation of dense communities of picoplankton that increase carbonate ion (Robbins and Blackwelder 1992). Calcification in microbial mats may serve as a mechanism of producing CO_2 or it results from an increase in the concentration of the carbonate ion. Due to the dense phototrophic biomass and high rates of photosynthesis and the alkaline conditions it is likely that mats become

depleted in CO_2. Another function that has been proposed is the detoxification of intracellular calcium. But whatever the function, calcification can be generally inferred from the changes in the concentration of inorganic carbon and from the low solubility product of calcium carbonate.

Calcium carbonate is rather insoluble. Aragonite, which is often thought to be a product of biological calcium carbonate precipitation, has a solubility product (K_{sp}) of $10^{-6.19}$, and the more stable form calcite $10^{-6.37}$ (at 25°C and salinity of 35) (Zeebe and Wolf-Gladrow 2001). This means that when the ion activity product (IAP) (molar concentrations multiplied by their activity coefficients) of $\{Ca^{2+}\}$ and $\{CO_3^{2-}\}$ in a solution exceeds $10^{-6.19}$ calcium carbonate is saturated, although several-fold supersaturation is normally required for spontaneous precipitation (Arp et al. 2001).

$$\left\{Ca^{2+}\right\} + \left\{CO_3^{2-}\right\} \rightarrow CaCO_3$$

The concentration of calcium-ion in seawater is about 10^{-2} M (Ehrlich 1996). When the concentration of calcium ion is assumed to be constant, then the carbonate concentration determines calcification. CO_2 reacts with water to form bicarbonate and this dissociates according to the following reversible reactions:

$$CO_2 + H_2O \leftrightarrow H^+ + HCO_3^-$$

$$HCO_3^- \leftrightarrow H^+ + CO_3^{2-}$$

Typical physicochemical processes that cause calcification are degassing of CO_2 and evaporation of water due to solar radiation. Evaporation leads to the formation of brines in which minerals precipitate, of which calcium carbonate is only a minor component (Yechieli and Wood 2002). In hot spring microbial mats degassing of CO_2 shifts the equilibrium towards the carbonate ion and to the formation of travertine deposits (Fouke et al. 2000).

In microbial mats, a number of biological processes influence the equilibriums of carboxy species and hence may control calcification (Dupraz et al. 2009). These include specific metabolic processes in which CO_2 is consumed such as photosynthesis, chemosynthesis and, less importantly, heterotrophic CO_2 fixation. Metabolisms in which CO_2 is produced such as respiration and fermentation may cause an acidification of the medium and eventually result in dissolution of calcium carbonate rather than precipitation. Nevertheless, Krumbein (1974) demonstrated the formation of aragonite on the surface of marine bacteria as the result of their metabolism of substrates such as glucose, sodium acetate and sodium lactate. However, this also strongly depends on the environmental conditions that apply (Canfield and Raiswell 1991). A variety of metabolic processes influence the equilibrium of inorganic carbon by the production of acids and bases.

Photosynthesis is an important process in the vast majority of microbial mats, at least in those occurring in illuminated environments. Because photosynthesis normally involves CO_2 fixation it has often been considered important for calcification (Golubić 1973; Krumbein and Giele 1979; Pentecost 1988). The fixation of CO_2 from a bicarbonate solution will result in an increase in carbonate ion:

$$2HCO_3^- \leftrightarrow CO_2 + H_2O + CO_3^{2-}$$

Other important metabolic processes in microbial mats which remove CO_2 are chemosynthesis and heterotrophic CO_2 fixation. Organisms that carry out chemosynthesis include colourless sulphide oxidizing bacteria, nitrifying bacteria, autotrophic sulphate-reducing bacteria as well as methanogenic- and acetogenic bacteria. Heterotrophic CO_2 fixation occurs in virtually all organisms but is limited and negligible compared to the CO_2 produced during the oxidation of organic compounds. This process is therefore not important for calcification. Many reports mention calcification as a result of photosynthesis by cyanobacteria (e.g. Golubić 1973; Krumbein and Giele 1979; Pentecost 1988; Pentecost and Bauld 1988). Calcification associated with anoxygenic photosynthesis or anoxygenic phototrophic bacteria has only been reported in one case in which the purple non-sulphur bacterium *Rhodopseudomonas palustris* was shown to stimulate calcification in a solution that was oversaturated with calcium carbonate (Bosak et al. 2007). Phototrophic sulphur bacteria produce sulphuric acid and they will therefore cause calcium carbonate dissolution rather than its precipitation although anaerobic sulphide oxidation has been reported to be involved in calcium carbonate precipitation in a marine stromatolite (Visscher et al. 1998). The same holds true for most chemosynthetic metabolisms and for heterotrophic CO_2 fixation. Precipitation of calcium carbonate may be indirectly associated with oxygenic photosynthesis and due to an increase of pH and/or a shift in the equilibrium of inorganic carbon (Golubić 1973; Krumbein and Cohen 1977). This was elegantly demonstrated in a hypersaline microbial mat (Ludwig et al. 2005). These authors showed that calcification was solely due to the increase of the carbonate ion as the result of photosynthesis and that the changes in the activity of calcium were not important. The contribution of heterotrophic bacteria was indirect as these organisms kept the concentration of dissolved inorganic carbon high in the pore water. Sulphate reducing bacteria did not change the pH and their effect was solely maintaining high concentrations of dissolved inorganic carbon. On the other hand, Chafetz and Buczynski (1992) found that calcification in stromatolithic microbial mats was associated with heterotrophic bacteria rather than

with the cyanobacteria and these seemingly contradictory observations emphasize the complexity of the process and the fact that the actual environmental conditions may cause quit different outcomes.

Aerobic or anaerobic oxidation of organic compounds results in the production of CO_2 and/or HCO_3^- and affects pH and consequently causes a shift in the equilibrium of inorganic carbon. Organic matter possessing the "Redfield" stoichiometry of C:N:P of 106:16:1 is oxidized by O_2, NO_3^- and SO_4^{2-} according to the following reactions (Boudreau and Canfield 1993):

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 138O_2 \rightarrow 122H_2O + 106\,CO_2 + 16\,NO_3^- + 16H^+ + H_3PO_4$$

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 94.4NO_3^- \rightarrow 84.8H_2O + 13.6\,CO_2 + 92.4\,HCO_3^- + 55.2N_2 + H_3PO_4$$

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 53SO_4^{2-} \rightarrow 106\,HCO_3^- + 53H_2S + 16\,NH_3 + H_3PO_4$$

The formation of CO_2 and the acidification of the medium could result in the dissolution of calcium carbonate rather than cause its precipitation. Anaerobic respiration results in the formation of bicarbonate and could give rise to supersaturation of calcium carbonate. The effects of the sequential oxidation of organic matter by the three electron acceptors oxygen, nitrate and sulphate on pore water pH and calcium carbonate saturation are complex and depend on the prevailing conditions (Boudreau and Canfield 1993).

In the microbial mats of Solar Lake (Sinai), it has been shown that sulphate reduction and $CaCO_3$ formation were stoichiometrically related and organic carbon was transformed into a number of different carbonate minerals (Jørgensen and Cohen 1977; Krumbein and Cohen 1977; Krumbein et al. 1977). However, whether sulphate reduction in microbial mats in reality results in calcium carbonate precipitation depends largely on a variety of conditions that prevail in these microbial mats. Most important is the development of alkaline conditions, the removal of excess CO_2 or the presence of a suitable buffer (Ehrlich 1996). The precipitation of sulphide as iron sulphide acts as a pH buffer. In the absence of iron, sulphate reduction produces equal amounts of H^+ and HCO_3^-, which will thus cause a decrease of carbonate saturation. In many microbial mats high rates of sulphate reduction occur but despite this, calcification is absent. In the modern stromatolites of the Exuma Cays the tightly associated sulphate reduction and anaerobic sulphide oxidation promoted calcification, while the couple oxygenic photosynthesis and aerobic respiration cause calcium carbonate dissolution (Visscher et al. 1998).

Modern microbial mats are often considered as the structural analogues of Precambrian stromatolites. By definition stromatolites are lithified laminated formations. Precambrian stromatolites were formed in shallow marine areas. Lithification of present day coastal microbial mats is extremely rare and it is still an enigma why this should be so. Kempe and Kazmierczak (1990a, b) and Kazmierczak et al. (1996) investigated stromatolites in the sea-linked Satonda Crater Lake in Indonesia and alkaline Lake Van in Turkey, both formed under extreme alkaline conditions. They hypothesized that the greater abundance of stromatolites during the Precambrian should be attributed to the much greater alkalinity of the marine environment during that era (Kempe and Kazmierczak 1990a). The hypothesis of a Precambrian soda ocean may certainly offer an explanation for the greater abundance of stromatolites and the discovery of modern calcifying stromatolites in alkaline seas supports this. Nevertheless, calcification in these stromatolites is still under biological control rather than being a spontaneous occurrence. Moreover, other recent stromatolites are formed under less alkaline or normal marine conditions such as those found in the French Polynesian atolls (Défarge et al. 1994a, b) or in the Bahamas (Reid and Browne 1991). Even if the early oceans were more alkaline, the marine environment today is still supersaturated with calcium carbonate. Furthermore, in microbial mats several biological processes predominate which presumably increase the concentration of carbonate ion, which theoretically should lead to calcium carbonate precipitation. As a result of active photosynthesis and CO_2 fixation in the top layer of cyanobacterial mats the pH in these mats may reach values as high as 9.5 (Fig. 4.3) (Revsbech et al. 1983). Although these conditions would normally promote calcification, this does not happen in most marine microbial mats.

There is also abundant evidence of non-lithifying Proterozoic microbial mats that are recognized as microbially induced sediment structures (MISS), suggesting that the past may not have been so different from the present (Noffke et al. 2006; Noffke 2009). An experiment in which a non-lithifying mat was transplanted into an environment with lithifying microbial mats showed that it was now capable of calcification (Dupraz et al. 2009). Kremer et al. (2008) showed abundant calcification in an otherwise typically non-lithifying coastal microbial mat. This indicates that calcification is probably not so unusual and that the absence of lithification of these mats may be due to a subsequent dissolution of the calcium carbonate.

Hence, spontaneous calcification does not seem to be important in stromatolites. Biological control of calcification may not only exist in the change of the carbonate ion concentration and equilibrium but also in a mechanism that inhibits calcification (anti-calcification) (Westbroek et al. 1994). Biologically controlled calcification must distinguish

between supersaturation of calcium carbonate in a solution (which is the thermodynamic force) and those factors that influence the kinetics of the process. The latter may be either inhibitory or stimulatory factors. Supersaturation of calcium carbonate in the ocean is the primary driving force of calcification that can be dramatically increased in the immediate vicinity of phototrophic organisms. Because uncontrolled calcification in or around organisms is unwanted it may be clear that some mechanism must exist that can inhibit the process. Crystal poisons such as Mg^{2+}, SO_4^{2-}, PO_4^{3-} that complex with CO_3^{2-} and Ca^{2+}, respectively, are not sufficient and additional mechanisms must be postulated. It is known that some small acidic molecules may inhibit crystallization. An example is the binding of Ca^{2+} to oxalate (Verrecchia et al. 1990). Acidic polysaccharides are also very effective in binding Ca^{2+} or interact with it (Dupraz and Visscher 2005; Braissant et al. 2009). These interactions will doubtless influence calcification. Figure 4.16 depicts the way in which such polysaccharides could influence crystallization or crystal growth (Westbroek et al. 1994). The association of a polyanion with Ca^{2+} ions will lower the activity of the latter below the saturation of calcium carbonate and prevent subsequent crystallization. Likewise, polyanions may associate with a growing calcium carbonate crystal and prevent its further growth. A layer of charged polymers may bind calcium carbonate crystal and arrest its growth. The structure of such polymers may determine crystal shape. Evidence has been obtained that this mechanism is involved in the formation and morphology of coccoliths in the coccolithophore *Emiliania huxleyi* (Borman et al. 1982, 1987).

In microbial mats it is hypothesized that extracellular polymeric substances (EPS) which are mainly composed of polysaccharides serve as agents that inhibit calcification. EPS produced by cyanobacteria are often rich in uronic acids and contain other acidic groups such as pyruvate, succinate, sulphate and phosphate groups and hence are negatively charged polyanions (Bertocchi et al. 1990; De Philippis et al. 2001; Sutherland 2001). Many microbial mats are composed of vast amounts of EPS in which the cyanobacteria and other organisms are embedded. It is possible that this EPS acts as an anti-calcification agent. Cyanobacterial EPS may bind 55–183 mg Ca g^{-1} EPS (Li et al. 2001; Ortega-Morales et al. 2006). When heterotrophic bacteria decompose this EPS, high concentrations of calcium carbonate may exist locally, leading to precipitation (Dupraz and Visscher 2005). In some non-lithifying microbial mats such as in Solar Lake (Sinai), aragonite needles are formed in the deeper layers of the mat,

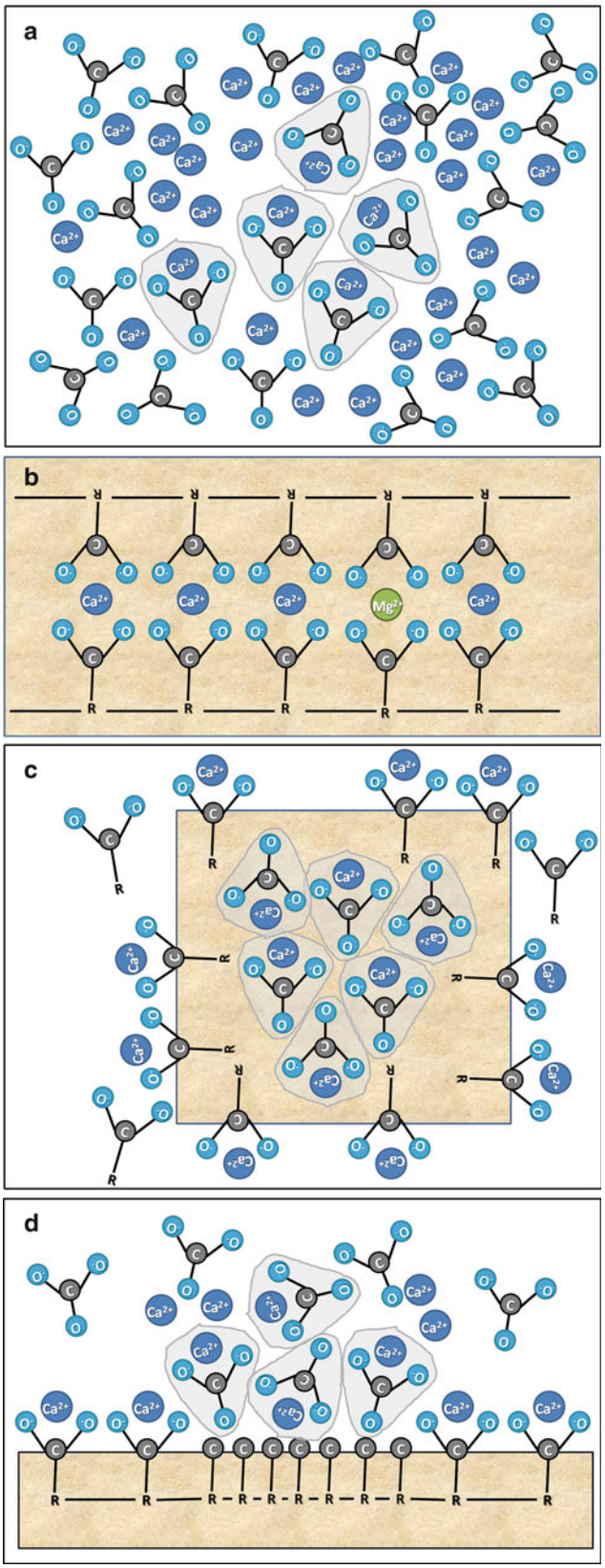

Fig. 4.16 Simplified model of the possible interactions of charged extracellular polymeric substances with calcium carbonate: (**a**) nucleation of calcium (Ca^{2+}) and carbonate (CO_3^{2-}) ions; (**b**) Inhibition of nucleation by a polyanion; (**c**) Inhibition of crystal growth by association of a crystallization nucleus with a polyanion; (**d**) Calcium carbonate crystal bound to a layer of charged polymers. The growth of the crystal may be arrested and the charged polymer may determine the eventual shape of the calcium carbonate crystal

where the organic matter is subject to degradation. Several others have observed the association of calcification with bacterial activity (Chafetz and Buczynski 1992; Krumbein 1979; Krumbein and Giele 1979).

Mucilage EPS is often produced by cyanobacteria as an overflow metabolism when they experience nutrient limitation. This is particularly the case under nitrogen depleted conditions, a situation common in the marine environment. In microbial mats, where extremely dense communities of cyanobacteria are present there is a high demand for nitrogen, while there is often a shortage of this important nutrient. Therefore, many microbial mats are diazotrophic, i.e. the cyanobacteria that build these mats fix atmospheric dinitrogen. However, most diazotrophic mats consist of non-heterocystous cyanobacteria. As argued in the section on N_2 fixation these cyanobacteria are not efficient N_2 fixers because the process is seriously hindered by oxygen. It is likely that such cyanobacteria in fact still are nitrogen limited. Cyanobacteria that grow under nitrogen limited conditions tend to produce a lot of mucilage (Ortega-Calvo and Stal 1994). It is possible that Precambrian calcifying microbial mats were not nitrogen-limited and that the growth of the organisms therefore might have been balanced with less overflow metabolism and mucilage production. This might also hold true for modern calcifying microbial mats. While some of these mats may rely on a sufficient external supply of combined nitrogen, others comprise heterocystous N_2-fixing cyanobacteria that satisfy their nitrogen demand. One example from freshwater environments is the Rivulariaceae. This group of heterocystous cyanobacteria produces extent calcium carbonate formations (Whitton 1987). The marine cyanobacterium *Calothrix* spp. belongs also to this taxonomic group and is known in some cases to form microbial mats but usually does not calcify.

A model for calcification and the development of stromatolites in the Exuma Cays, Bahamas is presented in Fig. 4.16. Subtidal and intertidal stromatolites that can be found in the Exuma Cays (Bahamas) are characterized by mats of the cyanobacterium *Schizothrix* sp. The model for calcification in these mats is based on a number of observations and assumptions. Two types of mats of *Schizothrix* can be distinguished. Lithifying microbial mats of *Schizothrix* are usually characterized by low ratios of photosynthesis over respiration while the opposite is true non-lithifying mats (Pinckney et al. 1995). Moreover, calcium carbonate was not associated with the cyanobacteria but with heterotrophic bacteria (Chafetz and Buczynski 1992; Chafetz 1994). It was further assumed that extracellular polymeric substances (EPS) may interfere by binding calcium and magnesium ions, thus locally inhibiting carbonate precipitation (Borman et al. 1982, 1987; Westbroek et al. 1994).

Exuma Cays stromatolites are formed at high energy sites. Intertidal stromatolites can be found on the Atlantic Ocean coast and are exposed to high wave energy. Subtidal stromatolites are almost exclusively encountered in channels with high currents. The cyanobacterium *Schizothrix* sp. is a filamentous organism composed of thin (often less than 1 μm wide) trichomes which are enveloped by a thin rigid polysaccharide sheath. This organism is capable of colonizing a solid substrate. Because grazing pressure will be low in these high-energy areas, a community of *Schizothrix* sp. may develop. Under conditions of low sedimentation rate a mat of *Schizothrix* sp. and associated microorganisms will develop (Fig. 4.17a). These mats are rigid and tightly associated with the underlying substrate. The cyanobacteria will grow and produce sheath material and possibly some mucus. It is assumed that this EPS will bind Ca^{2+} or that uronic acids prevent further growth of crystallization nuclei (Borman et al. 1982). During a period of sedimentation, *Schizothrix* sp. will move rapidly upwards by phototaxis and continue growth in the top layers where optimum light conditions prevail. The trichomes form a dense network in which carbonate sand grains (ooids) are trapped and agglutinated by EPS, while Ca^{2+} is further bound. Empty sheaths and other organic matter that has been abandoned deeper in the sediment will be decomposed (Fig. 4.17b). During a subsequent period of low rates of sedimentation a dense mat of *Schizothrix* sp. will develop in the top layer of the sediment. This layer is characterized by active growth of the cyanobacteria and may be associated with abundant production of EPS. The matrix of EPS in which the mat is embedded may bind Ca^{2+} efficiently and condenses EPS to the gel state (Rees 1969; Decho and Moriarty 1990; Decho 1994). It is conceived that this will lower the activity of this ion so that calcium carbonate will not precipitate. Depending on the physicochemical gradients that typically develop in microbial mats due to phototrophic and heterotrophic activities, some dissolution and re-precipitation of $CaCO_3$ and re-crystallization of the carbonate sediment grains may occur (Fig. 4.17c). During the next stage of development, *Schizothrix* sp. moves upward after another period of high rate of sedimentation. While growth of the cyanobacterium and the production of EPS in the new top layer trap and agglutinate the carbonate sand, the large amount organic matter that was left behind is decomposed by heterotrophic bacteria. Because EPS is also decomposed, Ca^{2+} that was bound will be released (Decho et al. 2005). This will locally result in supersaturation of calcium carbonate resulting in the formation of a microcrystalline crust of precipitated carbonate (Fig. 4.17d). A similar type of bacterial calcification occurs during the degradation of calcium oxalate. Oxalate is a product of metabolism of fungi and other organisms and is capable of immobilizing calcium (Verrecchia et al. 1990). In the Exuma Cay stromatolites another type of biologically influenced calcification occurs. The unicellular cyanobacterium *Solentia* bores in the calcium carbonate grains, dissolving them partly and the subsequent precipitation fuses the ooids, forming a solid lithified structure (Reid et al. 2000). This model explains that *in situ* calcium carbonate precipitation and lithification of the mat is

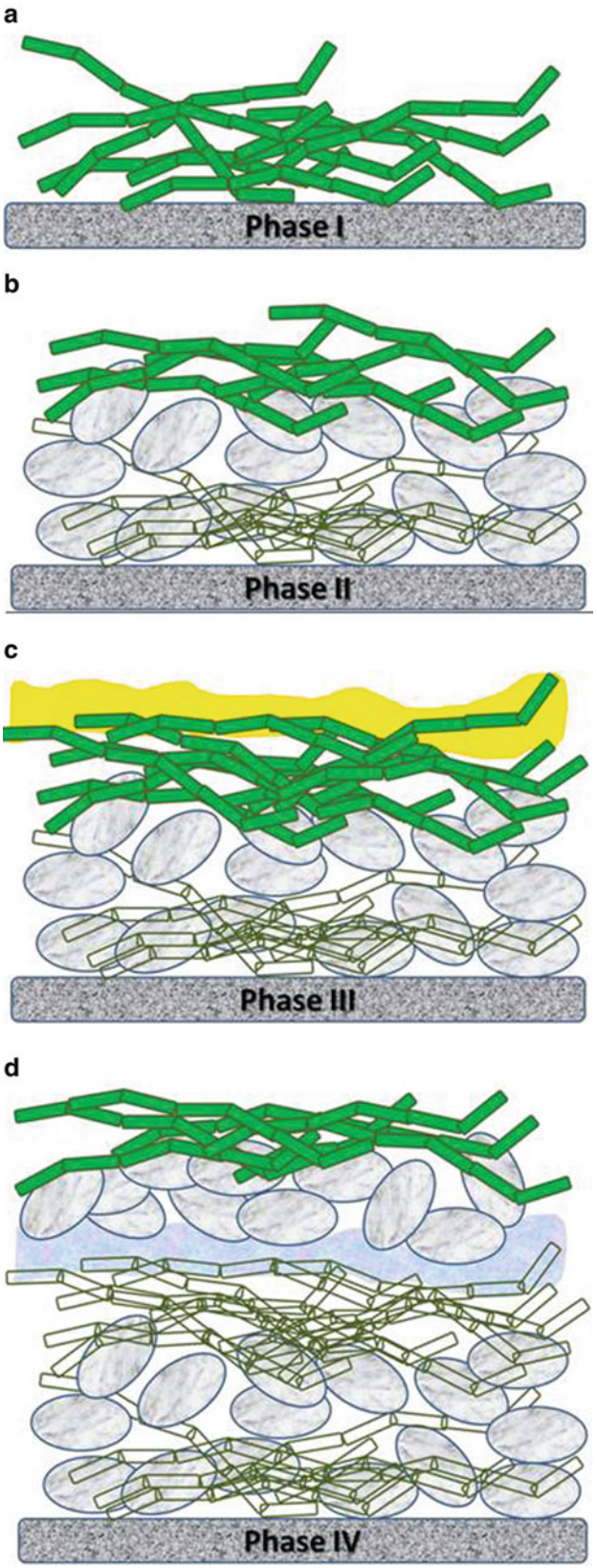

Fig. 4.17 Simplified scheme of the development of lithified micritic layers in Bahamas stromatolites. For explanation, see text

controlled by biology. It is indirectly associated with the cyanobacteria but degradation of organic matter, notably EPS, by heterotrophic bacteria is required for this process. Alternating periods with high and low rates of sedimentation are responsible for the formation of the laminated structure of the lithified microbial mats, which could therefore be termed stromatolites.

4.7 Nitrogen Metabolism and Nitrogen Fixation

4.7.1 Introduction

In cyanobacteria nitrogen content may amount up to about 10% of dry weight and is quantitatively the third most abundant element. Any shortage of it will immediately affect the amount of phycobiliproteins and, consequently, the efficiency of light harvesting for photosynthesis (Allen and Smith 1969). Cyanobacteria may produce a unique nitrogenous compound known as cyanophycin or multi-L-arginyl-poly(L-aspartic acid). Its high nitrogen content means that it can serve as a nitrogen reservoir (Mackerras et al. 1990a, b).

Cyanobacteria use a variety of nitrogen sources (Flores and Herrero 1994). Ammonia can be taken up by passive diffusion or the protonated form ammonium (NH_4^+) by a specific uptake system (Fig. 4.18). The amino acids arginine,

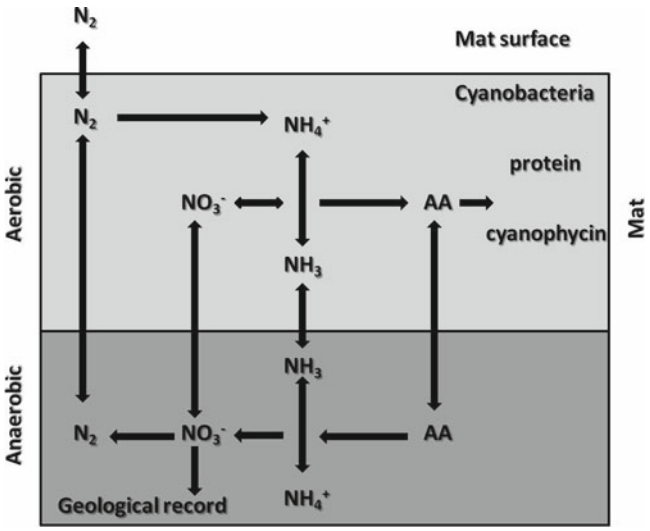

Fig. 4.18 The nitrogen cycle in a cyanobacterial mat. Cyanobacteria take up and assimilate ammonium into amino acids (*AA*) which are used for protein synthesis or can be stored as cyanophycin. Amino acids and ammonia may leak out the cells and oxidized to nitrate (nitrite) by nitrifying bacteria. In the anoxic part of the mat ammonium and nitrate (nitrite) is converted to dinitrogen by anammox and dinitrifying bacteria. Nitrate (nitrite) can be taken up by the cyanobacteria and assimilated. N_2-fixing cyanobacteria produce ammonium which is subsequently assimilated into cell material

asparagine and glutamine have been reported to serve as nitrogen sources in cyanobacteria (Flores and Herrero 1994). Nitrate and nitrite are important sources of nitrogen for cyanobacteria. This involves the uptake of nitrate or nitrite and its subsequent reduction to ammonia. This process involves ferredoxin as an electron donor and is therefore intimately associated with photosynthesis. Urea appears also to be a common nitrogen source for cyanobacteria (Moore et al. 2002; Valladares et al. 2002). Many cyanobacteria are capable of using dinitrogen (N_2) as the source of nitrogen.

4.7.2 The Nitrogen Cycle

Nitrogen occurs in different chemical oxidation states, varying from its most reduced form ammonia (NH_3) (−3), to hydroxylamine (NH_2OH) (−1), dinitrogen (N_2) (0), nitrous oxide (N_2O) (+1), nitric oxide (NO) (+2), nitrite (NO_2^-) (+3) to its most oxidized form nitrate (NO_3^-) (+5). All of these oxidation states are biologically significant and microorganisms may carry out reduction and oxidation reactions transforming one form into another. The element nitrogen therefore is subject to microbiological cycling in nature. In microbial mats all steps of the nitrogen cycle may be present and cyanobacteria play a particular important role (Fig. 4.17).

Ammonia is assimilated into amino acids that are used for the synthesis of proteins. Luxury uptake of nitrogen may occur and be stored as cyanophycin. Ammonia and amino acids may leak out of the cell. When oxygen is present, ammonium may be oxidized via nitrite to nitrate by nitrifying bacteria. Nitrate may be taken up by the cyanobacteria and assimilated or under anoxic conditions converted to gaseous nitrogen by denitrifying bacteria. Anaerobic ammonium oxidation (anammox) is another process that leads to the conversion of fixed nitrogen to N_2 (Jaeschke et al. 2009; Porubsky et al. 2009). Hence, these processes cause a loss of combined nitrogen, which is counteracted by the capacity of some cyanobacteria to fix dinitrogen (Joye and Paerl 1994). Nitrogen cycling in microbial mats contributes to the nutrient limitation patterns of mangrove trees. In dwarf habitats, microbial mats serve as a source of nitrogen via the fixation of dinitrogen, while in fringe and transition habitats, mats compete with the trees for nitrogen via denitrification (Lee and Joye 2006).

In microbial mats the decomposition of organic matter may be incomplete which could mean that part of the nitrogen is not recycled and so enters the geological record (Fig. 4.17). Hence this nitrogen is withdrawn from the microbial nitrogen cycle. It is not clear how important this process is since even in mats in which a net accretion of organic matter occurs, up to 99% of the produced organic matter may be recycled within the mat (Krumbein et al. 1977). In exceptional cases organic nitrogen produced by N_2-fixing cyanobacteria can be transformed to nitrate deposits known as guano or caliche nitrates (Ehrlich 1996).

Most of the nitrogen in the biosphere is present in the atmosphere in the form of dinitrogen (N_2), which amounts to 3.9×10^{18} kg N. In the oceans and on land the amount of combined nitrogen (organic and inorganic) amounts each about 10^{15} kg N. The amount of nitrogen in living biomass on earth amounts only 1.3×10^{13} kg (Ehrlich 1996). It is generally assumed that primary production in the marine environment is limited by nitrogen. In particular marine microbial mats with their dense and compressed biomass often experience a shortage of nitrogen. The majority of organisms cannot use the most abundant form of nitrogen, N_2. Only N_2-fixing organisms (diazotrophs) are capable of using dinitrogen. All these organisms possess nitrogenase. Cyanobacteria are among the most important diazotrophs and in all marine microbial mats that have been investigated to date, N_2 fixation has been observed.

4.7.3 Nitrogenase

In all N_2-fixing organisms the enzyme complex nitrogenase is present. This enzyme is similar in all organisms that contain it. The complex is composed of two enzymes: dinitrogenase reductase which is a dimer of identical subunits and also termed the iron-protein, which is encoded by *nifH*, and dinitrogenase, a tetramer composed of two different subunits (α_2 β_2), encoded by *nifDK*. Dinitrogenase is also known as the molybdenum-iron protein (Howard and Rees 1994).

Nitrogenase catalyzes the following reaction:

$$N_2 + 8e^- + 8H^+ + 16\,\text{MgATP} \rightarrow 2\,NH_3 + H_2 + 16\,\text{MgADP} + 16P_i$$

Reduced ferredoxin is the electron donor of nitrogenase. The equation shown above makes clear that the fixation of N_2 is at the expense of considerable amount of energy and low potential electrons. This high energy demand of nitrogenase presents often a problem for diazotrophic organisms except for cyanobacteria which produce reduced ferredoxin and convert light energy into chemical energy during photosynthesis. However, nitrogenase is extremely sensitive to oxygen and therefore diazotrophic organisms must provide an anaerobic environment in order to be able to fix N_2. Cyanobacteria as oxygenic phototrophic and principally aerobic organisms need special adaptations.

4.7.4 Dinitrogen-Fixing Cyanobacteria

Diazotrophic cyanobacteria are capable of using dinitrogen (N_2) as the sole source of nitrogen for growth. These organisms can be subdivided in three main groups (Table 4.3).

Table 4.3 Types and characteristics of N_2-fixing cyanobacteria

Type I Heterocystous cyanobacteria
Exclusively filamentous species that differentiate special cells: heterocysts
Strategy: spatial separation of N_2 fixation and oxygenic photosynthesis and protection of nitrogenase in the heterocyst
Diazotrophic growth under fully aerobic conditions
Examples: *Anabaena, Aphanizomenon, Calothrix, Fischerella, Mastigocladus, Nodularia, Nostoc, Scytonema*
Occurrence: waterblooms (freshwater lakes and brackish seas), paddy fields, various microbial mats, symbiotic with a variety of different organisms
Type II Anaerobic N_2-fixing non-heterocystous cyanobacteria
Filamentous and unicellular species
Strategy: avoidance (of oxygen)
Induction and maintenance of nitrogenase only under anoxia or low oxygen; sulhide may be necessary in order to inhibit oxygenic photosynthesis
Examples: *Geitlerinema, Leptolyngbya, Synechococcus*, many other cyanobacteria
Occurrence: many different environments, particularly microbial mats
Type III Aerobic N_2-fixing non-heterocystous cyanobacteria
Filamentous and unicellular species
Strategy: diverse and unknown (temporal separation of N_2 fixation and oxygenic photosynthesis in concert with oxygen protection mechanisms; or in case of *Trichodesmium* possibly a combination of temporal and spatial separation. Nitrogenase may or may not be confined to special cells termed 'diazocytes'; or lacking PS-II in uncultivated 'Group A')
Diazotrophic growth possible under fully aerobic conditions
Examples: *Crocosphaera*, *Cyanothece*, *Gloeothece*, *Lyngbya*, *Symploca*, *Synechococcus*, *Trichodesmium*
Occurrence: tropical ocean (*Crocosphaera, Cyanothece, Trichodesmium*), carbonate cave walls and paddy fields (*Gloeothece*), microbial mats (*Cyanothece*, *Gloeothece*, *Lyngbya*, *Symploca*, *Synechococcus*)

Group I consists of heterocystous cyanobacteria. These filamentous organisms differentiate special cells, heterocysts, which have lost the capacity of oxygenic photosynthesis and have evolved a modified thick cell envelope. Heterocysts are the site of N_2 fixation in these cyanobacteria. The thick cell wall contains special lipopolysaccharides and forms a diffusion barrier for gases, limiting the entry of oxygen. Respiration scavenges the little oxygen that enters the heterocyst. Since photosystem II is absent from the heterocyst, no photosynthetic oxygen is evolved by these cells. Therefore the heterocyst is virtually anoxic and provides an excellent environment for the oxygen-sensitive nitrogenase. Photosystem I-mediated conversion of light energy in the heterocyst provides nitrogenase with ATP. However, for reducing equivalents nitrogenase depends on the import of carbohydrates from the neighbouring vegetative cells. The strategy that heterocystous cyanobacteria have developed in order to be able to grow diazotrophically can be best described as the spatial separation of the two incompatible processes of N_2 fixation and oxygenic photosynthesis. Among the cyanobacteria heterocystous species are the ultimate adapted organisms for N_2 fixation. The vast majority of heterocystous cyanobacteria can be found in freshwater or terrestrial systems, both free-living and as symbionts.

Heterocystous cyanobacteria occur in some brackish basins but are rare in the marine environment, including microbial mats. *Fischerella* and *Mastigocladus* form mats in thermal springs. The heterocystous *Calothrix* sp. has been found as the dominant organism in microbial mats in the tidal area of the Pacific coast in Baja California Sur, Mexico. *Calothrix* is also known from a variety of other marine and brackish habitats such as the spray zone of rocky shores (Jones and Stewart 1969; Whitton and Potts 1982). Mats of the heterocystous cyanobacterium *Anabaena* have been found in a coastal lagoon in southwest France (Villbrandt and Stal 1996) and *Nodularia* occurs in coastal microbial mats of the Dutch barrier islands (Severin and Stal 2008). However, these are exceptions rather than a rule. The vast majority of microbial mats are built by non-heterocystous cyanobacteria, notwithstanding the facts that in many cases N_2 fixation is a crucial process in these systems.

Group II consists of filamentous and unicellular cyanobacteria that do not show cell differentiation and are capable of N_2 fixation only under virtually anoxic conditions with no oxygenic photosynthesis occurring. These organisms, although possessing the genetic capacity of synthesizing nitrogenase, have obviously not evolved a mechanism to protect effectively nitrogenase from oxygen inactivation. Consequently, their strategy can be characterized as avoidance of oxygenated environments. Such environments usually also prevent oxygenic photosynthesis. Among the non-heterocystous cyanobacteria up to about 50% may belong to this group of organisms but for virtually all of them it is uncertain whether they live diazotrophically in their natural environment. Non-heterocystous cyanobacteria that are capable of inducing nitrogenase activity under anaerobic conditions can be found in many environments, including microbial mats. However, most environments in which these cyanobacteria occur are permanently oxygenated and therefore diazotrophic growth is unlikely. In contrast, microbial mats are often characterized by steep and fluctuating gradients of oxygen and sulphide. Anoxia frequently occurs in microbial mats; this as a rule coincides with high levels of sulphide, a very potent inhibitor of oxygenic photosynthesis. Thus, it is not surprising that evidence hinted to anaerobic N_2-fixing cyanobacteria growing diazotrophically in microbial mats in which H_2S was present (Villbrandt and Stal 1996).

Group III cyanobacteria also comprise non-heterocystous filamentous and unicellular cyanobacteria but they are remarkable as they possess the capacity of inducing nitrogenase and growing diazotrophically under fully aerobic conditions. To date our knowledge of how these organisms are protecting their undoubtedly oxygen-sensitive nitrogenase

is incomplete. Although the number of species that we know that possess this capability is still relatively rare, their numbers are increasing at steady pace. Examples can be found in terrestrial environments such as cave-walls, paddy fields and thermal springs, and in the marine environment. Freshwater lakes apparently do not harbour aerobic N_2-fixing non-heterocystous cyanobacteria. In the ocean the planktonic colony-forming *Trichodesmium* spp. is known as an efficient diazotrophic growing, non-heterocystous cyanobacterium. In addition, several unicellular diazotrophic cyanobacteria occur in the oceans. These include *Crocosphaera* and *Cyanothece* (Needoba et al. 2007). The pico-sized and hitherto uncultivated 'Group A' cyanobacteria are abundant and metagenomic analyses suggest that these organisms may lack the oxygenic photosystem II (Zehr et al. 2008). Their mode of life is unclear and could either be photoheterotrophic or symbiotic. Strikingly, diazotrophic cyanobacteria in the oceans are confined to the tropical and subtropical regions with surface water temperature well above 20°C. In microbial mats aerobic N_2-fixing non-heterocystous cyanobacteria are reported to belong predominantly to the genera *Oscillatoria* and *Lyngbya*, which are morphologically and phylogenetically closely related to *Trichodesmium*. However, in a variety of environments unicellular N_2-fixing cyanobacteria such as *Cyanothece*, *Gloeothece* and *Synechococcus* are known to build microbial mats.

The first report of a culture of a filamentous non-heterocystous aerobic N_2-fixing cyanobacterium was by Pearson et al. (1979). This organism was originally identified as *Microcoleus chthonoplastes* but later renamed as *Symploca* sp. (Janson et al. 1998). *Symploca* sp. is also morphologically related to *Oscillatoria*, and was isolated from a tidal microbial mat (Pearson et al. 1979; Malin and Pearson 1988). It has been proposed that the strategy of aerobic non-heterocystous cyanobacteria, in analogy with the heterocystous species, is temporal separation of the incompatible processes of photosynthesis and N_2 fixation. The latter would than occur during the dark (Mullineaux et al. 1981; Stal and Krumbein 1987). However, not all species in this group follow this strategy. *Trichodesmium* spp. fix N_2 during the day (Capone et al. 1990). Moreover, all species that have been cultured are capable of growing diazotrophically under continuous light and, in the unicellular *Gloeothece* sp., culture conditions can be chosen under which N_2 fixation occurs during the light period of a light dark cycle (Ortega-Calvo and Stal 1991).

4.7.5 Daily Variation of N_2 Fixation

N_2 fixation has a high demand of energy and low-potential reducing equivalents. For the oxygenic phototrophic cyanobacteria light is the source of ATP generation and electrons are derived from water and transferred to ferredoxin mediated by photosynthetic electron transport. Thus, in cyanobacterial mats N_2 fixation ought to be directly linked to light. However, since oxygen exerts a negative effect on nitrogenase, daily variations of N_2 fixation in microbial mats can be expected. The patterns of these daily variations will depend on the type of diazotrophic cyanobacterium and on the dynamics of light and oxygen in the mat. In Fig. 4.19 five daily patterns of nitrogenase activity, measured in different microbial mats, are depicted.

N_2 fixation in heterocystous cyanobacteria is intimately linked to light. The heterocyst is not capable of CO_2 fixation and therefore does not accumulate storage carbohydrate, as is the case in vegetative cells. Dark energy generation in heterocysts will be limited because at one hand the reducing equivalents must be imported from the vegetative cells while at the other hand the oxygen entry in the heterocyst is limited as a result of the diffusion barrier provided by the cell wall (Walsby 1985). Therefore it is not surprising that daily variations of N_2 fixation in communities of heterocystous cyanobacteria are strongly light dependent (Griffiths et al. 1987; Storch et al. 1990; Stal 1995; Falcón et al. 2007) (Fig. 4.18a). However, considerable dark nitrogenase activity may occur in such communities. The ratio of light over dark nitrogenase activity in different populations of heterocystous cyanobacteria varies considerably and is possibly dependent on the species, the light history or other conditions.

In microbial mat communities composed of non-heterocystous cyanobacteria the daily pattern of N_2 fixation is less predictable (Paerl et al. 1989, 1996) (Fig. 4.18b–e). This depends largely on the type of organism and prevailing conditions in the mat. Moreover, due to the fact that these conditions may also vary from day to day (tidal movement, light and overcast, temperature and other factors), the daily pattern of N_2 fixation may change considerably.

The daily pattern of N_2 fixation in non-heterocystous cyanobacteria is the result of the combined effects of oxygen, light, and in some cases sulphide. As in heterocystous cyanobacteria, non-heterocystous species must supply nitrogenase with sufficient energy and low-potential reducing equivalents. This condition is satisfied in the light but the serious drawback is the evolution of oxygen. In such mats, photosynthesis obviously must occur at daytime and N_2 fixation is confined to the night (Fig. 4.18b). For instance this is the case in mats of *Gloeothece* and *Oscillatoria*. Whereas during the day microbial mats often become supersaturated with oxygen because the diffusion of this gas is limited, at night they may turn anoxic within minutes (Stal 1995). The microbial community, including the cyanobacteria, consumes oxygen in the dark by respiration. Obviously, anoxic conditions are ideal for N_2 fixation, but pose a problem with respect to the supply of energy and reducing equivalents. However, all cyanobacteria isolated from marine microbial mats and tested appeared to be

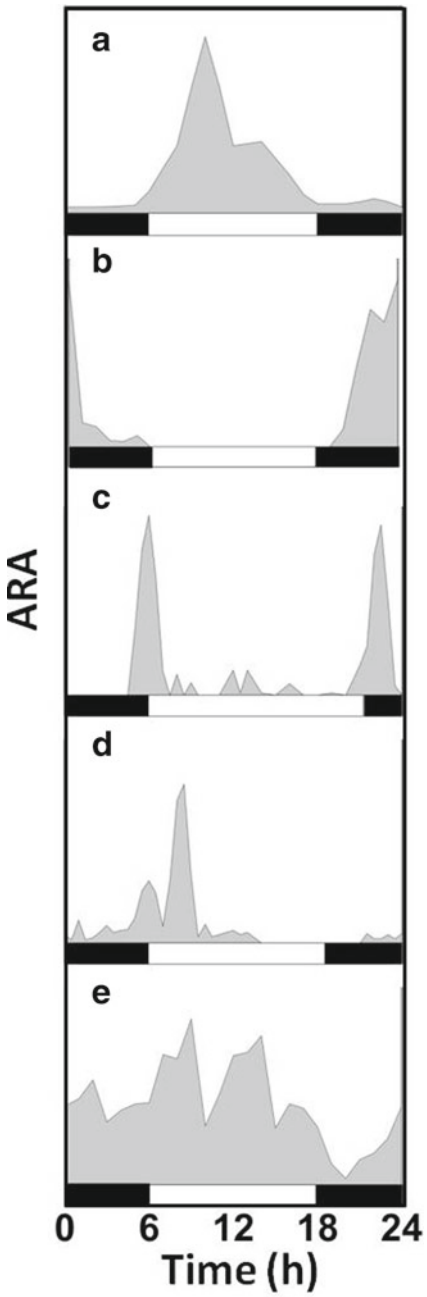

Fig. 4.19 Five typical patterns of daily variation of N_2 fixation (ARA, relative units) in microbial mats: (**a**) Mat of the heterocystous *Calothrix* sp. in Baja California, Mexico (Data from Stal et al. 1994); (**b**) Mat of the unicellular *Gloeothece* sp. on the wall of a carbonate cave (Data from Griffiths et al. 1987); (**c**) Mixed mat of the non-heterocystous *Microcoleus chthonoplastes* and *Lyngbya* sp. of a tidal flat of the North Sea island of Mellum (Data from Villbrandt et al. 1990); (**d**) Mat dominated by *Lyngbya* sp. (location as **c**) (Data from Villbrandt et al. 1990); (**e**) Mat of non-heterocystous *Lyngbya aestuarii* (location as **a**) (Author, unpublished)

capable of fermentation of endogenous storage carbohydrate (Stal and Moezelaar 1997). Although the energy generation by fermentation is undoubtedly small, it has been shown that it exceeds many times the extremely low maintenance requirements of cyanobacteria (Stal and Moezelaar 1997). It has also been shown that dark anoxic conditions supported considerable nitrogenase activity in the filamentous, non-heterocystous cyanobacterium *Oscillatoria limosa* (*Lyngbya aestuarii*) (Stal and Heyer 1987). In microbial mats in which this cyanobacterium occurred, daily patterns of N_2 fixation were found in which this activity was low but totally confined to the dark (Villbrandt et al. 1990). However, other patterns were also observed at different times in the same mats with the same organism. For instance, it could often be seen that nitrogenase activity peaked around sunset and sunrise (Fig. 4.18c). This was confirmed by experiments with *O. limosa* grown in the laboratory under an alternating light dark cycle and with anoxic conditions established 1 h after the onset of the dark period and aerobic conditions 1 h after the onset of the light period (Stal and Heyer 1987). Highest nitrogenase activities in these cultures were obtained in the light in the absence of oxygen. Also, in natural samples it has been observed that highest nitrogenase activities occurred at sunrise (Villbrandt et al. 1990) (Fig. 4.18d). Exactly the same observation was done for the hot spring unicellular cyanobacterium *Synechococcus* (Steunou et al. 2008). *NifH* was transcribed at the end of the day but the nitrogenase was present during the whole night and disappeared once the mat became enriched by oxygen the next day. Nevertheless, during most of the night nitrogenase activity was low, and revealed a small peak at sunset and a large peak at sunrise. This is because light is available while oxygen is still absent. After sunset, oxygen may have been present for some time, allowing energy generation through aerobic respiration. Once anoxic conditions are established only low rates of nitrogenase activity can be supported by the lower rate of fermentative energy generation. Vertical profiles of oxygen measured during a 24 h period have shown that the mat which possessed this type of fluctuating nitrogenase activity, oxygen was indeed present during the first hours after sunset and that it appeared again in the morning only hours after sunrise.

In freshly colonized sediments of North Sea tidal sand flats, *O. limosa* (*L. aestuarii*) is often the pioneer cyanobacterium (Stal et al. 1985). This is most likely because of its capacity to grow diazotrophically. In this pioneer state biomass is low and therefore so is the oxygen demand in the dark. Such sediments normally do not turn anoxic. However, during the light they may accumulate oxygen up to several fold saturation (Villbrandt et al. 1990). N_2 fixation in such systems is typically confined to the night, peaking at sunrise when light becomes available but at oxygen levels well below air saturation (Fig. 4.18d). In other systems such as in mats of the unicellular N_2-fixing *Gloeothece,* which grows on carbonate cave walls, a peak of nitrogenase activity is observed immediately after sunset and then decreasing gradually until hardly any activity is detectable at the end of the night

(Fig. 4.18b). This organism depends on oxygen for respiratory energy generation and it is possible that in the course of the dark period this organism depletes its endogenous storage carbohydrate (Maryan et al. 1986).

Another type of daily pattern of N_2 fixation in microbial mats of non-heterocystous cyanobacteria is more or less constant activity or fluctuations scattered throughout the day and night (Fig. 4.18e). This is often the case when Group 2 diazotrophic cyanobacteria are involved. These cyanobacteria are only capable of fixing N_2 under anoxic conditions or at least when oxygen concentrations are low and oxygenic photosynthesis is inhibited. Such a situation can be expected in microbial mats in which high concentrations of sulphide inhibit oxygenic photosynthesis. In the light, sulphide at the same time may serve as electron donor for nitrogenase in these situations. In most cases oxygenic photosynthesis is continuing in the surface layers of the mat. Sulphide in inhibitory concentrations for photosynthesis is usually present in the deeper layers where light intensity will also be low. In the dark, N_2 fixation may be supported by fermentation of endogenous storage carbohydrate. Thus both in the light and in the dark relatively low nitrogenase activities can be expected.

Severin and Stal (2008) recorded light-response curves of nitrogenase activity in coastal microbial mats. They observed changes in the fitted parameters of nitrogenase activity during a 24 h cycle and used these parameters and the monitored natural light intensities to calculate the daily amount of N_2 fixation. The daily variations of nitrogenase activity in the different types of microbial mats agreed with those that have been found previously and were typical for the cyanobacterial communities present in these mats (Fig. 4.20). Severin and Stal (2008) also integrated the daily amount of N_2 fixation during different days with different total daily irradiances and found that it was independent on the amount of light received by the microbial mat, even if nitrogenase activity had a strong light response. Also, the two types of microbial mats which were investigated and which were characterized by totally different daily patterns of nitrogenase activity, did not differ in their total daily integrated amount of N_2 fixation (Table 4.4). These authors concluded that N_2 fixation in these microbial mats was tuned to a maximum by the concerted action of a diverse diazotrophic community in which different components become active at different times as the result of the changing conditions.

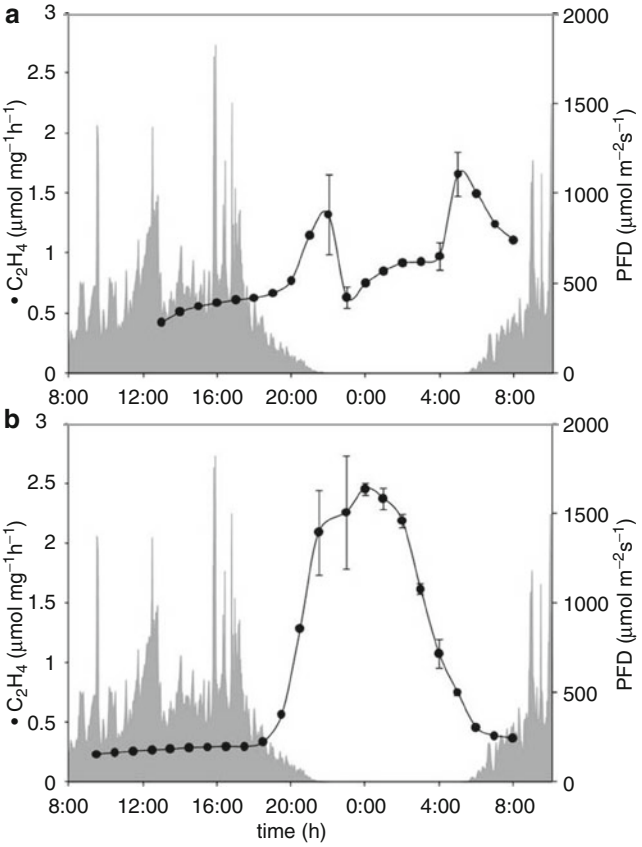

Fig. 4.20 **Daily patterns of N_2 fixation in two different types of microbial mat on the green beach of the North Sea island Schiermonnikoog:** (**a**) Mat containing a variety of filamentous cyanobacteria, including heterocystous species; (**b**) Mat containing predominantly *Lyngbya* sp. Nitrogenase activity (*circles*) was calculated from the actual ambient irradiance (*grey area*) and the light response curves (ARA) recorded for both mats at hourly intervals (From Severin and Stal 2008)

Table 4.4 Daily integrated nitrogenase activity (µmol C_2H_4 mg chl a^{-1}) and photon flux (µmol m^{-2}) for two microbial mats (After Severin and Stal 2008)

	Station I		Station II	
Date	Nitrogenase activity	Photon flux	Nitrogenase activity	Photon flux
28.05.06	17.9	26,295	20.4	23,993
29.05.06	18.0	29,399	17.5	29,275
30.05.06	17.7	23,866	17.5	29,443
31.05.06	17.8	15,839	17.5	14,052
Average	17.9		18.2	

4.7.6 Vertical Distribution of N_2 Fixation in Microbial Mats

Little is known about the vertical distribution of N_2 fixation in microbial mats, but the vertical distribution and dynamics of factors that control it such as light, oxygen and sulphide have been investigated in considerable detail. Light is attenuated strongly in microbial mats. The wavelengths that are absorbed by the cyanobacteria in the top layers are obviously attenuated most strongly. Far red light (<700 nm), however, is absorbed by the cyanobacteria to only a small extent; also the attenuation of this light in (wet) sediment is small compared to shorter wavelengths. Far red light does not support oxygenic

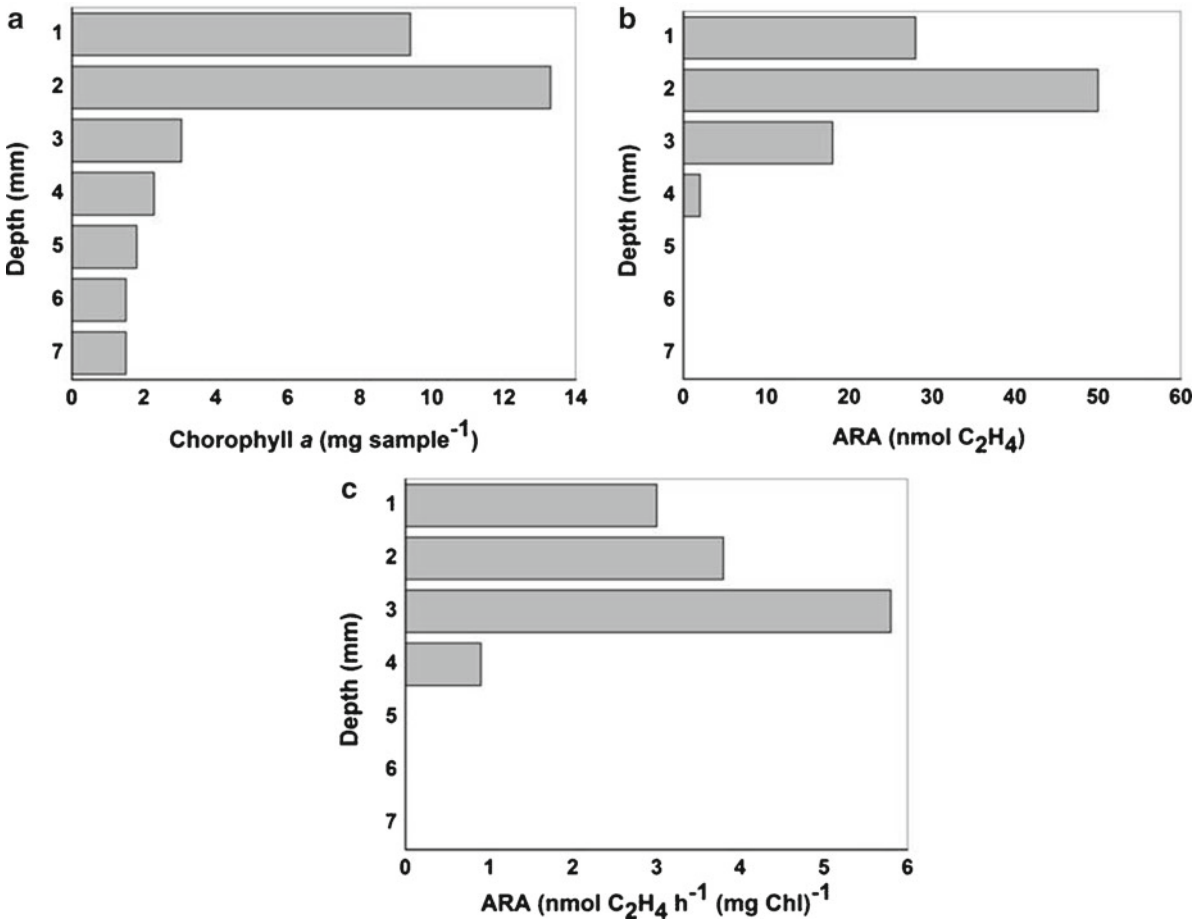

Fig. 4.21 Vertical distribution of chlorophyll *a* in: (**a**) potential nitrogenase activity (acetylene reduction, ARA); (**b**) specific, chlorophyll *a*-based ARA; (**c**) a microbial mat (Data from Stal et al. 1984)

photosynthesis but anoxygenic photosynthesis depends on it. It can thus be assumed that the cyanobacteria in the lower part of the mat are not capable of oxygenic photosynthesis. This has been shown by microelectrode measurements of oxygen concentration and photosynthesis. Such measurements have also shown that in some microbial mats sulphide is present in these layers. In an attempt to measure potential nitrogenase activity in microbial mats it was shown that in a mat of 3-mm maximum surface related nitrogenase activity occurred in the depth horizon of 1–2 mm (Stal et al. 1984). However, when nitrogenase activity was expressed on the basis of chlorophyll *a*, highest specific nitrogenase activity was present in the lowest layer of the cyanobacterial mat (2–3 mm) (Fig. 4.21). Cyanobacterial biomass was highest in the top layer, decreasing gradually until about 3 mm depth. Thus it is likely that a spatial separation of N_2 fixation and oxygenic photosynthesis had occurred in this mat. The top layer carries out oxygenic photosynthesis and CO_2 fixation, while N_2 is fixed in the lower layers.

4.7.7 Effects of Anoxia and Sulphide on N_2 Fixation in Microbial Mats

Among the non-heterocystous diazotrophic bacteria those that are capable of N_2 fixation under fully aerobic conditions are rare (Bergman et al. 1997). Since they grow by oxygenic photosynthesis, these organisms not normally perform N_2 fixation. It has been questioned whether this capacity of N_2 fixation is of any importance in the natural environment (Rippka and Waterbury 1977). Padan and Cohen (1982) mention that the facultative anoxygenic photosynthetic cyanobacterium *Oscillatoria limnetica* (*Geitlerinema*) is capable of N_2 fixation when carrying out sulphide-dependent anoxygenic photosynthesis. Villbrandt and Stal (1996) investigated the effect of sulphide on N_2 fixation in cyanobacterial mats and on cultures of cyanobacteria isolated from these mats. They compared a mat dominated by a heterocystous cyanobacterium (*Anabaena*) with another one dominated by non-heterocystous filamentous organisms (*Oscillatoria* and *Phormidium*). Sulphide inhibited

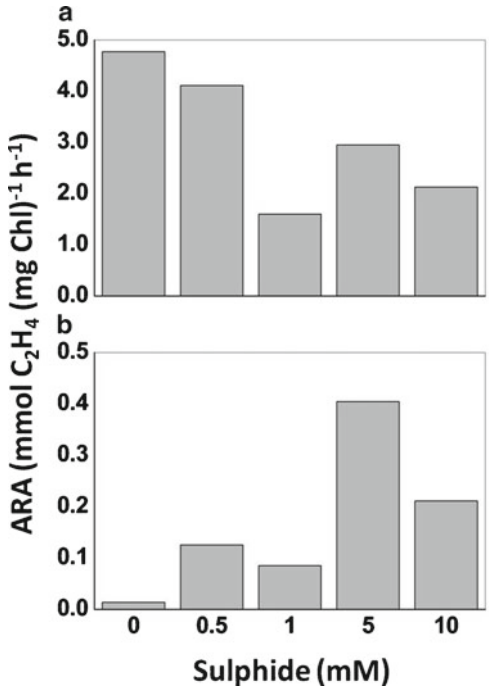

Fig. 4.22 Effect of sulphide on nitrogenase activity (acetylene reduction, ARA) in: (**a**) mat of the heterocystous *Anabaena* sp. (lagoon, Atlantic coast of France); (**b**) mat of non-heterocystous cyanobacteria (*Oscillatoria* sp. and *Phormidium* sp.) (lagoon, Mediterranean coast of France) (Data from Villbrandt and Stal 1996)

nitrogenase activity in the mat of *Anabaena*, but greatly stimulated it in the mat of non-heterocystous cyanobacteria (Fig. 4.22). Both light and dark nitrogenase activity was inhibited by sulphide in the mat of *Anabaena* but when DCMU was added in order to inhibit oxygenic photosynthesis virtually no effect of sulphide on N_2 fixation was seen in this mat. Therefore the effect of sulphide was mainly through the inhibition of oxygenic photosynthesis and respiration. Only the addition of 10 mM sulphide resulted in the almost complete inhibition of dark nitrogenase activity which depends on respiratory energy generation. In the light, this sulphide concentration resulted in a decrease of nitrogenase activity to the level obtained in the presence of DCMU, which in this case was about 40% of the control. This demonstrated that this amount of sulphide caused the complete inhibition of oxygenic photosynthesis. Due to the large amount of iron in this mat the actual concentration of free sulphide was probably much lower. Moreover, as was shown in a laboratory culture of *Anabaena* isolated from this mat, the effect of sulphide strongly depended on the pH. At pH 9.5 a total sulphide concentration of 5 mM had no effect on N_2 fixation but at pH 6.5 this concentration almost completely inhibited nitrogenase activity. This shows that the effect of sulphide is through the gaseous species H_2S. This gas will passively diffuse into the cell. Because the pH in these mats is usually high, very little H_2S will be present, even when the total concentration of sulphide is high.

In non-heterocystous mats the situation is totally different. In the control, without sulphide, nitrogenase activity is low in the light. In the dark or when oxygenic photosynthesis was inhibited by DCMU, nitrogenase activity was greatly stimulated. This can obviously be explained by the sensitivity of N_2 fixation in these organisms for photosynthetic and atmospheric oxygen. Sulphide stimulated nitrogenase activity in the light, in the dark and with DCMU. Stimulation was most marked in the light and reached a maximum at 5 mM (Fig. 4.21b). However, even at 10 mM sulphide nitrogenase activity was about tenfold the control. The stimulation in the dark was small (it doubled) and reached already maximum at 0.5 mM. Also, with DCMU, stimulation was maximal at 0.5 mM, the same order of magnitude as the effect in the dark (Villbrandt and Stal 1996). The effect of sulphide on the light activity of nitrogenase is best explained by its inhibition of oxygenic photosynthesis in concert with a lowering of environmental oxygen concentration.

None of the cyanobacteria isolated from this mat possessed the capacity for aerobic N_2 fixation, but all of the strains were capable of inducing nitrogenase under anaerobic conditions (Villbrandt and Stal 1996). In experiments with *Phormidium*, it was shown that sulphide (total concentration up to 8 mM) had no effect on nitrogenase activity when this was induced anaerobically with DCMU. Therefore it was concluded that sulphide did not act as an electron donor to nitrogenase and that the stimulatory effect observed in the mat was most probably due to the scavenging of environmental oxygen. Sulphide very efficiently induced nitrogenase in *Phormidium* and other non-heterocystous cyanobacteria with anaerobic nitrogenase. About 4 mM total sulphide was sufficient for full induction of nitrogenase. However, in contrast with what was seen with the heterocystous cyanobacterium and to what was expected, it appeared that induction of nitrogenase with sulphide was optimal at high pH, which means that the ions HS^- and/or S^{2-} were more efficient than the gas H_2S. This suggested that *Phormidium* could actively take up sulphide ion. Thus the uptake of sulphide ion may be essential to allow diazotrophic growth in these organisms.

4.7.8 Oxygen Protection of Nitrogenase in Microbial Mats

Because cyanobacterial mats often contain a high density of biomass and have low rates of molecular diffusion, they may become markedly supersaturated with oxygen. This poses the question of oxygen protection of nitrogenase in microbial mats. Although in heterocystous cyanobacteria nitrogenase is confined to the heterocysts, and protected from oxygen under normal atmospheric conditions, it has been shown that N_2 fixation may be seriously impaired at oxygen pressure well above atmospheric levels. In the majority of cases,

non-heterocystous cyanobacteria are the dominant organisms in mats. Many of these species possess only the capacity of anaerobic nitrogenase activity because the lack of an adequate oxygen protection mechanism. They may be able to grow diazotrophically under anaerobic conditions and when sulphide inhibits oxygenic photosynthesis. This may under circumstances lead to a vertical spatial separation of oxygenic photosynthesis in the top layer of the mat and N_2 fixation in the deeper parts. Paerl and Prufert (1987) and Paerl et al. (1995) emphasized the importance for N_2 fixation of anoxic microzones in microbial mats and other systems. In a few cases, microbial mats have been shown to be built by non-heterocystous cyanobacteria that are capable of N_2 fixation under fully aerobic conditions (Pearson et al. 1979; Stal et al. 1984; Villbrandt et al. 1990; Gallon et al. 1991; Paerl et al. 1991). Since nitrogenase in these organisms is as sensitive to oxygen as in any other organism, these cyanobacteria obviously must possess a protection mechanism. Despite a large amount of research on this problem the precise mechanism by which these species protect nitrogenase from oxygen inactivation is still not known (Bergman et al. 1997). In fact, in all cases in which aerobic N_2-fixing cyanobacteria form microbial mats, N_2 fixation is confined to the night. Thus, a temporal separation of N_2 fixation and photosynthesis (respectively during the night and during the day) is maintained (Stal 1995). Because these mats turn anoxic during the night, there is no need for oxygen protection. The problem of oxygen protection of nitrogenase in microbial mats is therefore hardly relevant.

Aerobic N_2-fixing non-heterocystous cyanobacteria isolated from microbial mats include the filamentous *Oscillatoria*, *Lyngbya*, and *Microcoleus*, and the unicellular *Gloeothece*, *Cyanothece* and *Synechococcus* (Bergman et al. 1997). Among the different mechanisms that have been proposed for oxygen protection of nitrogenase, the uptake and reduction of oxygen, seems to be the most promising. Such systems may act in concert with enzymes that remove oxygen radicals.

In Table 4.5 the effects of different treatments of a diazotrophic microbial mat composed of *Oscillatoria* on nitrogenase activity are shown. When these mats were incubated in the laboratory and exposed to elevated salinity, phosphate fertilization or to a tidal movement of the water, all these treatments resulted in a dramatic increase of nitrogenase activity. The application of a tidal movement (alternating immersion and emersion of the mat) resulted in a two orders of magnitude increase in nitrogenase activity. The vertical profiles of oxygen in these mats, measured at the same time, showed that the increase of N_2 fixation was probably the result of markedly decreased concentrations of oxygen. The reference showed oxygen supersaturation, peaking at about 250 μm depth, typical for these mats. When the mats were subject to increased salinity, phosphate fertilization or to a tidal movement, oxygen profiles decreased dramatically. The decrease of oxygen concentration was most pronounced in the mats subject to both phosphate fertilization and tidal movement. These treatments also resulted in the strongest stimulation of nitrogenase activity. It is obvious that the decreased oxygen concentration is associated with the increased potential to fix dinitrogen.

Table 4.5 Effect of different treatments of a nitrogen-fixing microbial mat of *Oscillatoria* sp. from the island of Texel, The Netherlands

Treatment	Nitrogenase activity (nmol C_2H_4 cm^{-2} h^{-1})
Reference	2 ± 1
High salinity	31 ± 5
Phosphate fertilization	164 ± 18
Tidal movement	234 ± 21

Sediment cores containing the mat were incubated in the laboratory in aquaria filled with seawater (Instant Ocean). The seawater was aerated. Illumination was by 75 W halogen lamps applied at a 16–8 h light–dark cycle. Heating of the mats was prevented by a heat filter and fans. The reference cores were incubated in such a way that the mat was just exposed while the water level was just underneath the mat surface. The mat surface was moist. This incubation mimics the natural situation most closely. In another aquarium the seawater was pumped in and out at 6-h intervals, mimicking a tidal movement. At each high water the mat was covered by 5 cm of water. The tidal range was about 15 cm. In the third incubation the salinity was increased to twice the normal value (3%). In the fourth treatment, phosphate concentration in the seawater was increased to 100 μM. The cores were incubated for 1 week and vertical oxygen profiles and nitrogenase activity (acetylene reduction) were measured

One possibility that must be investigated is the capacity of nitrogenase in these cyanobacteria to reduce oxygen (autoprotection) (Bergman et al. 1997). This causes the reduction of O_2 to H_2O_2, which can be further reduced by peroxidases.

4.7.9 Heterocystous Versus Non-heterocystous Cyanobacteria in Microbial Mats

There is no doubt that heterocystous cyanobacteria are particularly well adapted for diazotrophic growth. They can fix N_2 in the light while carrying out oxygenic photosynthesis. In this way they make optimal use of light energy to satisfy the large demands of nitrogenase. Oxygen protection of nitrogenase in these organisms is assured by the heterocyst. Anoxic conditions usually result in scarcely higher nitrogenase activities and the inhibition of oxygenic photosynthesis by DCMU invariably results in lower activities, apparently because it inhibits the flow of reduction equivalents from the vegetative cells. Non-heterocystous cyanobacteria either cannot fix N_2 at all in the presence of oxygen or those that can invariably can much better in the dark or when transferred to anoxic conditions (Stal 1995). Often the inhibition of oxygenic photosynthesis by DCMU also stimulates nitrogenase

activity considerably. Notwithstanding these facts, the vast majority of marine microbial mats are composed of non-heterocystous cyanobacteria. Thus the question is raised as to why heterocystous cyanobacteria are not more common in these mats.

On the tidal flats in Guerrero Negro, Baja California Sur, Mexico, two types of microbial mats can be found in close vicinity of each other (Stal et al. 1994; Stal 1995) (Fig. 4.7). The smooth mat is composed of the non-heterocystous cyanobacterium *Lyngbya aestuarii* and covers the lower areas of the tidal flat. On the upper tidal flat and on slightly elevated spots a pustular mat develops which is composed of the heterocystous cyanobacterium *Calothrix*. Both mats fix N_2 but show distinct differences in their daily nitrogenase patterns. *Calothrix* fixes predominantly during the day while nitrogenase activity in the mats of *L. aestuarii* is confined to the night. Due to their locations on the tidal flat the mats of *L. aestuarii* are covered more often and during longer periods of time at high tide than the mats of *Calothrix*. During inundation of the mats of *L. aestuarri* diffusion is limited. This causes oxygen supersaturation during the period of photosynthesis and anoxic conditions at night. These anoxic conditions also allow the development of a community of sulphate-reducing bacteria. This mat has a very dense biomass and is characterized by steep gradients of oxygen and sulphide, typical for microbial mats. Due to this dense mat structure the gradients of oxygen and sulphide exist, regardless whether the mat is inundated or not.

The situation in the mat of *Calothrix* is totally different. This pustular mat has a porous structure. Due to this structure in this mat there is a free exchange of oxygen with the atmosphere and oxygen supersaturation or anoxic conditions are not usually the case. In exceptional cases when the mat is inundated for a prolonged period of time anoxic conditions or oxygen supersaturation may occur but normally the mat will be inundated for short periods or not at all. Stal et al. (1994) hypothesized that heterocystous cyanobacteria would not be able to maintain themselves in an environment in which either dark anoxic conditions or high concentrations of sulphide occur. Cyanobacteria incapable of fermentation will die within 2–3 h of dark anoxic conditions (Stal and Moezelaar 1997). However, there is no reason why heterocystous cyanobacteria should be incapable of fermentation and this has been demonstrated in a number of symbiotic *Nostoc* spp. (Margheri and Allotta 1993; De Philippis et al. 1996).

In order to investigate the possibility of sulphide as a selecting factor, Villbrandt and Stal (1996) compared a heterocystous and a non-heterocystous N_2-fixing mat in two coastal lagoons in France. The mat of heterocystous cyanobacteria was found in a lagoon with exceptional high amounts of iron, while this was not the case in the other system (Stal et al. 1996). As a result of the high amount of iron the sediment on which this microbial mat was found it did not contain any free sulphide because it precipitated as iron sulphide (Schaub and Van Gemerden 1996). Villbrandt and Stal (1996) hypothesized that the absence of sulphide would allow the proliferation of heterocystous species. On the one hand it was indeed demonstrated that N_2 fixation in heterocystous cyanobacteria was sensitive to sulphide. But on the other hand, unrealistic high concentrations of sulphide (10 mM) were required to obtain full inhibition. Sulphide inhibition of nitrogenase in heterocystous cyanobacteria depended on H_2S, which in microbial mats is present in very low concentrations as a result of the alkaline conditions. However, in heterocystous cyanobacteria it is uncertain whether other metabolic processes than N_2 fixation are more severely influenced by sulphide. In microbial mats and living stromatolites from Cuatro Cienegas, Mexico, evidence for the presence of heterocystous N_2-fixing cyanobacteria was obtained from the pattern of nitrogenase activity with highest activities during the day and its inhibition of oxygenic photosynthesis (Falcón et al. 2007). The negative effect of molybdate addition on nitrogenase activity observed by these authors was interpreted as a contribution of sulphate reducing bacteria to N_2 fixation. Molybdate inhibits sulphate reduction and this would therefore decrease the sulphide production. The ecological effects of such experiments are complex and difficult to interpret.

Another reason why heterocystous cyanobacteria are absent from the majority of microbial mats could lie in the fact that such organisms generally are not motile and that the link between the heterocyst and the vegetative cell is weak (Stal et al. 1994). Non-heterocystous cyanobacteria that form microbial mats are mostly motile by gliding movement. This is an important property since it facilitates optimal vertical positioning. It allows the cyanobacteria to compensate for the rapidly shifting physicochemical gradients in microbial mats. Gliding motility is also important because microbial mats often develop in environments that are characterized by high rates of sedimentation. In the rare cases that heterocystous cyanobacteria dominate microbial mats their filaments are orientated in a uniform manner at the mat surface. The tapered trichomes of *Calothrix* are orientated vertically with the terminal heterocysts situated away from the surface. These filaments do not glide freely. The same is the case for another mat-building heterocystous cyanobacterium, *Scytonema*, which likewise reveals a vertical orientation. In this organism intercalary heterocysts are formed located in the centre of the aggregates. It is likely that shear forces produced during gliding in these highly compressed microbial mats would result in the breakage of the weak link between heterocysts and the neighbouring vegetative cells. Hence, although gliding motility is an essential property for cyanobacteria in microbial mats, it has at the same time a serious disadvantage for heterocystous species. It is therefore

expected that mats of such cyanobacteria can only develop in environments with low rates of sedimentation and relatively constant physicochemical gradients.

4.7.10 Other Diazotrophic Organisms in Microbial Mats and the Case of *Microcoleus chthonoplastes*

The capacity of N_2 fixation is widespread among bacteria and archaea and such organisms are among those that form the microbial community of microbial mats. The question is therefore relevant whether or not microorganisms other than cyanobacteria contribute to the fixation of N_2 in microbial mats and to what extent. Chemotrophic bacteria and archaea will be confronted with a limited supply of substrate to satisfy the energy demand of nitrogenase. Although they do not evolve O_2 they will still have to cope with an aerobic environment and oxygen supersaturation as the result of the oxygenic photosynthesis of the cyanobacteria. Or they avoid the aerobic environment but this would limit their energy generation capabilities.

Anoxygenic phototrophs will have ample energy (sunlight) but may be limited in their sources of electron donor (i.e. sulphide or sulphur, ferrous iron, organic compounds). The problem of non-heterocystous cyanobacteria is to provide an anoxic environment for nitrogenase. Steppe et al. (1996) proposed a joint venture between diazotrophic bacteria and cyanobacteria. The latter would provide the former with organic matter and oxygen and the bacteria provide CO_2 and fixed nitrogen to the cyanobacteria. This model evolved from the observation that cultures and natural samples of the common and cosmopolitan mat-building cyanobacterium *Microcoleus chthonoplastes* possessed nitrogenase genes belonging to the γ- or δ-*Proteobacteria*, while cyanobacterial *nif* genes were lacking.

Although in the literature *M. chthonoplastes* has repeatedly been presented as a diazotroph, this may have been due to wrong identification (Garcia-Pichel et al. 1996; Siegesmund et al. 2008). For instance, the aerobically N_2-fixing *M. chthonoplastes* isolated by Pearson (Pearson et al. 1979; Malin and Pearson 1988) was later identified as *Symploca* sp. (Janson et al. 1998) and the anaerobic N_2-fixing *M. chthonoplastes* 'strain 11' is related to *Geitlerinema* (Siegesmund et al. 2008) to which genus also the Solar Lake strain '*Oscillatoria limnetica*' belongs. Dubinin et al. (1992) and Sroga (1997) reported also on N_2-fixing *Microcoleus* but their correct assignments await confirmation. Rippka et al. (1979) were unable to detect nitrogenase activity in the type strain of *M. chthonoplastes* PCC7420 and Villbrandt and Stal (unpublished results) were unable to induce nitrogenase activity under strictly anaerobic conditions in the collection of the 'true' *M. chthonoplastes* (Garcia-Pichel et al. 1996).

Bolhuis et al. (2010) discovered that a collection of *M. chthonoplastes* from distant geographic locations possess the structural genes for nitrogenase (*nifHDK*), but that they were not typical cyanobacterial but rather belong to the δ-Proteobacteria. The type strain of *M. chthonoplastes* PCC7420 possesses a full nitrogenase operon. These authors were unable to express the nitrogenase genes in any of these strains, though they showed expression in a microbial mat, indicating that the laboratoy conditions used were inappropriate for expressing nitrogenase in this strain. It was conceived that *M. chthonoplastes* obtained the nitrogenase operon from a sulphate reducing bacterium through lateral gene transfer. Hence, the attribution of N_2 fixation to organisms other than cyanobacteria may have been erroneous in a number of reports.

Severin et al. (2010) showed that filamentous and unicellular cyanobacteria dominated the clone libraries of *nifH* and their transcripts in two coastal microbial mats, but that δ- and γ-Proteobacteria also contributed importantly. The *nifH* of the γ-Proteobacteria belonged predominantly to anoxygenic phototrophic purple sulphur bacteria. The *nifH* of the δ-Proteobacteria belonged partly to *M. chthonoplastes* and for the other part might have belonged to sulphate reducing bacteria. Other reports also mention the predominance of *nifH* belonging to δ- and γ-Proteobacteria (Zehr et al. 1995; Olson et al. 1999; Omoregie et al. 2004) in microbial mats and Steppe and Paerl (2002) showed the transcription of δ-proteobacterial *nifH*.

4.8 Cyanobacteria and the Sulphur Cycle in Microbial Mats

The sulphur cycle has a large impact on microbial mats either when sulphate is present and the end-oxidation of organic matter is carried out by sulphate reducing bacteria, or when the ecosystem receives primary sulphide as is the case in sulphur springs. Seawater contains abundant sulphate (28 mM) and therefore sulphate reduction is usually a dominant process in coastal and hypersaline microbial mats. Sulphate-reducing bacteria are essentially anaerobic micro-organisms that oxidize simple organic compounds using sulphate as electron acceptor, which results in the formation of sulphide. A variety of different chemotrophic microorganisms as well as cyanobacteria and purple sulphur bacteria are capable of reducing elemental sulphur to sulphide. Sulphide is eventually oxidized back to sulphate. This can be done anaerobically by anoxygenic phototrophic bacteria such as purple and green sulphur bacteria or also by some cyanobacteria. Elemental sulphur is produced as an intermediate in this process. The cycling between elemental sulphur (S_0) and sulphide (S^{2-}) is also called the 'mini sulphur cycle' and is probably a dominant process in microbial mats (Van Gemerden 1993).

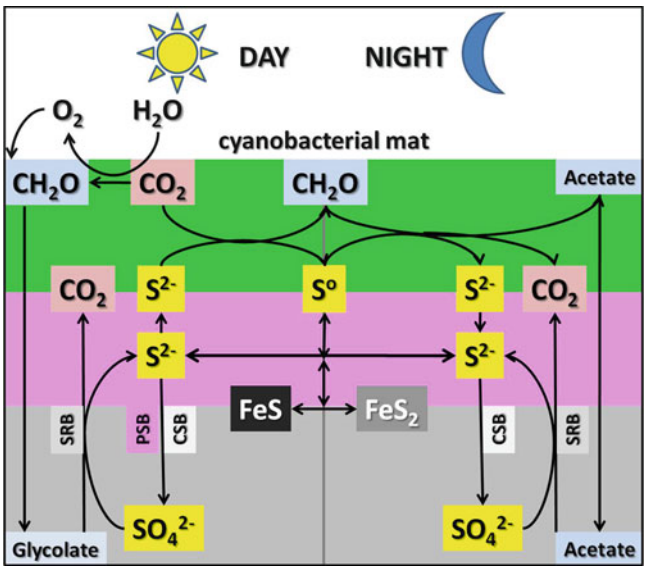

Fig. 4.23 A simplified scheme showing the role of cyanobacteria in the cycle of sulphur in a microbial mat. *SRB* sulphate-reducing bacteria, *CSB* colourless sulphur bacteria, *PSB* photosynthetic sulphur bacteria. For further explanation, see text

Sulphide-dependent anoxygenic photosynthesis by purple sulphur bacteria may account for more than 25% of the total photosynthetic carbon fixation as was found in microbial mats in the Ebro Delta in Spain (Martínez-Alonso et al. 2004). Some cyanobacteria are capable of sulphide-dependent anoxygenic photosynthesis, but oxidize sulphide only to elemental sulphur or to thiosulphate (Fig. 4.23). Colourless sulphur bacteria oxidize sulphide aerobically to sulphate but some species can carry out this oxidation anaerobically using nitrate as electron acceptor (denitrification). This anaerobic chemotrophic sulphide oxidation is probably not important because of the limited availability of nitrate in microbial mats. Hence, colourless sulphur bacteria and anoxygenic phototrophic bacteria compete for sulphide.

De Wit et al. (1995), using a mathematical model of a microbial mat, discovered a strikingly clear interaction between purple and colourless sulphur bacteria. Depending on the environmental parameters the model predicted that either the colourless sulphur bacteria dominate or that they coexist with purple sulphur bacteria. In the latter case purple sulphur bacteria can outweigh the colourless sulphur bacteria by more than an order of magnitude. This may explain why coastal or hypersaline microbial mats sometimes exhibit a purple layer and sometimes not, even when the black layer of FeS is present in both cases, pointing at anaerobic conditions and the presence and production of sulphide.

Another process that consumes considerable amounts of sulphide is when it reacts with ferric iron producing elemental sulphur. A peak of elemental sulphur has been observed underneath the aerobic zone where high rates of sulphate reduction occurred while sulphide remained very low due to oxidation by anoxygenic photosynthesis or by ferric iron (Wieland et al. 2005). Other processes in the sulphur cycle in microbial mats include the disproportionation of thiosulphate, sulphite and elemental sulphur. In these reactions one part of the molecule is oxidized while the other is reduced (Bak and Pfennig 1987; Canfield and Thamdrup 1996). The biomass of the major functional groups of microorganisms involved in the sulphur cycle, the purple sulphur bacteria, colourless sulphur bacteria and sulphate reducing bacteria may account for 40% of the total bacterial community (Visscher and Van Gemerden 1993).

In microbial mats most of the organic matter produced by photosynthetic CO_2 fixation is recycled. The organic matter (dissolved organic matter, DOM) is liberated into the mat environment by a variety of different mechanisms. Fermentation by the cyanobacteria results in the excretion of low-molecular organic carbon compounds (acetate, ethanol, and lactate) that serve directly as substrate for sulphate reducing bacteria. Photorespiration by cyanobacteria results in the formation and excretion of glycolate, which has also been shown to be used by sulphate-reducing bacteria (Fründ and Cohen 1992; Friedrich and Schink 1995) (Fig. 4.22). Degradation of more complex DOM, which is produced as a result of cell lysis or the exudation of extracellular polymeric substances (EPS), requires the combined action of several different microorganisms until it is eventually end-oxidized by sulphate reducing bacteria. Hence, the metabolic activity of the mat cyanobacteria can directly influence sulphate reduction.

It has been assumed that sulphate-reducing bacteria are present only below the euphotic depth in the microbial mat because only there, conditions are permanently anoxic. This layer is recognized by its black colour that indicates the presence of FeS. There are several lines of evidence that this may be incorrect. Sulphate reduction itself may take place under oxygenated conditions although the majority of sulphate-reducing bacteria are obligate anaerobes (Canfield and Des Marais 1991). However, sulphate-reducing bacteria are found throughout the microbial mat as is the case with sulphate reduction (Visscher et al. 1992; Stal 1993). In fact, 16S rRNA gene sequence analysis of microbial mats have demonstrated that the oxygen tolerant sulphate-reducing bacteria are predominantly found in the top layers of the mat while the obligate anaerobic species are found in the deeper layers of the mat (Risatti et al. 1994). Thus a vertical stratification of different groups of sulphate-reducing bacteria is likely and those in the top layer co-exist with cyanobacteria. Several reports have demonstrated the intimate association of cyanobacteria and sulphate reducing bacteria (Baumgartner et al. 2006). Throughout the hypersaline cyanobacterial mat of Solar Lake (Sinai, Egypt) sulphate-reducing bacteria were present and the rates of sulphate

Table 4.6 Groups of cyanobacteria with different types of adaptation to sulphide (After Cohen et al. 1986; Stal 1995)

Group 1.	Sulphide-sensitive oxygenic photosynthesis only
Group 2.	Sulphide-resistant oxygenic photosynthesis only
Group 3.	Sulphide-insensitive oxygenic photosynthesis concurrent with sulphide-dependent anoxygenic photosynthesis
Group 4.	Sulphide-sensitive oxygenic photosynthesis replaced by sulphide-dependent anoxygenic photosynthesis

reduction were sometimes higher in the oxygenated layer than in the deeper permanent anoxic parts of the mat (Teske et al. 1998). The dominant filamentous sulphate-reducing *Desulfonema* migrated during a day night cycle moving from the cyanobacterial layer and probably following the oxygen chemocline (Minz et al. 1999). This showed that some sulphate reducing bacteria tolerate substantial levels of oxygen. Facultative aerobic respiration and motility were considered as essential adaptations for these sulphate reducing bacteria to thrive in a microbial mat.

Many sulphate-reducing bacteria are much less oxygen-sensitive than had previously been assumed and sulphate-reducing bacteria are known that are even capable of aerobic respiration (Cypionka et al. 1985; Dilling and Cypionka 1990; Marschall et al. 1993). However, cultures that carry out dissimilatory sulphate reduction in the presence of oxygen have not been isolated thus far. It is possible that sulphate reduction in the oxygenated part of the microbial mat occurs in anoxic microniches, e.g. in aggregates.

Sulphate reduction also plays a crucial role in calcium carbonate precipitation and thereby controls the lithification process in stromatolites. This is supposed to be the result of two intertwined processes. First, the degradation of EPS liberates calcium that was bound to it and the sulphate reducing bacteria produce carbonate and raise the pH (Visscher et al. 2000; Dupraz et al. 2004).

Cohen et al. (1986) distinguished four groups of cyanobacteria with respect to the degree of sulphide inhibition and the possibility to carry out sulphide-dependent anoxygenic photosynthesis (Table 4.6). Cyanobacteria belonging to Group 1 are extremely sulphide sensitive. Oxygenic photosynthesis is inhibited at low levels of sulphide (<0.1 mM) and these species are not capable of anoxygenic photosynthesis. Cyanobacteria belonging to this group are evidently not important in marine microbial mats but are likely to be found in freshwater lakes, in the oceans or in terrestrial systems in which sulphide is absent or present at insignificant concentrations. Examples of such cyanobacteria are *Anacystis nidulans* (*Synechococcus elongatus*) and *Plectonema boryanum* (*Leptolyngbya boryana*) in which CO_2 fixation was inhibited at 60 and 75 μM (Cohen et al. 1986). In Group 2 cyanobacteria are represented that are incapable of anoxygenic photosynthesis but that resist considerable levels of sulphide. Oxygenic photosynthesis in these organisms is often stimulated at moderate (<1 mM) sulphide concentration. This type of adaptation is typical for marine microbial mats with fluctuating sulphide concentrations. The mat-forming and diazotrophic cyanobacterium *Oscillatoria limosa* (*Lyngbya aestuarii*) is a typical example of this group (Stal 1995). Also Group 3 cyanobacteria are typically found in marine microbial mats. These cyanobacteria are characterized by sulphide-insensitive oxygenic photosynthesis concurrent with sulphide-dependent anoxygenic photosynthesis. The cosmopolitan mat-forming cyanobacterium '*Microcoleus chthonoplastes*' (*Geitlerinema*) belongs to this group (De Wit and Van Gemerden 1988). Oxygenic photosynthesis in Group 4 cyanobacteria is as sensitive to sulphide as in those belonging to Group 1. The difference is in their capacity of carrying out sulphide-dependent anoxygenic photosynthesis. The sulphide tolerance of this group of cyanobacteria varies considerably from less than 1–10 mM. *Oscillatoria limnetica* (*Geitlerinema*) is the best-studied cyanobacterium belonging to that group. Photosystem II in this cyanobacterium is switched off when exposed to <0.1 mM of sulphide. Anoxygenic photosynthesis is induced in a process requiring protein synthesis. *O. limnetica* tolerates up to 9.5 mM sulphide but anoxygenic photosynthesis is gradually inhibited at concentrations exceeding 4 mM. A good example of how a mat community is structured with respect to sulphide was presented for microbial mats in Fuente Podrida, a cold sulphur spring located in East Spain (Camacho et al. 2005). Three filamentous cyanobacteria were found that fitted Group 1 (sensitive) UVFP3, in areas where sulphide was absent and Group 2 (tolerant) UVFP2 and Group 3 (anoxygenic photosynthesis) UVFP1. The latter isolate was related to *Planktothrix*, while the other cyanobacteria did not have close relatives. *Oscillatoria boryana* is also a typical Group 4 organism. This organism employs sulphide-dependent anoxygenic photosynthesis in the early morning which depleted sulphide locally (Castenholz et al. 1991). Sulphide concentrations of over 1 mM inhibit oxygenic photosynthesis completely. With increasing light intensity oxygenic photosynthesis became dominant. At low light sulphide-dependent anoxygenic photosynthesis remained the dominant mode. *O. boryana* is also able to photosynthesize at substantial rates over a wide range of sulphide concentrations by shifting between oxygenic and anoxygenic modes and possibly by combining both.

In microbial mats most of the sulphide is present as 'acid-volatile sulphide' (AVS) which is mostly in the form of ferrous sulphide (FeS) (Fig. 4.22). In this form sulphide is virtually insoluble. Only free sulphide may be toxic. Free dissolved sulphide occurs as hydrogen sulphide or sulphide ions in a pH-dependent equilibrium.

$$H_2S \leftrightarrow HS^- \leftrightarrow S^{2-}$$

Below pH 7, H_2S becomes gradually more important while above pH 9 it is S^{2-}. Between pH 7 and 9 virtually all sulphide is present as HS^-. H_2S is a gas that can enter the cell by passive diffusion. However, cyanobacteria capable of anoxygenic photosynthesis are apparently capable of uptake of the sulphide ion. Sulphide may also react with elemental sulphur to form polysulphides. It was thought that this process could occur only in microbial mats in which the amount of iron is not sufficient to keep the level of free sulphide low (Jørgensen and Cohen 1977). However, Visscher (1992) measured very high concentrations of polysulphides in a cyanobacterial mat, indicating that this compound may be more common than previously assumed. While on the one hand polysulphides are an order of magnitude more toxic for most organisms than sulphide, on the other hand it may serve as the form of elemental sulphur that is transported in cells (Steudel et al. 1990). The microbial mat purple sulphur bacterium *Thiocapsa roseopersicina* is capable of anoxygenic photosynthesis at the expense of polysulphide (Visscher et al. 1990).

4.9 Interactions of Cyanobacteria with Iron

Iron is one of the most abundant elements on Earth and it has several important functions in microbial mats. Iron occurs in three oxidation states: elemental iron Fe^o, ferrous (reduced iron), Fe^{2+} and ferric (oxidized iron), Fe^{3+}. Ferric iron is virtually insoluble and in the presence of oxygen ferrous iron is readily oxidized, except under acidic conditions (pH < 2). Elemental iron is not stable in nature because it will also be oxidized. Thus, in the presence of oxygen at physiological pH iron is hardly available for organisms and aerobic microorganisms often produce compounds that have a high affinity for ferric iron. These siderophores bind iron and transport it into the cells.

Iron is an essential micronutrient for all organisms and also its redox behaviour gives rise to its biological importance. Iron occurs in a number of enzymes that act as electron carriers, such as cytochromes, in respiratory electron transport chains and ferredoxins which serve as electron donors to a variety of processes (including N_2 fixation, nitrate- and sulphate reduction) in the cell, and indirectly, CO_2 fixation. Moreover, iron is an important co-factor in enzymes such as nitrogenase and nitrate reductase. In addition to this assimilatory metabolism of iron, the dissimilatory iron metabolism is of importance in microbial mats and other environments. Ferric iron may serve as electron acceptor in anaerobic respiration (Lovley 1991). Under acid conditions, ferrous iron can be oxidized aerobically by the chemolithotrophic, autotrophic bacterium *Thiobacillus ferrooxidans* (Leduc and Ferroni 1994). Under neutral conditions, ferrous iron is rapidly oxidized by oxygen (Druschel et al. 2008). However, *Gallionella ferruginea* and *Leptothrix ochracea* oxidize iron to support an autotrophic mode of metabolism (Hallbeck and Pedersen 1991; Carlile and Dudeney 2000). Instead of competing with the chemical reaction they rather seem to compete with the autocatalysis of iron oxidation as the result of their own activity (Rentz et al. 2007). Ferrous iron may also serve as electron donor in anoxygenic photosynthesis by specialized purple bacteria (Widdel et al. 1993; Ehrenreich and Widdel 1994). The fourth biologically controlled iron transformation is the formation of magnetite in magnetotactic bacteria and in a variety of other organisms (Stolz 1993).

In microbial mats iron is often present in high amounts (Wieland et al. 2005). In coastal and hypersaline microbial mats suspended iron oxides present in seawater precipitate in the sediment. Iron may precipitate either as oxides and hydroxides, siderite ($FeCO_3$) or as iron sulphide (FeS) and pyrite (FeS_2). Iron readily reacts with sulphide:

$$2Fe^{3+} + H_2S \rightarrow 2Fe^{2+} + S^o + 2H^+$$

$$2Fe^{2+} + 2H_2S \rightarrow 2FeS + 4H^+$$

$$2Fe^{3+} + 3H_2S \rightarrow 2FeS + S^o + 6H^+$$

FeS is virtually insoluble. Thus both ferric and ferrous iron is important in immobilizing the toxic sulphide. Ferrous iron including FeS will react with oxygen both chemically as well as biologically. In microbial mats oxygen supersaturation may present a problem for cyanobacterial growth and the presence of ferrous iron may aid in keeping the partial pressure of oxygen low (Wieland et al. 2005). Moreover, the oxidation of iron in siderite will result in the liberation of CO_2.

In coastal and hypersaline microbial mats a layer of oxidized iron is often observed between the layer of cyanobacteria and the anoxic layers below (Fig. 4.2). When purple sulphur bacteria are present, this layer of oxidized iron usually separates them from the cyanobacteria. The origin of this layer of oxidized iron is not precisely known. Since this layer is generally found at the transition of the oxic-anoxic layers, ferric iron may be produced by chemical oxidation of ferrous iron or biologically by iron-oxidizing bacteria. When oxygen is unavailable due to the continuously migrating oxic-anoxic transition zone, denitrifying bacteria could be responsible for the anaerobic iron oxidation (Straub et al. 1996) although in many microbial mats the amount of nitrate is probably too low to allow for this process. Cohen (1989) supposed that some mat-forming cyanobacteria were capable of iron-dependent anoxygenic photosynthesis. However, it is more likely that the iron is in fact oxidized by oxygen evolved by photosynthesis. Alternatively, anoxygenic phototrophic purple bacteria that use iron as electron donor may have produced the layer of ferric iron. Such organisms have been

isolated from freshwater and marine sediments, including intertidal mud (Widdel et al. 1993; Ehrenreich and Widdel 1994). Also green sulphur bacteria have been shown to use Fe^{2+} as an electron donor in anoxygenic photosynthesis (Crowe et al. 2008). Although such anoxygenic phototrophic bacteria may be responsible for the layer of ferric iron found in microbial mats this has not been demonstrated (Pierson and Parenteau 2000). Moreover, other evidence showed that the oxidation of iron in microbial mats was entirely the result of the oxygen production by the cyanobacteria (Trouwborst et al. 2007). In fact, reduced iron stimulated photosynthesis in cyanobacterial mats which led to higher oxygen levels and higher pH resulting in the precipitation of iron oxides (Pierson et al. 1999).

The formation of distinct layers of oxidized iron in microbial mats may well have resulted in the formation of so-called Banded Iron Formations (BIFs) formed during the Archean and Proterozoic ages. Banded Iron Formations are finely layered sedimentary rocks composed mainly of silica and iron oxides (James and Trendall 1982). BIFs were deposited over large areas and several thousands are known. Although the majority is only few meters thick and covers a limited area, others are several hundreds of meters thick and extend over many thousands of square kilometres (James and Trendall 1982). The iron content of BIFs is typically in the range of 24–35%, which is 5–7 times more than normally found in the crust. These iron formations are therefore of great economic importance. The silica content of BIF's is about 45%. Together, iron oxides and silica may make up to 90% of the weight of BIF. Iron oxides and silica (chert) occur in alternating layers. The cherty banded iron formation of Hamersley Basin, Australia, is one of the largest in the world and is characterized by stratification at different scales. At the millimetre scale microbands of iron minerals are recognized, separated at the centimetre scale by mesobands of chert. Regular banding is seen at the meter scale (macrobands) (James and Trendall 1982). Over 90% of the deposits are from the early Proterozoic age (2,500–1,900 Million years). Although it is tempting to assume a biological basis for the genesis of these cherty iron stromatolites, so far evidence of biogenesis has not emerged. Because of the fact that BIFs were overwhelmingly present during the early Proterozoic this has also been taken as evidence for the oxygenation of the earth's atmosphere which started 2,300 Million years ago. One mechanism for BIF formation may be the chemical oxidation of ferrous iron with oxygen evolved by oxygenic photosynthesis, most likely by cyanobacteria (Trouwborst et al. 2007). The huge amounts of ferrous iron in the earth's crust would act like a buffer and prevent the oxygenation of the atmosphere until most of the iron was oxidized. Although less abundant, the fact that BIF's are also known from the mid Archean ($3.4–2.9 \times 10^9$ years) might indicate other mechanisms. Geological evidence shows that until 2.0×10^9 years ago the oxygen level of the earth's atmosphere was still quite low. High energy solar UV irradiation (200–300 nm range) could freely reach the surface of the earth where it could be absorbed by ferrous iron, resulting in the formation of ferric iron and H_2 which escaped into the atmosphere (Cairns-Smith 1978). This reaction has been experimentally proven to be a possible explanation for the precipitation of ferric iron.

Ferrous and ferric iron strongly absorbs in the region 220–270 nm, UV light that is deleterious for organisms. It has been suggested that both ferrous and ferric iron play an important role in protection from UV irradiation because they provide an effective UV screen (Pierson and Olson 1989). It is assumed that the flux of UV irradiation that reached the earth surface during the early Precambrian was very high since the oxygen-free atmosphere would scarcely attenuate it. It is also known that microbial life developed on earth during this period, particularly in stromatolites. This life was apparently not arrested by the high UV flux. Although UV-C light does not reach the earth surface because it is completely absorbed by the earth's atmosphere, it is interesting that some mat-forming cyanobacteria such as *Microcoleus chthonoplastes* accumulate large amounts of iron at the outer polysaccharide sheath (Stal 1994). Iron is bound to negatively charged polysaccharides, particularly through the presence of uronic acids and precipitates at the sheath (Bender et al. 1994). Acidic extracellular polymeric substances containing carboxyl groups have been shown to mediate iron oxide mineralization (Chan et al. 2009). This has also been seen in a new cyanobacterium ('Chroogloeocystis siderophila') isolated from an iron-depositing hot spring microbial mat (Brown et al. 2005). This strain possesses elevated requirements for iron and also tolerates high levels of iron, making this organism well adapted to thrive in high iron environments. 'C. siderophila' failed to grow at low (8 μM) and at very high (1,000 μM) concentrations of Fe^{3+}. Although this iron is present in an insoluble form, its toxicity at high concentrations may be through the binding of iron precipitates to the outer sheath. Ferric iron is reduced through this EPS or through other cellular processes associated with it, or even under the influence of light. The ferrous iron produced in this way can be taken up by the cell but when in excess it may cause a problem inside the cell or it may draw excessively on the pool of reducing equivalents. Brown et al. (2005) suggested that the accumulation of iron served as a pool of iron for times of low iron availability but this seems unnecessary when thriving in a high iron environment. However, the iron precipitates bound to the outer sheath may present a way for the uptake of iron in organisms that lack siderophores. The sheath EPS would then serve as a siderophore.

There may be other advantages of accumulating iron precipitates by cyanobacteria. One of the possibilities is that it evolved from an ancient UV screen to serve new functions

for the cyanobacteria or for the ecosystem as a whole. For instance, the accumulation of iron by mat-forming cyanobacteria has been shown to protect the organism from sulphide produced either by sulphate-reducing bacteria living in the immediate vicinity of the cyanobacteria or produced by themselves through the reduction of elemental sulphur. Hence, the bound ferric hydroxides may represent a buffer against toxic sulphide, which reacts to produce ferrous iron and iron sulphide. Another possibility is that ferrous iron will react with oxygen, keeping its concentration low and minimizing photorespiration which would lead to a loss of fixed carbon. A low partial pressure of oxygen is essential for the cell in order to minimize the oxygenase reaction of the CO_2-fixing enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) The layer of ferric iron may therefore present an efficient barrier between the aerobic and anaerobic parts of the system (Stal 2001). Finally, *M. chthonoplastes* is also capable of reducing ferric iron probably using it as an electron acceptor during anaerobic dark metabolism (Stal 1994).

Iron oxides form complexes with phosphate which is then immobilized and unavailable as source of phosphate. It is liberated when the iron is reduced. Hence, the cycle of oxidation and reduction of iron may also be important for the temporal binding and storage of phosphate in a microbial mat.

4.10 Phosphorus in Microbial Mats

Few studies have addressed the role of phosphorus in microbial mats. This is remarkable because phosphate is involved in a variety of geochemical reactions that are important in mats and it is indispensible for growth and metabolic activity for all forms of life, including cyanobacteria. The almost complete ignorance of phosphorus in the study of microbial mats is also in strong contrast with the attention it receives in the study of phytoplankton. Generally, nitrogen or phosphorus limits growth of phytoplankton and most likely this applies also to cyanobacteria that form microbial mats.

Typically about 3% dry mass of cells consists of phosphorus, but some cells can store phosphate as polyphosphate, which increases their phosphorus content. Cyanobacteria take up orthophosphate (H_3PO_4) which is the most common form of inorganic phosphorus.

The solubility of orthophosphate is controlled by elements such as Ca^{2+}, Mg^{2+}, Fe^{2+}, Fe^{3+} and Al^{3+}. In seawater, the solubility of orthophosphate is predominantly controlled by Ca^{2+}, which at a suitable pH (7.4–8.1) produces the virtually insoluble hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; solubility product 1.53×10^{-112}) (Ehrlich 1996). In addition, phosphate may also form an insoluble precipitate with ferric iron ($FePO_4 \cdot 2H_2O$, strengite, solubility product 1.35×10^{-18}). Phosphate may be liberated from these insoluble minerals by microbial activity. The mechanisms include:

(i) production of organic acids
(ii) production of chelators
(iii) dissimilatory reduction of ferric iron
(iv) production of sulphide (Ehrlich 1996).

The latter can react with ferric iron phosphate according to:

$$2FePO_4 + 3H_2S \rightarrow 2FeS + 2H_3PO_4 + S^o$$

All these processes are likely to occur in microbial mats, but the phosphate liberated will be taken up immediately by the microbial community. Hence, the occurrence of free orthophosphate ion in microbial mats is expected to be negligible. Any organic phosphates must be cleaved hydrolytically by phosphatases.

As argued in Sect. 4.7, the growth of most cyanobacterial mats in coastal environments seems to be limited by nitrogen. However, microbial mats formed by heterocystous cyanobacteria are more likely to become phosphate-limited because N_2 fixation provides all the nitrogen needed for their growth. Mats built by non-heterocystous cyanobacteria are probably still nitrogen-limited as a result of impairment of N_2 fixation by oxygen. However, as is shown in Table 4.5, phosphate fertilization of such a mat resulted in a dramatic increase of N_2 fixation. Similar observations were made by Camacho and De Wit (2003) for hypersaline microbial mats and by Pinckney et al. (1995) in stromatolitic microbial mats in the Bahamas. While in hypersaline microbial mats the addition of nitrogen resulted in a shift in the community from cyanobacteria to diatoms without increasing the photosynthetic capacity of the mat, phosphate additions greatly stimulated the cyanobacterial community and their capacity of N_2 fixation. Obviously, these mats were co-limited by phosphate and nitrogen. It is known that N_2 fixation requires a certain amount of phosphate for optimal performance (de Nobel et al. 1997). The effect of phosphate fertilization on N_2 fixation may also have been indirect since it also caused a strong decrease in dissolved oxygen in the mat. The latter explanation is supported by the fact that N_2 fixation was also stimulated by other treatments that resulted in a decrease of oxygen (Table 4.5). Phosphate fertilization may also have stimulated heterotrophic bacterial activity and consequently oxygen uptake. The addition of phosphate stimulated gross photosynthesis and oxygen consumption equally well in the hypersaline microbial mat so that net photosynthesis remained unaltered (Ludwig et al. 2006). Hence, the microbial community as a whole was phosphate limited. However, high phosphate (1 mM) additions inhibited photosynthesis but not oxygen consumption. This was possibly due to chemical interactions of phosphate with iron or calcium ions, influencing their availability (Elser et al. 2005). Phosphate limitation caused very high C:P ratios which

constrains the quality of the food for herbivores and therefore minimizes grazing activity (Elser et al. 2005). Phosphate fertilization of coastal mats resulted in a considerable increase of chlorophyll *a* and a shift in cyanobacterial species composition from an *Oscillatoria*-dominated community to one with mainly *Phormidium*-type forms (Stal unpublished).

Although phosphorus may occur in other oxidation states (from +5 to −3), it is not important in redox reactions, as is the case with nitrogen and sulphur. Bacteria readily oxidize any reduced phosphorus, both aerobically and anaerobically. The reduction of orthophosphate is thermodynamically not favorable and is therefore not important for dissimilatory purposes. Hence, the microbial phosphorus cycle consists predominantly of the uptake of inorganic phosphate and the liberation by excretion or autolysis of organic phosphate, which is subsequently mineralized by phosphatases.

Although almost all phosphate on earth is present in the oxidized (+5) form, it has become clear that the more reduced form phosphonate (+3) plays an important role in many organisms (White and Metcalf 2007). Phosphonates are characterized by a very stable C-P bond. The potential of the use of phosphonate as a source of phosphorus has been proposed for the marine planktonic filamentous cyanobacterium *Trichodesmium* (Dyhrman et al. 2006) and the transporter gene *phnD* has been found in marine picocyanobacteria (IIikchyan et al. 2009). The genes that code for the enzymes that are capable of hydrolyzing this bond may have been spread by lateral gene transfer (Huang et al. 2005). In the ocean the source of phosphonates may in fact be the cyanobacterium *Trichodesmium*, of which 10% of the phosphorus is present as phosphonate (Dyhrman et al. 2009). Hot spring microbial mats may constitute up to 5% as phosphonate and may therefore represent an important source of phosphorus, when other sources become unavailable (Adam et al. 2008). The unicellular mat-forming cyanobacterium *Synechococcus* OS-B' possesses genes for the transport of metabolism of phosphonates which were transcribed upon phosphate starvation. This organism could become adapted to growth at the expense of phosphonate even when inorganic phosphate is the dominant source of phosphorus in these mats and phosphonate appeared to be inhibitory in the short term (Adam et al. 2008). The source of phosphonate in microbial mats remains unknown as well as whether it is common in microbial mats.

Phosphate may be stored in mineral deposits such as phosphorite, apatite, strengite, and other forms. Phosphorite deposits are usually found in coastal waters or shallow seas. They can be formed authigenically when soluble phosphate reacts with calcium to form calcium phosphate or by diagenesis when phosphate replaces carbonate in calcareous concretions (Ehrlich 1996). Both processes are probably biologically controlled. The model of Piper and Codespoti (1975) explains phosphorite formation in the marine environment from the mineralization of organic matter below the oxygen minimum layer, where it is coupled to denitrification. This results in excess inorganic phosphate compared to combined nitrogen. Upwelling transports phosphate to the sea surface, where it precipitates with calcium. This model could also apply to microbial mats where the same processes take place. Such phosphorite accumulation has been observed in cyanobacterial mats found on the bottom of small brackish ponds of atolls in French Polynesia called *kopara* (Rougerie et al. 1997). Dahanayake and Krumbein (1985) also reported phosphorite formed by a microbial mat, but concluded that fungi rather than cyanobacteria produced this particular fossil mat.

4.11 Conclusions

Laminated microbial mats are often considered to be recent analogues of fossil Precambrian stromatolites. Stromatolites are laminated lithified structures that have been formed by growth and metabolism of microorganisms. Studies of carbon isotope ratios provide evidence that photosynthesis was involved in the formation of stromatolites and the discovery of microfossils supports the idea that cyanobacteria have built these formations. However, modern microbial mats rarely lithify and doubts have been raised as to whether these systems really can be considered as analogues. Moreover, the sedimentary record may be biased because lithified mats have a greater potential of preservation. Nevertheless, non-lithifying mats have also left their traces in the fossil record and therefore we know that they have existed throughout the geological history. There are a limited number of examples of microbial mats that calcify and form more or less laminated lithified structures which have morphologies very similar to the Precambrian examples. The comparison of lithifying and non-lithifying microbial mats has provided deeper understanding of the factors that determine the processes leading to lithification.

In the majority of examples of microbial mats, cyanobacteria play a key role in their formation. Cyanobacteria are oxygenic phototrophic bacteria and many species are capable of using dinitrogen (N_2) as their only source of nitrogen. Hence, these organisms have a minimum requirement to proliferate, which is important considering the harsh conditions in which microbial mats often develop. Only extreme conditions will limit the biodiversity and exclude higher grazing organisms so that cyanobacteria accumulate to the dense community that produces a mat. Cyanobacteria have a number of additional properties that make them excellent model organisms for forming microbial mats. Many species are motile through gliding movement, which allows them to position themselves under optimal conditions. Light and possibly chemical factors serve as signals to direct the movement of the organisms. Many cyanobacteria are further characterized

by a high affinity for light and reach maximum rate of photosynthesis at very low light intensities and have low compensation points. The requirement for energy for maintenance purposes is low. Cyanobacteria often have high affinities for nutrients and perhaps even more important, they possess storage possibilities for a variety of growth factors. In addition, their metabolic versatility and reactivity are important properties of cyanobacteria. For instance, cyanobacteria are not only photoautotrophs that perform oxygenic photosynthesis but many are also capable of anoxygenic photosynthesis. The majority of mat-forming cyanobacteria is even capable of performing oxygenic and anoxygenic photosynthesis in concert, allowing maximum flexibility and reactivity to quickly changing environmental conditions. Whereas aerobic respiration of endogenous glycogen seems to be the normal metabolism in the dark, this does not usually occur in microbial mats, which often are devoid of oxygen during the night. However, most, if not all, mat forming cyanobacteria are capable of fermentation.

Growth and metabolic activity of the cyanobacteria introduce organic matter in the microbial mat system and its degradation will drive the growth of other micro-organisms in microbial mats. Although some organic matter may become liberated into the environment by death and lysis of the cyanobacteria, this seems not to be most important. In mature microbial mats there is hardly any net growth of cyanobacteria despite the high rates of photosynthesis. Organic matter may become liberated as a result of photorespiration, fermentation, excretion of organic solutes and the secretion of extracellular polymeric substances (EPS), notably polysaccharides. Cyanobacteria may produce a well-defined polysaccharide sheath. This is often a structural component of the cell envelope of cyanobacteria. However, cyanobacteria may also produce vast amounts of mucilage which is not or only partly associated with the organism. Mucilage is often composed of recalcitrant polysaccharides. It produces a matrix in which the microbial mat is embedded. This material is sticky and it will glue sediment particles and organisms together, giving stability to the sediment surface. Since this matrix cannot be mixed it presents a diffusive barrier. The polysaccharide matrix is therefore responsible for the accumulation and supersaturation with oxygen in the light and likewise for the anoxic conditions that occur during the night. Of particular importance is the role that EPS probably plays in calcification. It is likely that EPS inhibits this process and that it serves as an anti-calcification agent. This may be either by preventing growth of small calcite crystallization nuclei or by binding Ca^{2+}. Therefore cyanobacterial mats in which a high amount of mucus is produced will not calcify. The production of mucus in cyanobacteria may be related to unbalanced growth. Unbalanced growth occurs when one growth factor is in shortage. Nitrogen limitation is well known as a factor that stimulates the secretion of mucus. Marine microbial mats are often developing under conditions of nitrogen shortage. This is evidenced by the fact that these mats are built by N_2-fixing cyanobacteria. These mats usually consist of non-heterocystous diazotrophic cyanobacteria that are inefficient in fixing dinitrogen because they lack an effective mechanism to protect nitrogenase from oxygen. This is particularly the case when during the day very high levels of oxygen occur in the mat. At night oxygen is absent and therefore N_2 fixation in these mats occurs predominantly then. However, the limited amount of energy and low-potential electrons that can be generated under such conditions will not allow the fixation of ample dinitrogen. Heterocystous cyanobacteria are optimally equipped for N_2 fixation in the light and will not usually face nitrogen-limited growth. However, as stated above, such cyanobacteria are largely excluded from microbial mats. Possibly heterocystous cyanobacteria cannot tolerate high levels of sulphide or dark anoxic conditions. Also the absence of gliding motility in heterocystous cyanobacteria and the weak connection between the heterocyst and the vegetative cell may exclude these organisms from environments with high rates of sedimentation. It seems reasonable to assume that Precambrian microbial mats did not face nitrogen limitation and thus might have produced much less mucus, calcification therefore not being inhibited. Future research should investigate the nitrogen state of modern calcifying microbial mats and the role of EPS as anti-calcification agents in non-lithifying mats.

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References

Abed RMM, Garcia-Pichel F, Hernández-Mariné M (2002) Polyphasic characterization of benthic, moderately halophilic, moderately thermophilic cyanobacteria with very thin trichomes and the proposal of *Halomicronema excentricum* gen. nov., sp. nov. Arch Microbiol 177:361–370

Adam MM, Gómez-García MR, Grossman AR, Bhaya D (2008) Phosphorus deprivation responses and phosphonate utilization in a thermophilic *Synechococcus* sp. from microbial mats. J Bacteriol 190:8171–8184

Albertano P, Kovacik L (1996) Light and temperature responses of terrestrial sciaphilous strains of *Leptolyngbya* sp. in cross-gradient cultures. Algol Stud 83:17–28

Allen MM, Smith AJ (1969) Nitrogen chlorosis in blue-green algae. Arch Mikrobiol 69:114–120

Allewalt JP, Bateson MM, Revsbech NP, Slack K, Ward DM (2006) Effect of temperature and light on growth of and photosynthesis by *Synechococcus* isolates typical of those predominating in the Octopus Spring microbial mat community of Yellowstone National Park. Appl Environ Microbiol 72:544–550

Anderson KL, Tayne TA, Ward DM (1987) Formation and fate of fermentation products in hot spring cyanobacterial mats. Appl Environ Microbiol 53:2343–2352

Arp G, Reimer A, Reitner J (2001) Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. Science 292:1701–1704

Artus NN, Somerville SC, Somerville CR (1986) The biochemistry and cell biology of photorespiration. CRC Crit Rev Plant Sci 4:121–147

Awramik SM (1984) Ancient stromatolites and microbial mats. In: Cohen Y, Castenholz RW, Halvorson HO (eds) Microbial mats: stromatolites. Alan R. Liss Inc, New York, pp 1–22, 498 pp

Badger MR, Andrews TJ (1982) Photosynthesis and inorganic carbon usage by the marine cyanobacterium, *Synechococcus* sp. Plant Physiol 70:517–523

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

Bar-Or Y, Shilo M (1987) Characterization of macromolecular flocculants produced by *Phormidium* sp. strain J-1 and by *Anabaenopsis circularis* PCC 6720. Appl Environ Microbiol 53:2226–2230

Bar-Or Y, Shilo M (1988) The role of cell-bound flocculants in coflocculation of benthic cyanobacteria with clay particles. FEMS Microbiol Ecol 53:169–174

Bar-Or Y, Kessel M, Shilo M (1985) Modulation of cell surface hydrophobicity in the benthic cyanobacterium *Phormidium* J-1. Arch Microbiol 142:21–27

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

Bauer MR, Haddad RI, Des Marais DJ (1991) Method for determining stable isotope ratios of dissolved organic carbon in interstitial and other natural marine waters. Mar Chem 33:335–351

Bauersachs T, Kremer B, Schouten S, Sinninghe Damsté JS (2009) A biomarker and $\delta^{15}N$ study of thermally altered Silurian cyanobacterial mats. Org Geochem 40:149–157

Bauld J (1984) Microbial mats in marginal marine environments: Shark Bay, Western Australia, and Spencer Gulf, South Australia. In: Cohen Y, Castenholz RW, Halvorson HO (eds) Microbial mats: stromatolites. Alan R. Liss Inc, New York, pp 39–58, 498 pp

Baumgartner LK, Reid RP, Dupraz C, Decho AW, Buckley DH, Spear JR, Przekop KM, Visscher PT (2006) Sulfate reducing bacteria in microbial mats: changing paradigms, new discoveries. Sediment Geol 185:131–145

Bebout BM, Garcia-Pichel F (1995) UV B-induced vertical migrations of cyanobacteria in a microbial mat. Appl Environ Microbiol 61:4215–4222

Bender J, Rodriguezeaton S, Ekanemesang UM, Phillips P (1994) Characterization of metal-binding bioflocculants produced by the cyanobacterial component of mixed microbial mats. Appl Environ Microbiol 60:2311–2315

Bergman B, Gallon JR, Rai AN, Stal LJ (1997) N_2 fixation by non-heterocystous cyanobacteria. FEMS Microbiol Rev 19:139–185

Berner R, Jensen T (1982) Ultrastructure of two hypolithic cyanobacteria from the Negev desert of Israel. Cytobios 35:7–18

Bertocchi C, Navarini L, Cesaro A (1990) Polysaccharides from cyanobacteria. Carbohydr Polym 12:127–153

Bhaya D (2004) Light matters: phototaxis and signal transduction in unicellular cyanobacteria. Mol Microbiol 53:745–754

Bolhuis H, Severin I, Confurius-Guns V, Wollenzien UIA, Stal LJ (2010) Horizontal transfer of the nitrogen fixation gene cluster in the cyanobacterium *Microcoleus chthonoplastes*. ISME J 4:121–130

Borman AH, De Jong EW, Huizinga M, Kok DJ, Westbroek P, Bosch L (1982) The role in $CaCO_3$ crystallization of an acid Ca^{2+}-binding polysaccharide associated with coccliths of *Emiliania huxleyi*. Eur J Biochem 129:179–183

Borman AH, De Jong EW, Thierry R, Westbroek P, Bosch L (1987) Coccolith-associated polysaccharides from cells of *Emiliania huxleyi* (Haptophyceae). J Phycol 23:118–123

Bosak T, Greene SE, Newman DK (2007) A likely role for anoxygenic photosynthetic microbes in the formation of ancient stromatolites. Geobiology 5:119–126

Boudreau BP, Canfield DE (1993) A comparison of closed- and open-system models for porewater pH and calcite-saturation state. Geochim Cosmochim Acta 57:317–334

Braissant O, Decho AW, Przekop KM, Gallagher KL, Glunk C, Dupraz C, Visscher PT (2009) Characteristics and turnover of exopolymeric substances in a hypersaline microbial mat. FEMS Microbiol Ecol 67:293–307

Brock TD (1975) Effect of water potential on a *Microcoleus* (Cyanophyceae) from a desert crust. J Phycol 11:316–320

Brock TD (1978) Thermophilic microorganisms and life at high temperatures. Springer, New York, 465 pp

Brocks JJ, Logan GA, Buick R, Summons RE (1999) Archean molecular fossils and the early rise of eukaryotes. Science 285:1033–1036

Brown II, Mummey D, Cooksey KE (2005) A novel cyanobacterium exhibiting an elevated tolerance for iron. FEMS Microbiol Ecol 52:307–314

Budinoff CR, Hollibaugh JT (2008) Arsenite-dependent photoautotrophy by an *Ectothiorhodospira*-dominated consortium. ISME J 2:340–343

Caiola MG, Ocampo-Friedmann R, Friedmann EI (1993) Cytology of long-term desiccation in the desert cyanobacterium *Chroococcidiopsis* (Chroococcales). Phycologia 32:315–322

Caiola MG, Billi D, Friedmann EI (1996) Effect of desiccation on envelopes of the cyanobacterium *Chroococcidiopsis* sp. (Chroococcales). Eur J Phycol 31:99–105

Cairns-Smith AG (1978) Precambrian solution photochemistry, inverse segregation, and banded iron formations. Nature 276:807–808

Camacho A, de Wit R (2003) Effect of nitrogen and phosphorus additions on a benthic microbial mat from a hypersaline lake. Aquat Microb Ecol 32:261–273

Camacho A, Rochera C, Silvestre JJ, Vicente E, Hahn MW (2005) Spatial dominance and inorganic carbon assimilation by conspicuous autotrophic biofilms in a physical and chemical gradient of a cold sulfurous spring: the role of differential ecological strategies. Microb Ecol 50:172–184

Campbell SE (1979) Soil stabilization by a prokaryotic desert crust: implications for Precambrian land biota. Orig Life 9:335–348

Canfield DE, Des Marais DJ (1991) Aerobic sulfate reduction in microbial mats. Science 251:1471–1473

Canfield DE, Des Marais DJ (1994) Cycling of carbon, sulfur, oxygen and nutrients in a microbial mat. In: Stal LJ, Caumette P (eds) Microbial mats. Structure, development and environmental significance. Springer, Heidelberg, pp 255–263, 463 pp

Canfield DE, Raiswell R (1991) Carbonate precipitation and dissolution. Its relevance to fossil preservation. In: Allison PA, Briggs DEG (eds) Taphonomy: releasing the data locked in the fossil record. Topics in geobiology, vol 9. Plenum Press, New York, pp 411–453, 546 pp

Canfield DE, Thamdrup B (1996) Fate of elemental sulfur in an intertidal sediment. FEMS Microbiol Ecol 19:95–103

Capone DG, O'Neil JM, Zehr J, Carpenter EJ (1990) Basis for diel variation in nitrogenase activity in the marine planktonic cyanobacterium *Trichodesmium thiebautii*. Appl Environ Microbiol 56:3532–3536

Carlile MJ, Dudeney AWL (2000) A microbial mat composed of iron bacteria. Microbiology 146:2092–2093

Castenholz RW (1973) Movements. In: Carr NG, Whitton BA (eds) The biology of blue-green algae. Blackwell Scientific Publications, Oxford, pp 320–339, 676 pp

Castenholz RW (1976) The effect of sulfide on the blue green algae of hot springs. I. New Zealand and Iceland. J Phycol 12:54–68

Castenholz RW (1977) The effect of sulfide on the blue-green algae of hot springs. II. Yellowstone National Park. Microb Ecol 3:79–105

Castenholz RW (1982) Motility and taxes. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publishers, Oxford, pp 413–439, 688 pp

Castenholz RW, Utkilen HC (1984) Physiology of sulfide tolerance in a thermophilic *Oscillatoria*. Arch Microbiol 138:299–305

Castenholz RW, Jørgensen BB, Damelio E, Bauld J (1991) Photosynthetic and behavioral versatility of the cyanobacterium *Oscillatoria boryana* in a sulfide-rich microbial mat. FEMS Microbiol Ecol 86:43–58

Chafetz HS (1994) Bacterially induced precipitates of calcium carbonate and lithification of microbial mats. In: Krumbein WE, Paterson DM, Stal LJ (eds) Biostabilization of sediments. BIS Verlag, Oldenburg, pp 149–163, 526 pp

Chafetz HS, Buczynski C (1992) Bacterially induced lithification of microbial mats. Palaios 7:277–293

Chan CS, Fakra SC, Edwards DC, Emerson D, Banfield JF (2009) Iron oxyhydroxide mineralization on microbial extracellular polysaccharides. Geochim Cosmochim Acta 73:3807–3818

Chen M, Schliep M, Willows RD, Cai Z-L, Neilan BA, Scheer H (2010) A red-shifted chlorophyll. Science. doi:10.1126/science.1191127

Choi JS, Chung YH, Moon YJ, Kim C, Watanabe M, Song P-S, Joe C-O, Bogorad L, Park YM (1999) Photomovement of the gliding cyanobacterium *Synechocystis* sp. PCC 6803. Photochem Photobiol 70:95–102

Christensen BE, Kjosbakken J, Smidsrod O (1985) Partial chemical and physical characterization of two extracellular polysaccharides produced by marine periphytic *Pseudomonas* sp. strain NCMB 2021. Appl Environ Microbiol 50:837–845

Cloud PE, Semikhatov MA (1969) Proterozoic stromatolite zonation. Am J Sci 267:1017–1061

Cohen Y (1989) Photosynthesis in cyanobacterial mats and its relation to the sulfur cycle: a model for microbial sulfur interactions. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 22–36, 511 pp

Cohen Y, Jørgensen BB, Revsbech NP, Poplawski R (1986) Adaptation to hydrogen sulfide of oxygenic and anoxygenic photosynthesis among cyanobacteria. Appl Environ Microbiol 51:398–407

Cox G, Benson D, Dwarte DM (1981) Ultrastructure of a cave-wall cyanophyte *Gloeocapsa* NS4. Arch Microbiol 130:165–174

Crowe SA, Jones C, Katsev S, Magen C, O'Neill AH, Sturm A, Canfield DE, Haffner GD, Mucci A, Sundby B, Fowle DA (2008) Photoferrotrophs thrive in an Archean ocean analogue. Proc Natl Acad Sci USA 105:15938–15943

Cullen JJ, Neale PJ (1994) Ultraviolet radiation, ozone depletion, and marine photosynthesis. Photosynth Res 39:303–320

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

D'Antoni D'Amelio E, Cohen Y, Des Marais DJ (1989) Comparative functional ultrastructure of two hypersaline submerged cyanobacterial mats: Guerrero Negro, Baja California Sur, Mexico, and Solar Lake, Sinai, Egypt. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 97–113, 511 pp

Dahanayake K, Krumbein WE (1985) Ultrastructure of a microbial mat-generated phosphorite. Miner Deposita 20:260–265

Davey A (1983) Effects of abiotic factors on nitrogen fixation by blue-green algae in Antarctica. Polar Biol 2:95–100

Davey MC, Clarke KJ (1992) Fine structure of a terrestrial cyanobacterial mat from Antarctica. J Phycol 28:199–202

Davey A, Marchant HJ (1983) Seasonal variation in nitrogen fixation by *Nostoc commune* Vaucher at the Vesthold Hills, Antarctica. Phycologia 22:377–385

De Nobel WT, Snoep JL, Westerhoff HV, Mur LR (1997) Interaction of nitrogen fixation and phosphorus limitation in *Aphanizomenon flos-aquae* (Cyanophyceae). J Phycol 33:794–799

De Philippis R, Vincenzini M (1998) Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiol Rev 22:151–175

De Philippis R, Margheri MC, Vincenzini M (1996) Fermentation in symbiotic and free-living cyanobacteria. Arch Hydrobiol 83:459–468

De Philippis R, Sili C, Paperi R, Vincenzini M (2001) Exopolysaccharide-producing cyanobacteria and their possible exploitation: a review. J Appl Phycol 13:293–299

De Winder B, Matthijs HCP, Mur LR (1989a) The role of water retaining substrata on the photosynthetic response of three drought tolerant phototrophic micro-organisms isolated from a terrestrial habitat. Arch Microbiol 152:458–462

De Winder B, Pluis J, De Reus L, Mur LR (1989b) Characterization of a cyanobacterial, algal crust in the coastal dunes of the Netherlands. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 77–83, 511 pp

De Winder B, Stal LJ, Mur LR (1990) *Crinalium epipsammum* sp. nov.: a filamentous cyanobacterium with trichomes composed of elliptical cells and containing poly-beta-(1,4) glucan (cellulose). J Gen Microbiol 136:1645–1653

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

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

De Wit R, Van Boekel WHM, Van Gemerden H (1988) Growth of the cyanobacterium *Microcoleus chtonoplastes* on sulfide. FEMS Microbiol Ecol 53:203–209

De Wit R, van den Ende FP, van Gemerden H (1995) Mathematical simulation of the interactions among cyanobacteria, purple sulfur bacteria and chemotrophic sulfur bacteria in microbial mat communities. FEMS Microbiol Ecol 17:117–135

Decho AW (1990) Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. Oceanogr Mar Biol Annu Rev 28:73–153

Decho AW (1994) Molecular-scale events influencing the macroscale cohesiveness of exopolymers. In: Krumbein WE, Paterson DM, Stal LJ (eds) Biostabilization of sediments. BIS Verlag, Oldenburg, pp 135–148, 526 pp

Decho AW (2000) Microbial biofilms in intertidal systems: an overview. Cont Shelf Res 20:1257–1273

Decho AW, Moriarty DJW (1990) Bacterial exopolymer utilization by a harpacticoid copepod: a methodology and results. Limnol Oceanogr 35:1039–1049

Decho AW, Visscher PT, Reid RP (2005) Production and cycling of natural microbial exopolymers (EPS) within a marine stromatolite. Palaeogr Palaeoclim Palaeoecol 219:71–86

Défarge C, Trichet J, Couté A (1994a) On the appearance of cyanobacterial calcification in modern stromatolites. Sediment Geol 94:11–19

Défarge C, Trichet J, Maurin A, Hucher M (1994b) Kopara in Polynesian atolls: early stages of formation of calcareous stromatolites. Sediment Geol 89:9–23

Des Marais DJ, Canfield DE (1994) The carbon isotope biogeochemistry of microbial mats. In: Stal LJ, Caumette P (eds) Microbial mats. Structure, development and environmental significance. Springer, Heidelberg, pp 289–298, 463 pp

Des Marais DJ, D'Amelio E, Farmer JD, Jørgensen BB, Palmisano AC, Pierson BK (1992) Case study of a modern microbial mat-building community: the submerged cyanobacterial mats of Guerrero Negro, Baja California Sur, Mexico. In: Schopf JW, Klein C (eds) The proterozoic biosphere. A multidisciplinary study. Cambridge University Press, New York, pp 325–333, 1348 pp

Dilling W, Cypionka H (1990) Aerobic respiration in sulfate-reducing bacteria. FEMS Microbiol Lett 71:123–127

Dillon JG, Miller S, Bebout B, Hullar M, Pinel N, Stahl DA (2009) Spatial and temporal variability in a stratified hypersaline microbial mat. FEMS Microbiol Ecol 68:46–58

Donkor V, Häder DP (1991) Effects of solar and ultraviolet radiation on motility, photomovement and pigmentation in filamentous, gliding cyanobacteria. FEMS Microbiol Ecol 86:159–168

Donkor VA, Amewowor DHAK, Häder DP (1993) Effects of tropical solar radiation on the motility of filamentous cyanobacteria. FEMS Microbiol Ecol 12:143–148

Dor I, Paz N (1989) Temporal and spatial distribution of mat microalgae in the experimental solar ponds, Dead Sea area, Israel. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 114–122, 511 pp

Druschel GK, Emerson D, Sutka R, Suchecki P, Luther GW III (2008) Low-oxygen and chemical kinetic constraints on the geochemical niche of neutrophilic iron(II) oxidizing microorganisms. Geochim Cosmochim Acta 72:3358–3370

Dubinin AV, Gerasimenko LM, Zavarzin GA (1992) Nitrogen fixation by cyanobacterium *Microcoleus chthonoplastes* from hypersaline lagoons of lake Sivash. Microbiology 61:593–597

Dupraz C, Visscher PT (2005) Microbial lithification in marine stromatolites and hypersaline mats. Trends Microbiol 13:429–438

Dupraz C, Visscher PT, Baumgartner LK, Reid RP (2004) Microbe-mineral interactions: early carbonate precipitation in a hypersaline lake (Eleuthera Island, Bahamas). Sedimentology 51:745–765

Dupraz C, Reid RP, Braissant O, Decho AW, Norman RS, Visscher PT (2009) Processes of carbonate precipitation in modern microbial mats. Earth Sci Rev 96:141–162

Dyhrman ST, Chappell PD, Haley ST, Moffett JW, Orchard ED, Waterbury JB, Webb EA (2006) Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*. Nature 439:68–71

Dyhrman ST, Benitez-Nelson CR, Orchard ED, Haley ST, Pellechia PJ (2009) A microbial source of phosphonates in oligotrophic marine systems. Nat Geosci 2:696–699

Ehrenberg CG (1838) Über das im Jahre 1686 in Curland vom Himmel gefallene Meteorpapier und über dessen Zusammensetzung aus Conferven und Infusorien. Abh Kgl Akad Wiss Berlin, pp 45–58

Ehrenreich A, Widdel F (1994) Anaerobic oxidation of ferrous iron by purple bacteria, a new type of phototrophic metabolism. Appl Environ Microbiol 60:4517–4526

Ehrlich HL (1996) Geomicrobiology. Marcel Dekker Inc, Basel, 719 pp

Eisenhut M, Ruth W, Haimovich M, Bauwe H, Kaplan A, Hagemann M (2008) The photorespiratory glycolate metabolism is essential for cyanobacteria and might have been conveyed endosymbiontically to plants. Proc Natl Acad Sci USA 105:17199–17204

Eloff JN, Steinitz Y, Shilo M (1976) Photooxidation of cyanobacteria in natural conditions. Appl Environ Microbiol 31:119–126

Elser JJ, Schampel JH, Garcia-Pichel F, Wade BD, Souza V, Eguiarte L, Escalante A, Farmer JD (2005) Effects of phosphorus enrichment and grazing snails on modern stromatolitic microbial communities. Freshw Biol 50:1808–1825

Emerson D, Revsbech NP (1994a) Investigation of an iron-oxidizing microbial mat community located near Aarhus, Denmark: field studies. Appl Environ Microbiol 60:4022–4031

Emerson D, Revsbech NP (1994b) Investigation of an iron-oxidizing microbial mat community located near Aarhus, Denmark: laboratory studies. Appl Environ Microbiol 60:4032–4038

Falcón LI, Cerritos R, Eguiarte LE, Souza V (2007) Nitrogen fixation in microbial mat and stromatolite communities from Cuatro Cienegas, Mexico. Microb Ecol 54:363–373

Fechner R (1915) Die Chemotaxis der Oscillarien und ihre Bewegungserscheinungen überhaupt. Z Bot 7:289–364

Ferris MJ, Ruff Roberts AL, Kopczynski ED, Bateson MM, Ward DM (1996) Enrichment culture and microscopy conceal diverse thermophilic *Synechococcus* populations in a single hot spring microbial mat habitat. Appl Environ Microbiol 62:1045–1050

Flores E, Herrero A (1994) Assimilatory nitrogen metabolism and its regulation. In: Bryant DA (ed) The molecular biology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, pp 487–517, 881 pp

Fouke BW, Farmer JD, Des Marais DJ, Pratt L, Sturchio NC, Burns PC, Discipulo MK (2000) Depositional facies and aqueous-solid geochemistry of travertine-depositing hot springs (Angel Terrace, Mammoth Hot Springs, Yellowstone National Park, USA). J Sediment Res 70:565–585

Friedman I, Lipkin Y, Ocampo-Paus R (1967) Desert algae of the Negev (Israel). Phycologia 6:185–200

Friedrich M, Schink B (1993) Hydrogen formation from glycolate driven by reversed electron transport in membrane vesicles of a syntrophic glycolate-oxidizing bacterium. Eur J Biochem 217: 233–240

Friedrich M, Schink B (1995) Isolation and characterization of a desulforubidin-containing sulfate-reducing bacterium growing with glycolate. Arch Microbiol 164:271–279

Fründ C, Cohen Y (1992) Diurnal cycles of sulfate reduction under oxic conditions in cyanobacterial mats. Appl Environ Microbiol 58:70–77

Gallon JR, Hashem MA, Chaplin AE (1991) Nitrogen fixation by *Oscillatoria* Spp under autotrophic and photoheterotrophic conditions. J Gen Microbiol 137:31–39

Garcia-Pichel F, Bebout BM (1996) Penetration of ultraviolet radiation into shallow water sediments: high exposure for photosynthetic communities. Mar Ecol Prog Ser 131:257–262

Garcia-Pichel F, Castenholz RW (1991) Characterization and biological implications od scytonemin, a cyanobacterial sheath pigment. J Phycol 27:395–409

Garcia-Pichel F, Castenholz RW (1994) On the significance of solar ultraviolet radiation for the ecology of microbial mats. In: Stal LJ, Caumette P (eds) Microbial mats. Structure development and environmental significance. Springer, Heidelberg, pp 77–84, 463 pp

Garcia-Pichel F, Pringault O (2001) Cyanobacteria track water in desert soils. Nature 413:380–381

Garcia-Pichel F, Wojciechowski MF (2009) The evolution of a capacity to build supra-cellular ropes enabled filamentous cyanobacteria to colonize highly erodible substrates. PLoS One 4:e7801

Garcia-Pichel F, Mechling M, Castenholz RW (1994) Diel migrations of microorganisms within a benthic, hypersaline mat community. Appl Environ Microbiol 60:1500–1511

Garcia-Pichel F, Prufert-Bebout L, Muyzer G (1996) Phenotypic and phylogenetic analyses show *Microcoleus chthonoplastes* to be a cosmopolitan cyanobacterium. Appl Environ Microbiol 62:3284–3291

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

Gebelein CD (1976) Open marine subtidal and intertidal stromatolites (Florida, The Bahamas and Bermuda). In: Walter MR (ed) Stromatolites. Elsevier, Amsterdam, pp 381–388, 790 pp

Gerbaud A, Andre M (1987) An evaluation of the recycling in measurements of photorespiration. Plant Physiol 83:933–937

Gingras M, Hagadorn JW, Seilacher A, Lalonde SV, Pecoits E, Petrash D, Konhauser KO (2011) Possible evolution of mobile animals in association with microbial mats. Nat Geosci. doi:10.1038/NGO1142

Golubić S (1973) The relationship between blue-green algae and carbonate deposits. In: Carr NG, Whitton BA (eds) The biology of blue-green algae. Blackwell Scientific Publications/Univeristy of California Press, Oxford/Berkeley, pp 434–473, 676 pp

Griffin BM, Schott J, Schink B (2007) Nitrite, an electron donor for anoxygenic photosynthesis. Science 316:1870

Griffiths MSH, Gallon JR, Chaplin AE (1987) The diurnal pattern of dinitrogen fixation by cyanobacteria in situ. New Phytol 107:649–657

Guerrero R, Mas J (1989) Multilayered microbial communities in aquatic ecosystems: growth and loss factors. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 37–51, 511 pp

Häder D-P (1987a) Photomovement. In: Fay P, Van Baalen C (eds) The cyanobacteria. Elsevier, Amsterdam, pp 325–345, 534 pp

Häder D-P (1987b) Photosensory behavior in procaryotes. Microbiol Rev 51:1–21

Häder D-P (1988) Signal perception and amplification in photoresponses of cyanobacteria. Biophys Chem 29:155–159

Häder DP, Kumar HD, Smith RC, Worrest RC (1998) Effects on aquatic ecosystems. J Photochem Photobiol B 46:53–68

Halfen LN, Castenholz RW (1971) Gliding motility in the blue-green alga *Oscillatoria princeps*. J Phycol 7:133–145

Hallbeck L, Pedersen K (1991) Autotrophic and mixotrophic growth *of Gallionella ferruginea*. J Gen Microbiol 137:2657–2661

Hedges SB, Chen H, Kumar S, Wang DY-C, Thompsom AS, Watanabe H (2001) A genomic timescale for the origin of eukaryotes. BMC Evol Biol 1:4

Heijthuijsen JHFG, Hansen TA (1989) Betaine fermentation and oxidation by marine *Desulfuromonas* strains. Appl Environ Microbiol 55:965–969

Heyer H, Stal LJ, Krumbein WE (1989) Simultaneous heterolactic and acetate fermentation in the marine cyanobacterium *Oscillatoria limosa* incubated anaerobically in the dark. Arch Microbiol 151:558–564

Hoehler TM, Albert DB, Alperin MJ, Martens CS (1999) Acetogenesis from CO_2 in an anoxic marine sediment. Limnol Oceanogr 44:662–667

Hoehler TM, Bebout BM, Des Marais DJ (2001) The role of microbial mats in the production of reduced gases on the early Earth. Nature 412:324–327

Hoehler TM, Albert DB, Alperin MJ, Bebout BM, Martens CS, Des Marais DJ (2002) Comparative ecology of H_2 cycling in sedimentary and phototrophic ecosystems. Antonie Van Leeuwenhoek 81:575–585

Hoiczyk E (2000) Gliding motility in cyanobacteria: observations and possible explanations. Arch Microbiol 174:11–17

Howard JB, Rees DC (1994) Nitrogenase: a nucleotide-dependent molecular switch. Annu Rev Biochem 63:235–264

Huang J, Su Z, Xu Y (2005) The evolution of microbial phosphonate degradative pathways. J Mol Evol 61:682–690

Husic DW, Husic HD, Tolbert NE (1987) The oxidative photosynthetic carbon cycle or C_2 cycle. CRC Crit Rev Plant Sci 5:45–100

Ilikchyan IN, Mckay RML, Zehr JP, Dyhrman ST, Bullerjahn GS (2009) Detection and expression of the phosphonate transporter gene *phnD* in marine and freshwater picocyanobacteria. Environ Microbiol 11:1314–1324

Jaeschke A, Op den Camp HJM, Harhangi H, Klimiuk A, Hopmans EC, Jetten MSM, Schouten S, Sinninghe Damsté JS (2009) 16S rRNA gene and lipid biomarker evidence for anaerobic ammonium-oxidizing bacteria (anammox) in California and Nevada hot springs. FEMS Microbiol Ecol 67:343–350

James HL, Trendall AF (1982) Banded iron-formation: distribution in time and paleoenvironmental significance. In: Holland HD, Schidlowski M (eds) Mineral deposits and the evolution of the biosphere. Springer, Heidelberg, pp 199–217, 333 pp

Janson S, Matveyev A, Bergman B (1998) The presence and expression of *hetR* in the non-heterocystous cyanobacterium *Symploca* PCC 8002. FEMS Microbiol Lett 168:173–179

Jones K, Stewart WDP (1969) Nitrogen turnover in marine and brackish habitats. III. The production of extracellular nitrogen by Calothrix scopulorum. J Mar Biol Assoc UK 49:475–488

Jonkers HM, Ludwig R, de Wit R, Pringault O, Muyzer G, Niemann H, Finke N, de Beer D (2003) Structural and functional analysis of a microbial mat ecosystem from a unique permanent hypersaline inland lake: 'La Salada de Chiprana' (NE Spain). FEMS Microbiol Ecol 44:175–189

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

Jørgensen BB, Des Marais DJ (1988) Optical properties of benthic photosynthetic communities: fiber-optic studies of cyanobacterial mats. Limnol Oceanogr 33:99–113

Jørgensen BB, Nelson DC (1988) Bacterial zonation, photosynthesis, and spectral light distribution in hot spring microbial mats of Iceland. Microb Ecol 16:133–147

Jørgensen BB, Revsbech NP, Blackburn TH, Cohen Y (1979) Diurnal cycle of oxygen and sulfide microgradients and microbial photosynthesis in a cyanobacterial mat sediment. Appl Environ Microbiol 38:46–58

Jørgensen BB, Revsbech NP, Cohen Y (1983) Photosynthesis and structure of benthic microbial mats: micro-electrode and SEM studies of four cyanobacterial communities. Limnol Oceanogr 28:1075–1093

Jørgensen BB, Cohen Y, Revsbech NP (1986) Transition from anoxygenic to oxygenic photosynthesis in *a Microcoleus chthonoplastes* cyanobacterial mat. Appl Environ Microbiol 51:408–417

Jørgensen BB, Cohen Y, Des Marais DJ (1987) Photosynthetic action spectra and adaptation to spectral light distribution in a benthic cyanobacterial mat. Appl Environ Microbiol 53:879–886

Joye SB, Paerl HW (1994) Nitrogen cycling in microbial mats: rates and patterns of denitrification and nitrogen fixation. Mar Biol 119:285–295

Kalkowsky E (1908) Oolith und Stromatolith im Norddeutschen Buntsandstein. Z Dtsch Geol Ges 60:68–125

Kaplan A, Schwarz R, Lieman-Hurwitz J, Ronen-Tarazi M, Reinhold L (1994) Physiological and molecular studies on the response of cyanobacteria to changes in the ambient inorganic carbon concentration. In: Bryant DA (ed) The molecular biology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, pp 469–485, 881 pp

Karsten U (1996) Growth and organic osmolytes of geographically different isolates of *Microcoleus chthonoplastes* (cyanobacteria) from benthic microbial mats: response to salinity change. J Phycol 32:501–506

Kazmierczak J, Coleman ML, Gruszczynski M, Kempe S (1996) Cyanobacterial key to the genesis of micritic and peloidal limestones in ancient seas. Acta Palaeontol Pol 41:319–338

Kempe S, Kazmierczak J (1990a) Chemistry and stromatolites of the sea-linked Satonda Crater Lake, Indonesia: a recent model for the Precambrian sea? Chem Geol 81:299–310

Kempe S, Kazmierczak J (1990b) Calcium carbonate supersaturation and the formation of in situ calcified stromatolites. In: Ittekkot V, Kempe S, Michaelis W, Spitzy A (eds) Facets of modern biogeochemistry. Springer, Heidelberg, pp 255–278, 433 pp

Kempe S, Kazmierczak J (1993) Satonda crater lake, Indonesia. Hydrogeochemistry and biocarbonates. Facies 28:1–32

Kempe S, Kazmierczak J, Landmann G, Konuk T, Reimer A, Lipp A (1991) Largest known microbialites discovered in Lake Van, Turkey. Nature 349:605–608

Kennard JM, James NP (1986) Thrombolites and stromatolites: two distinct types of microbial structures. Palaios 1:492–503

Kenyon CN, Rippka R, Stanier RY (1972) Fatty acid composition and physiological properties of some filamentous blue-green algae. Arch Microbiol 83:216–236

Kidron GJ, Yaalon DH, Vonshak A (1999) Two causes for runoff initiation on microbiotic crusts: hydrophobicity and pore clogging. Soil Sci 164:18–27

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

Kremer B, Kazmierczak J, Stal LJ (2008) Calcium carbonate precipitation in cyanobacterial mats from sandy tidal flats of the North Sea. Geobiology 6:46–56

Krumbein WE (1974) On the precipitation of aragonite on the surface of marine bacteria. Naturwissenschaften 61:167

Krumbein WE (1979) Photolithotropic and chemoorganotrophic activity of bacteria and algae as related to beachrock formation and degradation (Gulf of Aqaba, Sinai). Geomicrobiol J 1:139–203

Krumbein WE (1983) Stromatolites.The challenge of a term in space and time. Precambrian Res 20:493–531

Krumbein WE, Cohen Y (1977) Primary production, mat formation and lithification: contribution of oxygenic and facultative anoxygenic cyanobacteria. In: Flügel E (ed) Fossil algae. Springer, Berlin, pp 38–56, 375 pp

Krumbein WE, Giele C (1979) Calcification in a coccoid cyanobacterium accociated with the formation of desert stromatolites. Sedimentology 26:593–604

Krumbein WE, Jens K (1981) Biogenic rock varnishes of the Negev Desert (Israel) an ecological study of iron and manganese transformation by cyanobacteria and fungi. Oecologia 50:25–38

Krumbein WE, Cohen Y, Shilo M (1977) Solar Lake (Sinai).4. Stromatolitic cyanobacterial mats. Limnol Oceanogr 22:635–656

Kühl M, Jørgensen BB (1992) Spectral light measurements in microbenthic phototrophic communities with a fiber-optic microprobe coupled to a sensitive diode array detector. Limnol Oceanogr 37:1813–1823

Kulp TR, Hoeft SE, Asao M, Madigan MT, Hollibaugh JT, Fisher JC, Stolz JF, Culbertson CW, Miller LG, Oremland RS (2008) Arsenic(III) fuels anoxygenic photosynthesis in hot spring biofilms from Mono Lake, California. Science 321:967–970

Lange W (1976) Speculations on a possible essential function of the gelatinous sheath of blue-green algae. Can J Microbiol 22:1181–1185

Lassen C, Ploug H, Jørgensen BB (1992a) Microalgal photosynthesis and spectral scalar irradiance in coastal marine sediments of Limfjorden, Denmark. Limnol Oceanogr 37:760–772

Lassen C, Ploug H, Jorgensen BB (1992b) A fibre-optic scalar irradiance microsensor – application for spectral light measurements in sediments. FEMS Microbiol Ecol 86:247–254

Leduc LG, Ferroni GD (1994) The chemolithotrophic bacterium *Thiobacillus ferrooxidans*. FEMS Microbiol Rev 14:103–119

Lee RY, Joye SB (2006) Seasonal patterns of nitrogen fixation and denitrification in oceanic mangrove habitats. Mar Ecol Prog Ser 307:127–141

Lehmann M, Wöber G (1976) Accumulation, mobilization and turnover of glycogen in the blue-green bacterium *Anacystis nidulans*. Arch Microbiol 111:93–97

Ley RE, Harris JK, Wilcox J, Spear JR, Miller SR, Bebout BM, Maresca JA, Bryant DA, Sogin ML, Pace NR (2006) Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl Environ Microbiol 72:3685–3695

Li P, Liu Z, Xu R (2001) Chemical characterization of the released polysaccharide from the cyanobacterium *Aphanothece halophytica* GR02. J Appl Phycol 13:71–77

Logan BW (1961) Cryptozoon and associate stromatolites from the recent of Shark Bay, Western Australia. J Geol 69:517–533

Lorenz MG, Wackernagel W (1990) Natural genetic transformation of *Pseudomonas stutzeri* by sand-adsorbed DNA. Arch Microbiol 154:380–385

Lorenz MG, Wackernagel W (1994) Bacterial gene transfer by natural genetic transformation in the environment. Microbiol Rev 58:563–602

Lorimer GH (1981) The carboxylation and oxygenation of ribulose 1,5-biphosphate: the primary events in photosynthesis and photorespiration. Annu Rev Plant Physiol 32:349–383

Lorimer GH, Andrews TJ, Tolbert NE (1973) Ribulose diphosphate oxygenase.II. Further proof of reaction products and mechanisms of action. Biochemistry 12:18–23

Lovley DR (1991) Dissimilatory Fe(III) and Mn(IV) reduction. Microbiol Rev 55:259–287

Lowe DR (1980) Stromatolites 3,400-Myr old from the Archean of Western Australia. Nature 284:441–443

Lu W-D, Chi Z-M, Su C-D (2006) Identification of glycine betaine as compatible solute in *Synechococcus* sp. WH8102 and characterization of its *N*-methyltransferase genes involved in betaine synthesis. Arch Microbiol 186:495–506

Ludwig R, Al-Horani FA, de Beer D, Jonkers HM (2005) Photosynthesis-controlled calcification in a hypersaline microbial mat. Limnol Oceanogr 50:1836–1843

Ludwig R, Pringault O, de Wit R, de Beer D, Jonkers HM (2006) Limitation of oxygenic photosynthesis and oxygen consumption by phosphate and organic nitrogen in a hypersaline microbial mat: a microsensor study. FEMS Microbiol Ecol 57:9–17

Mackay MA, Norton RS, Borowitzka LJ (1984) Organic osmoregulatory solutes in cyanobacteria. J Gen Microbiol 130:2177–2191

Mackerras AH, Youens BN, Weir RC, Smith GD (1990a) Is cyanophycin involved in the integration of nitrogen and carbon metabolism in the cyanobacteria *Anabaena cylindrica* and *Gloeothece* grown on light/dark cycles? J Gen Microbiol 136:2049–2056

Mackerras AH, De Chazal NM, Smith GD (1990b) Transient accumulations of cyanophycin in *Anabaena cylindrica* and *Synechocystis* 6308. J Gen Microbiol 136:2057–2065

Malin G, Pearson HW (1988) Aerobic nitrogen fixation in aggregate-forming cultures of the nonheterocystous cyanobacterium *Microcoleus chthonoplastes*. J Gen Microbiol 134:1755–1763

Malin G, Walsby AE (1985) Chemotaxis of a cyanobacterium on concentration gradients of carbondioxide, bicarbonate and oxygen. J Gen Microbiol 131:2643–2652

Margheri MC, Allotta G (1993) Homoacetic fermentation in the cyanobacterium *Nostoc* sp. strain Cc from *Cycas circinalis*. FEMS Microbiol Lett 111:213–217

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

Martin JP (1971) Decomposition and binding action of polysaccharides in soil. Soil Biol Biochem 3:33–34

Martínez-Alonso M, Mir J, Caumette P, Gaju N, Guerrero R, Esteve I (2004) Distribution of phototrophic populations and primary production in a microbial mat from the Ebro Delta, Spain. Int Microbiol 7:19–25

Maryan PS, Eady RR, Chaplin AE, Gallon JR (1986) Nitrogen fixation by *Gloeothece* sp. PCC 6909: respiration and not photosynthesis supports nitrogenase activity in the light. J Gen Microbiol 132:789–796

Mason TR, Von Brunn V (1977) 3-Gyr-old stromatolites from South Africa. Nature 266:47–49

Mazor G, Kidron GJ, Vonshak A, Abeliovich A (1996) The role of cyanobacterial exopolysaccharides in structuring desert microbial crusts. FEMS Microbiol Ecol 21:121–130

McCarren J, Heuser J, Roth R, Yamada N, Martone M, Brahamsha B (2005) Inactivation of *swmA* results in the loss of an outer cell layer in a swimming *Synechococcus* strain. J Bacteriol 187:224–230

McKay RML, Gibbs SP, Espie GS (1992) Effect of dissolved inorganic carbon on the expression of carboxysomes, localization of RubisCO and the mode of inorganic carbon transport in cells of the cyanobacterium *Synechocystis* UTEX 625. Arch Microbiol 159:21–29

Milliman JD (1993) Production and accumulation of calcium carbonate in the ocean: budget of a nonsteady state. Glob Biogeochem Cycle 7:927–957

Minz D, Fishbain S, Green SJ, Muyzer G, Cohen Y, Rittman BE, Stahl DA (1999) Unexpected population distribution in a microbial mat community: sulfate-reducing bacteria localized to the highly oxic chemocline in contrast to a eukaryotic preference for anoxia. Appl Environ Microbiol 65:4659–4665

Miziorko HM, Lorimer GH (1983) Ribulose-1,5-biphosphate carboxylase-oxygenase. Annu Rev Biochem 52:507–535

Moezelaar R, Bijvank SM, Stal LJ (1996) Fermentation and sulfur reduction in the mat-building cyanobacterium *Microcoleus chthonoplastes*. Appl Environ Microbiol 62:1752–1758

Monty CLV (1976) The origin and development of cryptalgal fabrics. In: Walter MR (ed) Stromatolites. Elsevier, Amsterdam, pp 139–249, 790 pp

Moore LR, Post AF, Rocap G, Chisholm SW (2002) Utilization of different nitrogen sources by the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. Limnol Oceanogr 47:989–996

Mullineaux PM, Gallon JR, Chaplin AE (1981) Acetylene reduction (nitrogen fixation) by cyanobacteria grown under alternating light-dark cycles. FEMS Microbiol Lett 10:245–247

Needoba JA, Foster RA, Sakamoto C, Zehr JP, Johnson KS (2007) Nitrogen fixation by unicellular diazotrophic cyanobacteria in the temperate oligotrophic North Pacific Ocean. Limnol Oceanogr 52:1317–1327

Neu TR (1992) Microbial "footprints" and the general ability of microorganisms to label interfaces. Can J Microbiol 38:1005–1008

Ng W-O, Grossman AR, Bhaya D (2003) Multiple light inputs control phototaxis in *Synechocystis* sp. starin PCC6803. J Bacteriol 185:1599–1607

Nicholson JAM, Stolz JF, Pierson BK (1987) Structure of a microbial mat at Great Sippewissett Marsh, Cape Cod, Massachusetts. FEMS Microbiol Ecol 45:343–364

Nilsson M, Rasmussen U, Bergman B (2006) Cyanobacterial chemotaxis to extracts of host and nonhost plants. FEMS Microbiol Ecol 55:382–390

Noffke N (2009) The criteria for the biogeneicity of microbially induced sedimentary structures (MISS) in Archean and younger, sandy deposits. Earth Sci Rev 96:173–180

Noffke N, Beukes N, Gutzmer J, Hazen R (2006) Spatial and temporal distribution of microbially induced sedimentary structures: a case study from siliciclastic storm deposits of the 2.9 Ga Witwatersrand Supergroup, South Africa. Precambrian Res 146:35–44

Nold SC, Ward DM (1996) Photosynthate partitioning and fermentation in hot spring microbial mat communities. Appl Environ Microbiol 62:4598–4607

Olson JM (2006) Photosynthesis in the Archean Era. Photosynth Res 88:109–117

Olson JB, Litaker RW, Paerl HW (1999) Ubiquity of heterotrophic diazotrophs in marine microbial mats. Aquat Microb Ecol 19:29–36

Omoregie EO, Crumbliss LL, Bebout BM, Zehr JP (2004) Determination of nitrogen-fixing phylotypes in *Lyngbya* sp. and *Microcoleus chthonoplastes* cyanobacterial mats from Guerrero Negro, Baja California, Mexico. Appl Environ Microbiol 70:2119–2128

Oren A (1988) The microbial ecology of the Dead Sea. Adv Microb Ecol 10:193–229

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

Oren A, Padan E, Avron M (1977) Quantum yields for oxygenic and anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. Proc Natl Acad Sci USA 74:2152–2156

Orpen JL, Wilson JF (1981) Stromatolites at ca. 3,500 Myr and a greenstone graniteunconformity in the Zimbabwean Archaean. Nature 291:218–220

Ortega-Calvo JJ, Stal LJ (1991) Diazotrophic growth of the unicellular cyanobacterium *Gloeothece* sp PCC 6909 in continuous culture. J Gen Microbiol 137:1789–1797

Ortega-Calvo JJ, Stal LJ (1994) Sulphate-limited growth in the N_2-fixing unicellular cyanobacterium *Gloeothece* (Nageli) sp PCC 6909. New Phytol 128:273–281

Ortega-Morales BO, Santiago-Garcia JL, Chan-Bacab MJ, Moppert X, Miranda-Tello E, Fardeau ML, Carrero JC, Bartolo-Perez P, Valadez-Gonzalez A, Guezennec J (2006) Characterization of extracellular polymers synthesized by tropical intertidal biofilm bacteria. J Appl Microbiol 102:254–264

Padan E, Cohen Y (1982) Anoxygenic photosynthesis. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publications, Oxford, pp 215–235, 688 pp

Paerl HW, Prufert LE (1987) Oxygen-poor microzones as potential sites of microbial N_2 fixation in nitrogen-depleted aerobic marine waters. Appl Environ Microbiol 53:1078–1087

Paerl HW, Bebout BM, Prufert LE (1989) Naturally occurring patterns of oxygenic photosynthesis and N_2 fixation in a marine microbial mat: physiological and ecological ramifications. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 326–341, 511 pp

Paerl HW, Prufert LE, Ambrose WW (1991) Contemporaneous N_2 fixation and oxygenic photosynthesis in the nonheterocystous mat-forming cyanobacterium *Lyngbya aestuarii*. Appl Environ Microbiol 57:3086–3092

Paerl HW, Pinckney JL, Kucera SA (1995) Clarification of the structural and functional roles of heterocysts and anoxic microzones in the control of pelagic nitrogen fixation. Limnol Oceanogr 40:634–638

Paerl HW, Fitzpatrick M, Bebout BM (1996) Seasonal nitrogen fixation dynamics in a marine microbial mat: potential roles of cyanobacteria and microheterotrophs. Limnol Oceanogr 41:419–427

Palinska KA, Liesack W, Rhiel E, Krumbein WE (1996) Phenotype variability of identical genotypes: the need for a combined approach in cyanobacterial taxonomy demonstrated on *Merismopedia*-like isolates. Arch Microbiol 166:224–233

Papineau D, Walker JJ, Mojzsis SJ, Pace NR (2005) Composition and structure of microbial communities from stromatolites of Hamelin Pool in Shark Bay, Western Australia. Appl Environ Microbiol 71:4822–4832

Paterson DM (1989) Short-term changes in the erodibility of intertidal cohesive sediments related to the migratory behaviour of epipelic diatoms. Limnol Oceanogr 34:223–234

Pearson HW, Howsley R, Kjeldsen CK, Walsby AE (1979) Aerobic nitrogenase activity associated with a non-heterocystous filamentous cyanobacterium. FEMS Microbiol Lett 5:163–169

Pentecost A (1984) Effects of sedimentation and light intensity on mat-forming Oscillatoriaceae with particular reference to *Microcoleus lyngbyaceus* Gomont. J Gen Microbiol 130:983–990

Pentecost A (1988) Growth and calcification of the cyanobacterium *Homoeothrix crustacea*. J Gen Microbiol 134:2665–2671

Pentecost A, Bauld J (1988) Nucleation of calcite on the sheaths of cyanobacteria using a simple diffusion cell. Geomicrobiol J 6:129–135

Pereira S, Zille A, Micheletti E, Moradas-Ferreira P, De Philippis R, Tamagnini P (2009) Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly. FEMS Microbiol Rev 33:917–941

Peschek GA (1978) Reduced sulfur and nitrogen compounds and molecular hydrogen as electron donors for anaerobic CO_2 photoreduction in *Anacystis nidulans*. Arch Microbiol 119:313–322

Pierce J (1988) Prospects for manipulating the substrate specificity of ribulose bisphosphate carboxylase/oxygenase. Physiol Plant 72:690–698

Pierson BK (1992) Introduction. In: Schopf JW, Klein C (eds) The proterozoic biosphere. A multidisciplinary study. Cambridge University Press, New York, pp 247–251, 1348 pp

Pierson BK, Olson JM (1989) Evolution of photosynthesis in anoxygenic photosynthetic procaryotes. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 402–427, 511 pp

Pierson BK, Parenteau MN (2000) Phototrophs in high iron microbial mats: microstructure of mats in iron-depositing hot springs. FEMS Microbiol Ecol 32:181–196

Pierson B, Oesterle A, Murphy GL (1987) Pigments, light penetration, and photosynthetic activity in the multi-layered microbial mats of Great Sippewisset salt marsh, Massachusetts. FEMS Microbiol Ecol 45:365–376

Pierson BK, Sands VM, Frederick JL (1990) Spectral irradiance and distribution of pigments in a highly layered marine microbial mat. Appl Environ Microbiol 56:2327–2340

Pierson BK, Parenteau MN, Griffin BM (1999) Phototrophs in high-iron-concentration microbial mats: physiological ecology of phototrophs in an iron-depositing hot spring. Appl Environ Microbiol 65:5474–5483

Pinckney J, Paerl HW, Reid RP, Bebout B (1995) Ecophysiology of stromatolitic microbial mats, Stocking Island, Exuma Cays, Bahamas. Microb Ecol 29:19–37

Piper DZ, Codespoti LA (1975) Marine phosphorite deposits and the nitrogen cycle. Science 179:564–565

Ploug H, Lassen C, Jørgensen BB (1993) Action spectra of microalgal photosynthesis and depth distribution of spectral scalar irradiance in a coastal marine sediment of Limfjorden, Denmark. FEMS Microbiol Ecol 102:261–270

Porubsky WP, Weston NB, Joye SB (2009) Benthic metabolism and the fate of dissolved inorganic nitrogen in intertidal sediments. Estuar Coast Shelf Sci 83:392–402

Potts M (1979) Nitrogen fixation (acetylene reduction) associated with communities of heterocystous and non-heterocystous blue-green algae in mangrove forests of Sinai. Oecologia 39:359–373

Potts M (1994) Desiccation tolerance of prokaryotes. Microbiol Rev 58:755–805

Potts M, Friedman EI (1981) Effects of water stress on crypto-endolithic cyanobacteria from hot dessert rocks. Arch Microbiol 130:267–271

Potts M, Ocampo-Friedmann R, Bowman MA, Tozun B (1983) *Chroococcus* S24 and *Chroococcus* N41 (cyanobacteria): morphological, biochemical and genetic characterization and effects of water stress on ultra structure. Arch Microbiol 135:81–90

Pringault O, Garcia-Pichel F (2004) Hydrotaxis of cyanobacteria in desert crusts. Microb Ecol 47:366–373

Rabenstein A, Rethmeier J, Fischer U (1995) Sulphite as intermediate sulphur compound in anaerobic sulphide oxidation to thiosulphate by marine cyanobacteria. Z Naturforsch C 50:769–774

Ramsing NB, Prufert-Bebout L (1994) Motility of *Microcoleus chthonoplastes* subjected to different light intensities quantified by digital image analysis. In: Stal LJ, Caumette P (eds) Microbial mats. Structure, development and environmental significance. Springer, Heidelberg, pp 183–191

Raven JA, Cockell CS, De La Rocha CL (2008) The evolution of inorganic carbon concentrating mechanisms in photosynthesis. Philos Trans R Soc Lond B 363:2641–2650

Reed RH, Stewart WDP (1983) Physiological responses of *Rivularia atra* to salinity: osmotic adjustment in hyposaline media. New Phytol 95:595–603

Reed RH, Stewart WDP (1988) The responses of cyanobacteria to salt stress. In: Rogers LJ, Gallon JR (eds) Biochemistry of algae and cyanobacteria. Clarendon, Oxford, pp 217–231, 374 pp

Reed RH, Borowitzka LJ, Mackay MA, Chudek JA, Foster R, Warr SCR, Moore DJ, Stewart WDP (1986a) Organic solute accumulation in osmotically stressed cyanobacteria. FEMS Microbiol Rev 39:51–56

Reed RH, Warr SRC, Kerby NW, Stewart WDP (1986b) Osmotic shock-induced release of low molecular weight metabolites from free-living and immobilized cyanobacteria. Enzyme Microbiol Technol 8:101–104

Rees DA (1969) Structure, conformation and mechanism in the formation of polysaccharide gels and network. Adv Carbohydr Chem Biochem 24:267–332

Reid RP, Browne KM (1991) Intertidal stromatolites in a fringing Holocene reef complex, Bahamas. Geology 19:15–18

Reid RP, Visscher PT, Decho AW, Stolz JF, Bebout BM, Dupraz C, Macintyre IG, Paerl HW, Pinckney JL, Prufert-Bebout L, Steppe TF, DesMarais DJ (2000) The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. Nature 406:989–992

Reinhold L, Kosloff R, Kaplan A (1991) A model for inorganic carbon fluxes and photosynthesis in cyanobacterial carboxysomes. Can J Bot 69:984–988

Renstrom-Kellner E, Bergman B (1989) Glycolate metabolism in cyanobacteria. III. Nitrogen controls excretion and metabolism of glycolate in Anabaena cylindrica. Physiol Plant 77:46–51

Renstrom-Kellner E, Bergman B (1990) Glycolate metabolism in cyanobacteria. IV. Uptake, growth and metabolic pathways. Physiol Plant 78:285–292

Rentz JA, Kraiya C, Luther GW III, Emerson D (2007) Control of ferrous iron oxidation within circumneutral microbial iron mats by cellular activity and autocatalysis. Environ Sci Technol 41:6084–6089

Revsbech NP, Jørgensen BB, Blackburn TH, Cohen Y (1983) Microelectrode studies of the photosynthesis and O_2, H_2S and pH profiles of a microbial mat. Limnol Oceanogr 28:1062–1074

Richardson LL, Castenholz RW (1987a) Diel vertical movements of the cyanobacterium *Oscillatoria terebriformis* in a sulfide-rich hot spring microbial mat. Appl Environ Microbiol 53:2142–2150

Richardson LL, Castenholz RW (1987b) Enhanced survival of the cyanobacterium *Oscillatoria terebriformis* in darkness under anaerobic conditions. Appl Environ Microbiol 53:2151–2158

Richardson LL, Castenholz RW (1989) Chemokinetic motility responses of the cyanobacterium *Oscillatoria terebriformis*. Appl Environ Microbiol 55:261–263

Rippka R, Waterbury JB (1977) The synthesis of nitrogenase by non-heterocystous cyanobacteria. FEMS Microbiol Lett 2:83–86

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61

Risatti JB, Capman WC, Stahl DA (1994) Community structure of a microbial mat: the phylogenetic dimension. Proc Natl Acad Sci USA 91:10173–10177

Robbins LL, Blackwelder PL (1992) Biochemical and ultrastructural evidence for the origin of whitings: a biologically induced calcium carbonate precipitation mechanism. Geology 20:464–468

Robins RJ, Hall DO, Shi DJ, Turner RJ, Rhodes MJC (1986) Mucilage acts to adhere cyanobacteria and cultured plant cells to biological and inert surfaces. FEMS Microbiol Lett 34:155–160

Roeske CA, O'Leary M (1984) Carbon isotope effects on the enzyme-catalyzed carboxylation of ribulose bisphosphate. Biochemistry 23:6275–6284

Romanowski G, Lorenz MG, Wackernagel W (1991) Adsorption of plasmid DNA to mineral surfaces and protection against DNAse-I. Appl Environ Microbiol 57:1057–1061

Rougerie F, Jehl C, Trichet J (1997) Phosphorus pathways in atolls: interstitial nutrient pool, cyanobacterial accumulation and Carbonate-Fluoro-Apatite (CFA) precipitation. Mar Geol 139:201–217

Ruff-Roberts AL, Kuenen JG, Ward DM (1994) Distribution of cultivated and uncultivated cyanobacteria and *Chloroflexus*-like bacteria in hot spring microbial mats. Appl Environ Microbiol 60:697–704

Schau L, Benson AA, Bassham JA, Calvin M (1950) The path of carbon in photosynthesis. XI. The role of glycolic acid. Physiol Plant 3:487–495

Schaub BEM, Van Gemerden H (1996) Sulfur bacteria in sediments of two coastal ecosystems: the Bassin d'Arcachon and the Étang du Prevost, France. Hydrobiologia 329:199–210

Schmetterer G (1994) Cyanobacterial respiration. In: Bryant DA (ed) The molecular biology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, pp 409–435, 881 pp

Schopf JW, Walter MR (1982) Origin and early evolution of cyanobacteria: the geological evidence. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publications, Oxford, pp 543–564, 688 pp

Serôdio J, Marques da Silva J, Catarino F (1997) Nondestructive tracing of migratory rhythms of intertidal benthic microalgae using *in vivo* chlorophyll *a* fluorescence. J Phycol 33:542–553

Severin I, Stal LJ (2008) Light dependency of nitrogen fixation in a coastal cyanobacterial mat. ISME J 2:1077–1088
Severin I, Acinas SG, Stal LJ (2010) Diversity of nitrogen-fixing bacteria in cyanobacterial mats. FEMS Microbiol Ecol 73:514–525
Siegesmund M, Johansen JR, Karsten U, Friedl T (2008) *Coleofasciculus* gen. nov. (Cyanobacteria): morphological and molecular criteria for revision of the genus *Microcoleus* Gomont. J Phycol 44:1572–1585
Smith AJ (1982) Modes of cyanobacterial carbon metabolism. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell, Oxford, pp 47–85, 688 pp
Smith RC, Prezelin BB, Baker KS, Bidigare RR, Boucher NP, Coley T, Karentz D, Macintyre S, Matlick HA, Menzies D, Ondrusek M, Wan Z, Waters KJ (1992) Ozone depletion – ultraviolet radiation and phytoplankton biology in Antarctic waters. Science 255:952–959
Srivastava P (2005) Vindhyan akinetes: an indicator of mesoproterozoic biosphere evolution. Orig Life Evol Biosph 35:175–185
Sroga GE (1997) Regulation of nitrogen fixation by different nitrogen sources in the filamentous non-heterocystous cyanobacterium *Microcoleus* sp. FEMS Microbiol Lett 153:11–15
Stal LJ (1993) Mikrobielle Matten. In: Meyer-Reil L-A, Köster M (eds) Mikrobiologie des Meeresbodens. Gustav Fischer, Jena, pp 196–220, 290 pp
Stal LJ (1994) Microbial mats in coastal environments. In: Stal LJ, Caumette P (eds) Microbial mats. Structure, development and environmental significance. Springer, Heidelberg, pp 21–32
Stal LJ (1995) Physiological ecology of cyanobacteria in microbial mats and other communities. New Phytol 131:1–32
Stal LJ (2000) Cyanobacterial mats and stromatolites. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 61–120, 669 pp
Stal LJ (2001) Coastal microbial mats: the physiology of a small-scale ecosystem. S Afr J Bot 67:399–410
Stal LJ (2010) Microphytobenthos as a biogeo-morphological force in intertidal sediment stabilization. Ecol Eng 36:236–245
Stal LJ, Heyer H (1987) Dark anaerobic nitrogen fixation (acetylene reduction) in the cyanobacterium *Oscillatoria* sp. FEMS Microbiol Ecol 45:227–232
Stal LJ, Krumbein WE (1981) Aerobic nitrogen fixation in pure cultures of a benthic marine *Oscillatoria* (cyanobacteria). FEMS Microbiol Lett 11:295–298
Stal LJ, Krumbein WE (1985) Isolation and characterization of cyanobacteria from a marine microbial mat. Bot Mar 28:351–365
Stal LJ, Krumbein WE (1987) Temporal separation of nitrogen fixation and photosynthesis in the filamentous, non-heterocystous cyanobacterium *Oscillatoria* sp. Arch Microbiol 149:76–80
Stal LJ, Moezelaar R (1997) Fermentation in cyanobacteria. FEMS Microbiol Rev 21:179–211
Stal LJ, Reed RH (1987) Low-molecular mass carbohydrate accumulation in cyanobacteria from a marine microbial mat in response to salt. FEMS Microbiol Ecol 45:305–312
Stal LJ, Grossberger S, Krumbein WE (1984) Nitrogen fixation associated with the cyanobacterial mat of a marine laminated microbial ecosystem. Mar Biol 82:217–224
Stal LJ, Van Gemerden H, Krumbein WE (1985) Structure and development of a benthic marine microbial mat. FEMS Microbiol Ecol 31:111–125
Stal LJ, Paerl HW, Bebout B, Villbrandt M (1994) Heterocystous versus non-heterocystous cyanobacteria in microbial mats. In: Stal LJ, Caumette P (eds) Microbial mats. Structure, development and environmental significance. Springer, Heidelberg, pp 403–414
Stal LJ, Behrens SB, Villbrandt M, Van Bergeijk S, Kruyning F (1996) The biogeochemistry of two eutrophic marine lagoons and its effect on microphytobenthic communities. Hydrobiologia 329:185–198
Steppe TF, Paerl HW (2002) Potential N_2 fixation by sulfate-reducing bacteria in a marine intertidal microbial mat. Aquat Microb Ecol 28:1–12
Steppe TF, Olson JB, Paerl HW, Litaker RW, Belnap J (1996) Consortial N_2 fixation: a strategy for meting nitrogen requirements of marine and terrestrial cyanobacterial mats. FEMS Microbiol Ecol 21:149–156
Steudel R, Holdt G, Visscher PT, Van Gemerden H (1990) Search for polythionates in cultures of *Chromatium vinosum* after sulfide incubation. Arch Microbiol 153:432–437
Steunou A-S, Bhaya D, Bateson M, Melendrez M, Ward D, Brecht E, Peters JW, Kühl M, Grossman A (2006) *In situ* analysis of nitrogen fixation and metabolic switching in unicellular thermophilic cyanobacteria inhabiting hot spring microbial mats. Proc Natl Acad Sci USA 103:2398–2403
Steunou A-S, Jensen SI, Brecht E, Becraft ED, Bateson MM, Kilian O, Bhaya D, Ward DM, Peters JW, Grossman AR, Kühl M (2008) Regulation of nif gene expression and the energetics of N_2 fixation over the diel cycle in a hot spring microbial mat. ISME J 2:364–378
Stolz JF (1993) Magnetosomes. J Gen Microbiol 139:1663–1670
Storch TA, Saunders GW, Ostrofsky ML (1990) Diel nitrogen fixation by cyanobacterial surface blooms in Sanctuary Lake, Pennsylvania. Appl Environ Microbiol 56:466–471
Straub KL, Benz M, Schink B, Widdel F (1996) Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. Appl Environ Microbiol 62:1458–1460
Sutherland IW (2001) Biofilm exopolysaccharides: a strong and sticky framework. Microbiology 147:3–9
Tabita FR (1988) Molecular and cellular regulation of autotrophic carbon dioxide fixation in microorganisms. Microbiol Rev 52:155–189
Tago Y, Aida K (1977) Exocellular mucopolysaccharide closely related to bacterial floc formation. Appl Environ Microbiol 34:308–314
Taiz L (1984) Plant cell expansion: regulation of cell wall mechanical properties. Annu Rev Plant Physiol 35:585–657
Talbot MMB, Bate GC, Campbell EE (1990) A review of the ecology of surf-zone diatoms, with special reference to *Anulus australis*. Oceanogr Mar Biol Annu Rev 28:155–175
Tamagnini P, Axelsson R, Lindberg P, Oxelfelt F, Wünschiers R, Lindblad P (2002) Hydrogenases and hydrogen metabolism of cyanobacteria. Microbiol Mol Biol Rev 66:1–20
Tamagnini P, Leitão E, Oliveira P, Ferreira D, Pinto F, Harris DJ, Heidorn T, Lindblad P (2007) Cyanobacterial hydrogenases: diversity, regulation and applications. FEMS Microbiol Rev 31:692–720
Tease B, Jürgens UJ, Golecki JR, Heinrich UR, Rippka R, Weckesser J (1991) Fine-structural and chemical analyses on inner and outer sheath of the cyanobacterium *Gloeothece* sp. PCC-6909. Antonie Van Leeuwenhoek Int J Gen Mol Microbiol 59:27–34
Teske A, Ramsing NB, Habicht K, Fukui M, Küver J, Jørgensen BB, Cohen Y (1998) Sulfate-reducing bacteria and their activities in cyanobacterial mats of Solar Lake (Sinai, Egypt). Appl Environ Microbiol 64:2943–2951
Trouwborst RE, Johnston A, Koch G, Luther GW III, Pierson BK (2007) Biogeochemistry of Fe(II) oxidation in a photosynthetic microbial mat: implications for Precambrian Fe(II) oxidation. Geochim Cosmochim Acta 71:4629–4643
Trüper HG, Galinski EA (1989) Compatible solutes in halophilic phototrophic procaryotes. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 342–348, 511 pp
Turner SM, Malin G, Liss PS, Harbour DS, Holligan PM (1988) The seasonal variation of dimethyl sulfide and dimethylsulfonio-propionate concentrations in nearshore waters. Limnol Oceanogr 33:364–375
Turpin DH, Miller AG, Canvin DT (1984) Carboxysome content of *Synechococcus leopoliensis* (Cyanophyta) in response to inorganic carbon. J Phycol 20:249–253
Utkilen HC (1976) Thiosulphate as electron donor in the blue-green alga *Anacystis nidulans*. J Gen Microbiol 95:177–180
Valladares A, Montesinos ML, Herrero A, Flores E (2002) An ABC-type, high-affinity urea permease identified in cyanobacteria. Mol Microbiol 43:703–715

Van Bergeijk SA, Stal LJ (1996) The role of oxygenic phototrophic microorganisms in production and conversion of dimethylsulfoniopropionate and dimethylsulfide in microbial mats. In: Kiene RP, Visscher PT, Keller MD, Kirst GO (eds) Biological and environmental chemistry of DMSP and related sulfonium compounds. Plenum Press, New York, pp 369–379, 430 pp

Van Gemerden H (1987) Competition between purple sulfur bacteria and green sulfur bacteria: role of sulfide, sulfur and polysulfides. Acta Acad Abo 47:13–27

Van Gemerden H (1993) Microbial mats: a joint venture. Mar Geol 113:3–25

Van Gemerden H, Tughan CS, De Wit R, Herbert RA (1989) Laminated microbial ecosystems on sheltered beaches in Scapa Flow, Orkney Islands. FEMS Microbiol Ecol 62:87–102

Van Liere L, Mur LR (1979) Growth kinetics *of Oscillatoria agardhii* Gomont in continuous culture, limited in its growth by the light energy supply. J Gen Microbiol 115:153–160

Verrecchia EP, Dumant J-L, Collins KE (1990) Do fungi building limestone exist in semi-arid regions? Naturwissenschaften 77:584–586

Vila-Costa M, Simó R, Harada H, Gasol JM, Slezak D, Kiene RP (2006) Dimethylsulfoniopropionate uptake by marine phytoplankton. Science 314:652–654

Villbrandt M (1992) Interactions of nitrogen fixation and photosynthesis in marine cyanobacterial mats (Mellum, Southern North Sea). PhD thesis, University of Oldenburg, Oldenburg, Germany, 163 pp

Villbrandt M, Stal LJ (1996) The effect of sulfide on nitrogen fixation in heterocystous and non-heterocystous cyanobacterial mat communities. Algol Stud 83:549–563

Villbrandt M, Stal LJ, Krumbein WE (1990) Interactions between nitrogen fixation and oxygenic photosynthesis in a marine cyanobacterial mat. FEMS Microbiol Ecol 74:59–72

Vincent WF, Castenholz RW, Downes MT, Howard-Williams C (1993a) Antarctic cyanobacteria – light, nutrients, and photosynthesis in the microbial mat environment. J Phycol 29:745–755

Vincent WF, Downes MT, Castenholz RW, Howard-Williams C (1993b) Community structure and pigment organisation of cyanobacteria-dominated microbial mats in Antarctica. Eur J Phycol 28:213–221

Visscher PT (1992) Microbial sulfur cycling in laminated marine ecosystems. PhD thesis, University of Groningen, Groningen, 113 pp

Visscher PT, Van Gemerden H (1991) Production and consumption of dimethylsulfoniopropionate in marine microbial mats. Appl Environ Microbiol 57:3237–3242

Visscher PT, Van Gemerden H (1993) Sulfur cycling in laminated marine microbial ecosystems. In: Oremland RS (ed) Biogeochemistry of global change: radiatively active trace gases. Chapman and Hall, New York, pp 672–690, 879 pp

Visscher PT, Nijburg JW, Van Gemerden H (1990) Polysulfide utilization by *Thiocapsa roseopersicina*. Arch Microbiol 155:75–81

Visscher PT, Prins RA, Van Gemerden H (1992) Rates of sulfate reduction and thiosulfate consumption in a marine microbial mat. FEMS Microbiol Ecol 86:283–293

Visscher PT, Reid RP, Bebout BM, Hoeft SE, Macintyre IG, Thompson JA (1998) Formation of lithified micritic lamninae in modern marine stromatolites (Bahamas): the role of sulfur cycling. Am Mineral 83:1482–1493

Visscher PT, Reid RP, Bebout BM (2000) Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. Geology 28:919–922

Vogt C, Rabenstein A, Rethmeier J, Fischer U (1998) Alkali-labile precursors of dimethyl sulfide in marine benthic cyanobacteria. Arch Microbiol 169:263–266

Walsby AE (1985) The permeability of heterocysts to the gases nitrogen and oxygen. Proc R Soc Lond B 226:345–366

Walter MR (ed) (1976) Stromatolites. Elsevier, Amsterdam, 790 pp

Walter MR, Heys GR (1985) Links between the rise of metazoa and the decline of stromatolites. Precambrian Res 29:149–174

Walter MR, Buick R, Dunlop JSR (1980) Stromatolites 3,400-3,500 Myr. old from the North Pole area, Western Australia. Nature 284:443–445

Walter MR, Grotzinger JP, Schopf JW (1992) Proterozoic stromatolites. In: Schopf JW, Klein C (eds) The proterozoic biosphere. A multidisciplinary study. Cambridge University Press, New York, pp 253–260, 1348 pp

Warburg O (1920) Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen.II. Biochem Z 103: 188–217

Warburg O, Krippahl G (1960) Glykolsäurebildung in *Chlorella*. Z Naturforsch B15:197–199

Ward DM, Weller R, Shiea J, Castenholz RW, Cohen Y (1989) Hot spring microbial mats: anoxygenic and oxygenic mats of possible evolutionary significance. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 3–15, 511 pp

Ward DM, Ferris MJ, Nold SC, Bateson MM, Kopcynski ED, Ruff-Roberts AL (1994) Species diversity in hot spring microbial mats as revealed by both molecular and enrichment culture approaches – relationship between biodiversity and community structure. In: Stal LJ, Caumette P (eds) Microbial mats. Structure, development and environmental significance. Springer, Heidelberg, pp 33–44, 463 pp

Warr SCR, Reed RH, Stewart WDP (1984) Osmotic adjustment of cyanobacteria: the effects of NaCl, KCl, Sucrose and glycine betaine on glutamine synthetase activity in a marine and a halotolerant strain. J Gen Microbiol 130:2169–2175

Warr SRC, Reed RH, Stewart WDP (1988) The compatibility of osmotica in cyanobacteria. Plant Cell Environ 11:137–142

Waterbury JB, Rippka R (1989) Subsection I. Order Chroococcales. In: Staley JT, Bryant MP, Pfennig N, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 3. Williams and Wilkins, Baltimore, pp 1728–1746, 744 pp

Waterbury JB, Willey JM, Franks DG, Valois FW, Watson SW (1985) A cyanobacterium capable of swimming motility. Science 230:74–76

Westbroek P, Buddemeier B, Coleman M, Kok DJ, Fautin D, Stal LJ (1994) Strategies for the study of climate forcing by calcification. In: Doumenge F (ed) Past and presence biomineralization processes. Musée Oceanographique, Monaco, pp 37–60

Whale GF, Walsby AE (1984) Motility of the cyanobacterium *Microcoleus chthonoplastes* in mud. Br Phycol J 19:117–123

White AK, Metcalf WW (2007) Microbial metabolism of reduced phosphorus compounds. Annu Rev Microbiol 61:379–400

Whitton BA (1987) The biology of Rivulariaceae. In: Fay P, Van Baalen C (eds) The cyanobacteria. Elsevier, Amsterdam, pp 513–534, 534 pp

Whitton BA, Potts M (1982) Marine littoral. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publications, Oxford, pp 515–542, 688 pp

Widdel F, Schnell S, Heising S, Ehrenreich A, Assmus B, Schink B (1993) Ferrous iron oxidation by anoxygenic phototrophic bacteria. Nature 362:834–836

Wieland A, Zopfi J, Benthien M, Kühl M (2005) Biogeochemistry of an iron-rich hypersaline microbial mat (Camargue, France). Microb Ecol 49:34–49

Wuichet K, Zhulin IB (2003) Molecular evolution of sensory domains in cyanobacterial chemoreceptors. Trends Microbiol 11:200–203

Yallop ML, De Winder B, Paterson DM, Stal LJ (1994) Comparative structure, primary production and biogenic stabilization of cohesive and non-cohesive marine sediments inhabited by microphytobenthos. Estuar Coast Shelf Sci 39:565–582Yechieli Y, Wood WW (2002)

Hydrogeologic processes in saline systems: playas, sabkhas, and saline lakes. Earth Sci Rev 58:343–365

Zavarzin GA, Gerasimenko LM, Zhilina TN (1993) Cyanobacterial communities in hypersaline lagoons of Lake Sivash. Microbiology 62:645–652

Zeebe RE, Wolf-Gladrow D (2001) CO_2 in seawater: equilibrium, kinetics and isotopes. Elsevier, New York, 346 pp

Zehr JP, Mellon M, Braun S, Litaker W, Steppe T, Paerl HW (1995) Diversity of heterotrophic nitrogen fixation genes in a marine cyanobacterial mat. Appl Environ Microbiol 61:2527–2532

Zehr JP, Bench SR, Carter BJ, Hewson I, Niazi F, Shi T, Tripp HJ, Affourtit JP (2008) Globally distributed uncultivated oceanic N_2-fixing cyanobacteria lack oxygenic photosystem II. Science 322:1110–1112