CHAPTER TWENTY

Geomicrobiology of Sulfur

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CONTENTS

20.1 INTRODUCTION

Sulfur is the 14th most abundant element in the Earth's crust and can exist in redox states from −2 to +6 (Table 20.1). Inorganic sulfur occurs most commonly in the −2, −1, 0, and +6 oxidation states, represented by sulfides (e.g., HS^-/H_2S and pyrite, FeS_2), elemental sulfur (S^0), and sulfate (SO_4^{2-}), respectively (Roy and Trudinger, 1970). The dominant forms of sulfur in the geologic record are base-metal sulfide minerals (i.e., particularly pyrite) and sulfates, preserved both in sulfate evaporite minerals (gypsum and anhydrite) (Holser, 1997) and as carbonate-associated sulfate, where sulfate substitutes into the carbonate mineral lattice at ~100 to 1000 ppm levels (Burdett et al., 1989).

Sulfur biogeochemical cycling plays a major role in regulating the redox state of the oceans and atmosphere (Holland, 1973; Berner et al., 1983), in large part due to the wide range in oxidation states that sulfur compounds can possess. These diverse redox states allow for S compounds to play key roles in a range of microbial metabolic processes (Figure 20.1), predominantly sulfate reduction (Peck, 1959), sulfide and sulfur oxidation (Dahl and Truper, 1994), and the disproportionation (Bak and Pfennig, 1987) of intermediate valence compounds (e.g., elemental sulfur, thiosulfate, sulfite), which are intimately coupled to the carbon and oxygen cycles (Holland, 1973; Berner et al. 1983; Berner and Canfield, 1989; Hayes and Waldbauer, 2006). These various metabolic processes can leave diagnostic geochemical and isotopic fingerprints in the environments occupied by the microbes (c.f. Szabo et al., 1950; Canfield, 2001a; Johnston et al., 2005a; Zerkle et al., 2009). Thus, sulfur-bearing phases preserved in sedimentary rocks throughout the geologic past record the evolution of both paleoenvironmental conditions—i.e., the redox state of the oceans and atmosphere—and microbial evolution (i.e., the appearance and ecological significance of different sulfur-cycling metabolic pathways) over Earth history (Canfield and Teske, 1996; Canfield, 2001a).

Sulfur is an essential element for life and is used both in essential biological molecules and in respiratory oxidation–reduction transformations. These transformations are fundamental to the biogeochemical sulfur cycle and affect the concentration, oxidation state, and isotopic composition of sulfur species in the environment. Microorganisms catalyze the transformation of sulfur for both biomass building (assimilatory) and energy metabolism (dissimilatory) and purposes.

TABLE 20.1
Geomicrobially important forms of sulfur and their oxidation state(s).

Compound	Formula	Oxidation state(s)
Hydrogen sulfide/bisulfide	H_2S/HS^-	(−2)
Pyrite	FeS_2	(−1)
Polysulfide(s)[a]	S_N^{2-}	2 (−1); N-2 (0)
Elemental sulfur[b]	S_8, S_N^0	(0)
Dithionite (hyposulfite)	$(O_2S–SO_2)^{2-}$	2 (+3)
Bisulfite/sulfite[c]	HSO_3^-/SO_3^{2-}	(+4)
Thiosulfate[c]	$(S–SO_3)^{2-}$	(−1); (+5)
Dithionate	$(O_3S–SO_3)^{2-}$	2 (+5)
Trithionate	$(O_3S–S–SO_3)^{2-}$	(0); 2 (+5)
Tetrathionate	$(SO_3–S–S–SO_3)^{2-}$	2 (0); 2 (+5)
Sulfate	SO_4^{2-}	(+6)

SOURCE: Vairavamurthy, A et al., *Geochim Cosmochim Acta*, 57, 1619, 1993.
[a] The terminal sulfurs are oxidation state −1, while central sulfurs are 0.
[b] Occurs in an octagonal ring in crystalline form, or in equilibrium with polysulfides.
[c] One sulfur has an oxidation state of −1, the other sulfur has an oxidation state of +5.

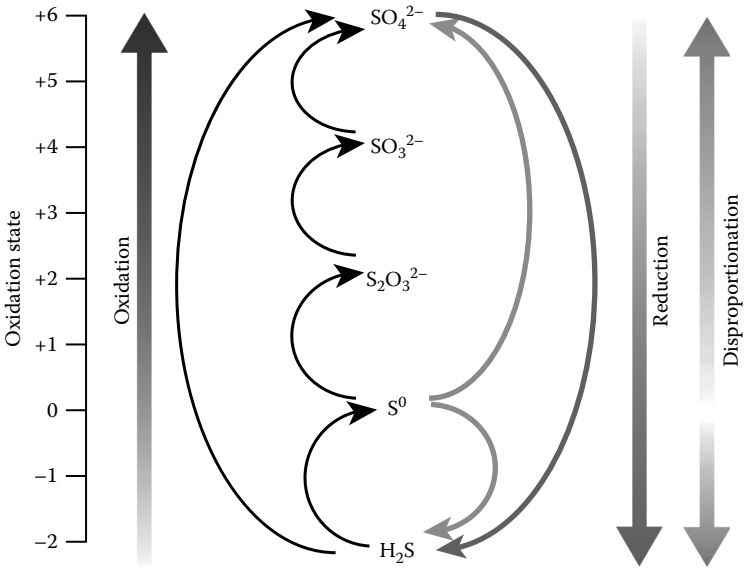


Figure 20.1. Major microbial sulfur-cycling metabolisms and associated redox transformations: sulfate reduction transforms sulfate to hydrogen sulfide, sulfide oxidation transforms sulfide to a more oxidized state between elemental sulfur (S^0) and sulfate, and disproportionation of intermediate-valence sulfur species (e.g., S^0) transforms these compounds to both H_2S and SO_4^{2-}.

Assimilatory sulfur transformations are those responsible for incorporation of inorganic sulfur (such as sulfate) into biomolecules. Most significantly, sulfur forms part of the structure of the amino acids cysteine and methionine. However, it also plays many other roles in the cell, including in electron transport (Fe-S complexes) and in coenzymes and cosubstrates such as coenzyme A, S-adenosyl methionine, biotin, and glutathione. Sulfur typically comprises less than 1% by weight of biomass (Zehnder and Zinder, 1970); S/C ratios can range from 0.02 to 0.08 (Fagerbakke et al., 1996).

Dissimilatory sulfur transformations are those in which sulfur is not directly incorporated by microorganisms but used for other processes such as anaerobic respiration. Dissimilatory processes are responsible for the overwhelming majority of sulfur transformations in geomicrobiological processes. In these reactions, oxidized forms of sulfur, especially sulfate, but also elemental sulfur and other species, can serve as terminal electron acceptors. Reduced forms of sulfur, such as hydrogen sulfide, can serve as sources of electrons (reducing power) to generate energy, coupled to the oxidation of compounds such as oxygen or nitrate (e.g., Dahl and Truper, 1994).

20.2 MICROBIAL SULFATE REDUCTION

The dissimilatory energy metabolism of microbial sulfate reduction (MSR) is an important component of global biogeochemical cycles and is catalyzed by a diversity of Archaea and Bacteria (Pereira et al., 2011; Rabus et al., 2013). The process is of particular importance in the oceans, where abundant sulfate (at ~28 mM) comprises an oxidant pool that is 10 times larger than atmospheric oxygen (Hayes and Waldbauer, 2006). MSR is coupled to the oxidation of organic compounds or hydrogen and plays a large role in both the carbon and sulfur cycles.

20.2.1 Microbial Sulfate Reducers

Most cultured sulfate-reducing microorganisms belong to the Domain Bacteria, while several belong to Archaea (Pereira et al., 2011). As first described, the sulfate reducers were thought to be represented by only three genera of the Bacteria, *Desulfovibrio*, *Desulfotomaculum* (originally classified as *Clostridium* because of its ability to form endospores), and *Desulfomonas*. These organisms are nutritionally specialized in that among organic energy sources, they can use only lactate, pyruvate,

fumarate, malate, and ethanol. Furthermore, none of these organisms are able to degrade their organic energy sources beyond acetate (Postgate, 1984), i.e., they are "incomplete oxidizers" (Rabus et al., 2013). The importance of the sulfate reducers in the anaerobic mineralization of organic matter in sulfate-rich environments remained unappreciated before the discovery of the "complete oxidizers," capable of the oxidation of organic matter to CO_2. This restricted view of sulfate reducers changed rapidly with the discovery of a sulfate reducer, *Desulfotomaculum acetoxidans* (Widdel and Pfennig, 1977, 1981), which is able to oxidize acetate anaerobically to CO_2 and H_2O with sulfate. Subsequently, a wide variety of other sulfate reducers were discovered that differed in the nature of the energy sources they were capable of using, including a wide range of aliphatic, aromatic, and heterocyclic compounds. In many cases, the organisms are able to completely remineralize the substrate to bicarbonate/carbon dioxide and each substrate by a specific group of sulfate reducers (e.g., Pfennig et al., 1981; Imhoff-Stuckle and Pfennig, 1983; Braun and Stolp, 1985; Bak and Widdel, 1986a,b; Szewzyk and Pfennig, 1987; Platen et al., 1990; Zellner et al., 1990; Aeckersberg et al., 1991; Boopathy and Daniels, 1991; Qatabi et al., 1991; Schnell and Schink, 1991; Tasaki et al., 1991, 1992; Kuever et al., 1993; Rueter et al., 1994; Janssen and Schink, 1995; Rees et al., 1998; Londry et al., 1999; Meckenstock et al., 2000). Some of these sulfate reducers were also found to use H_2 as an energy source. Most require an organic carbon source, but some can grow autotrophically on hydrogen. Table 20.2 presents a list of some of the different kinds of sulfate reducers in the domain Bacteria. While most sulfate reducers discovered to date are mesophiles, thermophilic types are also now in culture (e.g., Pfennig et al., 1981; Zeikus et al., 1983; Stetter et al., 1987; Burggraf et al., 1990; Itoh et al., 1999). At least one moderate psychrophile, *Desulforhopalus vacuolatus* (optimal growth at 10°C–19°C), has been described (Isaksen and Teske, 1996)—isolated from sediment in Kysing Fjord, Denmark, at 10°C.

Sulfate reducers are morphologically diverse and are known to include cocci, sarcinae, rods, vibrios, spirilla, and filaments (Figure 20.2). The cultured representatives in the domain Bacteria are primarily gram-negative, though some gram-positive relatives of the *Clostridiales* are now known (Pereira et al., 2011 and references therein).

TABLE 20.2
Some sulfate-reducing bacteria.[a]

Heterotrophs	Autotrophs[b]
Desulfovibrio desulfuricans[c,d]	*Desulfovibrio baarsii*
Desulfovibrio vulgaris	*Desulfobacter hydrogenophilus*
Desulfovibrio gigas	*Desulfosarcina variabilis*
Desulfovibrio fructosovorans	*Desulfonema limicola*
Desulfovibrio sulfodismutans	
Desulfomonas pigra	
Desulfotomaculum nigrificans	
Desulfotomaculum acetoxidans	
Desulfotomaculum orientis[d]	
Desulfobacter postgatei	
Desulfobulbus propionicus	
Desulfobacterium phenolicum[e]	
Desulfobacterium indolicum[f]	
Desulfobacterium catecholicum[g]	

[a] For a more detailed description of sulfate reducers, see Pfennig et al. (1981), Postgate (1984), Dworkin (2001), and Rabus et al. (2013).
[b] Autotrophic growth on H_2 and CO_2.
[c] Some strains can grow mixotrophically on H_2 and CO_2 and acetate.
[d] At least one strain can grow autotrophically on H_2 and CO_2.
[e] Bak and Widdel (1986b).
[f] Bak and Widdel (1986a).
[g] Szewzyk and Pfennig (1987).

The first sulfate reducers described from the Archaeal domain were the Euryarchaeota *Archaeoglobus fulgidus* (Stetter et al., 1987; Speich and Trüper, 1988) and *Archaeoglobus profundus* (Burggraf et al., 1990). Following these early discoveries, facultative sulfate reducers from the Crenarchaeota *Caldivirga maquilingensis* and *Thermocladium modestius* were reported (Itoh et al., 1999) and more known from genome sequence in recent years (Pereira et al., 2011). The two Archaeal sulfate reducers that were discovered by Stetter et al. (1987) and Burggraf et al. (1990) are extremely thermophilic, anaerobic, gram-negative, irregularly shaped cocci. *A. fulgidus* strains were found to grow naturally in a hydrothermal system at temperatures between 70°C and 100°C in the vicinities of Vulcano and Stufe di Nerone, Italy. Under laboratory conditions, the cultures grow anaerobically in marine mineral salts medium supplemented with yeast extract. In this medium, they produce a large amount of hydrogen sulfide and some methane. Thiosulfate, but not elemental sulfur, can act as alternative electron acceptor. Energy sources

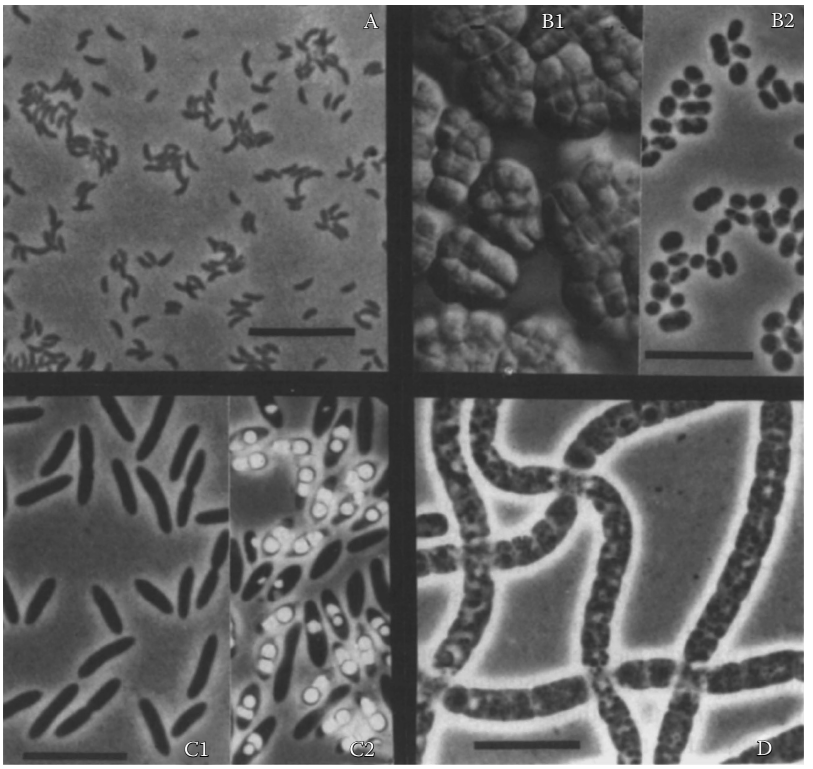


Figure 20.2. Sulfate-reducing bacteria. (A) Desulfovibrio desulfuricans (phase contrast). (B1, B2) Desulfosarcina variabilis: (B1) sarcina packets (interference contrast); (B2) free-living cells (phase contrast). (C1, C2) Desulfotomaculum acetoxidans: (C1) vegetative cells (phase contrast); (C2) cells with spherical spores and gas vacuoles (phase contrast). (D) Desulfonema limicola (phase contrast). (With kind permission from Springer Science+Business Media: *The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria*, Vol. I, Copyright 1981, Pfennig, N, Widdel, F, and Trüper, HG, Springer, Berlin, Germany.)

include hydrogen and some simple organic molecules as well as glucose, yeast extract, and other more complex substrates. Cells contain a number of compounds such as 8-OH-5-deazaflavin and methanopterin previously found only in methanogens, which are also members of the domain Archaea, but 2-mercaptoethanesulfonic acid and factor F430, which are found in methanogens, were absent (Stetter et al., 1987).

A. profundus was isolated from the Guaymas hot vent area (Gulf of California, also known as the Sea of Cortez). It grows anaerobically at temperatures between 65°C and 95°C (optimum 82°C) in a pH range of 4.5–7.5 at NaCl concentrations in the range of 0.9%–3.6%. Unlike *A. fulgidus*, it is an obligate mixotroph that requires H_2 as an energy source. Its organic carbon requirement can be satisfied by acetate, lactate, pyruvate, yeast extract, beef extract, peptone, or acetate-containing crude oil. As for *A. fulgidus*, sulfate, thiosulfate, and sulfate can serve as terminal electron acceptors for growth. Although S^0 is reduced by resting cells, it does not support growth (Burggraf et al., 1990). Interestingly, the primary sulfate reduction genes in fully sequenced Archaeal sulfate reducers appear to have derived from Bacterial genomes (Wagner et al., 1998; Pereira et al., 2011).

The presence of as-yet-unidentified, extremely thermophilic sulfate reducers was detected in hot deep-sea sediments at the hydrothermal vents of the tectonic spreading center of Guaymas Basin (Sea of Cortez or Gulf of California). Sulfate-reducing activity was measurable between 100°C and 110°C (optimum 103°C–106°C). The responsible organisms are probably examples of Archaea (Jørgensen et al., 1992).

The Crenarchaeotal strains *C. maquilingensis* and *T. modestius* described by Itoh et al. (1999) were also thermophiles, growing over a temperature range of 60°C–92°C with an optimum at 85°C.

These were provided a variety of complex carbon sources such as gelatin, glycogen, beef or yeast extract, peptone, and tryptone. In addition to being able to employ sulfate as a terminal electron acceptor, these strains also were able to utilize sulfur or thiosulfate.

Sulfate reducers play several important roles in geomicrobiology in relation to other elemental cycles. In arsenic-contaminated groundwater, sulfide produced by sulfate reducers can precipitate arsenic phases, removing it from solution (Kirk et al., 2004). Sulfate reducers also play a role in the anaerobic oxidation of methane (AOM; Boetius et al., 2000). This process, which oxidizes methane to CO_2 in marine sediments and methane seeps, is postulated to be catalyzed by a consortium of methane cycling archaea and sulfate-reducing bacteria. The bacterial partner in these consortia typically derives from the *Desulfosarcina*, *Desulfococcus*, or *Desulfobulbus* group within the δ-proteobacteria (Knittel and Boetius, 2009). AOM is thought to operate close to thermodynamic equilibrium, and many details of this process are as yet unclear—including whether a syntrophic relationship between the archaea and sulfate-reducing (or other) bacterial partner is obligate. Furthermore, sulfate reducers play an integral role in the cycling of environmentally relevant iron oxide phases (Flynn et al., 2014).

20.2.2 Coupling to Carbon and Nitrogen Cycles

20.2.2.1 Autotrophy

Autotrophic sulfate reducers use hydrogen as an electron donor. Although the ability of *Desulfovibrio desulfuricans* to grow autotrophically with hydrogen (H_2) as an energy source had been previously suggested, experiments by Mechalas and Rittenberg (1960) failed to demonstrate it. Seitz and Cypionka (1986), however, obtained autotrophic growth of *D. desulfuricans* strain Essex 6 with hydrogen, but the growth yield was less when sulfate was the terminal electron acceptor. Better yields were obtained with nitrate or nitrite as terminal electron acceptor, presumably because the latter two acceptors did not need to be activated by ATP, which is a requirement for sulfate reduction (see in the following text), and the oxidation of hydrogen with the N-anions yields greater free energy of reaction than with sulfate (Thauer et al., 1977). *Desulfotomaculum orientis* also has the ability to grow autotrophically with hydrogen as energy source using sulfate, thiosulfate, or sulfite as the terminal electron acceptor (Cypionka and Pfennig, 1986). Under optimal conditions, better growth yields were obtained with this organism than had been reported for *D. desulfuricans* (12.4 versus 9.4 g of dry cell mass per mole of sulfate reduced). This may be because *Desulfotomaculum* can utilize inorganic pyrophosphate generated in sulfate activation as an energy source whereas *Desulfovibrio* cannot. *D. orientis* gave better growth yields when thiosulfate or sulfite was the terminal electron acceptor than when sulfate was; this is also consistent with the ATP required for sulfate activation. The organism excreted acetate that was formed as part of its CO_2 fixation process (Cypionka and Pfennig, 1986). The acetate may have been formed via the activated acetate pathway in which it is formed directly from two molecules of CO_2 as is the case in methanogens and homoacetogens (see Chapters 7 and 23), and as has now been shown to occur in *Desulfovibrio baarsii*, which can also grow with hydrogen and sulfate (Jansen et al., 1984) and in *Desulfobacterium autotrophicum* (Schauder et al., 1989). *Desulfobacter hydrogenophilus*, by contrast, assimilates CO_2 by a reductive tricarboxylic acid cycle when growing autotrophically with H_2 as energy source and sulfate as terminal electron acceptor (Schauder et al., 1987). Other sulfate reducers able to grow autotrophically on hydrogen as energy source and sulfate as terminal electron acceptor include *Desulfonema limicola*, *Desulfonema ishimotoi*, *Desulfosarcina variabilis* (Pfennig et al., 1981; Fukui et al., 1999), and *D. autotrophicum* (Schauder et al., 1989). The pathways for CO_2-fixation vary among these strains, as reviewed by Rabus et al. (2013), and include the reductive citric acid cycle and the Ljungdahl–Wood pathway.

20.2.2.2 Mixotrophy

D. desulfuricans has been shown to grow mixotrophically with any one of several different compounds as sole energy source, including hydrogen, formate, and isobutanol. The carbon in the organic energy sources was not assimilated. It was derived instead from substances as complex as yeast extract or as simple as acetate and CO_2. Sulfate was the terminal electron acceptor in all instances (Mechalas and Rittenberg, 1960; Sorokin, 1966a–d; Badziong and Thauer,

1978; Badziong et al., 1978; Brandis and Thauer, 1981). A strain of *D. desulfuricans* used by Sorokin (1966a) was able to derive as much as 50% of its carbon from CO_2 when it grew on hydrogen as the energy source and acetate and CO_2 as carbon sources, whereas on lactate and CO_2, it derived only 30% of its carbon from CO_2. Badziong et al. (1978), using a different strain of *Desulfovibrio*, found that 30% of its carbon was derived from CO_2 when it grew on hydrogen, acetate, and CO_2. Members of some other genera of sulfate-reducing bacteria can also grow mixotrophically on hydrogen and acetate and CO_2 (Pfennig et al., 1981). In all the instances, ATP is generated chemiosmotically from hydrogen oxidation in the periplasm.

20.2.2.3 Heterotrophy

The great majority of autotrophic sulfate reducers can grow heterotrophically with sulfate as terminal electron acceptor. In general, sulfate reducers specialize with respect to the carbon or energy source they can utilize (see also Pfennig et al., 1981). When acetate serves as energy source, it may be completely oxidized anaerobically via the tricarboxylic acid cycle, as in the case of *Desulfobacter postgatei* (Brandis-Heep et al., 1983; Gebhardt et al., 1983). More commonly, however, sulfate reducers oxidize acetate by reversal of the active-acetate-synthesis pathway (Schauder et al., 1986). Assimilation of acetate most likely involves carboxylation to pyruvate. ATP synthesis in the heterotrophic mode of sulfate reduction, insofar as it is understood, is mainly by oxidative phosphorylation (chemiosmotically) involving transfer of hydrogen abstracted from an organic substrate into the periplasm followed by its oxidation (Odom and Peck, 1981; but see also Kramer et al., 1987; Odom and Wall, 1987). In the case of lactate, this hydrogen transfer from the cytoplasm to the periplasm across the plasma membrane appears to be energy driven (Pankhania et al., 1988). Some ATP may be formed by substrate-level phosphorylation.

Organic carbon oxidation by many strains is incomplete, producing acetate, although some strains are capable of complete oxidation of organic substrates to CO_2. Those organisms capable of complete versus incomplete oxidation are reviewed by Rabus et al. (2013). The most common substrate for cultivation of substrate reducers in pure culture is lactate, although depending on the strain a wide variety of substrates can be used including methanol, ethanol, acetate, propionate, succinate, fumarate, fatty acids, or sugars.

20.2.2.4 Nitrogen

Most sulfate reducers derive their nitrogen needs from assimilation of ammonium (Rabus et al., 2013). Some sulfate reducers that are also capable of reducing nitrate derive nitrogen from the ammonium produced through this pathway. A few species of sulfate reducers are capable of nitrogen fixation, including *Desulfovibrio*, *Desulfotomaculum*, and *Desulfobacter* species (Rabus et al., 2013). Sulfate reducing bacteria have been shown to be an important source of nitrogen in benthic hypoxic zones, where nitrogen is lost due to denitrification (Bertics et al., 2013). Despite the large energy requirement imposed during nitrogen fixation, consortia of methane oxidizing archaea and sulfate-reducing bacteria performing AOM (see Section 19.8) have also been demonstrated to be capable of nitrogen fixation (Dekas et al., 2009).

20.2.3 Sulfate Reduction Pathways

Scientific knowledge regarding the biochemistry of sulfate reduction has undergone substantial progress in the last two decades. Much of this progress has come about through the characterization of protein crystal structures, notably that of dissimilatory sulfite reductase, which have improved our mechanistic understanding of the intermediate steps of sulfate reduction (c.f. Oliveira et al., 2008a,b; Schiffer et al., 2008; Parey et al., 2010; Venceslau et al., 2014).

Sulfate is imported to cells through sulfate transporters that operate as symporters transferring sulfate into the cell along with cations (Figure 20.3). The cations are generally protons in freshwater species of sulfate-reducing bacteria and sodium ions in the case of marine species (Cypionka, 1995). Expression of transporters is regulated, and strains are thought to have versions of these transporters adapted for high-affinity accumulation of sulfate (under sulfate limitation) and for low-affinity accumulation (under high sulfate). These transporters are thought to be uncoupled from direct consumption of ATP. This contrasts with sulfate permeases used in sulfate

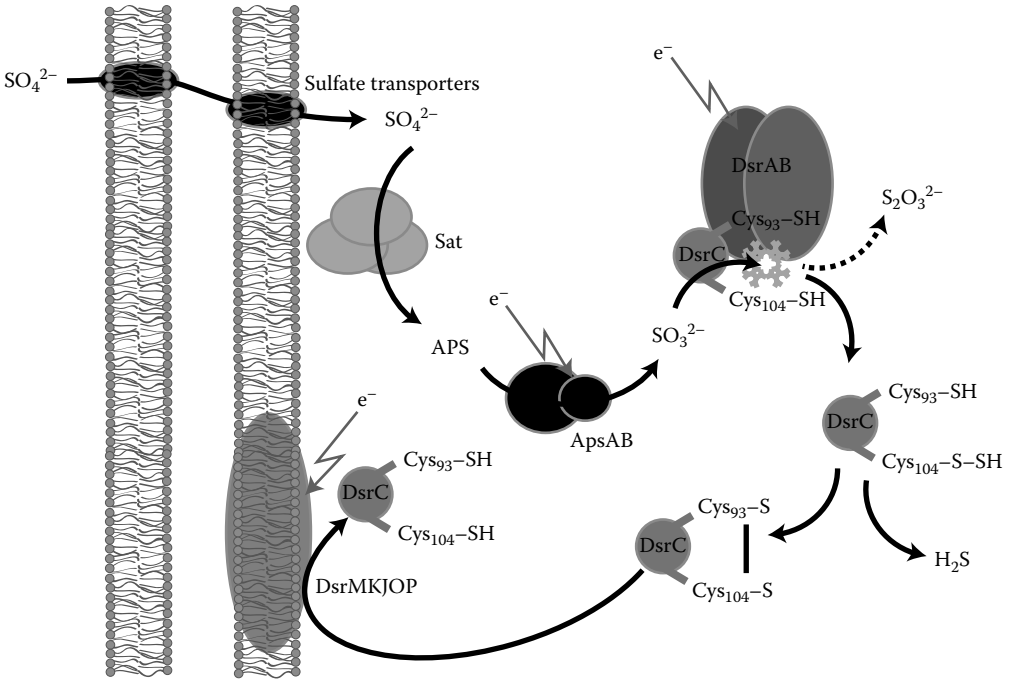


Figure 20.3. Schematic of the processes involved in dissimilatory sulfate reduction. These are as follows: (1) sulfate is transported into the cells through the cell envelope and into the cytoplasm; (2) Sat activates sulfate to APS; (3) APS reductase reduces APS to sulfite; and (4) sulfite interacts with the DsrABC complex. This interaction is complex and in vitro can produce a range of direct products, including trithionate, thiosulfate, and sulfide. Partially reduced sulfur bound to DsrAB can yield thiosulfate. Generally, four electrons are transferred by DsrAB, and zero-valent sulfur is bound to DsrC that undocks from DsrAB and may form a heterodisulfide while releasing H2S. The DsrC acts as an electron acceptor that interacts with the energy-conserving complex in the cell membrane (DsrMKJOP), regenerating reduced DsrC that can redock with DsrAB. Sites of electron donation are indicated by orange arrows.

assimilation by a wide variety of microbes (Piłsyk and Paszewski, 2009), which are ATP dependent.

After import into the cell, sulfate is "activated" by ATP sulfurylase, consuming a molecule of ATP, and generating the "activated" form of sulfate APS (adenosine 5′-phosphosulfate) (Figure 20.3). Direct reduction of sulfate to sulfite is thermodynamically unfavorable at standard state, and the formation of the APS intermediate allows an exergonic reaction from APS to sulfite. However, the formation of APS itself is endergonic, despite the investment of a molecule of ATP to form APS (Cypionka, 1995):

$$SO_4^{2-} + ATP \xrightarrow{\text{ATP sulfurylase}} APS + PP_i \quad (20.1)$$

In members of the genus *Desulfovibrio*, pyrophosphate (PP_i) is hydrolyzed to inorganic phosphate (P_i), which helps to pull Reaction 20.1 in the direction of APS:

$$PP_i + H_2O \xrightarrow{\text{Pyrophosphatase}} 2P_i \quad (20.2)$$

Unlike in assimilatory sulfate reduction (see Section 20.7), APS, once formed, is reduced directly to sulfite and adenylic acid (AMP):

$$APS + 2e \xrightarrow{\text{APS reductase}} SO_3^{2-} + AMP \quad (20.3)$$

The APS reductase, unlike PAPS reductase, does not require NADP as a cofactor but, like PAPS reductase, contains bound flavine adenine dinucleotide and iron (for further discussion see, for instance, Peck, 1993). This is a highly exothermic reaction that is coupled to energy conservation via the QmoABC complex in sulfate-reducing bacteria (Ramos et al., 2012).

The subsequent details on the reduction of sulfite to sulfide were the subject of controversy for many years. Two models competed for several

decades: a step-wise reduction of sulfite to sulfide via trithionate and thiosulfate (termed the trithionate pathway), and a direct, six-electron reduction of sulfite (Bandurski et al., 1956; Harrison and Thode, 1958; Peck 1959, 1962; Kobayashi et al., 1969). These proposed pathways are reviewed in detail by Rabus et al. (2013) and Bradley et al. (2011). More recent evidence has come to light suggesting that neither of these two pathways is correct in detail. The reduction of sulfite to sulfide is carried out by the enzyme complex dissimilatory sulfite reductase (Dsr), which consists of three subunits. Crystal structures (Oliveira et al., 2008a; Parey et al., 2010) show that it consists of two subunits, DsrA and DsrB in a symmetrical $\alpha_2\beta_2$ arrangement, with each subunit bound to a third subunit DsrC. Recent work has demonstrated that sulfite is likely to be reduced in two, two-electron transfers (Lui et al., 1993; Parey et al., 2010), after which a zero-valent sulfur bound to DsrC dissociates from the DsrAB complex (Oliveira et al., 2008a) (Figure 20.3). The DsrC subsequently releases sulfide and transfers oxidizing power to the membrane-bound DsrMKJOP complex in the membrane, where energy is conserved.

20.2.4 Oxygen Tolerance of Sulfate Reducers

In general, sulfate reducers are considered strict anaerobes, yet some show limited oxygen tolerance (Abdollahi and Wimpenny, 1990; Wall et al., 1990; Marshall et al., 1993; Minz et al., 1999; Baumgartner et al., 2006). Indeed, *D. desulfuricans*, *Desulfovibrio vulgaris*, *Desulfovibrio desulfodismutans*, *D. autotrophicum*, *Desulfobulbus propionicus*, and *Desulfococcus multivorans* have shown an ability to use oxygen as terminal electron acceptor, that is, to respire microaerophilically (<10 μM dissolved O_2) without being able to grow under these conditions (Dilling and Cypionka, 1990; Baumgartner et al., 2001). These organisms may have several mechanisms for responding to oxidative stress, such as *bd*- and *cox*-oxidases (Ramel et al., 2013).

Some evidence has been presented in support of aerobic growth of sulfate-reducing bacteria (Canfield and Des Marais, 1991; Jørgensen and Bak, 1991; Fründ and Cohen, 1992). Responses of various *Desulfovibrio* species to oxygen exposure have been investigated (Cypionka, 2000; Faraleira et al., 2003). A chemostat study of a coculture of *Desulfovibrio oxyclinae* and *Marinobacter* strain MB isolated from a mat from Solar Lake in the Sinai Peninsula, showed *D. oxyclinae* is able to grow slowly on lactate in the presence of air and the concurrent absence of sulfate or thiosulfate. The lactate is oxidized to acetate by *D. oxyclinae* (Krekeler et al., 1997; Sigalevich and Cohen, 2000; Sigalevich et al., 2000a). *Marinobacter* strain MB is a facultatively aerobic heterotroph. When grown on lactate in the presence of sulfate in a chemostat supplied with oxygen after an initial anaerobic growth phase, a pure culture of *D. oxyclinae* tended to form clumps after ~149 h of exposure to oxygen (Sigalevich et al., 2000b). Such clumps were not formed in coculture with *Marinobacter* strain MB (Sigalevich et al., 2000b). The clumping may represent a defense mechanism against exposure to oxygen for sulfate-reducing bacteria in general because the interior of active clumps >3 μm in size will become anoxic.

20.3 OTHER REDUCTION DISSIMILATORY METABOLISMS

In addition to reduction of sulfate, many microbes are capable of the reduction of other sulfur species such as sulfite, thiosulfate, elemental sulfur, or polysulfides. Most, if not all, sulfate-reducing bacteria such as *Desulfovibrio* are capable of sulfite and thiosulfate reduction. Inducible sulfite reduction has also been observed with obligate and facultative anaerobes *Clostridium pasteurianum* and *Shewanella oneidensis* MR-1 (Burns and DiChristina, 2009), respectively. Neither is capable of dissimilatory sulfate reduction, though both can reduce sulfite or thiosulfate to sulfide for energy conservation. In the absence of added selenite, whole *C. pasteurianum* cells did not release detectable amounts of trithionate or thiosulfate when reducing sulfite, but in the presence of selenite, they do. Selenite was found to inhibit thiosulfate reductase but not trithionate reductase in whole cells, but inhibited both in cell extracts (Harrison et al., 1980). A purified sulfite reductase from *C. pasteurianum* produced sulfide from sulfite. It was also able to reduce NH_2OH, SeO_3^{2-}, and NO_2^- but did not reduce trithionate or thiosulfate (Harrison et al., 1984). Several physical and chemical properties of this enzyme differed from those of bisulfite reductases in sulfate reducers. Its role in *C. pasteurianum* may be in detoxification when excess bisulfite is present (Harrison et al., 1984). Peck (1993) referred to the enzymes involved in the

transformation of bisulfite to sulfide collectively as bisulfite reductase. Distinct sulfite, trithionite, and thiosulfate reductases were also identified (reviewed in Peck and LeGall, 1982). However, at the time, they did not visualize a major role for these enzymes in sulfite reduction to sulfide. Furthermore, *S. oneidensis* MR-1 is not known to contain a Dsr-like sulfite reductase, but does carry an alternative octahaem *c* cytochrome (SirA) required for sulfite reduction (Shirodkar et al., 2010), while polysulfide reductase is (Psr) is required for thiosulfate reduction (Burns and DiChristina, 2009).

20.3.1 Thiosulfate Reduction

Thiosulfate reducers play an important role in some environments (Jorgensen, 1990a). Liang et al. (2014) demonstrated that *Anaerobaculum* species were abundant in oil pipelines and contributed to biocorrosion, although this was ameliorated during syntrophic growth with methanogens. *Shewanella* has been shown to couple H_2 oxidation to both concurrent reduction of MnO_2 and thiosulfate, indicating a potential coupling with the biogeochemical manganese cycle (Lee et al., 2011).

Growth and growth yield of some members of the anaerobic and thermophilic and hyperthermophilic *Thermotogales* were shown to be stimulated in the presence of thiosulfate (Ravot et al., 1995). The test organisms included *Fervidobacterium islandicum*, *Thermosipho africanus*, *Thermotoga maritima*, *Thermotoga neapolitana*, and *Thermotoga* sp. SERB 2665. The last named was isolated from an oil field. All reduced thiosulfate to sulfide. The *Thermotogales* in this group are able to ferment glucose among various energy-yielding substrates. Thiosulfate, like elemental sulfur (see, e.g., Janssen and Morgan, 1992), appears to serve as an electron sink by suppressing H_2 accumulation in the fermentation of glucose, for instance. This accumulation has an inhibitory effect on the growth of these organisms. The biochemical mechanism by which they reduce thiosulfate remains to be elucidated. *Pyrobaculum islandicum* is able to mineralize peptone by way of the tricarboxylic acid cycle, using thiosulfate as terminal electron acceptor, producing CO_2 and H_2S in a ratio of 1:1 (Selig and Schönheit, 1994). In *Salmonella enterica*, a membrane-bound thiosulfate reductase catalyzes thiosulfate reduction (Stoffels et al., 2012).

20.3.2 Elemental Sulfur Reduction

Elemental sulfur can be used anaerobically as terminal electron acceptor in bacterial respiration or as an electron sink for disposal of excess reducing power. The product of S^0 reduction in either case is sulfide. Polysulfide may be an intermediate in respiration (Fauque et al., 1991; Schauder and Müller, 1993). Some members of both Bacteria and Archaea can respire on sulfur (Schauder and Kröger, 1993; Bonch-Osmolovskaya, 1994; Ma et al., 2000). Examples of Bacteria include *Desulfuromonas acetoxidans*, *Desulfovibrio gigas*, and some other sulfate reducers (Pfennig and Biebl, 1976; Biebl and Pfennig, 1977; Fauque et al., 1991); examples of Archaea include *Pyrococcus furiosus* (Schicho et al., 1993), *Pyrodictium* (Stetter et al., 1983; Stetter 1985), *Pyrobaculum* (Huber et al., 1987), *Acidianus*, *Caldisphaera*, and *Acidilobus* (Boyd et al., 2007).

Organisms that use S^0 reduction as an electron sink include *Thermotoga* spp. in the domain Bacteria and *Thermoproteus*, *Desulfurococcus*, and *Thermofilum* in the domain Archaea (Jannasch et al., 1988a,b). These organisms are fermenters that dispose in this way excess of H_2 they produce, which would otherwise inhibit their growth (Bonch-Osmolovskaya et al., 1990; Janssen and Morgan, 1992; Bonch-Osmolovskaya, 1994). It is possible that these organisms can salvage some energy in the disposal of H_2 (e.g., Schicho et al., 1993). Some fungi, for example, *Rhodotorula* and *Trichosporon* (Ehrlich and Fox, 1967), can also reduce sulfur to H_2S with glucose as electron donor. This is probably not a form of respiration.

The energy source for the sulfur-respiring Archaea is sometimes hydrogen and methane but more often organic molecules such as glucose and small peptides (e.g., Boyd et al., 2007), whereas that for Bacteria may be simple organic compounds (e.g., ethanol, acetate, propanol) or more complex organics. In the case of *D. acetoxidans* (domain Bacteria), an electron transport pathway including cytochromes appears to be involved (Pfennig and Biebl, 1976). When acetate is used as an energy source, oxidation proceeds anaerobically by way of the tricarboxylic acid cycle (see Chapter 7). The oxaloacetate required for initiation of the cycle is formed by carboxylation of pyruvate, which arises from carboxylation of acetate (Gebhardt et al., 1985). Energy is gained in the oxidation of isocitrate and 2-ketoglutarate. Membrane preparations were

shown to oxidize succinate using S^0 or NAD as electron acceptor by an ATP-dependent reaction. Similar membrane preparations reduced fumarate to succinate with H_2S as electron donor by an ADP-independent reaction. Menaquinone mediated hydrogen transfer. Protonophores and uncouplers of phosphorylation inhibited reduction of S^0 but not fumarate. The compound 2-n-nonyl-4-hydroxyquinoline N-oxide inhibited electron transport to S^0 and fumarate. Together these observations support the notion that S^0 reduction in *D. acetoxidans* involves a membrane-bound electron transport system and the ATP is formed chemiosmotically, that is, by oxidative phosphorylation, when growing on acetate (Paulsen et al., 1986).

The hyperthermophilic Archaea, *Thermoproteus tenax* and *P. islandicum*, growing on S^0 and glucose or casamino acids in the case of the former and on peptone in the case of the latter, mineralized their carbon substrates completely. They produced CO_2 and H_2S in a ratio of 1:2 using the tricarboxylic acid cycle (Selig and Schönheit, 1994).

Shewanella can reduce S^0 using similar enzymatic machinery to thiosulfate reduction, encoded by the *phs* gene cluster (Burns and DiChristina, 2009). The ability to reduce S^0 may play an important role in alkaline groundwaters, where sulfate- and S^0-reduction produces sulfide that can subsequently reduce iron phases such as goethite, regenerating S^0. Under these conditions, S^0 acts as an iron shuttle promoting iron reduction in conditions under which it would otherwise be thermodynamically unfavorable (Flynn et al., 2014).

20.4 OXIDATIVE PROCESSES

Oxidation of reduced sulfur compounds is a major process in geomicrobiology. Oxidation of sulfide or sulfur, usually coupled to reduction of O_2, is a process that occurs wherever reduced sulfur encounters oxygen-replete environments, such as in marine sediments, oxygen minimum zones (OMZs), and in hydrothermal systems. Bacteria capable of sulfide oxidation are in many cases autotrophs and can even coexist as carbon-fixing symbionts in animals such as mussels or worms (see Section 19.8). Some phototrophic bacteria are also capable of sulfide oxidation and use H_2S or S^0 as an electron donor for CO_2 reduction.

20.4.1 Physiology and Biochemistry of Microbial Oxidation of Reduced Forms of Sulfur

20.4.1.1 Oxidation of Sulfide

20.4.1.1.1 AEROBIC OXIDATION OF SULFIDE

Many aerobic bacteria that oxidize sulfide are obligate or facultative chemosynthetic autotrophs (chemolithoautotrophs). When growing in the autotrophic mode, they use sulfide as an energy source to assimilate CO_2. Most of them oxidize the sulfide to sulfate, regardless of the level of oxygen tension (e.g., *Acidithiobacillus thiooxidans* London and Rittenberg, 1964). However, some like *Thiobacillus thioparus* form elemental sulfur (S^0) if the pH of their milieu is initially alkaline and the rH_2* is 12, that is, if the milieu is partially reduced due to an oxygen tension below saturation. Thus, *T. thioparus* T5, isolated from a microbial mat, produces elemental sulfur in continuous culture in a chemostat under conditions of oxygen limitation. In this case, small amounts of thiosulfate together with even smaller amounts of tetrathionate and polysulfide are also formed (van den Ende and van Gemerden, 1993). In batch culture under oxygen limitation, *T. thioparus* has been observed to produce initially a slight increase in pH followed by a drop to 7.5 in 4 days and a rise in rH_2 to 20 (Sokolova and Karavaiko, 1968). The reaction leading to the formation of elemental sulfur can be summarized as

$$HS^- + 0.5O_2 + H^+ \rightarrow S^0 + H_2O \qquad (20.4)$$

Wirsen et al. (2002) described an autotrophic, microaerophilic sulfide-oxidizing organism from a coastal marine environment, *Candidatus Arcobacter sulfidicus* that produced filamentous elemental sulfur. It fixed CO_2 via the reductive tricarboxylic acid cycle rather than the Calvin–Benson–Bassham cycle (Hügler et al., 2005). The organism was able to fix nitrogen (Wirsen et al., 2002). *Thiovulum* sp. is another example of a member of the domain Bacteria that oxidizes sulfide to sulfur under reduced oxygen concentrations (Wirsen and Jannasch, 1978).

* $rH_2 = -\log[H_2] = (Eh/0.029) + 2pH$, because $Eh = -0.029 \log[H_2] + 0.058 \log[H^+]$.

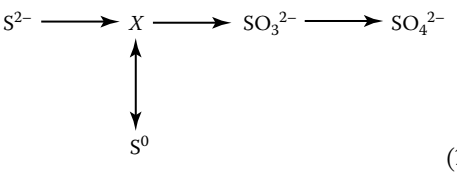
Under conditions of high oxygen tension (at or near saturation), *T. thioparus* will oxidize soluble sulfide all the way to sulfate (London and Rittenberg, 1964; Sokolova and Karavaiko, 1968; van den Ende and van Gemerden, 1993):

$$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+ \qquad (20.5)$$

London and Rittenberg (1964) (see also Vishniac and Santer, 1957) suggested that the intermediate steps in the oxidation of sulfide to sulfate involved

$$4S^{2-} \rightarrow 2S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow SO_3^{2-} + S_3O_6^{2-} \rightarrow 4SO_3^{2-} \rightarrow 4SO_4^{2-} \qquad (20.6)$$

However, this reaction sequence does not explain the formation of elemental sulfur at reduced oxygen tension. Unless this occurs by way of a specialized pathway, which seems doubtful, a more attractive model of the pathway that explains both the processes, the formation of S^0 and SO_4^{2-}, in a unified way is the one proposed by Roy and Trudinger (1970) (see also Suzuki et al., 1994; Yamanaka, 1996; Suzuki, 1999):



(20.7)

Here, X represents a common intermediate in the oxidation of sulfide and elemental sulfur to sulfite. Roy and Trudinger visualized X as a derivative of glutathione or a membrane-bound thiol. It may also be a representative of the *intermediate sulfur* described by Pronk et al. (1990). The scheme of Roy and Trudinger (1970) permits integration of a mechanism for elemental sulfur oxidation into a unified pathway for oxidizing reduced forms of sulfur. Hallberg et al. (1996) found this mechanism consistent with the action of *Acidithiobacillus caldus* on reduced forms of sulfur.

Sorokin (1970) questioned the sulfide-oxidizing ability of Thiobacilli, believing that they oxidize only thiosulfate resulting from chemical oxidation of sulfide by oxygen and that any elemental sulfur formed by Thiobacilli from sulfide is due to the chemical interaction of bacterial oxidation products with S^{2-} and $S_2O_3^{2-}$, as previously proposed by Nathansohn (1902) and Vishniac (1952). This view is not accepted today. Indeed, Vainshtein (1977) and others have presented clear evidence to the contrary. Nübel et al. (2000) showed that hyperthermophilic, microaerophilic, chemolithotrophic *Aquifex aeolicus* VF5 oxidizes sulfide to elemental sulfur using a membrane-bound electron transport pathway that conveys electrons from the oxidation of sulfide to oxygen. The pathway includes a quinone pool, a cytochrome bc_1 complex, and cytochrome oxidase.

20.4.1.1.2 ANAEROBIC OXIDATION OF SULFIDE

Most bacteria that oxidize sulfide anaerobically are photosynthetic autotrophs, but a few, like the facultative anaerobes *Thiobacillus denitrificans* and *Thermothrix thiopara*, are chemosynthetic autotrophs (chemolithoautotrophs). In the presence of nonlimiting concentrations of sulfide, most photosynthetic autotrophs oxidize sulfide to elemental sulfur, using the reducing power from this reaction in the assimilation of CO_2. However, some exceptional organisms exist that never form elemental sulfur. When elemental sulfur is formed, it is usually accumulated intracellularly by purple sulfur bacteria (PSB) and extracellularly by green sulfur bacteria and cyanobacteria. Elemental sulfur accumulated extracellularly by *Chlorobium* appears to be readily available to the cell that formed it, but not to other individuals in the population of the same organism or to other photosynthetic bacteria that can oxidize elemental sulfur. The sulfur is apparently attached to the cell surface (van Gemerden, 1986). Recent study by environmental scanning electron microscopy suggests that the extracellularly deposited sulfur is associated with spinae on the cell surface (Douglas and Douglas, 2000). Spinae are helically arrayed proteins, which form a hollow tube protruding from the cell surface (Easterbrook and Coombs, 1976). Details of the biochemistry of sulfide oxidation by the photosynthetic autotrophs remain to be explored.

The chemosynthetic autotroph *T. denitrificans* can oxidize sulfide to sulfate anaerobically with nitrate as terminal electron acceptor. As sulfide is oxidized, nitrate is reduced via nitrite to nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen (N_2) (Baalsrud and Baalsrud, 1954; Milhaud et al., 1958; Peeters and Aleem, 1970; Adams et al., 1971; Aminuddin and Nicholas, 1973). Acetylene has been found to cause accumulation of sulfur rather

than sulfate in gradient culture of a strain of *T. denitrificans* using nitrous oxide as terminal electron acceptor. In the absence of acetylene, the gradient culture, unlike a batch culture, did not even accumulate sulfur transiently. It was suggested that acetylene prevents the transformation of S^0 to SO_3^{2-} in this culture (Daalsgaard and Bak, 1992). Polysulfide ($S_{n-1}SH^-$), but not free sulfur, appears to be an intermediate in sulfide oxidation to sulfate by this organism (Aminuddin and Nicholas, 1973). The polysulfide appears to be oxidized to sulfite and thence to sulfate (Aminuddin and Nicholas, 1973, 1974a,b).

Oil field brine from the Coleville oil field in Saskatchewan, Canada, yielded two microaerophilic strains of bacteria, one (strain CVO) resembling *Thiomicrospira denitrificans* and the other (strain FWKO B) resembling *Arcobacter*. Both of these strains can oxidize sulfide anaerobically with nitrate as terminal electron acceptor (Gevertz et al., 2000). Each can grow autotrophically, but strain CVO can also use acetate in the place of CO_2. Strain CVO produces elemental sulfur or sulfate, depending on the sulfide concentration, while reducing nitrate or nitrite to dinitrogen. Strain FWKO B produces only sulfur and reduces nitrate only to nitrite. Anaerobic sulfide oxidation linked to nitrate reduction to nitrate has also been implicated in OMZs, where a "cryptic" sulfur cycle may exist (Canfield et al., 2010), with sulfate being reduced to sulfide and rapidly reoxidized to sulfate. A similar cryptic cycle may exist in the methane zone of marine sediments, driven by iron (Holmkvist et al., 2011).

20.4.1.1.3 HETEROTROPHIC AND MIXOTROPHIC OXIDATION OF SULFIDE

Hydrogen sulfide oxidation is not limited to autotrophs. Most known strains of *Beggiatoa* grow mixotrophically or heterotrophically on sulfide. In the former instance, the organisms derive energy from oxidation of the H_2S. In the latter, they apparently use sulfide oxidation to eliminate metabolically formed hydrogen peroxide in the absence of catalase (Burton and Morita, 1964). *Beggiatoa* deposits sulfur granules resulting from sulfide oxidation in its cells external to the cytoplasmic membrane in invaginated, double-layered membrane pockets (Strohl and Larkin, 1978; see also discussion by Ehrlich, 1999). The sulfur can be further oxidized to sulfate under sulfide limitation (Pringsheim, 1967). At least one strain of *Beggiatoa*, isolated from the marine environment, has proven to be autotrophic (Nelson and Jannasch, 1983; see also Jannasch et al., 1989). The heterotrophs *Sphaerotilus natans* (prokaryote, domain Bacteria), *Alternaria*, and yeast (eukaryotes, fungi) have also been reported to oxidize H_2S to elemental sulfur (Skerman et al., 1957a,b), using a pathway that includes reverse dissimilatory sulfite reductase (rDsr) coupled to energy metabolism (Belousova et al., 2013).

20.4.1.2 Oxidation of Elemental Sulfur

20.4.1.2.1 AEROBIC OXIDATION OF ELEMENTAL SULFUR

Elemental sulfur may be enzymatically oxidized to sulfuric acid by certain members of the Bacteria and Archaea. The overall reaction may be written as

$$S^0 + 1.5O_2 + H_2O \rightarrow H_2SO_4 \qquad (20.8)$$

Cell extract of *A. thiooxidans*, to which catalytic amounts of glutathione were added, oxidized sulfur to sulfite (Suzuki and Silver, 1966). Sulfite was also shown to be accumulated when sulfur was oxidized by *A. thiooxidans* in the presence of 2-*n*-heptyl-4-hydroxyquinoline N-oxide (HQNO), which has been shown to inhibit sulfite oxidation. The stoichiometry when the availability of sulfur was limited was 1 mol sulfite accumulated per mole each of sulfur and oxygen consumed (Suzuki et al., 1992). A sulfur-oxidizing enzyme in *T. thioparus* used glutathione as a cofactor to produce sulfite (Suzuki and Silver, 1966). The enzyme in both organisms contained nonheme iron and was classed as an oxygenase. The mechanism of sulfur oxidation is consistent with the model described in Reaction 20.7. The glutathione in this instance forms a polysulfide (compound X in Reaction 20.7) with the substrate sulfur, which is then converted to sulfite by the introduction of molecular oxygen. This reaction appears not to yield useful energy to the cell. Sulfur oxidation to sulfite that does not involve oxygenase but an oxidase with a potential for energy conservation has also been considered. Some experimental evidence supports such a mechanism (see Pronk et al., 1990).

20.4.1.2.2 ANAEROBIC OXIDATION OF ELEMENTAL SULFUR

Details are emerging regarding how elemental sulfur is oxidized in anaerobes, especially

photosynthetic autotrophs. In these organisms, the *sox* pathway plays an important role, although the precise details require further elucidation (Friedrich et al., 2001). *T. denitrificans* appears to follow the reaction sequence in Reaction 20.7 except that oxidized forms of nitrogen substitute for oxygen as terminal electron acceptor. *Acidithiobacillus ferrooxidans* has the capacity to oxidize elemental sulfur anaerobically using ferric iron as terminal electron acceptor (Brock and Gustafson, 1976; Corbett and Ingledew, 1987). The anaerobic oxidation yields enough energy to support growth at a doubling time of 24 h (Pronk et al., 1991, 1992).

20.4.1.3 Oxidation of Sulfite

20.4.1.3.1 AEROBIC OXIDATION OF SULFITE

Sulfite may be oxidized by two different mechanisms, one of which includes substrate-level phosphorylation whereas the other does not, although both yield useful energy through oxidative phosphorylation by the intact cell (see, e.g., review by Wood, 1988). In substrate-level phosphorylation, sulfite reacts oxidatively with adenylic acid (AMP) to give APS:

$$SO_3^{2-} + AMP \xrightarrow{\text{APS oxido-reductase}} APS + 2e \quad (20.9)$$

The sulfate of APS is then exchanged for phosphate:

$$APS + P_i \xrightarrow{\text{ADP sulfurylase}} ADP + SO_4^{2-} \quad (20.10)$$

ADP can then be converted to ATP as follows:

$$2ADPS \xrightarrow{\text{ADP sulfurylase}} ATP + AMP \quad (20.11)$$

Hence, the oxidation of 1 mol of sulfite yields 0.5 mol of ATP formed by substrate-level phosphorylation. However, most energy conserved as ATP is gained from shuttling electrons in Reaction 20.9 through the membrane-bound electron transport system to oxygen (Davis and Johnson, 1967).

A number of Thiobacilli appear to use an AMP-independent sulfite oxidase system (Roy and Trudinger, 1970, p. 214). These systems do not all seem to be alike. The AMP-independent sulfite oxidase of autotrophically grown Thiobacillus novellus may use the following electron transport pathway (Charles and Suzuki, 1966):

$$SO_3^{2-} \rightarrow \text{Cytochrome c} \rightarrow \text{Cytochrome oxidase} \rightarrow O_2 \quad (20.12)$$

The sulfite oxidase of *Thiobacillus neapolitanus* can be pictured as a single enzyme complex that may react either with sulfite and AMP in an oxidation that gives rise to APS and sulfate or with sulfite and water followed by oxidation to sulfate (Roy and Trudinger, 1970). The enzyme complex then transfers the reducing power that is generated to oxygen. Sulfite-oxidizing enzymes that do not require the presence of AMP have also been detected in *A. thiooxidans*, *T. denitrificans*, and *T. thioparus*. *Thiobacillus concretivorus* (now considered a strain of *A. thiooxidans*) was reported to shuttle electrons from SO_3^{2-} oxidation via the following pathway to oxygen (Moriarty and Nicholas, 1970):

$$SO_3^{2-} \rightarrow (\text{Flavin?}) \rightarrow \text{Coenzyme Q} \rightarrow \text{Cytochrome } b \rightarrow \text{Cytochrome } c \rightarrow \text{Cytochrome } a_1 \rightarrow O_2 \quad (20.13)$$

The archaeon *Acidianus ambivalens* appears to possess both an ADP-dependent and an ADP-independent pathway. The former occurs in the cytosol, whereas the latter is membrane associated (Zimmermann et al., 1999).

20.4.1.3.2 ANAEROBIC OXIDATION OF SULFITE

T. denitrificans is able to form APS reductase that is not membrane bound (Bowen et al., 1966), as well as a membrane-bound AMP-independent sulfite oxidase (Aminuddin and Nicholas, 1973, 1974a,b). Both enzyme systems appear to be active in anaerobically grown cells (Aminuddin, 1980). The electron transport pathway under anaerobic conditions terminates in cytochrome *d*, whereas under aerobic conditions it terminates in cytochromes aa_3 and *d*. Nitrate but not nitrite acts as electron acceptor anaerobically when sulfite is the electron donor (Aminuddin and Nicholas, 1974b).

20.4.1.4 Oxidation of Thiosulfate

Most chemosynthetic autotrophic bacteria that can oxidize elemental sulfur can also oxidize thiosulfate to sulfate. The photosynthetic, autotrophic, and purple and green sulfur bacteria and some purple nonsulfur bacteria oxidize thiosulfate

to sulfate as a source of reducing power for CO_2 assimilation (e.g., Trüper 1978; Neutzling et al., 1985). However, the mechanism of thiosulfate oxidation is probably not the same in all these organisms. The chemosynthetic, aerobic autotrophic *T. thioparus* will transiently accumulate elemental sulfur outside its cells when growing in excess thiosulfate in batch culture but only sulfate when growing in limited amounts of thiosulfate. *T. denitrificans* will do the same anaerobically with nitrate as terminal electron acceptor (Schedel and Trüper, 1980). The photosynthetic purple bacteria may also accumulate sulfur transiently, but some green sulfur bacteria (Chlorobiaceae) do not (see discussion by Trüper, 1978). Several of the purple nonsulfur bacteria (Rhodospirillaceae) when growing photoautotrophically with thiosulfate do not accumulate sulfur in their cells (Neutzling et al., 1985). Some mixotrophic bacteria oxidize thiosulfate only to tetrathionate.

Thiosulfate is a reduced sulfur compound with sulfur in a mixed valence state. Current evidence indicates that the two sulfurs are covalently linked, the outer or sulfane sulfur of $S_2O_3^{2-}$ having a valence of −1 and the inner or sulfone sulfur having a valence of +5. An older view was that the sulfane sulfur had a valence of −2 and the sulfone sulfur +6.

Charles and Suzuki (1966) proposed that when thiosulfate is oxidized, it is first cleaved according to the reaction:

$$S_2O_3^{2-} \rightarrow SO_3^{2-} + S^0. \qquad (20.14)$$

The sulfite is then oxidized to sulfate:

$$SO_3^{2-} + H_2O \rightarrow SO_4^{2-} + 2H^+ + 2e, \qquad (20.15)$$

and the sulfur is oxidized to sulfate via sulfite as previously described:

$$S^0 \rightarrow SO_3^{2-} \rightarrow SO_4^{2-} \qquad (20.16)$$

Alternatively, thiosulfate oxidation may be preceded by a reduction reaction, resulting in the formation of sulfite from the sulfone sulfur and sulfide from the sulfane sulfur:

$$S_2O_3^{2-} + 2e \rightarrow S^{2-} + SO_3^{2-} \qquad (20.17)$$

These products are then each oxidized to sulfate (Peck, 1962). In the latter case, it is conceivable that sulfur could accumulate transiently by the mechanisms suggested by Reaction 20.7, but sulfur could also result from asymmetric hydrolysis of tetrathionate resulting from direct oxidation of thiosulfate (see Roy and Trudinger, 1970 for detailed discussion):

$$2S_2O_3^{2-} + 2H^+ + 0.5O_2 \rightarrow S_4O_6^{2-} + H_2O \qquad (20.18)$$

$$S_4O_6^{2-} + OH^- \rightarrow S_2O_3^{2-} + S^0 + HSO_4^- \qquad (20.19)$$

The direct oxidation reaction may involve the enzymes thiosulfate oxidase and thiosulfate cytochrome *c* reductase, a thiosulfate-activating enzyme (Trudinger, 1961; Aleem, 1965). The thiosulfate oxidase may use glutathione as a coenzyme (see summary by Roy and Trudinger, 1970; Wood, 1988).

Thiosulfate may also be cleaved by the enzyme rhodanese, which is found in most sulfur-oxidizing bacteria. For instance, it can transfer sulfane sulfur to acceptor molecules such as cyanide to form thiocyanate. This enzyme may also play a role in thiosulfate oxidation. In anaerobically growing *T. denitrificans* strain RT, for instance, rhodanese initiates thiosulfate oxidation by forming sulfite from the sulfone sulfur, which is then oxidized to sulfate. The sulfane sulfur accumulates transiently as elemental sulfur outside the cells, and when the sulfone sulfur is depleted, the sulfane sulfur is rapidly oxidized to sulfate (Schedel and Trüper, 1980). In another strain of *T. denitrificans*, however, thiosulfate reductase rather than rhodanese catalyzes the initial step of thiosulfate oxidation, and both the sulfane and sulfone sulfur are attacked concurrently (Peeters and Aleem, 1970). *Thiobacillus versutus* (formerly *Thiobacillus* A_2) seems to oxidize thiosulfate to sulfate by a unique pathway (Lu and Kelly, 1983) that involves a thiosulfate multienzyme system that has a periplasmic location (Lu, 1986). No free intermediates appear to be formed from either the reduced ($S–SO_3^{2-}$) or sulfonate ($S–SO_3^{2-}$) sulfurs of thiosulfate.

Pronk et al. (1990) summarized the evidence that supports a model in which *A. ferrooxidans*, *A. thiooxidans*, and *Acidiphilium acidophilum* oxidize thiosulfate by forming tetrathionate in an initial step:

$$2S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 2e^- \qquad (20.20)$$

This is followed in the model by a series of hydrolytic and oxidative steps whereby tetrathionate is transformed into sulfate with transient accumulation of intermediary sulfur from polythionates. Thiosulfate dehydrogenase from *A. acidophilum*, which catalyzes the oxidation of thiosulfate to tetrathionate, was purified and partially characterized by Meulenberg et al. (1993).

20.4.1.5 Oxidation of Tetrathionate

Although bacterial oxidation of tetrathionate has been reported, the mechanism of oxidation is still not certain (see Roy and Trudinger, 1970; Kelly, 1982). It may involve disproportionation (see Section 20.5) and hydrolysis reactions. A more detailed scheme was described by Pronk et al. (1990), which was mentioned earlier in connection with thiosulfate oxidation.

20.4.2 Common Mechanism for Oxidizing Reduced Inorganic Sulfur Compounds in Domain Bacteria

Friedrich et al. (2001) suggested that the mechanism for oxidizing inorganic reduced sulfur compounds by aerobic and anaerobic sulfur-oxidizing bacteria, including anoxygenic phototrophic bacteria, have certain common features. Their suggestion is based on molecular comparisons of the *Sox* genes and the proteins they encode between those in *Paracoccus pantotrophus* and those in other bacteria capable of oxidizing inorganic reduced sulfur compounds. The *Sox* enzyme system in the archeon *Sulfolobus solfataricus* appears to differ from that in Bacteria on the basis of genomic analysis.

20.4.3 Ecology of Reduced S Oxidizers

Bacteria and Archaea comprise most of the geomicrobiologically important microorganisms that oxidize reduced forms of sulfur in relatively large quantities. These include aerobes, facultative organisms, and anaerobes. Most are obligate or facultative autotrophs or mixotrophs. Among aerobes in the domain Archaea, one of the most widely studied groups consists of the genera *Sulfolobus* and *Acidianus* (Table 20.3). Among the aerobes in Bacteria, one of the most important groups in terrestrial environments is that of the *Thiobacillaceae* (Table 20.3). This group includes obligate and facultative autotrophs as well as mixotrophs. Another bacterial group that oxidizes sulfide and is important in some freshwater and marine environments is the family *Beggiatoaceae*. Most cultured members of the group use hydrogen sulfide mixotrophically or heterotrophically. In the latter instance, they employ H_2S oxidation as protection against metabolically produced H_2O_2 in the absence of catalase (Nelson and Castenholz, 1981; Kuenen and Beudecker, 1982), but at least

TABLE 20.3
Some aerobic sulfur-oxidizing bacteria.[a,b]

Autotrophic	Mixotrophic	Heterotrophic
Acidithiobacillus albertensis[c]	*Pseudomonas* spp.	*Beggiatoa* spp.
Acidithiobacillus caldus[c]	*Thiobacillus intermedius*	*Thiobacillus perometabolis*
Acidithiobacillus ferrooxidans[c]	*Thiobacillus organoparus*	
Acidithiobacillus thiooxidans[c]	*Thiobacillus versutus*[d]	
Acidianus brierleyi[e]		
Alicyclobacillus disulfidooxidans[f,g]		
Alicyclobacillus tolerans[f,g]		
Beggiatoa alba MS-81-6		
Sulfolobus acidocaldarius[e]		
Thermothrix thiopara		
Thiobacillus denitrificans[h]		
Thiobacillus neapolitanus		
Thiobacillus novellus		
Thiobacillus tepidarius		
Thiobacillus thioparus		

[a] A more complete survey of aerobic sulfur-oxidizing bacteria can be found in Balows et al. (1992) and Dworkin (2001).
[b] All members of the domain Bacteria in this table are gram-negative except for *Alicyclobacillus disulfidooxidans and A. tolerans*.
[c] Formerly assigned to the genus *Thiobacillus* (see Kelly and Wood, 2000).
[d] Can also grow autotrophically and heterotrophically.
[e] Archeon.
[f] *Alicyclobacillus disulfidooxidans* formerly known as *Sulfobacillus disulfidooxidans* and *Alicyclobacillus tolerans* formerly known as *Sulfobacillus thermosulfidooxidans* subsp. *thermotolerans* (see Karavaiko et al., 2005).
[g] Facultative autotroph.
[h] Facultative anaerobe.

one marine strain, *Beggiatoa alba* MS-81-6, can grow autotrophically (Nelson and Jannasch, 1983). Other hydrogen sulfide oxidizers found in aquatic environments include *Thiovulum* (autotrophic) (e.g., Wirsen and Jannasch, 1978), *Achromatium*, *Thiothrix*, *Thiobacterium* (LaRiviere and Schmidt, 1981), and *Thiomicrospira* (Kuenen and Tuovinen, 1981). Of all these groups, only the Thiobacilli produce sulfate directly without accumulating elemental sulfur when oxidizing H_2S in the presence of abundant oxygen. The other groups accumulate sulfur (S^0), which they may oxidize to sulfate when the supply of H_2S is limited or depleted.

Among members of the domain Bacteria, *T. thioparus* oxidizes S^0 slowly to sulfate; this process becomes inhibited as the pH drops below 4.5. *Halothiobacillus halophilus* (formerly *T. halophilus*) is another neutrophilic, but extremely halophilic, obligate chemolithotroph that oxidizes elemental sulfur to sulfate (Wood and Kelly, 1991). By contrast, *A. thiooxidans*, *Acidithiobacillus albertensis* (formerly *T. albertis*) (Bryant et al., 1983), and *A. ferrooxidans* readily oxidize elemental sulfur to sulfate. This reaction produces protons, and being acidophilic, they may lower the pH as low as 1.0 in batch culture. All these organisms are strict autotrophs.

The Archaea *Sulfolobus* spp. and *Acidianus* spp. are also able to oxidize elemental sulfur to sulfate. Both the genera are extremely thermophilic. *S. acidocaldarius* will oxidize sulfur between 55°C and 85°C (70°C–75°C optimum) in a pH range of 0.9–5.8 (pH 2–3 optimum) (Brock et al., 1972; Shivvers and Brock, 1973). The organisms are facultative autotrophs. *Acidianus* (formerly *Sulfolobus*) *brierleyi* has traits similar to those of *S. acidocaldarius* but can also reduce S^0 anaerobically with H_2 and has a different GC (guanine + cytosine) content (31 versus 37 mol.%) (Brierley and Brierley, 1973; Segerer et al., 1986).

Moderately, thermophilic bacteria capable of oxidizing sulfur have also been observed—some were isolated from sulfurous hot springs, others from ore deposits. One of these, *Thiobacillus thermophilica* Imshenetskii, is a motile rod and is a facultative autotroph capable of oxidizing various sulfides and organic compounds besides elemental sulfur (Egorova and Deryugina, 1963). Another is an aerobic, gram-positive, facultative thermophile capable of sporulation, which is able to oxidize not only elemental sulfur but also Fe^{2+} and metal sulfides mixotrophically. It was originally named *Sulfobacillus thermosulfidooxidans* subsp. *thermotolerans* strain K1 (Golovacheva and Karavaiko, 1978; Bogdanova et al., 1990), and later renamed *Alicyclobacillus tolerans* (Karavaiko et al., 2005). Still, another is a gram-negative, facultatively autotrophic *Thiobacillus* sp. capable of growth at 50°C and 55°C with a pH optimum of 5.6 (range 4.8–8) (Williams and Hoare, 1972). Other thermophilic *Thiobacillus*-like bacteria have been isolated that can grow on thiosulfate at 60°C and 75°C and a pH range of 4.8–7.5 (LeRoux et al., 1977). A moderately thermophilic acidophile, *A. caldus* (formerly *T. caldus*), with an optimum growth temperature of ·45°C was isolated by Hallberg and Lindström (1994) and found capable of oxidizing S^{2-}, S^0, SO_3^{2-}, $S_2O_3^{2-}$, and $S_4O_6^{2-}$ (Hallberg et al., 1996).

A number of heterotrophs, including bacteria and fungi, have been reported to be able to oxidize reduced sulfur in the form of elemental sulfur, thiosulfate, and tetrathionate. A diversity of heterotrophic thiosulfate-oxidizing bacteria have been detected in marine sediments and around hydrothermal vents (Teske et al., 2000). Many bacteria that oxidize elemental sulfur oxidize it to thiosulfate, whereas others oxidize thiosulfate to sulfuric acid (Guittonneau, 1927; Guittonneau and Keiling, 1927; Grayston and Wainright, 1988; see also Roy and Trudinger, 1970, pp. 248–249). Some marine Pseudomonadaceae can gain useful energy from thiosulfate oxidation by using it as a supplemental energy source (Tuttle et al., 1974; Tuttle and Ehrlich, 1986).

Two examples of facultatively anaerobic sulfur oxidizers in the domain Bacteria are *T. denitrificans* (e.g., Justin and Kelly, 1978) and *T. thiopara* (Caldwell et al., 1976; Brannan and Caldwell, 1980), the former a mesophile and the latter a thermophile. The genome of *T. denitrificans* was sequenced (Beller et al., 2006). Anaerobically, both organisms use nitrate as terminal electron acceptor and reduce it to oxides of nitrogen and dinitrogen, with nitrite being an intermediate product. They can use sulfur in various oxidation states as an energy source. *T. denitrificans* is an obligate autotroph whereas *T. thiopara* is a facultative autotroph.

The strictly anaerobic sulfur oxidizers are represented by photosynthetic purple and green bacteria (Pfennig, 1977) and certain cyanobacteria (Table 20.4). All Cyanobacteria grow aerobically, but not all oxidize reduced sulfur compounds directly. The PSB (Chromatiaceae) (Figure 20.4) are obligate anaerobes that oxidize reduced

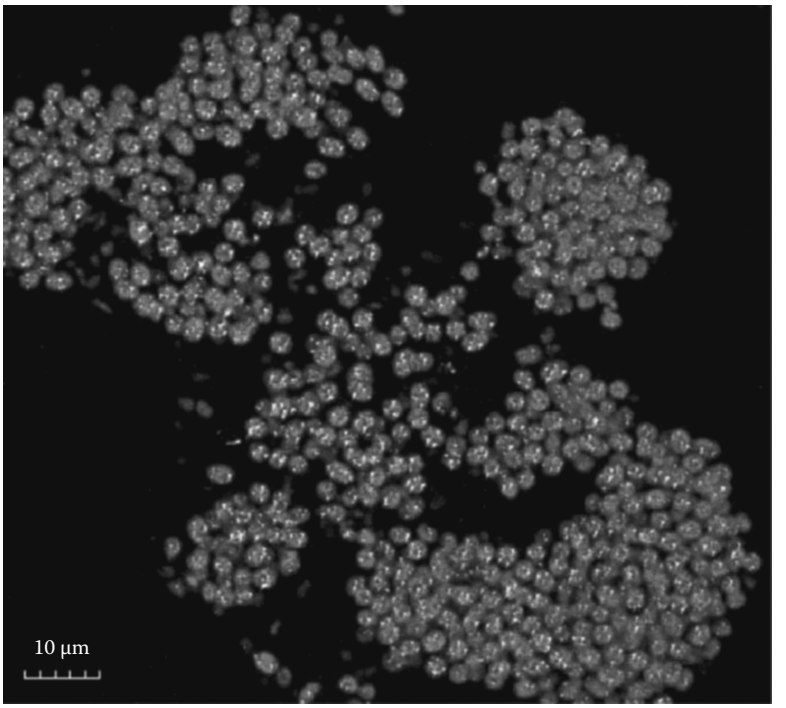


Figure 20.4. **(See color insert.)** Epireflective confocal microscopy of sectioned "pink berry" microbial consortium from Sippewissett Salt Marsh, Cape Cod, MA. Consortia mass is dominated by purple sulfur bacteria (PSB) belonging to the Halochromatium–Thiohalocapsa lineage of the Chromatiaceae. The bright reflective signals are refractile elemental sulfur inclusions within the PSB cells. (Reprinted from Wilbanks, EG et al., *Environ Microbiol*, 2014.)

sulfur, especially H_2S, and use it as a source of reducing power for CO_2 assimilation. Despite the terminology, several purple nonsulfur bacteria (Rhodospirillaceae) can also grow autotrophically on H_2S as a source of reducing power for CO_2 assimilation, but for the most part, they tolerate only low concentrations of sulfide, in contrast to PSB. In the laboratory, purple nonsulfur bacteria can also grow photoheterotrophically, using reduced carbon compounds as a carbon source. Most sulfur-oxidizing phototrophs, when growing on H_2S, oxidize it to S^0, which they deposit intracellularly (Figure 20.3), but *Ectothiorhodospira* spp. deposit it extracellularly. Under conditions of H_2S limitation, these strains oxidize the elemental sulfur they accumulate further to sulfate. Among the purple nonsulfur bacteria, *Rhodopseudomonas palustris* and *Rhodopseudomonas sulfidophila* do not form elemental sulfur as an intermediate from H_2S but oxidize sulfide directly to sulfate (Hansen and van Gemerden, 1972; Hansen and Veldkamp, 1973). In contrast, *Rhodospirillum rubrum*, *Rhodospirillum capsulata*, and *Rhodopseudomonas spheroides* form elemental sulfur from sulfide, which they deposit extracellularly (Hansen and van Gemerden, 1972). *R. sulfidophila* differs from most purple nonsulfur bacteria in being more tolerant of high concentrations of sulfide.

Green sulfur bacteria (Chlorobiaceae) are strictly anaerobic photoautotrophs that oxidize H_2S by using it as a source of reducing power in CO_2 fixation. They deposit the sulfur (S^0) they produce extracellularly. Under H_2S limitation, they oxidize the sulfur further to sulfate. At least a few strains of *Chlorobium limicola* forma *thiosulfatophilum* do not accumulate sulfur but oxidize H_2S directly to sulfate (Ivanov, 1968, p. 137; Paschinger et al., 1974). Many of these bacteria can also use thiosulfate as electron donor in the place of hydrogen sulfide.

Filamentous gliding green bacteria (Chloroflexacea) grow photoheterotrophically under anaerobic conditions, but at least some can also grow photoautotrophically with H_2S as electron donor under anaerobic conditions (Brock and Madigan, 1988).

A few filamentous Cyanobacteria, including some members of the genera *Oscillatoria*, *Lyngbya*, *Aphanothece*, *Microcoleus*, and *Phormidium*, which are oxygenic

TABLE 20.4
Some anaerobic sulfur-oxidizing bacteria.[a]

Photolithotrophs	Chemolithotrophs
Chromatinum spp.	*Thermothrix thiopara*[b,c]
Chlorobium spp.	*Thiobacillus denitrificans*[c]
Ectothiorhodospira spp.	
Rhodopseudomonas spp.[b]	
Chloroflexus aurantiacus[b]	
Oscillatoria sp.[c]	
Lyngbya spp.[c]	
Aphanothece spp.[c]	
Microcoleus spp.[c]	
Phormidium spp.[c]	

[a] For a more complete description of anaerobic sulfur-oxidizing bacteria, see Holt (1984) and Dworkin (2001).
[b] Facultatively autotrophic.
[c] Facultatively anaerobic.

photoautotrophs, can grow photosynthetically under anaerobic conditions with H_2S as a source of reducing power (Cohen et al., 1975; Garlick et al., 1977). They oxidize H_2S to elemental sulfur and deposit it extracellularly. In the dark, they can re-reduce the sulfur they produce using internal reserves of polyglucose as reductant (Oren and Shilo, 1979). At this time there is no evidence that these organisms can oxidize the sulfur they produce anaerobically further to sulfate under H_2S limitation.

Capability for sulfide-oxidation may also be encoded in viral genomes, some of which have been reported to encode genes for reverse dissimilatory sulfite reductase (*rdsr*; Anantharamn et al., 2014). This suggests a possibility for the transfer of these capabilities among microorganisms.

20.5 DISPROPORTIONATION

20.5.1 Elemental Sulfur Disproportionation

Anaerobic marine enrichment cultures consisting predominantly of slightly curved bacterial rods have been shown to contain chemolithotrophic bacteria that were able to grow on sulfur by disproportionating it into H_2S and SO_4^{2-}, but only in the presence of sulfide scavengers such as FeOOH, $FeCO_3$, or MnO_2 (Thamdrup et al., 1993; see also Janssen et al., 1996). The disproportionation reaction can be summarized as

$$4S^0 + 4H_2O \rightarrow SO_4^{2-} + 3H_2S + 2H^+ \quad (20.21)$$

Added ferrous iron scavenges the sulfide by forming FeS, whereas added MnO_2 scavenges sulfide in a redox reaction in which MnO_2 is reduced to Mn^{2+} by the sulfide, producing SO_4^{2-}, with S^0 a probable intermediate (Thamdrup et al., 1994). The scavenging action is needed to propel the reaction in the direction of sulfur disproportionation. In the disproportionation reaction, three pairs of electrons from one atom of sulfur are transferred via an as-yet-undefined electron transport pathway to three other atoms of sulfur, generating H_2S in Reaction 20.21. The sulfur atom yielding the electrons is transformed into sulfate. The transfer of the three pairs of electrons is the source of the energy conserved by the organism for growth and reproduction. This sulfur disproportionation reaction is similar to the one that has been observed under laboratory conditions with the photolithotrophic green sulfur bacteria *C. limicola* subspecies *thiosulfaticum* and *Chlorobium vibrioforme* under an inert atmosphere in the light in the absence of CO_2. To keep the reaction going, the H_2S produced had to be removed by continuous flushing with nitrogen (see Trüper, 1984).

A study of sulfur isotope fractionation as a result of sulfur disproportionation by enrichment cultures from Åarhus Bay, Denmark, and other sediment sources revealed that the sulfide produced may be depleted in ^{34}S by as much as 7.3%–8.6‰ and the corresponding sulfate produced may be enriched by as much as 12.6%–15.3‰. Similar fractionation is obtained from laboratory experiments (Habicht et al., 1998).

20.5.2 Thiosulfate Disproportionation

It has been demonstrated experimentally that some bacteria, like *Desulfovibrio sulfodismutans*, can obtain energy anaerobically by disproportionating thiosulfate into sulfate and sulfide (Bak and Cypionka, 1987; Bak and Pfennig, 1987; Jørgensen, 1990a,b):

$$S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+ \quad (\Delta G^0 = -21.9 \text{ kJ mol}^{-1}) \quad (20.22)$$

The energy from this reaction enables the organisms to assimilate carbon from a combination of CO_2 and acetate. Energy conservation by thiosulfate disproportionation seems, however,

paradoxical if the oxidation state of the sulfane sulfur is −2 and that of the sulfone sulfur is +6, as formerly believed, because no redox reaction would be required to generate a mole of sulfate and sulfide each per mole of thiosulfate. A solution to this paradox has been provided by the report of Vairavamurthy et al. (1993), which demonstrated spectroscopically that the charge density of the sulfane sulfur in thiosulfate is really −1 and that of the sulfone sulfur is +5. Based on this finding, the formation of sulfide and sulfate by disproportionation of thiosulfate requires a redox reaction. Another organism able to disproportionate thiosulfate is *Desulfotomaculum thermobenzoicum* (Jackson and McInerney, 2000). The addition of acetate to the growth medium stimulated thiosulfate disproportionation by this organism. Thiosulfate disproportionation has also been observed with *Desulfocapsa thiozymogenes* (Janssen et al., 1996).

D. desulfodismutans can also generate useful energy from the disproportionation of sulfite and dithionite to sulfide and sulfate (Bak and Pfennig, 1987). The overall reaction for sulfite disproportionation is

$$4SO_3^{2-} + H^+ \rightarrow 3SO_4^{2-} + HS^- \quad (\Delta G^0 = -58.9 \text{ kJ mol}^{-1}) \qquad (20.23)$$

D. sulfodismutans can also grow on lactate, ethanol, propanol, and butanol as energy sources and sulfate as terminal electron acceptor, like typical sulfate reducers, but growth is slower than by disproportionation of partially reduced sulfur compounds. Bak and Pfennig (1987) suggested that from an evolutionary standpoint, *D. sulfodismutans*-type sulfate reducers could be representative of the progenitors of typical sulfate reducers.

Perry et al. (1993) suggested that *Shewanella putrefaciens* MR-4, which they isolated from the Black Sea, disproportionates thiosulfate into either sulfide and sulfite or elemental sulfur and sulfite. They never detected any sulfate among the products in these reactions. These disproportionations are, however, endergonic (+30.98 and +16.10 kJ mol^{-1} at pH 7, 1 atm, and 25°C, respectively). Perry and coworkers suggested that in *S. putrefaciens* MR-4 these reactions must be coupled to exergonic reactions such as carbon oxidation.

Thiosulfate disproportionation seems to play a significant role in the sulfur cycle in marine environments (Jørgensen, 1990a). In Kysing Fjord (Denmark) sediment, thiosulfate was identified as a major intermediate product of anaerobic sulfide oxidation that was simultaneously reduced to sulfide, oxidized to sulfate, and disproportionated to sulfide and sulfate. This occurred at a rapid rate as reflected by a small thiosulfate pool. The metabolic fate of thiosulfate in these experiments was determined by adding differentially labeled ^{35}S-thiosulfate and following the consumption of the thiosulfate and the isotopic distribution in sulfide and sulfate formed from the sulfane and sulfone sulfur atoms of the labeled thiosulfate over time in separate experiments. According to Jørgensen (1990a), the disproportionation reaction can explain the observed large difference in ^{34}S/^{32}S in sulfate and sulfide in the sediments. These findings were extended to anoxic sulfur transformations in further experiments with Kysing Fjord sediments and with sediments from Braband Lake, Åarhus Bay, and Aggersund by Elsgaard and Jørgensen (1992). They showed a significant contribution made by thiosulfate disproportionation in anaerobic production of sulfate from sulfide. Addition of nitrate stimulated anoxic oxidation of sulfide to sulfate. Addition of iron in the form of lepidocrocite (FeOOH) caused partial oxidation of sulfide with the formation of pyrite and sulfur and precipitation of iron sulfides.

20.6 REDUCED FORMS OF SULFUR AS AN ELECTRON DONOR

All evidence to date indicates that to conserve energy, chemosynthetic autotrophic and mixotrophic bacteria that oxidize reduced forms of sulfur feed the reducing power (electrons) into a membrane-bound electron transport system whether oxygen, nitrate, or nitrite is the terminal electron acceptor (Peeters and Aleem, 1970; Sadler and Johnson, 1972; Aminuddin and Nicholas, 1974b; Loya et al., 1982; Lu and Kelly, 1983; Smith and Strohl, 1991; Kelly et al., 1993; also see review by Kelly, 1982). However, the components of the electron transport system in the plasma membrane, that is, the cytochromes, quinones, and nonheme iron proteins, are not identical in all organisms. Whatever the electron transport chain makeup in the plasma membrane, it is the oxidation state of a particular sulfur compound being oxidized, or more exactly the midpoint potential of its redox couple at physiological pH, that determines the entry point into the electron transport chain of the electrons removed during

the oxidation of the sulfur compound. Thus, the electrons from elemental sulfur are generally thought to enter the transport chain at the level of a cytochrome bc_1 complex or equivalent. As pointed out earlier, the first step in the oxidation of sulfur to sulfate can be the formation of sulfite by an oxygenation involving direct interaction with oxygen without involvement of the cytochrome system. Only in the subsequent oxidation of sulfite to sulfate is the electron transport system directly involved starting at the level of the cytochrome bc_1 complex or equivalent. Also, as discussed earlier, sulfite may be oxidized by an AMP-dependent or AMP-independent pathway. In either case, electrons are passed into the electron transport system at the level of a cytochrome bc_1 complex. In the AMP-dependent pathway, most of the energy coupling can be assumed to be chemiosmotic, that is, on average 1 or 2 mol of ATP can be formed per electron pair passed to oxygen by the electron transport system, but in addition, 0.5 mL of ATP can be formed via substrate-level phosphorylation (Reactions 20.9 and 20.10). By contrast, only 1 or 2 mol of ATP can be formed on average per electron pair passed to oxygen by the AMP-independent pathway.

Chemiosmosis is best explained if it is assumed that the sulfite oxidation half-reaction occurs at the exterior of the plasma membrane (in the periplasm):

$$SO_3^{2-} + H_2O \rightarrow SO_4^{2-} + 2H^+ + 2e \qquad (20.24)$$

and the oxygen reduction half-reaction on the inner surface of the plasma membrane (cytoplasmic side):

$$0.5O_2 + 2H^+ + 2e \rightarrow H_2O \qquad (20.25)$$

In *T. versutus*, a thiosulfate-oxidizing, multienzyme system has been located in the periplasm (Lu, 1986).

The pH gradient resulting from sulfite oxidation and any proton pumping associated with electron transport in the plasma membrane together with any electrochemical gradient provide the proton motive force for ATP generation via F_0F_1 ATP synthase. Proton translocation during thiosulfate oxidation has been observed in *T. versutus* (Lu and Kelly, 1988). Involvement of energy coupling via chemiosmosis is also indicated for *T. neapolitanus* using thiosulfate as energy source. The evidence for this is (1) inhibition of CO_2 uptake by the uncouplers carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone (FCCP) and (2) an increase in transmembrane electrochemical potential and CO_2 uptake in response to nigericin (Holthuijzen et al., 1987).

20.6.1 Autotrophs

20.6.1.1 Chemosynthetic Autotrophs

Reduced sulfur is not only an energy source but also a source of reducing power for chemosynthetic autotrophs that oxidize it. Because the midpoint potential for pyridine nucleotides (e.g., NAD(P)H) is lower than that for reduced sulfur compounds that could serve as potential electron donors, *reverse electron transport* from the electron-donating sulfur substrate to pyridine nucleotide is required (see Chapter 7). Electrons must travel up the electron transport chain, that is, against the redox gradient, to NADP with consumption of ATP providing the needed energy. This applies to both aerobes and anaerobes that use nitrate as terminal electron acceptor (denitrifiers).

Insofar as studied, thiobacilli (domain Bacteria) generally fix CO_2 by the Calvin–Benson–Bassham cycle (see Chapter 7), that is, by means of ribulose 1.5-bisphosphate carboxylase. In at least some Thiobacilli, this enzyme is detected in both the cytosol and the cytoplasmic polyhedral bodies called carboxysomes (Shively et al., 1973). The carboxysomes may represent a means of regulating the level of carboxylase activity in the cytosol (Beudecker et al., 1980, 1981; Holthuijzen et al., 1986a,b). *Sulfolobus* (domain Archaea) assimilates CO_2 via a reverse, that is, a reductive tricarboxylic acid cycle (see Brock and Madigan, 1988), like green sulfur bacteria (domain Bacteria) (see Chapter 7).

20.6.1.1.1 PHOTOSYNTHETIC AUTOTROPHS

In purple sulfur and nonsulfur bacteria, reverse electron transport, a light-independent sequence, is used to generate reduced pyridine nucleotide (NADPH) using ATP from photophosphorylation to provide the needed energy. In green sulfur bacteria as well as Cyanobacteria, photochemical electron transport is used to generate NADPH (Stanier et al., 1986) (see discussion in Chapter 7).

PSB, purple nonsulfur bacteria, and those cyanobacteria capable of anoxygenic photosynthesis fix CO_2 by the Calvin–Benson–Bassham cycle, that is, via the ribulose 1,5-bisphosphate carboxylate pathway, when growing photoautotrophically on reduced sulfur (see Chapter 7). Green sulfur bacteria, however, use a reverse, that is, a reductive tricarboxylic acid cycle mechanism (Stanier et al., 1986). However, *Chloroflexus aurantiacus*, a filamentous green nonsulfur bacterium, uses a 3-hydroxypropionate cycle (see discussion in Chapter 7).

20.6.2 Mixotrophy

Some sulfur-oxidizing chemosynthetic autotrophs can also grow mixotrophically (e.g., Smith et al., 1980). Among oxidizers of reduced sulfur, *T. versutus* is a good model for studying autotrophy, mixotrophy, and heterotrophy. It can also grow anaerobically on nitrate (e.g., Wood and Kelly, 1983; Claassen et al., 1987). The organism can use each of these forms of metabolism depending on medium composition (see review by Kelly, 1982). Another well-studied example is *A. acidophilum* growing on tetrathionate (Mason and Kelly, 1988).

Thiobacillus intermedius, which grows poorly as an autotroph in a thiosulfate–mineral salts medium, grows well if the medium is supplemented with yeast extract, glucose, glutamate, or other organic additive (London, 1963; London and Rittenberg, 1966). The organic matter seems to repress the CO_2-assimilating mechanism in this organism but not its ability to generate energy from thiosulfate oxidation (London and Rittenberg, 1966). *T. intermedius* also grows well heterotrophically in a medium containing glucose with yeast extract or glutathione but not in a glucose–mineral salts medium without thiosulfate (London and Rittenberg, 1966). It needs thiosulfate or organic sulfur compounds because it cannot assimilate sulfate (Smith and Rittenberg, 1974). A nutritionally similar organism is *Thiobacillus organoparus*, an acidophilic, facultatively heterotrophic bacterium, which resembles *A. acidophilum* (Wood and Kelly, 1978). *T. organoparus* was first isolated from acid mine water in copper deposits in Alaverdi (former Armenian SSR). It was found to grow autotrophically and mixotrophically with reduced sulfur compounds (Markosyan, 1973).

Thiobacillus perometabolis cannot grow at all autotrophically in thiosulfate–mineral salts medium but requires the addition of yeast extract, casein hydrolysate, or an appropriate organic compound to utilize thiosulfate as an energy source (London and Rittenberg, 1967). Growth on yeast extract or casein hydrolysate is much less luxuriant in the absence of thiosulfate.

Some marine pseudomonads, which are ordinarily considered to grow heterotrophically, have been shown to grow mixotrophically on reduced sulfur compounds (Tuttle et al., 1974). Growth of the cultures on yeast extract was stimulated by the addition of thiosulfate. The bacteria oxidized it to tetrathionate. The growth stimulation by thiosulfate oxidation manifested itself in increased organic carbon assimilation. A number of other heterotrophic bacteria, actinomycetes, and filamentous fungi are also able to oxidize thiosulfate to tetrathionate (Trautwein, 1921; Guittonneau and Keiling, 1927; Starkey, 1934), but whether the growth of any of these is enhanced by this oxidation is unknown at this time. Even if it is not, these organisms may play a role in the sulfur cycle in soils (Vishniac and Santer, 1957).

20.7 SULFUR ASSIMILATION

Inorganic sulfur is obtained for biosynthesis through uptake and reduction of sulfate by algae, plants, and most microorganisms. One possible pathway of assimilation in bacteria is the reduction of sulfate to sulfide and its subsequent reaction with serine to form cysteine, as in *Salmonella typhimurium* (see Freney, 1967, p. 239)[2*]:

$$SO_4^{2-} + ATP \xrightarrow{\text{ATP sulfurylase}} APS + PP_i \quad (20.26)$$

$$APS + ATP \xrightarrow{\text{APS kinase}} PAPS + ADP \quad (20.27)$$

$$PAPS + 2e \xrightarrow{\text{PAPS reductase}} SO_3^{2-} + PAP \quad (20.28)$$

$$SO_3^{2-} + 7H^+ + 6e \xrightarrow{\text{Sulfite reductase}} HS^- + 3H_2O \quad (20.29)$$

* APS, adenosine 5′-phosphatosulfate; PAPS, adenosine 3′-phosphate-5′-sulfatophosphate; PPi, inorganic pyrophosphate; PAP, adenosine 3′,5′-diphosphate.

$$HS^- + \text{Serine} \xrightarrow{\text{Cysteine synthase}} \text{Cysteine} + H_2O \quad (20.30)$$

This reaction sequence has also been found in *Bacillus subtilis*, *Aspergillus niger*, *Micrococcus aureus*, and *Enterobacter aerogenes* (Roy and Trudinger, 1970). Reaction 20.30 may be replaced by the sequence

$$\text{Serine} + \text{acetyl} \sim \text{SCoA} \longrightarrow \underset{\text{O-acetylserine}}{\begin{array}{l} CH_2OOCCH_3 \\ | \\ CHNH_2 \\ | \\ COOH \end{array}} + \text{CoASH} \quad (20.31)$$

$$\text{O-Acetylserine} + H_2S \rightarrow \text{Cysteine} + \text{Acetate} + H^+ \quad (20.32)$$

This latter sequence has been observed in *Escherichia coli* and *S. typhimurium* (Roy and Trudinger, 1970). The reduction of sulfate to *active thiosulfate* and its incorporation into serine from cysteine is also possible for some organisms, such as *E. coli* (Freney, 1967). In some organisms, such as *Allochromatium vinosum*, sulfate may be assimilated via reduction of APS instead of PAPS (Neumann et al., 2000).

20.8 SULFUR-CYCLING MICROBIAL CONSORTIA

Sulfur-metabolizing microbes are commonly found in intricate consortia with other microbes and in symbiotic relationships with a variety of eukaryotic hosts, including animals.

The most well-known example of microbial consortia involving sulfur cycling is the AOM. AOM can occur by a variety of terminal electron acceptors, of which sulfate appears to be the most environmentally significant. Here, the oxidation of methane to bicarbonate is coupled to the parallel, stoichiometric reduction of sulfate to hydrogen sulfide:

$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O \quad (20.33)$$

In this case, AOM is mediated by a consortium of methane-oxidizing archaea (e.g., *Methanosarcinales*) and sulfate-reducing bacteria (e.g., a member of the *Desulfosarcina–Desulfococcus* clade) (Boetius et al., 2000; Orphan et al., 2001, 2002). These two partners form clusters or aggregates of cells, often characterized by a spatial organization with the sulfate-reducing bacteria on the outside clustered around the methanotrophic archaea in the interior (Orphan et al., 2001). The metabolic intermediary between the sulfate-reducing bacteria and the methanotrophic archael partner remains unknown, although suggestions of methane thiol (Moran et al., 2007) and, more recently, disulfide (Milucka et al., 2012) have been made.

Another example of a microbial sulfur cycling consortium can be found in the so-called pink berries of Sippewissett Salt Marsh in Cape Cod, MA. These consortia (Figure 20.4), which can reach ~1 cm in diameter, are composed primarily of photosynthetic PSB (predominantly members of the *Halochromatium–Thiohalocapsa* lineage of the *Chromatiaceae*) and sulfate-reducing bacteria, closely related to *Desulfofustis glycolicus* in the family Desulfobulbaceae (Wilbanks et al., 2014). Here, the two partners form a metabolic cycle with the sulfate reducers producing hydrogen sulfide, which the PSB oxidize to either elemental S (which can be stored internally) or eventually back to sulfate (Wilbanks et al., 2014). A less intimate, but still close physical association can be found between sulfate-reducing bacteria and cyanobacteria within photosynthetic microbial mats (Fike et al., 2008, 2009). Here, small filamentous sulfate-reducing bacteria are observed to wrap around and intertwine with larger filamentous cyanobacteria (Figure 20.5). A metabolic connection between these two organisms remains speculative and may possibly involve sulfate-reducing bacteria coping with oxygen stress in order to use excess organic carbon (i.e., photosynthate) produced by the cyanobacteria as an electron donor or cyanobacteria acting as a sink for hydrogen sulfide produced during sulfate reduction.

Symbioses between sulfur-cycling microbes and animals are particularly common in marine environments, particularly those associated with hydrothermal vents and cold seeps. Remarkable consortia involving invertebrates and autotrophic sulfide-oxidizing bacteria have been discovered in submarine hydrothermal vent communities (Jannasch, 1984; Jannasch and Taylor, 1984; Jannasch and Mottl, 1985). Vestimentiferan tube worms (*Riftia pachyptila*), which grow around the submarine vents, especially white smokers, lack a mouth and digestive tract, and harbor special organelles in their

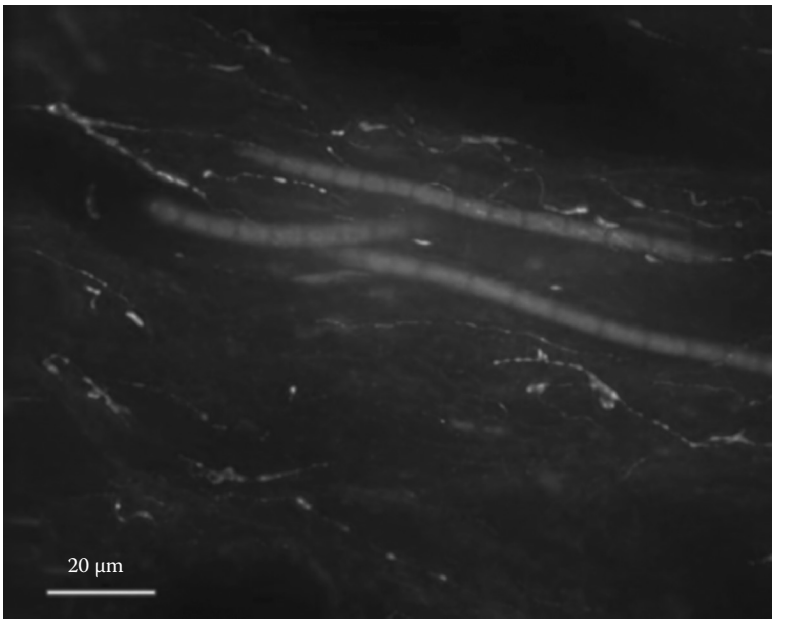


Figure 20.5. **(See color insert.)** CARD-FISH image of the upper portion (ca. 1.5 mm beneath the surface) of a microbial mat from Guerrero Negro, Baja California Sur, Mexico. This highlights the close physical association of cyanobacteria (large filamentous cells in red, chlorophyll autofluorescence) and a group of sulfate-reducing bacteria (thin filamentous cells in green, DSS 658 probe), which morphologically resemble the genus *Desulfonema*. Blue indicates the DNA stain DAPI. (Reprinted from *Geochim et Cosmochim Acta*, 73, Fike, DA, Finke, N, Zha, J, Blake, G, Hoehler, TM, and Orphan, VJ, The effect of sulfate concentration on (sub)millimeter-scale sulfide $\delta^{34}S$ in hypersaline cyanobacterial mats over the diel cycle, 6187–6204, Copyright 2009, with permission from Elsevier.)

body cavity called a trophosome. These organelles when viewed in section under a transmission electron microscope contain tightly packed bacteria (Cavanaugh et al., 1981). Metabolic evidence indicates that these are chemosynthetic, autotrophic bacteria (Felbeck, 1981; Felbeck et al., 1981; Rau, 1981; Williams et al., 1988). The bacteria in the trophosomes appear to be autotrophic sulfur-oxidizing bacteria that share the carbon they fix with the worm. The worm absorbs sulfide (HS) and oxygen from the water through a special organ at its anterior end consisting of a tentacular plume attached to a central supporting obturaculum (Jones, 1981; Goffredi et al., 1997) and transmits these via its circulatory system to the trophosome. The blood of the worm contains hemoglobin for reversible binding of oxygen and another special protein for reversible binding of sulfide. The latter protein prevents reaction of sulfide with the hemoglobin and its consequent destruction (Arp and Childress, 1983; Powell and Somero, 1983). The bound hydrogen sulfide and oxygen are released at the site of the trophosome.

Somewhat less intimate consortia around hydrothermal vents are formed by giant clams and mussels (*Mollusca*) with autotrophic sulfide-oxidizing bacteria. The bacteria in these instances reside not in the gut of the animals but on their gills (see Jannasch and Taylor, 1984, for discussion; also Rau and Hedges, 1979). These looser consortia involving autotrophic sulfide-oxidizing bacteria and mollusks are not restricted to hydrothermal vent communities but also occur in shallow water environments rich in hydrogen sulfide (Cavanaugh, 1983). Another unique symbiosis is found in the scaly foot snail (Waren et al., 2003), which has a symbiotic relationship with gammaproteobacteria within its esophageal gland and a nutritional dependence on chemoautotrophic inputs (Goffredi et al., 2004). Most notably, this organism has a set of mineralized scales (composed of a mix of pyrite and greigite) on its foot. These scales house abundant epsilon- and deltaproteobacteria that may have a role in the precipitation of these scales (Goffredi et al., 2004). Long, filamentous threads of sulfur cycling symbionts are also known to colonize the outer surfaces of the yeti crab (*Kiwa hirsuta*), giving the creature its name

(Goffredi et al., 2008). These organisms are found around deep-sea hydrothermal systems, where sulfide oxidation by microbial symbionts helps detoxify the environment for the crabs (Goffredi et al., 2008).

20.9 EVOLUTION OF SULFUR CYCLING OVER EARTH HISTORY

Microbial sulfur cycling leaves diagnostic chemical fingerprints in the environment, particularly in the stable isotopic composition of metabolites (Szabo et al., 1950; Holland, 1973; Canfield and Teske, 1996; Habicht et al., 1998; Canfield, 2001a,b; Habicht et al., 2002; Farquhar et al., 2003; Johnston et al., 2005a,b; Canfield et al., 2010). The record of this metabolic activity can be preserved in sedimentary rocks up to billions of years old. Of the various S-bearing compounds utilized by microbes, both sulfate salts (e.g., gypsum) and sulfide (particularly pyrite [FeS_2]) are common in the rock record. Building predominantly on the marine sedimentary record, the long-term evolution of sulfur cycling can be reconstructed (see in particular Canfield, 2001b). The patterns have been used to argue for the antiquity of MSR (Shen et al., 2001) and sulfur disproportionation (Canfield and Teske, 1996; Johnston et al., 2005a; Philippot et al., 2007), as well as to reconstruct the redox conditions of the ocean and atmosphere (Farquhar et al., 2000; Farquhar and Wing, 2003; Kah et al., 2004; Kampschulte and Strauss, 2004; Hurtgen et al., 2005; Fike et al., 2006; Riccardi et al., 2006; Fike and Grotzinger, 2008).

20.10 SUMMARY

Sulfur cycling plays a critical role in enabling and regulating a diverse suite of microbial metabolic pathways and has had a profound influence on the evolution of Earth's surface environment. Sulfur, which occurs in myriad organic and inorganic forms in nature, is essential to life and is taken up through *assimilatory* processes. In addition, myriad microbes make use of *dissimilatory* sulfur transformations (most notably sulfate reduction) in which oxidation or reduction of sulfur-bearing compounds is coupled to that of another compound in an energy-yielding reaction to drive biochemical processes. These *dissimilatory* processes are responsible for the overwhelming majority of sulfur transformations in geomicrobiological processes. In these reactions, oxidized forms of sulfur, especially sulfate, but also elemental sulfur and thiosulfate, serve as terminal electron acceptors. Reduced forms of sulfur such as hydrogen sulfide and elemental sulfur can serve as sources of electrons to generate energy and for reducing power. Diverse microorganisms (both Bacteria and Archaea) spanning chemolithoautotrophs, anoxygenic and oxygenic (cyanobacteria) photolithotrophs, mixotrophs, and heterotrophs together play important roles in global sulfur cycling (Figure 20.1). These processes leave behind diagnostic geochemical fingerprints (particularly in the isotopic fractionation between the stable isotopes of sulfur (^{32}S, ^{33}S, ^{34}S, and ^{36}S) that can be used to track the evolution and ecological impact of these sulfur cycling metabolisms over Earth history.

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