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Chapter 10
Hydrogen Sulfide: A Toxic Gas Produced by Dissimilatory Sulfate and Sulfur Reduction and Consumed by Microbial Oxidation

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Abstract Sulfur is an essential element for the synthesis of cysteine, methionine, and other organo-sulfur compounds needed by living organisms. Additionally, some prokaryotes are capable of exploiting oxidation or reduction of inorganic sulfur compounds to energize cellular growth. Several anaerobic genera of Bacteria and Archaea produce hydrogen sulfide (H_2S), as a result of using sulfate (SO_4^{2-}), elemental sulfur (S^0), thiosulfate ($S_2O_3^{2-}$), and tetrathionate ($S_4O_6^{2-}$) as terminal electron acceptors. Some phototrophic and aerobic sulfur bacteria are capable of using electrons from oxidation of sulfide to support chemolithotrophic growth. For the most part, biosulfur reduction or oxidation requires unique enzymatic activities with metal cofactors participating in electron transfer. This review provides an examination of cytochromes, iron-sulfur proteins, and sirohemes participating in electron movement in diverse groups of sulfate-reducing, sulfur-reducing, and sulfide-oxidizing Bacteria and Archaea.

Keywords hydrogen sulfide production • sulfate reduction • sulfide oxidation • sulfite reduction • sulfur cycle

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1 Introduction

Sulfur is one of the most versatile elements in life due to its reactivity in different reduction and oxidation states. Sulfur is the element with the highest number of allotropes (about 30), but only a few are found in nature and occur in biological

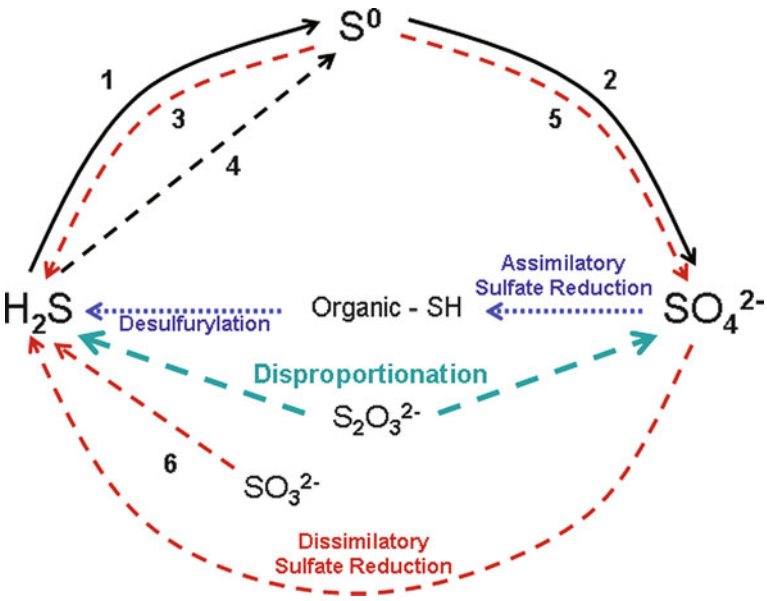

Figure 1 The biological sulfur cycle with roles of bacteria identified. Solid lines indicate aerobic reactions, dashed lines indicate anaerobic reactions, and dotted lines indicate both aerobic and anaerobic activity. **Desulfurylation** by many aerobic and anaerobic prokaryotes, **assimilatory sulfate reduction** by many aerobic and anaerobic microorganisms, **dissimilatory sulfate reduction** by anaerobic organisms listed in Table 1 of this chapter and in Table 1 of Ref. [25], and **disproportionation** of thiosulfate by *Desulfovibrio* and *Desulfocapsa*. **1** and **2**: Sulfide and sulfur oxidation by colorless sulfur bacteria. **3**: Sulfur reduction by the anaerobic microorganisms listed in Table 1 of this chapter. **4** and **5**: Anaerobic sulfide and sulfur oxidation by purple sulfur bacteria and green sulfur bacteria. **6**: Sulfite-reducing bacteria.

systems. The inorganic sulfur compounds of biological relevance which occur in the biological sulfur cycle are elemental sulfur, sulfate, sulfite, thiosulfate, polythionates, sulfide, and polysulfides (Figure 1).

Sulfur can adopt many oxidation states, ranging from −2 to +6. Inorganic sulfur compounds of intermediate oxidation states can serve as electron acceptors or donors in redox processes. In contrast, sulfate and sulfide cannot be further oxidized or reduced, respectively, and they are therefore the final products of most sulfur oxidation or reduction pathways. The biological roles of inorganic sulfur compounds are rather restricted: either they serve as acceptors or donors of electrons for dissimilatory energy-generating electron transport (almost exclusively among prokaryotes), or they are employed as sources for sulfur assimilation, very common in prokaryotes as well as in algae, fungi, and plants.

Despite its toxicity (5-fold higher than CO), H_2S is a fundamental molecule in both anaerobic and aerobic organisms. Since the first description of hydrogen sulfide toxicity by Ramazzini in 1713, most studies about H_2S have been devoted to its toxic effects with little attention paid to its physiological function [1]. The liberation of H_2S is controlled not only by the rate of its production by sulfate- and sulfur-reducing prokaryotes, but also by its tendency to rapidly precipitate as metal sulfides, its

pH-dependent speciation, and its fast biological and chemical oxidation. Only the protonated compound hydrogen sulfide (H_2S) is volatile, whereas sulfide (S^{2-}) dominates under alkaline conditions, and at neutral pH, most of inorganic sulfide is present as bisulfide anion (systematically named sulfanide, HS^-).

1.1 Overview of Bacteria and Archaea Associated with Hydrogen Sulfide Metabolism

1.1.1 Sulfate-Reducing Bacteria and Archaea

Dissimilatory sulfate reduction (also known as anaerobic sulfate respiration) is an essential step in the global sulfur cycle and is exclusively mediated by the sulfate-reducing prokaryotes (SRP), a physiologically and phylogenetically versatile group of microorganisms [2, 3]. SRP are of major functional and numerical importance in many ecosystems and they can grow under different physico-chemical conditions. SRP are found in almost all ordinary environments on this planet: they are present in geothermal areas and hot springs, soils, fresh, marine, brackish, and artesian waters, estuarine muds, cyanobacterial microbial mats, oil and natural gas wells, anaerobic sludge, digestive tracts of humans and animals [3–7]. Dissimilatory sulfate reduction has evolved approximately 3.47 billion years ago [8] and sulfate-reducing bacteria (SRB) should be considered as ancestral microorganisms, which have contributed to the primordial biogeochemical cycle for sulfur as soon as life emerged on the planet [9]. SRB contribute to the complete oxidation of organic matter and participate through metal reduction and sulfide production to the overall biogeochemistry of these extreme environments [2, 10].

As of 2012, 65 genera containing 250 species of SRP have been isolated and characterized [2]. They belong to five phyla within the Bacteria [the *Deltaproteobacteria* (the most frequently represented lineage among SRB), the spore-forming *Desulfovirgula*, *Desulfotomaculum*, *Desulfurispora*, *Desulfosporomusa*, and *Desulfosporosinus* species within the phylum *Firmicutes*, the *Thermodesulfovibrio* species within the phylum *Nitrospirae* and two phyla represented by *Thermodesulfobium narugense* and *Thermodesulfobacterium*/*Thermodesulfatator* species], and two divisions within the Archaea [the euryarchaeotal genus *Archaeoglobus* and the three crenarchaeotal genera *Vulcanisaeta*, *Thermocladium*, and *Caldivirga*, affiliated with the *Thermoproteales*] (Table 1) [3, 6, 7, 11–13].

As of June 2013, a total of 101 genomes of SRP were available at the Integrated Microbial Genomes website including 36 *Desulfovibrio* species, 10 *Desulfotomaculum* species, 8 *Desulfobulbus* species, 5 *Desulfosporosinus* species and 4 *Archaeoglobus* species.

SRP may have a heterotrophic, autotrophic, lithoautotrophic, or respiration-type of life under anaerobiosis and their possible microaerophilic nature has also been reported [2, 3, 10, 14]. More than one hundred compounds including H_2, sugars, amino acids, mono- and dicarboxylic acids, alcohols, and aromatic compounds are

Table 1 Genera of prokaryotes displaying dissimilatory sulfate and sulfur reduction (list incomplete).

	Sulfate Reducers		Sulfur Reducers
Archaea		Archaea	
	Archaeoglobus fulgidus		***Acidianus ambivalens***
	Caldivirga		*Acidilobus*
	Vulcanisaeta		*Caldisphaera*
			Caldivirga
Bacteria	*Ammonifex*		*Caldococcus*
	Candidatus desulforudis		*Desulfolobus*
	Desulfacinum		*Desulfurococcus*
	Desulfobacter		*Hyperthermus*
	Desulfobacterium autotrophicum		*Methanobacterium*
	Desulfobulbus		*Methanococcus*
	Desulfocapsa		***Methanosarcina barkeri***
	Desulfococcus		*Pyrobaculum*
	Desulfocurvus		***Pyrococcus furiosus***
	Desulfofustis		***Pyrodictium abyssi***
	Desulfohalobium		*Staphylothermus*
	Desulfoluna		*Stetteria*
	Desulfomicrobium norvegicum		*Stygiolobus*
	Desulfonatronovibrio		*Thermococcus*
	Desulfosarcina		
	Desulfosporosinus	Bacteria	
	Desulfotomaculum acetoxidans		*Campylobacter*
	Desulfovibrio gigas		*Desulfomicrobium*[a]
	***Desulfovibrio vulgaris* H**		*Desulfotomaculum*[a]
	Desulfovirga		*Desulfovibrio*[a]
	Syntrophobacter		*Desulfurella*
	Thermodesulfatator		*Desulfurobacterium*
	Thermodesulfobacterium commune		***Desulfuromonas acetoxidans***
	Thermodesulfobium		*Salmonella*
	Thermodesulfovibrio		***Sulfurospirillum deleyianum***
			Shewanella
			Wolinella succinogenes

Model organisms and/or major species studied are written in bold.
[a]Only some thiophilic species are able to reduce elemental sulfur.

potential electron donors for SRP [2, 3, 15]. SRP are the anaerobic microorganisms that reduce the greatest number of different terminal electron acceptors including inorganic sulfur compounds and various other organic and inorganic substrates [3, 4, 6, 10, 15–17]. The contribution of SRP to the total carbon mineralization process in marine sediments, where sulfate is not limiting, was estimated to be up to 50 % [3, 4].

The most extensive biochemical and physiological studies have been done with SRB of the genus *Desulfovibrio* which are the most rapidly and easily cultivated

sulfate reducers. Dissimilatory sulfate reduction in *Desulfovibrio* species is linked to electron transport-coupled phosphorylation because substrate level phosphorylation is inadequate to support their growth [18]. The SRB belonging to the genus *Desulfovibrio* possess a number of unique physiological and biochemical characteristics such as the requirement for ATP to reduce sulfate [18], the cytoplasmic localization of two key enzymes [adenosine 5'-phosphosulfate (APS) reductase and dissimilatory-type sulfite reductase] involved in the pathway of dissimilatory sulfate reduction [19, 20], the periplasmic localization of some hydrogenases [21, 22], and the abundance of multiheme *c*-type cytochromes [15, 16, 23].

A set of unique membrane-bound respiratory complexes are involved in sulfate respiration. The Dsr (dissimilatory sulfite reductase) MKJOP and the Qmo (quinone-interacting membrane oxidoreductase) ABC complexes are present in all SRP and are deemed essential for dissimilatory sulfate reduction [24, 25]. A group of other complexes (Hmc, high-molecular weight cytochrome; Nhc, nineheme cytochrome; Ohc, octaheme cytochrome; Qrc, quinone reductase complex; Tmc, tetraheme membrane cytochrome) are present only in sulfate reducers that are characterized by a high content of multiheme cytochromes *c* (mainly the deltaproteobacterial SRB) [24]. A model reflecting organization of membrane-bound respiratory complexes associated with electron transport and cell energetics in *Desulfovibrio* species is given in Figure 2 [25].

1.1.2 Sulfur-Reducing Bacteria and Archaea

Compared with SRP, the environmental distribution of elemental sulfur reducers, and their quantitative role in carbon cycling, is poorly understood. The mechanism of elemental sulfur reduction (characterization of enzymes and electron carriers) has been much less studied than that of dissimilatory sulfate reduction [25–30].

1.1.2.1 Eubacterial Sulfur Reduction

Elemental sulfur is probably the most widespread sulfur species in sediments and geological deposits. Many biological and chemical oxidation processes of H_2S do not directly produce sulfate but rather elemental sulfur, which may accumulate [3]. Elemental sulfur is relatively reactive and in contrast to sulfate, it requires no energy-dependent activation before a reduction can take place. The problem in the utilization of elemental sulfur mainly concerns the low solubility of sulfur flower in water (0.16 µmole per liter at 25 °C) [31]. The so-called “hydrophilic sulfur” is probably the form available in aqueous medium; it consists of elemental sulfur associated with small portions of oxocompounds such as polythionates.

Several genera of the domain Bacteria are able to grow by a dissimilatory reduction of elemental sulfur to sulfide in a respiratory type of metabolism [2, 3, 15, 27, 29, 30, 32–36]. The facultative sulfur-reducing eubacteria, such as the SRB, utilize elemental (or colloidal) sulfur as a respiratory substrate in the absence of

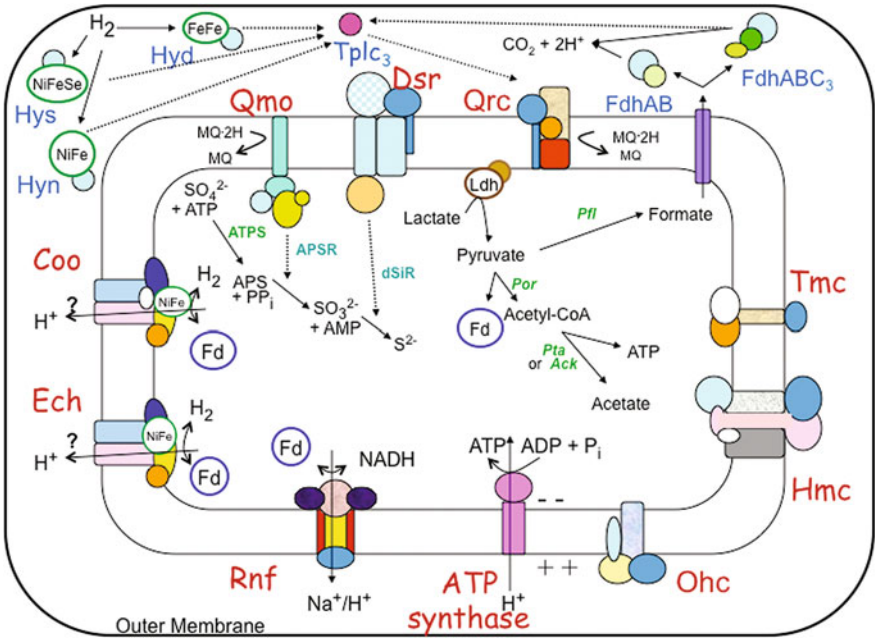

Figure 2 Model reflecting the organization of protein complexes associated with electron transport and cell energetics in sulfate-reducing bacteria. Abbreviations are as follows: Qmo = QmoABC complex, Qrc = QrcABCD complex, Dsr = DsrKMJOP complex, Tmc = TmcABCD complex, Hmc = HmcABCDEF complex, ATP synthase = proton-driven ATP synthase, Coo = carbon monoxide dehydrogenase-hydrogenase membrane complex system, Ech = multi-subunit membrane-bound hydrogenase, Fd = ferredoxin, Fdh = formate dehydrogenase, Hyd = periplasmic [Fe] hydrogenase, Hyn = periplasmic [NiFe] hydrogenase, Hys = periplasmic [NiFeSe] hydrogenase, Ohc = octaheme cytochrome *c* membrane complex, Rnf = NADH:quinone oxidoreductase membrane complex, $Tplc_3$ = periplasmic type I cytochrome c_3. Cytoplasmic enzymes are as follows: Ack = acetate kinase, APSR = adenylylsulfate reductase, ATPS = ATP sulfurylase, dSiR = dissimilatory sulfite reductase, Ldh = lactate dehydrogenase, Pfl = pyruvate formate lyase, Por = pyruvate:ferredoxin oxidoreductase, and Pta phosphotransacetylase. Reproduced by permission from [25]; copyright 2012, Academic Press.

other possible terminal electron acceptors such as sulfate, sulfite, thiosulfate, nitrate or nitrite. The growth of many species of SRB in the presence of sulfate is inhibited by elemental sulfur, probably because sulfur as an oxidant shifts the potential of redox couples in the medium and cells to unfavorable and positive values [3, 30]. Even if most of the SRB are not able to grow by dissimilatory elemental sulfur reduction, some thiophilic species of SRB, belonging to fifteen genera (such as *Desulfomicrobium*, *Desulfovibrio*, *Desulfohalobium*, *Desulfosporosinus*, *Desulfotomaculum*) can use elemental sulfur as an alternative electron acceptor (Table 1) [25, 37–39]. The eubacterial sulfur reducers comprise both facultative and true (or strict) respiratory microorganims. Sulfur reducers use many different types of metabolic systems for oxidizing organic compounds. Both complete and

incomplete oxidation of organic electron donors has been reported for sulfur-reducing eubacteria.

The sulfur reductase (SR) (EC 1.12.98.4-sulfhydrogenase, formerly EC 1.97.1.3-sulfur reductase) is a constitutive enzyme in sulfur-reducing eubacteria: *Desulfuromonas* (*Drm.*) *acetoxidans*, *Wolinella* (*W.*) *succinogenes*, *Sulfurospirillum* (*S.*) *deleyianum*, *Desulfomicrobium* (*Dsm.*), and *Desulfovibrio* (*D.*) species [26, 27]. From genome organization, it appears that a multisubunit polysulfide reductase (PSR) (α, β, and γ) is found in 8 Bacteria genera and 3 Archaea genera [25].

1.1.2.2 Archaeal Sulfur Reduction

Many genera of Archaea are able to grow with elemental sulfur as terminal electron acceptor in the energy metabolism [40]. The dissimilatory reduction of elemental sulfur to hydrogen sulfide is linked with energy conservation as evidenced by growth on H_2 and S^0 ($E^{0'}S^0/SH^- = -270$ mV) [41]. SR is also a constitutive enzyme in the Archaea: *Methanosarcina* (*Ms.*) *barkeri* 227, *Methanococcus thermolithotrophicus*, and *Methanobacterium thermoautotrophicum* Marburg [30].

All archaeal sulfur reducers are extremely thermophilic, whereas sulfur-reducing eubacteria may be mesophilic or moderately thermophilic. The reduction of S^0 is widespread among members of the Archaea, including deep-branching hyperthermophilic genera. In the *Euryarchaeota*, sulfur reduction is present in the orders *Thermoplasmatales*, *Thermococcales*, and many methanogens; in the *Crenarchaeota*, sulfur reduction is found in the orders *Desulfurococcales*, *Sulfolobales*, and *Thermoproteales* [29, 30].

Four mechanisms of S^0 reduction are known in Archaea: (a) The most widespread metabolism consists in the facultative or obligate chemolitho-autotrophic reduction of S^0 with H_2, accomplished by many hyperthermophiles from the *Crenarchaeota*, including members of the genera *Thermoproteus*, *Sulfolobus*, *Stygiolobus*, *Pyrobaculum*, *Ignicoccus*, *Acidianus, Thermoplasma* (Table 1) [28, 30]. (b) Some members of the Archaea including representatives from the genera *Thermococcus, Thermodiscus, Hyperthermus, Stetteria, Thermocladium*, *Pyrodictium*, *Pyrococcus*, and *Desulfurococcus* utilize S^0 reduction as a H_2 sink during fermentative metabolism (Table 1) [29, 30]. (c) Some members of the order *Thermoproteales,* and *Pyrobaculum* (*Pyb.*) *islandicum*, can respire heterotrophically with S^0 in an apparently energy-gaining metabolism [30, 42]. (d) Some hydrogen-oxidizing methanogenic Archaea like *Methanococcus*, *Methanosarcina*, *Methanobacterium*, *Methanothermus,* and *Methanopyrus* can also reduce S^0 with H_2 or methanol [43].

1.1.3 Sulfur-, Sulfite-, and Thiosulfate-Disproportionating Bacteria

The disproportionation (or dismutation) of inorganic sulfur intermediates (also called "inorganic sulfur compound fermentation") at moderate temperatures (0–80 °C) consists of a microbiologically catalyzed chemolithotrophic process in which compounds such as sulfite, thiosulfate, and S^0 serve as both electron donor and acceptor to produce sulfate plus sulfide [44]. Reactions involving disproportionation of thiosulfate, sulfite, and S^0 are listed in equations (1), (2), and (3), respectively.

$$S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+ \qquad \Delta G^{0'} = -21.9 \text{ kJ mol}^{-1} S_2O_3^{2-} [41] \tag{1}$$

$$4\, SO_3^{2-} + H^+ \rightarrow 3\, SO_4^{2-} + HS^- \qquad \Delta G^{0'} = -58.9 \text{ kJ mol}^{-1} SO_3^{2-} [41] \tag{2}$$

$$4\, S^0 + 4\, H_2O \rightarrow SO_4^{2-} + 3\, HS^- + 5\, H^+ \qquad \Delta G^{0'} = +10.2 \text{ kJ mol}^{-1}\ S^0 [41] \tag{3}$$

Desulfovibrio sulfodismutans DSM 3696 was the first bacterium isolated able to carry out the disproportionation of sulfite and thiosulfate to sulfide plus sulfate [45]. *Desulfocapsa sulfoexigens* was the first microorganism able to disproportionate S^0 to sulfate and sulfide [46]. The disproportionation of sulfur compounds is associated with only small free energy changes [47]. Only three *Desulfocapsa* species are able to disproportionate S^0, sulfite, and thiosulfate with growth.

The capacities of dissimilatory sulfate reduction and of sulfite and thiosulfate disproportionation are constitutively present in *D. desulfuricans* CSN (DSM 9104) and *D. sulfodismutans*. In contrast, ATP sulfurylase and sulfite oxidoreductase activities were not detected in these last two strains. During sulfite and thiosulfate dismutation, sulfate is formed via APS reductase and ATP sulfurylase, but not by sulfite oxidoreductase [47]. Elemental sulfur-disproportionating bacteria can be traced back in time as long as 3.5 billion years indicating that elemental sulfur dismutation would be one of the oldest biological processes on Earth [44, 48].

1.1.4 Sulfide-, Sulfur-, Sulfite-, and Thiosulfate-Oxidizing Bacteria

Biological oxidation of hydrogen sulfide to sulfate is one of the major reactions of the biological sulfur cycle (Figure 1). Chemotrophic sulfur-oxidizing bacteria (SOB) are found in four classes of the Proteobacteria (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, and Epsilonproteobacteria) whereas anoxygenic phototrophic sulfur bacteria are only present in Gammaproteobacteria [49].

1.1.4.1 Anoxygenic Phototrophic Bacteria

Reduced inorganic sulfur compounds play an important role as electron donors for photosynthetic carbon dioxide fixation in anoxygenic phototrophic sulfur bacteria. Four major phylogenetic groups of anoxygenic phototrophic bacteria can be distinguished: (a) The green sulfur bacteria (GSB) (family *Chlorobiaceae*), (b) the purple sulfur (PSB) and purple non-sulfur bacteria (PNSB), (c) the Gram-positive Heliobacteria, (d) the filamentous and gliding green bacteria (*Chloroflexaceae*) [50, 51].

Dissimilatory sulfur metabolism (i.e., the use of inorganic sulfur compounds, such as sulfide, elemental sulfur, polysulfides, sulfite, or thiosulfate, as sources or sinks of electrons) has been mainly investigated in the PSB of the families *Ectothiorhodospiraceae* and *Chromatiaceae*. Many of the PNSB can also utilize inorganic sulfur compounds as a source of electrons. The GSB of the family *Chlorobiaceae*, some cyanobacteria, and some members of the filamentous anoxygenic phototrophic bacteria (family *Chloroflexaceae*) are also able to grow phototrophically using reduced sulfur compounds as electron donors [50, 51].

Anoxygenic phototrophic PSB constitute a major group of bacteria widely distributed in nature, primarily in aquatic environments. The two families of PSB, the *Ectothiorhodospiraceae* and *Chromatiaceae*, respectively, produce external and internal sulfur granules. Typical habitats of PSB of the family *Chromatiaceae* are freshwater lakes and intertidal sandflats. The family *Ectothiorhodospiraceae* is found mainly in hypersaline waters. Many species of PSB are "extremophilic", growing at high salt and/or pH. PNSB have been isolated from almost every environment, including freshwater, marine systems, soils, plants, and activated sludge. PSB (more than 30 genera) consist of a variety of morphological types and belong to the Gammaproteobacteria (order *Chromatiales*) (Table 2). GSB are found in various types of aquatic habitats such as the pelagial of lagoons or lakes, bacterial mats in hot springs, or bottom layers of bacterial mats in intertidal sediments.

More than twenty genera of PNSB are now recognized (Table 2). PNSB constitute a physiologically versatile group of purple bacteria that can grow well both in darkness and phototrophically.

1.1.4.2 Colorless Sulfur Bacteria

The name "colorless sulfur bacteria" (CSB) has been utilized since the time of Winogradsky [49] to designate microorganisms able to use reduced sulfur compounds (e.g., sulfide, elemental sulfur, and organic sulfides) as sources of energy for growth. The adjective "colorless" is utilized because of the lack of photopigments in these organisms, although colonies and dense cultures of these bacteria could actually be brown or pink due to their high cytochrome content [49]. CSB play an essential role in the oxidative side of the biological sulfur cycle (Figure 1).

Table 2 Anoxygenic phototrophic sulfur bacteria using reduced inorganic sulfur compounds as electron donors (list incomplete).

Purple Sulfur Bacteria	Green Sulfur Bacteria	Purple Non-Sulfur Bacteria
Allochromatium vinosum	*Ancalochloris*	*Blastochloris*
Amoebobacter	***Chlorobaculum tepidum***	*Phaeovibrio*
Chromatium	*Chlorobium*	*Rhodobaca*
Ectothiorhodosinus	*Chloroherpeton*	***Rhodobacter capsulatus***
Ectothiorhodospira	*Clathrochloris*	*Rhodobium*
Halochromatium	*Pelodictyon*	*Rhodomicrobium*
Halorhodospira halophila	*Prosthecochloris*	*Rhodopila*
Isochromatium		*Rhodoplanes*
Lamprobacter		***Rhodopseudomonas palustris***
Lamprocystis		*Rhodospira*
Marichromatium		***Rhodospirillum rubrum***
Rhabdochromatium		*Rhodothallasium*
Thermochromatium		*Rhodovibrio*
Thioalkalicoccus		*Rhodovivax*
Thiobaca		*Rhodovulum*
Thiocapsa roseopersicina		*Roseospira*
		Roseospirillum
Thiococcus		*Rubrivivax*
Thiodictyon		
Thiocystis		
Thioflavicoccus		
Thiolamprovum		
Thiopedia		
Thiophaeococcus		
Thiorhodococcus		
Thiorhodovibrio		
Thiorhodospira		
Thiospirillum		

Model organisms and/or major species studied are written in bold.

A wide variety of CSB oxidize various inorganic sulfur compounds in nature. Most of these bacteria are chemolithoautotrophs coupling sulfide oxidation with nitrate or oxygen reduction. Based on comparative analysis of 16 rRNA sequences, the known CSB are grouped into four phylogenetic lineages, one within the Archaea and three within the Bacteria [49]. Most of the CSB belong to the phylum Proteobacteria, in particular the class Gammaproteobacteria (*Thiomicrospira, Thioalkalimicrobium, Thioalkalivibrio, Thiothrix, Thiohalospira, Thiohalomonas, Halothiobacillus,* and the *Acidithiobacillaceae*), the class Betaproteobacteria (*5 Thiobacillus* spp. and *Sulfuricella denitrificans*), the class Alphaproteobacteria (*2 Starkeya* spp. and *Thioclava pacifica*), and the class Epsilonproteobacteria (3 *Sulfurimonas* spp., *Sulfurovulum*, and *Thiovulum*) (Table 3).

Table 3 Colorless sulfur bacteria: obligately and facultative chemolithoautotrophic genera able to gain energy from oxidizing inorganic sulfur compounds (list incomplete).

Obligately Chemolithoautotrophic Genera	Facultative Chemolithoautotrophic Genera
Acidianus*	***Acidianus****
*Acidithiobacillus**	*Acidiphilium*
Aquifex	*Acidithiobacillus**
Arcobacter	*Alkalilimnicola*
*Beggiatoa**	*Aquaspirillum*
Halothiobacillus	*Beggiatoa**
Hydrogenivirga	*Hydrogenobacter*
Hydrogenovibrio	*Magnetospirillum*
Sulfolobus*	*Paracoccus*
Sulfuricella	*Pseudaminobacter*
Sulfuricurvum	*Sphaerotilus*
*Sulfurihydrogenibium**	*Starkeya*
Sulfurimonas	*Stygiolobus*
Sulfurivirga	*Sulfobacillus*
Sulfurovum	***Sulfolobus****
*Thermithiobacillus**	*Sulfurihydrogenibium**
*Thermothrix**	***Sulfurisphaera***
Thioalkalibacter	*Sulfuritalea*
Thioalkalimicrobium	***Sulfurococcus***
Thioalkalispira	*Tetrathiobacter*
Thioalkalivibrio	*Thermithiobacillus**
*Thiobacillus**	*Thermocrinis*
Thiobacter	*Thermothrix**
Thiofaba	*Thiobacillus**
Thiohalobacter	*Thioclava*
Thiohalomonas	*Thiomargarita*
Thiohalophilus	*Thiomonas*
Thiohalorhabdus	*Thioploca*
Thiohalospira	*Thiosphaera*
Thiomicrospira	*Thiospira*
Thioprofundum	*Thiothrix*
Thiovirga	
Thiovulum	

Archaebacteria are written in bold.
*Genera containing both facultative and obligately chemolithoautotrophic species.

In addition to the Proteobacteria, five *Sulfobacillus* species belong to the phylum *Firmicutes* and five *Sulfurihydrogenibium* species belong to the phylum *Aquificae*. Within the Archaea, *Sulfurisphaera ohwakuensis*, four *Acidianus* species and six *Sulfolobus* species belong to the family *Sulfolobaceae* within the phylum *Crenarchaeota* [49]. Haloalkaliphilic and neutrophilic halophilic chemolithoautotrophic SOB comprise four and seven different groups, respectively, within the

Gammaproteobacteria [52]. CSB can be found wherever reduced sulfur compounds are available (e.g., in soils sediments, at aerobic/anaerobic interfaces in water, and at volcanic sources such as the hydrothermal vents). CSB growing at neutral to slightly alkaline pH values are found in soils, freshwater, and marine sediments [52]. The acidophilic CSB are mainly found in acid mine-drainage water.

1.2 Properties and Toxicity of Hydrogen Sulfide

The toxicological effect of H_2S was first described in 1713 by Ramazzini and Scheele was the first one to synthesize H_2S gas [1]. H_2S is a small gaseous molecule freely permeable through a membrane. Like CO and NO, H_2S fulfills all of the criteria to define a gasotransmitter of clinical relevance [1, 53]. H_2S is produced endogenously by 3 enzymes in mammalian cells and plays important roles in physiological and pathophysiological conditions [1].

H_2S is the only product excreted from the dissimilatory sulfur metabolism of SRB. If sulfate is the energetically stable form of sulfur under aerobic conditions, H_2S is the stable form under anaerobic reduced conditions. Intermediary oxidation states of sulfur (such as sulfite, thiosulfate, and elemental sulfur) may be formed in natural habitats by incomplete biological or chemical oxidation of sulfide, or during anaerobic sulfide oxidation by purple and green sulfur microorganisms.

Under ambient temperature and pressure, H_2S (CAS registry number: 7783-06-4) is a colorless and flammable gas heavier than air ($d = 1.19$), slightly soluble in water, with a molecular weight of 34.08. The smell of H_2S is characteristic of rotten eggs or the obnoxious odor of a blocked sewer. The melting point of H_2S is -82.3 °C, its boiling point is -60.3 °C, and its freezing point is -86 °C. H_2S is a weak acid in aqueous solution with an acid dissociation constant (pK_a) of 6.76 at 37 °C. H_2S is a highly lipophilic molecule and can diffuse through cell membranes without facilitation of membrane channels. The half-life of H_2S in air varies from 12 to 37 hours.

Ambient air H_2S comes from two different sources. Inorganic H_2S sources include volcanic gases, sulfur deposits, petroleum refinery, natural gas, manure pits, pulp and paper mill industry, and sulfur springs. Organic H_2S sources include bacteria and decomposition of organic matters such as released from sewers, water treatment plants, or septic tanks.

Sulfide is a well-known toxin with the potential to harm organisms through, for example, mitochondrial depolarization [54], decreased hemoglobin oxygen affinity [55], inhibition of twenty enzymes involved in aerobic metabolism [56], and reversible inhibition of cytochrome *c* oxidase [57]. H_2S is as toxic as hydrogen cyanide (HCN) because of the capacity of their corresponding anions S^{2-} and CN^- to coordinate and precipitate metal cations [58]. H_2S is the primary toxic form of the compound because of its ability to diffuse across cellular membranes.

1.3 *Effects of Hydrogen Sulfide on Gene Expression and Physiology of Desulfovibrio vulgaris Hildenborough*

H_2S is toxic to most life forms including the SRB themselves for which its presence presents a stress, which these organisms must overcome. The activity of SRB is influenced by the presence of metals such as iron, manganese, copper, cadmium, nickel, lead, and zinc. Inhibitory or even lethal effects are observed at high concentrations of heavy metals, while a low concentration could promote the SRB activity [59]. Most SRB tolerate more than 10 mM sulfide and sulfate reducers growing on aromatic hydrocarbons formed as much as 25 mM sulfide before growth ceased. Some *Desulfotomaculum* species are more sensitive to sulfide, which affects their growth at concentrations of 4–7 mM [59]. Sulfidic sulfur can be present in three different forms (H_2S, HS^-, and S^{2-}) of which the relative fractions depend on the pH of the environment, see reactions (4) and (5) [60].

$$H_2S \rightarrow H^+ + HS^- \qquad pK_a1 = 6.97\ (25\ ^\circ C) \tag{4}$$

$$HS^- \rightarrow H^+ + S^{2-} \qquad pK_a2 = 12.9\ (25\ ^\circ C) \tag{5}$$

The response of *Desulfovibrio vulgaris* Hildenborough (hereafter referred to as *D. vulgaris* H) cells to high sulfide stress has been determined [60]. The growth of *D. vulgaris* H cells was compared in an open system, where sulfide was removed as H_2S by continuous gassing (low sulfide, 1 mM), with a closed system, where sulfide was accumulated (high sulfide, 10 mM). High sulfide decreased the final cell density by 33 % and the specific growth rate constant by 52 %, indicating a decrease in bioenergetics fitness. Under high sulfide conditions the transcription of ribosomal protein-encoding genes was decreased, in agreement with the lower *D. vulgaris* H growth rate. The expression of the DsrD gene, located downstream of the Dsr genes was also strongly down-regulated. In contrast, the expression of many genes involved in proteolysis, stress response, and iron accumulation were increased. High sulfide represents a significant stress condition, in which the bioavailability of metals like iron may be lowered [60].

2 Enzymology of Hydrogen Sulfide Production from Sulfate

2.1 *Enzymology of Dissimilatory Sulfate Reduction*

The hallmark characteristic of microorganisms utilizing dissimilatory sulfate reduction is the production of copious amounts of H_2S. Prior to reduction, sulfate must be activated by ATP sulfurylase to produce adenosine 5'-phosphosulfate (APS) plus pyrophosphate (PP_i), reaction (6).

$$SO_4^{2-} + 2\,H^+ + ATP \rightarrow APS + PP_i \quad \Delta G^{0'} = +46.0\ \text{kJ/mol}\ [41] \quad (6)$$

The sulfate activation reaction is thermodynamically unfavorable and depletion of end products (APS and PP_i) is required to favor APS production. Hydrolysis of PP_i by a cytoplasmic inorganic pyrophosphatase (EC 3.6.1.1) producing inorganic phosphate, enhances the production of APS, reaction (7).

$$PP_i + H_2O \rightarrow 2\,P_i \quad \Delta G^{0'} = -21.9\ \text{kJ/mol}\ [41] \quad (7)$$

Metal ions (Zn^{2+}, Mn^{2+}, Co^{2+}, Mg^{2+}) are known to stabilize inorganic pyrophosphatases of SRB with greatest activity observed with Mg^{2+} [61, 62].

SRB use APS reductase to catalyze the production of HSO_3^- from APS. The reaction of APS/AMP + HSO_3^- is slightly exergonic with $E^{0'} = -60$ mV [41]. Production of H_2S from APS is a two step process. APS reductase catalyzes the two electron reduction of APS to hydrogen sulfite (reaction 8) [41] which is followed by the six electron reduction converting hydrogen sulfite to sulfide by dissimilatory sulfite reductase (reaction 9).

$$APS + H_2 \rightarrow HSO_3^- + AMP + H^+ \quad \Delta G^{0'} = -68.9\ \text{kJ/mol}\ [41] \quad (8)$$

$$HSO_3^- + 3\,H_2 \rightarrow HS^- + 3\,H_2O \quad \Delta G^{0'} = -171.7\ \text{kJ/mol}\ [41] \quad (9)$$

While dissimilatory sulfate reduction proceeds, SRB also use the assimilatory sulfate reduction pathway to synthesize the amino acid cysteine. In both sulfate reduction pathways, sulfate is activated to APS by ATP sulfurylase.

Initially, it was proposed that a trithionate pathway was used to metabolize intermediates produced by the dissimilatory sulfate reduction and several reports with cell-free systems supported this [63–71]. However, there is no convincing evidence for the presence of trithionate reductases in SRB, either by genome analysis or biochemically, and thiosulfate reductases have been reported only in a few SRB. It is probable that under general growth conditions trithionate and thiosulfate are not produced as intermediates in dissimilatory sulfite reduction [72–74].

2.2 ATP Sulfurylase

ATP sulfurylase (ATPS) is also known as adenylylsulfate pyrophosphorylase or sulfate adenylyltransferase (EC 2.7.7.4) and is a product of the *ppa* gene. The pathway utilizing ATPS is indicated in Figure 3.

For optimal activity, ATPS from *Desulfotomaculum* (*Dst.*) (formerly *Clostridium*) *nigrificans* and *D. desulfuricans* strain 8303 require Mg^{2+} to neutralize the

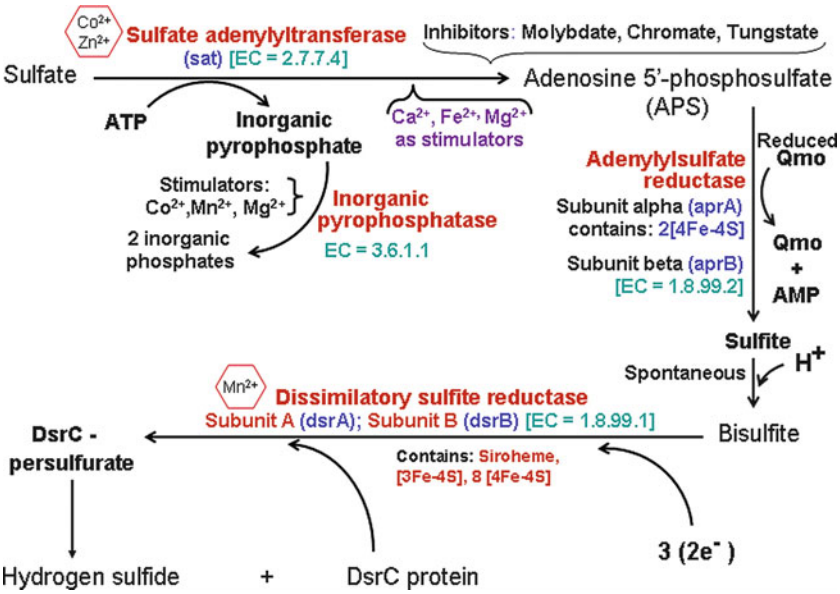

Figure 3 Pathway for sulfide production from dissimilatory sulfate reduction by *Desulfovibrio* species.

charge on ATP and PP_i (reaction 10) [75, 76]. Inhibitors of ATP sulfurylase include CrO_4^{2-}, MoO_4^{2-}, and WO_4^{2-} which produce unstable intermediates.

$$\text{ATP-Mg} + SO_4^{2-} \rightarrow \text{APS} + PP_i\text{-Mg} \tag{10}$$

The ATPS from *Archaeoglobus* (*Ar.*) *fulgidus* DSM 4304 has a molecular mass of 150 kDa (53.1 kDa subunits) compared to 141 kDa (46.9 kDa subunits) for the enzyme from *D. desulfuricans* ATCC 27774 [77, 78]. Using extended X-ray absorption fine structure (EXAFS) and electron paramagnetic resonance (EPR) spectroscopies, Co^{2+} and Zn^{2+} were found to bind to three sulfur atoms and one nitrogen atom in a tetrahedral coordination in the enzymes from *D. desulfuricans* and *D. gigas* [78]. Such a tetrahedral Zn^{2+} site was observed in the crystal structure of *Thermus thermophilus* ATPS [79] and analysis of the crystal structure of ATPS from the sulfur-oxidizing purple sulfur bacterium *Alc. vinosum* reveals that three cysteine residues and one histidine are involved in the zinc-binding site [75]. Similarly, four coordinating amino residues are conserved in the ATPS of *Ar. fulgidus, Pyrodictium* (*P.*) *abyssi, Sulfolobus solfataricus, D. desulfuricans,* and *D. gigas* [75, 80] and may be the site for Zn^{2+} binding. ATPS occurs in dissimilatory sulfate-reducing microorganisms as homotrimers with a zinc ion bound to each monomer [78] and in dissimilatory SOB, ATPS is a homodimer [75]. From structural and genetic analysis, adjacent monomers of ATPS from *Alc. vinosum* have the GXXKXXD sequence and zinc ion stabilizes the APS and PP_i binding sites

[75]. The binding of cobalt to ATPS is unresolved at this time but may contribute to stability of the polymeric structure [78].

2.3 *Dissimilatory Adenylylsulfate Reductase*

In the pathway of sulfate reduction, APS is reduced to sulfite by APS reductase (APSR) (adenylylsulfate reductase, EC 1.8.99.2). A αβ heterodimer forms the basic enzyme unit with the active site FAD located in the AprA subunit and two ferredoxin-like [4Fe-4S] clusters in the AprB subunit. The crystal structure of APSR has been reported for *D. gigas* at 3.1 Å [81] and for *Ar. fulgidus* at 1.6 Å [82]. Using homology modeling of 20 different species, Meyer and Kuever [83] have compared the dissimilatory APSR (AprBA) of SRP and SOB and report that the protein matrix around the [4Fe-4S] clusters and the FAD cofactor show high similarity.

The *D. gigas* APSR has a hexamer structure consisting of six αβ heterodimers [81]. The α subunit of *D. gigas* APSR has a distinct region that binds FAD; another area is the capping attributed to the specific molecular configuration and the third is the helical domain [81]. FAD is attached to the α subunit domain by six hydrogen bonds and a shallow cleft is formed in the α subunit by the action of the FAD and helical domains. The β subunit of *D. gigas* APSR has a domain for binding the [4Fe-4S] cluster, a segment that contains the β-sheet protein and the C-terminal region. In formation of the dimer, the domain of the β subunit containing the [4Fe-4S] clusters is buried in the shallow cleft occurring in the α subunit. The β-sheet protein and the C-terminal domains stabilize the interaction between the α subunit and β subunit of APSR. Cluster I [4Fe-4S] is buried in the β subunit while cluster II [4Fe-4S] is located on the surface of the β subunit where it presumably acquires electrons from the electron donor [81]. The redox potentials for the cluster I and cluster II are 0 and −400 mV, respectively [84]. The electron donor for the dissimilatory APSR in SRB is the membrane QmoABC complex [85]. Using deletion mutants of *D. vulgaris* H, it has been shown that the Qmo complex (*qmoABC* genes) is not essential for sulfite or thiosulfate reduction [86]. The proximity of *AprBA* genes to *QmoABC* for *D. gigas* and *D. vulgaris* H are given in Figure 4.

A comparison of the *D. gigas* APSR crystal structure with the structure of the *Ar. fulgidus* enzyme reveals considerable similarity [81]. The α and β subunits from *Ar. fulgidus* APSR have the same three domains in each subunit as the *D. gigas* APSR. In the β subunit from *Ar. fulgidus* APSR, the redox potential for cluster I [4Fe-4S] is −60 mV while that for cluster II [4Fe-4S] is –520 mV [82]. The C-terminus of the *D. gigas* APSR β subunit is longer than the C-terminus of *Ar. fulgidus* APSR.

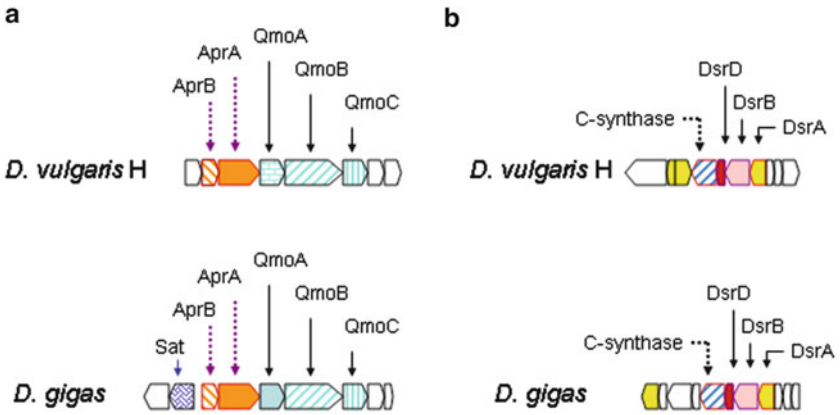

Figure 4 Genes for dissimilatory sulfate reduction in *D. vulgaris* Hildenborough and *D. gigas* DSM 1382. (**a**) Loci of genes for adenylyl sulfate reductase (AprAB), sulfate adenylyl transferase (Sat), and heterodisulfide reductase (QmoABC). (**b**) Loci of genes for dissimilatory sulfite reduction (DsrABD) and cobyrinic acid a,c–diamide synthase (C-synthase). Draft sequence is used for *D. gigas*.

2.4 Sulfite Reductases

Two classes of sulfite reductases can be defined in SRP on the basis of their physiological function. The first class comprises the low-molecular-mass and low-spin sulfite reductases, also called assimilatory-type sulfite reductases (aSiR) (EC 1.8.99.1). They have a molecular mass around 27 kDa, consist of a single polypeptide chain and contain one [4Fe-4S] cluster coupled to a siroheme in a low-spin state [87]. The second class is constituted by the high-spin dissimilatory sulfite reductases (EC 1.8.99.3) dSiR, which possess a molecular mass around 200 kDa and a complex molecular architecture hosting siroheme centers and [4Fe-4S] clusters. While dSiR is preferred it had been customary to refer to dissimilatory sulfite reductase as Dsr and currently the genes for dSiR are designated as *dsr* and their gene products as Dsr. Both aSiR and dSiR enzymes have iron-sulfur clusters and an iron-chelating sirohydrochlorin referred to as siroheme [88].

2.4.1 Dissimilatory High-Spin Sulfite Reductase

High-spin dSiR has either an $\alpha_2\beta_2$ structure, as in the case of *Ar. fulgidus* [89] while some SRB have an $\alpha_2\beta_2\gamma_m\delta_n$ multimeric structure, with α 50 kDa, β 45 kDa, γ 11 kDa, and δ 8 kDa [90, 91]. Catalytic activity of dSiR is attributed to the $\alpha_2\beta_2$ structure and the third protein, γ subunit (DsrC), is an independent protein that functions as a sulfur carrier protein to facilitate the release of H_2S following reduction of sulfite (Figure 3) [92, 93]. The reduced sulfur atom forms a persulfide with a conserved cysteine on DsrC, the γ subunit, and with the release of sulfide, the

intramolecular disulfide linkage of DsrC is reformed by interaction with DsrK, a subunit of the DsrMKJOP transmembrane complex [94–96]. The DsrD, δ subunit, lacks cysteine residues [97] and has no function in electron transport.

Five different types of enzymes belonging to the high-spin DSR class (desulfoviridin, desulfofuscidin, desulforubidin, P-582, and Archaeal) have been isolated and characterized from different genera of SRP [2, 15, 16]. These five enzymes differ mainly by the behavior of their siroheme moieties, their major optical absorption, and EPR spectra [2, 15, 16] (Table 4).

Metals as cofactors are important in dSiR with each $\alpha_2\beta_2$ structure having associated with it two sirohemes, a [4Fe-4S] cluster closely associated with each siroheme and four ferredoxin-type [4Fe-4S] clusters at some distance from the sirohemes. The binding of a siroheme and the [4Fe-4S] cluster to the α subunit and β subunit would be attributed to the (Cys-X_5–Cys)–X_n–(Cys-X_3–Cys) arrangement [98]. Binding of the ferredoxin-type [4Fe-4S] clusters to the α and β subunits is predicted to follow the arrangement of Cys-X_2–Cys-X_2–Cys that is preceded or followed by a Cys-Pro sequence [99–101].

2.4.1.1 Desulfoviridin-Type Sulfite Reductase

The green protein, desulfoviridin, is the dSiR characteristic of the genus *Desulfovibrio* but it has also been found in some species of the genera *Desulfococcus, Desulfomonile, Desulforegula,* and *Desulfonema* [15, 90, 102–105]. The structure of the dSiR of *D. vulgaris* H was reported to be a $\alpha_2\beta_2\gamma_2$ structure [67] and similar to the $\alpha_2\beta_2\gamma_2$ structure found in *D. vulgaris oxamicus* (Monticello), *D. gigas*, and *D. desulfuricans* ATCC 27774 [106]. DsrA and DsrB in *D. vulgaris* H are the products of the *dsrAB* operon while *dsrC*, which encodes for DsrC, is located at another site on the chromosome [95, 96].

Associated with the $\alpha_2\beta_2$ structure are two sirohemes and two sirohydrochlorins which are positioned at the interface of DsrA and DsrB. The sirohydrochlorin accounts for the absorption maximum at 628 nm. The sulfur atom from sulfite, the substrate, is bound to the iron atom of siroheme in DsrA and this region is surrounded by basic amino acids. The other side of the siroheme is surrounded by residues from DsrB. Access to this catalytic site is through a positively charged channel; however, a similar channel is lacking in the region where sirohydrochlorin is bound to DsrA [95, 96]. The *D. vulgaris* H DsrC subunit is in close proximity to the cleft formed between DsrA and DsrB. The C-terminus of DsrC contains a cysteine moiety which may interface with the siroheme and may participate in the catalytic reaction. Oliveira et al. [95, 96] propose that the initial reduction of sulfite is a four and not a six electron step with S^0 formed as an intermediate product. S^0 would interact with the terminal cysteine on DsrC to form a persulfide which would be reduced to sulfide. Reduction of DsrC could be achieved by heterodisulfide reductase activity of the membrane-bound DsrMKJOP which has an appropriate iron-sulfur center for this reduction process [107].

Table 4 Characteristics of selected bacterial and archaeal high-spin and low-spin sulfite reductases. Iron compounds are listed for intact enzyme structure.

Protein	λmax (nm)	Organism	M_r(kDa)	Non-heme Iron	Siroheme (sirohydrochlorin)	[4Fe-4S] Cluster	Reference
High-spin "dissimilatory-type" sulfite reductase							
Desulfoviridin	628	*D. gigas*	200	34	2(2)	8	[64, 92, 93]
	630	*D. vulgaris* H	200	34	2(2)	8	[95, 96, 165]
P-582	582	*Dst. nigrificans*	194	16	1.3	4	[65, 111]
	582	*Dst. thermocisternum*[a]	196	16	2	4	[112]
Desulfofuscidin	576	*T. commune*[b]	167	20–21	4	4	[69, 120]
	578	*T. mobile*	190	32	4	8	[119]
Desulforubidin	545	*Dsm. novegicum*[c]	225	36	4	8	[15, 93, 102, 113]
	545	*Ds. variabilis*	208	15	2	8	[166]
Archaeal	593	*Ar. fulgidus*	218	36	4	8	[89, 117]
	NR[d]	*Ar. profundus*	198	24	2	6	[112]
Low-spin "assimilatory-type" sulfite reductase							
	590	*D. vulgaris* H	27.2	5	1	1	[67, 143]
	587	*Drm. acetoxidans*	23.6	5	1	1	[145]
	590	*Ms. barkeri*	23	5	1	1	[87, 144]

[a]Calculated from gene analysis.
[b]Formerly known as *D. thermophilus*.
[c]Formerly known as *D. desulfuricans* Norway 4 and *D. baculatus* Norway 4.
[d]NR: not reported

Adjacent to the genes for dissimilatory sulfite reduction in *D. vulgaris* H is a gene that encodes for DsvD, a peptide of only 78 amino acids [108]. A similar gene encoding 77 amino acids is also present in the genome of *Ar. fulgidus* and it is downstream of the dsrB gene. The DsvD, also known as DsrD, is not the γ subunit and would not be associated with electron transfer because it lacks cysteine residues [97]. DsvD has structural homology to DNA-binding proteins and may have a role in transcription or translation [109].

D. gigas is a unique sulfate reducer in the sense that it has a multimeric dissimilatory sulfite reductase. Dsr-I and Dsr-II have enzymatic activity while Dsr-III is inactive [92]. The $\alpha_2\beta_2\gamma_2$ structure of Dsr-I type from *D. gigas* contains eight [4Fe-4S] clusters, two planar sirohydrochlorins and two saddle-shaped sirohemes while the Dsr-II type contains two sirohemes, two hydrochlorins, two [3Fe-4S] clusters, and six [4Fe-4S] clusters [92]. Dsr-III from *D. gigas* has iron-sulfur clusters similar to Dsr-II and the inactivity of Dsr-III is attributed to the absence of iron in the siroheme [92].

Analysis of the sirohydrochlorin from several *Desulfovibrio* species reveals that it is different from that isolated from *Escherichia* (*E.*) *coli*. Notably, one of the eight carboxylates of the tetrapyrrole moiety is replaced by an amide group at the 2'-acetate [110]. Additional [4Fe-4S] clusters, referred to as remote iron-sulfur clusters, are located on the surfaces of the α and β subunits. In *D. gigas*, the S atom of sulfite binds to the Fe of the siroheme and a positively charged arginine along with two lysine moieties in the α subunit has an electrostatic interaction with the three oxygen atoms on sulfite. Another arginine forms hydrogen bonding with sulfite and directs the release of sulfide and water molecules through a positively charged water channel.

The γ subunit of *D. gigas* Dsr-I is aligned adjacent to the α/β subunits and the C-terminus of the γ subunit is proposed to have multiple conformations that may influence catalytic function. It has been proposed that activity of the C-terminus of the γ subunit could explain the mechanism for three products (sulfide, thiosulfate, and trithionate) produced by Dsr [92]. The association of dsr genes in *D. gigas* and *D. vulgaris* H is given in Figure 4.

2.4.1.2 P-582-Type Sulfite Reductase

P-582-type sulfite reductase has only been reported in several species of the spore-forming SRB genera *Desulfosporosinus* and *Desulfotomaculum* [15, 25, 65, 111]. The structure of dSiR from *Dst. thermocisternum,* a Gram-positive thermophilic sulfate reducer, has been deduced from gene analysis [112]. From cloning and sequence analysis, it is suggested that the *dsr* operon in *Dst. thermocisternum* is similar to that of *Ar. fulgidus* and *D. vulgaris* H in terms of sequence and gene organization.

The *dsrA* gene encodes for a protein of 54.1 kDa with a 76 % similarity to *Ar. fulgidus* DsrA and an 83 % similarity to DsrA of *D. vulgaris* H. Down-stream of the *dsrA* gene in *Dst. thermocisternum* is the *dsrB* gene and it encodes a protein of

44.2 kDa with an 89 % similarity to the *Ar. fulgidus* DsrB. Down-stream from the *dsrB* is the *dsrD* gene which encodes for a peptide of 11.1 kDa and the DsrD, a hypothetical δ-polypeptide, has a projected sequence identity of 38 % with *Ar. fulgidus* DsrD and 41 % identity to *D. vulgaris* H DsrD. The proposed operon for dissimilatory sulfite reductase in *Dst. thermocisternum* consists of *dsrABD*.

2.4.1.3 Desulforubidin-Type Sulfite Reductase

The red brown protein, desulforubidin, belongs to the genera *Desulfohalobium*, *Desulfosarcina*, *Desulfomicrobium*, *Desulfocurvus, Desulfobulbus, Desulfofustis*, and *Desulfobacter* [2, 39, 113, 114]. The crystal structure at 2.5 Å resolution of the *Desulfomicrobium* (*Dsm.*) *norvegicum* dSiR was reported by Oliveira et al. [93]. Due to the distinctive spectral characteristics, the dSiR is a desulforubidin-type with the $\alpha_2\beta_2\gamma_2$ structure and the isolated complex contains two DsrC proteins. The *Dsm. norvegicum* dSiR contains four siroheme groups and eight [4Fe-4S] clusters.

One of the cofactors in the dSiR is a siroheme bound to a [4Fe-4S] cluster and each of the peptides making up the $\alpha_2\beta_2$ structure contains a siroheme-[4Fe-4S] moiety. The siroheme-[4Fe-4S] component is at the interface of the DsrA and DsrB structures with the [4Fe-4S] cluster bound into the DsrB subunit. It is suggested by Oliveira et al. [93] that the two siroheme-[4Fe-4S] cofactors bound in the DsrA structures are not involved in the enzymatic sulfite reduction because they are not readily accessible. In comparison, the site where the possibly inactive siroheme-[4Fe-4S] is bound in *Dsm. norvegicum* is occupied by a sirohydrochlorin in *D. vulgaris* H and *D. gigas*. The DsrC of *Dsm. norvegicum* has a helical structure where the C-terminal segment of the protein is flexible and is inserted in the cleft between DsrA and DsrB.

The dSiR produced by *Desulfobacter* (*Dba.*) *vibrioformis* and *Desulfobulbus* (*Dbu.*) *rhabdoformis* is of the desulforubidin-type and the operons for dSiR were characterized by gene analysis [115]. As in the case of dSiR from the genera *Desulfovibrio, Desulfotomaculum*, *Desulfomicrobium,* and *Archaeoglobus,* the α and β subunits of dSiR in *Dba. vibrioformis* and *Dbu. rhabdoformis* are encoded on *dsrA* and *dsrB*, respectively. The polypeptide encoded on *dsrA* is 48–50 kDa and for *dsrB* is 42–43 kDa in *Dba. vibrioformis* and *Dbu. rhabdoformis*, respectively, and is comparable to polypeptides reported for other siroheme-containing sulfite reductases. Important characteristics of siroheme-containing dSiR are the homology region of H1-H5 and the binding site for the ferredoxin motif [116, 117] and these are also present in *Dba. vibrioformis* and *Dbu. rhabdoformis*. High sequence identity was reported when *dsrA* and *dsrB* from *Dba. vibrioformis* and *Dbu. rhabdoformis* were compared to respective genes found in *D. vulgaris* H, *Dst. thermocisternum*. *Ar. fulgidus, Ar. profundus, Pyb. islandicum,* and *Allochromatium* (*Alc.*) *vinosum*.

Also in the *dsr* operons of *Dba. vibrioformis* and *Dbu. rhabdoformis* was *dsr*D which would encode a polypeptide of 9.8–8.7 kDa [115]. While the DsrD function is unknown, its distribution appears to be only in sulfate-reducing microorganisms. Very good sequence similarity of DsrD between *Dba. vibrioformis, Dbu. rhabdoformis, D. vulgaris* H, *Dst. thermocisternum, Ar. fulgidus,* and *Ar. profundus,* was reported. A gene comparable to *dsrD* appears absent in *Pyb. islandicum* and *Alc. vinosum.* The *dsr* operons of *Dba. vibrioformis* and *Dbu. rhabdoformis* contain a gene, *dnrN,* that encodes for a 53 kDa protein of unknown function. The *dsrN* shows considerable homology to *cbiA*, a gene for amination of cobyrinic acid to cobyrinic acid a,c-diamine [118]. Larsen et al. [115] suggest a possible role for DsrN in sulfate reducers as the amidation of siroheme since siroamide is a prosthetic group present in sulfite reductase of *Desulfovibrio* species [110].

2.4.1.4 Desulfofuscidin-Type Sulfite Reductase

The dark brown-colored protein, desulfofuscidin is the dSiR of thermophilic eubacterial sulfate reducers such as *Thermodesulfovibrio* strains *hydrogeniphilus* and *yellowstoni* and *Thermodesulfobacterium* (*T.*) *commune* and *T. mobile* [119–121]. The reduction of sulfite by *T. mobile* and *T. commune* is accomplished by a sulfite reductase that has the $\alpha_2\beta_2$ subunit structure.

The tetrameric dSiR from *T. commune* has a molecular mass of 167 kDa and consists of nonidentical subunits of approximately 47 kDa [120], compared to 175 kDa (gel filtration) or 190 kDa (sedimentation equilibrium) found for the dSiR from *T. mobile*, with subunits of 38–44 kDa [119]. Spectral absorption maxima of dSiR from *T. commune* are at 576, 389, and 279 nm while for dSiR *T. mobile* these are at 578, 392, and 281 nm. Four siroheme groups are found in the dSiR of *T. mobile* and *T. commune* with four [4Fe-4S] clusters in *T. commune* [69] and eight [4Fe-4S] clusters in *T. mobile* [119]. The EPR spectrum of desulfofuscidin exhibits resonances assigned to high-spin Fe(III) heme centers, with *g* values of 7.02, 4.81, and 1.91 for the enzyme from *T. commune* [120], and 7.26, 4.78, and 1.92 for the enzyme from *T. mobile* [119].

2.4.1.5 Archaeal-Type Sulfite Reductase

The dissimilatory *Ar. fulgidus* sulfite reductase accounts for 0.5 % of the soluble proteins and the isolated enzyme occurs as a tetramer with an $\alpha_2\beta_2$ structure [117]. The oxidized enzyme has an α-absorption maximum at 593 nm; upon reduction with dithionite, the maximum shifts to 598 nm. In the oxidized protein an absorption band with a maximum at 715 nm is observed; however, this band disappears upon dithionite reduction suggesting that the siroheme is in the high-spin state [117, 122].

Ar. veneficus grows with dissimilatory sulfite or thiosulfate reduction but is unable to couple growth to the transfer of electrons from the electron donor to sulfate [123]. The Dsr gene locus of *Ar. profundus* has *dsrA* contiguous to *dsrB* [112]. The DsrD, as product of *Ar. profundus dsrD*, is projected to be about 9.3 kDa and would be comparable to the γ subunit present in the dSiR of *Desulfovibrio* species where it functions in sulfite-binding [108]. Downstream of *dsrABD* in both *Archaeoglobus* species is a gene for ferredoxin and this is significant because ferredoxin may be the electron donor for enzymatic sulfite reduction.

2.4.2 Oxy-Sulfur Reductases in Non-sulfate Reducers

There are several genera of bacteria that are capable of reducing tetrathionate, thiosulfate, and sulfite to H_2S (see reactions 11, 12, and 13), respectively. Sulfite, an intermediate of dissimilatory sulfate reduction, is readily utilized by SRB and the metabolism of tetrathionate or thiosulfate with the production of sulfide is covered in Section 2.1. In the environment, thiosulfate is found in marine and freshwater sediments [124] and in the lumen of mammalian large intestine [125]. Tetrathionate is present in soils, where SRB are growing [126], and is produced in the gut as a result of inflammatory response [127]. Several microorganisms that are taxonomically unrelated have enzymes that function under anaerobic conditions as facilitating sulfite respiration (reactions 11, 12, and 13) [20].

$$S_4O_6^{2-} + 2\,e^- \rightarrow 2\,S_2O_3^{2-} \qquad E^{0'} = +170\ \text{mV}\ [41] \qquad (11)$$

$$S_2O_3^{2-} + 2\,H^+ + 2\,e^- \rightarrow SO_3^{2-} + H_2S \qquad E^{0'} = -402\ \text{mV}\ [41] \qquad (12)$$

$$SO_3^{2-} + 8\,H^+ + 6\,e^- \rightarrow H_2S + 3\,H_2O \qquad E^{0'} = -160\ \text{mV}\ [41] \qquad (13)$$

The sulfite reductase, Fsr, present in *Methanocaldococcus jannaschii* (M_r 70 kDa) could have arisen from gene fusion since the N-terminal part of the enzyme is a homolog of the β subunit of coenzyme F_{420}-reducing hydrogenase (FpoF or FqoF) while the C-terminal half has homology to DsrA and DsrB of the siroheme-containing dSiR [128]. A homolog of this bifunctional sulfite reductase from *Methanocaldococcus jannaschii* is also present in *Methanopyrus kandleri* and *Methanococcoides burtonii* [129].

The dissimilatory sulfite reductase, dSiR, of *Bilophila wadsworthia* has a $\alpha_2\beta_2\gamma_n$ ($n \geq 2$) multimeric structure. This enzyme has an absorption maximum at 630 nm [130] indicating that it is a desulfoviridin-type enzyme. The α subunit (M_r 49 kDa) contains the siroheme-[4Fe-4S] while the β subunit polypeptide is 54 kDa. The β subunit is a fusion protein resulting from fused *dsrB* and *dsrD* genes. Based on phylogenic analysis, *Bilophila wadsworthia* is closely related to *D. desulfuricans* Essex 6 and at one time may have been a dissimilatory sulfate reducer [130].

An inducible dissimilatory sulfite reductase in *Clostridium pasteurianum* couples growth to the reduction of sulfite [131]. The isolated enzyme (M_r 83.6 kDa), has an

absorption maximum at 585 nm, but has no siroheme cofactor. Using reduced methyl viologen as the electron donor, thiosulfate or trithionate could not replace sulfite; however, nitrite and hydroxylamine were slowly reduced by the sulfite reductase.

Respiratory-linked tetrathionate reduction is present in soil bacterial communities [132] and species of *Citrobacter, Proteus, Salmonella,* and *Pseudomonas* [133]. In *Salmonella* (*Sal.*) *enterica* Serovar Typhimurium, tetrathionate reductase is encoded on the *ttr* operon with expression controlled by the global regulator OxrA. This membrane-bound tetrathionate reductase is induced by tetrathionate and has a bis-molybdopterin guanine dinucleotide (MGD) cofactor [134].

Dissimilatory thiosulfate reduction is found in numerous environmental bacteria including *Shewanella oneidensis* [135] and enteric bacterial pathogens [125] such as *Sal. enterica*. Anaerobic thiosulfate reductase activity (reaction 12; EC 1.97.1-) in *Sal. enterica* is linked to the plasma membrane and the enzyme is a product of the *phsABC* operon. Subunit phsA contains the active site and the cofactor MGD [136]. The cytochrome *b* subunit, phsC, is an integral membrane protein that contains two heme cofactors and a site to interact with the electron donor, naphthoquinone-8 [125]. Subunit phsB has four iron-sulfur clusters which transfer electrons between the subunits phsA and phsC.

Sulfite is reduced to sulfide by a cytoplasmic sulfite reductase and in *Sal. enterica* the anaerobic sulfite reductase is a product of the chromosomal *asr* (anaerobic sulfite reduction) operon [137, 138] with *asrA, asrB*, and *asrC* encoding for peptides of 40, 31, and 37 kDa, respectively. From genome analysis, it is predicted that a [4Fe-4S] ferredoxin is present in both the asrA and asrC peptides and a siroheme in the asrC subunit. NADH is proposed to be bound into the asrB subunit. This anaerobic sulfite reductase forms a large complex of about 360,000 M_r. An even larger complex has been detected for the assimilatory sulfite reductase (670,000 M_r) which has an $\alpha_8\beta_4$ structure with a flavin moiety in the α subunit and siroheme plus [4Fe-4S] clusters in the β subunit [139].

Dissimilatory reduction of sulfite to sulfide by *W. succinogenes* is attributed to an octaheme cytochrome *c* without the involvement of a coupled siroheme-[4Fe-4S] cofactor [140]. The octaheme cytochrome *c*, MccA, contains a heme bound by the unique motif of $CX_{15}CH$, and is encoded on the *mccABCD* gene cluster. An octaheme cytochrome *c*, SirA, in *Shewanella oneidensis* MR-1 also displays dissimilatory sulfite reductase activity [141].

2.4.3 Low-Molecular-Mass and Low-Spin Assimilatory-Type Sulfite Reductase from *Desulfovibrio vulgaris* H, *Desulfuromonas acetoxidans*, and *Methanosarcina barkeri*

A low-molecular-mass assimilatory-type sulfite reductase has been purified and characterized from *D. vulgaris* H [67, 142, 143]. This enzyme has a molecular mass of 27.2 kDa and its optical spectrum exhibits maxima at 405, 545, and 590 nm (Table 4). This hemoprotein is able to reduce sulfite to sulfide in the presence of reduced methyl viologen. The specific sulfite reductase activity was 900 mU/mg protein [87].

The *D. vulgaris* H assimilatory-type sulfite reductase has been studied by chemical, EPR, and Mössbauer techniques [143]. This protein contains one siroheme and a single [4Fe-4S] cluster. As purified, the siroheme is in a low-spin Fe(III) state (S = 1/2) which exhibits characteristic EPR resonances at g = 2.44, 2.36, and 1.77. Hereby, the iron-sulfur cluster is in the $[4Fe\text{-}4S]^{2+}$ state. Similar to the hemoprotein subunit of *E. coli* sulfite reductase, low-temperature Mössbauer spectra of *D. vulgaris* H sulfite reductase also show evidence for an exchange-coupled siroheme-[4Fe-4S] unit [143]. The presence of an assimilatory-type sulfite reductase in *D. vulgaris* H is surprising because this strain produces large amounts of sulfide during normal growth on sulfate and also because the enzymes responsible for dissimilatory sulfate reduction are constitutive.

Two low molecular mass hemoproteins with sulfite reductase activity (named P_{590}) have been purified and characterized from two sulfur reducers: *Ms. barkeri* and *Drm. acetoxidans* [87, 144, 145]. Both monomeric hemoproteins present visible spectra similar to that of the low-molecular-mass and low-spin assimilatory-type sulfite reductase of *D. vulgaris* H. The *Drm. acetoxidans* sulfite reductase has a molecular mass of 23.5 kDa and exhibits absorption maxima at 405, 545, and 587 nm [144]. The *Ms. barkeri* P_{590} has a molecular mass of 23 kDa and its optical visible spectrum exhibits maxima at 395, 545 and 590 nm (Table 4) [145]. EPR spectra of the enzyme as isolated show that the siroheme is in a low-spin Fe(III) state (S = 1/2) with g-values at 2.40, 2.30, and 1.88 for the *Ms. barkeri* P_{590} enzyme and g-values at 2.44, 2.33, and 1.81 for the *Drm. acetoxidans* enzyme [144].

Chemical analysis shows that both hemoproteins contain one siroheme and one [4Fe-4S] cluster per polypeptidic chain [144]. The specific sulfite reductase activity was 906 mU/mg protein for the *Drm. acetoxidans* P_{590} enzyme and 2,790 mU/mg protein for the *Ms. barkeri* enzyme [87, 144]. The M*s. barkeri* P_{590} enzyme has a higher specific sulfite reductase activity than that reported for the high-spin dSiR from SRB such as desulforubidin, desulfofuscidin, and desulfoviridin [25]. The two assimilatory-type sulfite reductases of sulfur reducers contain 5 labile sulfur atoms and 5 iron atoms; as it is the case for the *D. vulgaris* H enzyme, it was postulated that the extra sulfur atom could be the bridging ligand between the [4Fe-4S] center and the siroheme [87]. Both hemoproteins catalyze the direct six-electron reduction of sulfite to sulfide without the formation of free intermediates (thiosulfate and trithionate).

3 Enzymology of Hydrogen Sulfide Production from Elemental Sulfur

3.1 Eubacterial Sulfur Reductase

3.1.1 Sulfur Reductase in *Desulfovibrio* and *Desulfomicrobium* Species

The tetraheme cytochrome c_3 has the function of an elemental sulfur reductase in several *Desulfomicrobium* and *Desulfovibrio* species from which the sulfur

reductase activity can be copurified with the tetrahemoprotein [26, 146, 147]. An exposed, low-potential heme of the tetraheme cytochrome c_3 from *Dsm. norvegicum* Norway 4 has been proposed to play an important mechanistic role. The polysulfide chains of colloidal S^0 are attacked by the reduced tetraheme cytochrome c_3, leading to a collapse of the micelles with the precipitation of S_8 molecules [148].

The sulfide produced by polysulfide reduction opens up the S_8 rings by a nucleophilic attack, leading to the production of new molecules of polysulfides, which are themselves quickly reduced to sulfide by *Dsm. norvegicum* tetraheme cytochrome c_3 [148]. Membranes isolated from *D. gigas* and *Dsm. norvegicum* contained hydrogenase and *c*-type cytochromes and catalyzed the dissimilatory sulfur reduction to sulfide. Sufficient hydrogenase and tetrahemic cytochrome c_3 must be linked with the *D. gigas* cytoplasmic membrane in the correct conformation to generate proton translocation sufficient for the chemiosmotic synthesis of ATP [149].

3.1.2 Polysulfide Reductase from *Wolinella succinogenes*

The mechanism of polysulfide respiration has been mainly investigated in the epsilon proteobacterial subclass *W. succinogenes* [27, 32]. The membrane fraction isolated from *W. succinogenes* cells grown with formate and either fumarate or polysulfide, as electron acceptor, catalyzes the polysulfide reduction by formate or H_2. The corresponding electron transport chain consists of 8-methyl-menaquinone, polysulfide reductase, and either hydrogenase or formate dehydrogenase. The isolated polysulfide reductase consists of the three subunits predicted by the *psrABC* operon, and contains a molybdenum ion coordinated by two molecules of MGD. A model of this membrane association with coupled proton pumping during sulfur respiration is given in Figure 5.

The PsrA subunit is the catalytic unit, PsrB is an iron-sulfur protein, and PsrC is an integral membrane protein that serves to anchor the other subunits on the membrane. Energy conservation via polysulfide respiration in Archaea and Bacteria appears to be similar. Membrane-bound respiratory chains produce a chemiosmotic potential, which is used by membrane-bound ATP synthases to form ATP [29, 32].

3.1.3 Polysulfide Reductase from *Desulfuromonas acetoxidans*

The final draft genome of *Drm. acetoxidans* codes for a "cytochromome" of 47 putative multiheme cytochromes *c* [150]. This strain contains several multiheme *c*-type cytochromes, the most abundant being the triheme cytochrome c_7. Polysulfides are formed in solution from the reaction of elemental sulfur with sulfide and are probably the *in vivo* substrate utilized by sulfur-reducing eubacteria.

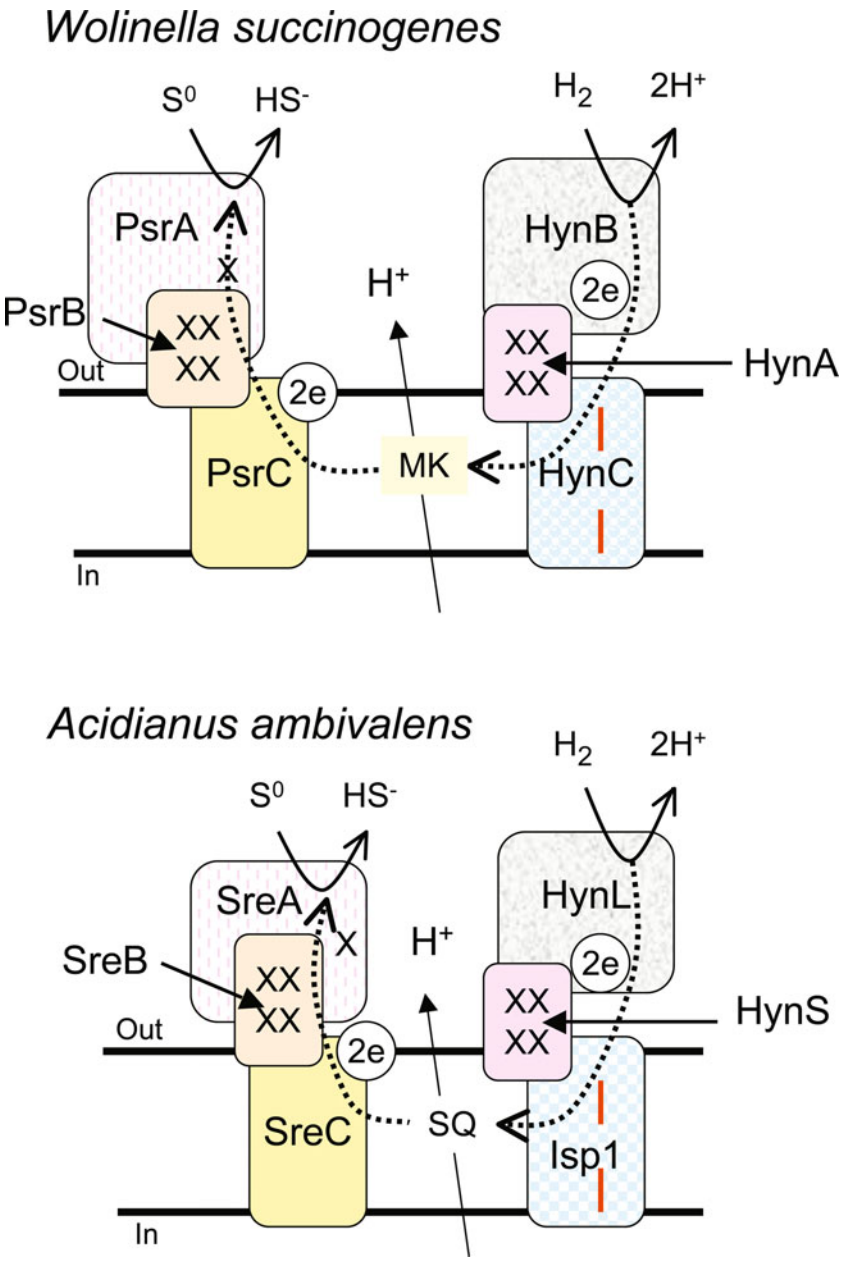

Figure 5 Model of sulfur-reducing complexes in membranes of *A. ambivalens* and *W. succinogenes*. PsrABC are sulfur-reducing subunits and HynABC are hydrogenase subunits of *W. succinogenes*. MK = menaquinone, SreABC are sulfur-reducing subunits and HynL, HynS, and Isp1 are hydrogenase subunits of *A. ambivalens*, SQ = sulfolobusquinone, X = [4Fe-4S] center, 2e = 2 electrons, and dashed lines indicates electron flow. The two vertical red lines in HynC and Isp1 indicate the presence of two b-type hemes. Out = periplasm and In = cytoplasm. Reproduced by permission from [25]; copyright 2012, Academic Press.

The *Drm. acetoxidans* triheme cytochrome c_7 is rapidly reduced by the *D. vulgaris* H [Fe] hydrogenase and it can completely reduce polysulfide with a very high specific activity of 20 μmoles of hydrogen consumed/min/mg protein [151]. This activity is twice as high as that reported for the purified tetraheme cytochrome c_3 with the highest specific SR activity from *Dsm. norvegicum* [26, 147]. These results indicate that cytochrome c_7 is probably the true terminal sulfur reductase in *Drm. acetoxidans* but the physiological electron donor for this triheme protein remains to be found.

3.1.4 Sulfur Oxidoreductase from *Sulfurospirillum deleyianum*

The *S. deleyianum* DSM 6946 sulfur oxidoreductase (SR) is a constitutive cytoplasmic enzyme. The *S. deleyianum* SR is energized by a [Ni-Fe] hydrogenase and is several times more active in crude extracts than in other sulfur-reducing eubacteria [27]. Sulfur reduction is enhanced by the presence of thiols and the SR contains at least one [4Fe-4S] center but no *b*- or *c*-type cytochromes [27].

3.2 Archaebacterial Sulfur Reductase

3.2.1 Membraneous Sulfur Reductase Complex from *Acidianus ambivalens*

The acidophilic *Acidianus* (*A.*) *ambivalens* DSM 3772 grows on elemental sulfur at 80 °C. A hydrogenase-sulfur reductase complex (SR) has been isolated from the membrane of *A. ambivalens* and the Sr subunits are similar to those found in *W. succinogenes* [25] (Figure 5). SR is a molybdoenzyme belonging to the DMSO reductase family [152]. The SR gene cluster consists of 5 subunits. The s*reA* gene produces a 110 kDa protein that has binding motifs for a [4Fe-4S] center. The *sreB* gene encodes for a protein rich in cysteines and could coordinate a [4Fe-4S] center. SreC is a hydrophobic protein that stabilizes the SR into the membrane and the role for SreD is unresolved at this time. The hydrogenase gene cluster consists of 12 genes and 3 of these encode for structural proteins. The HynL subunit contains nickel and the subunit HynS contains several [Fe-S] binding motifs, making this a [Ni-Fe] hydrogenase. The third structural gene, Isp1, is an integral membrane protein and serves to bind the 2 other subunits into the membrane. The quinone, presumed to be sulfolobusquinone, transfers electrons from hydrogenase to the SR.

3.2.2 Sulfur-Reducing Complex from *Pyrodictium abyssi*

Extensive studies on sulfur reduction have been performed with *Pyrococcus* (*P.*) *furiosus*, *A. ambivalens*, *Py. abyssi*, and *Py. brockii* as model organisms. The mechanism of sulfur reduction with H_2, in some members of the *Crenarchaeota*

(e.g., *Pyrodictium* species and *A. ambivalens*) is similar to that of some eubacteria, such as *W. succinogenes*. This dissimilatory process involves two multi-subunits, membrane-bound enzymes: a nickel-iron-containing hydrogenase and a SR or PSR. These two enzymes together reduce sulfur to H_2S with H_2 as electron donor.

The composition of the described electron transfer chain shows participations of similar [NiFe] hydrogenases and similar PSR in the case of *A. ambivalens* and *W. succinogenes*, whereas the *Py. abyssi* SR seems to be different. Nine major subunits constitute the hydrogenase-sulfur multienzyme complex isolated from *Py. abyssi* DSM 6158 [153] and the subunits from *Py. abyssi* range from 24 to 82 kDa with 550 kDa for the entire complex. Analysis of this complex reveals the presence of a [NiFe] hydrogenase, a *c*-type heme, one or two *b*-type hemes, and an undetermined number of [Fe-S] centers.

3.2.3 Sulfur Reductase from *Pyrococcus furiosus*

P. furiosus has been studied as the model organism for the mechanism of the fermentation-based sulfur reduction. Two enzymes play key roles in the sulfur metabolism: a membrane-bound oxidoreductase complex (MBX) and a cytoplasmic coenzyme A-dependent NADPH sulfur oxidoreductase (NSR). MBX is encoded by an operon with 13 open reading frames and plays an essential role in mediating electron flow to sulfur [154]. NSR is a homodimeric flavoprotein (M_r 100,000) and reduces elemental sulfur to H_2S with NADPH as the electron donor [154]. This MBX-NSR complex sulfur-reduction system has only been reported so far in the *Thermococcales*.

4 Microbial Oxidation of Hydrogen Sulfide to Sulfate

The oxidative inorganic sulfur metabolism has been recently and extensively described both in eubacterial and archaebacterial microorganisms [28, 29, 49, 155]. Here, we briefly describe enzymes or multienzyme systems involved in sulfur compound oxidation in Bacteria and Archaea.

4.1 Archaebacterial Inorganic Sulfur Compound Oxidation

Aerobic dissimilatory sulfur oxidation is common in the order *Sulfolobales* of the *Crenarchaeota* which frequently thrive in continental solfataric fields [29, 155]. Mechanisms of archaeal inorganic sulfur compound oxidation were almost exclusively studied in *Acidianus* species with the thermoacidophilic *A. ambivalens* as the model organism [28, 29].

The initial step of *A. ambivalens* sulfur oxidation involves a cytoplasmic sulfur oxygenase reductase (SOR) (EC 1.13.11.55) catalyzing the oxygen-dependent sulfur disproportionation to form sulfide plus hydrogen sulfite [155] (reaction 14):

$$4\,S^0 + O_2 + 4\,H_2O \rightarrow 2\,H_2S + 2\,HSO_3^- + 2\,H^+ \qquad (14)$$

Then thiosulfate is likely produced from sulfur and hydrogen sulfite in a non-enzymatic reaction.

SOR of *A. ambivalens* is a homo-oligomer composed of 24 identical monomers and the catalytic pocket of each subunit contains a low-potential mononuclear non-heme iron center and three conserved cysteinyl residues. The iron center is likely the site for both sulfur reduction and oxidation [156]. The three products of the *A. ambivalens* sulfur oxidation step (sulfite, thiosulfate, and sulfide) are presumably further oxidized to sulfate for energy conservation [29, 155].

Two sulfite oxidation pathways are present in *A. ambivalens*: (a) A membrane-bound sulfite:acceptor oxidoreductase as part of the Sox complex; (b) An alternative indirect soluble sulfite oxidation pathway coupled to substrate-level phosphorylation via APS reductase and APS:phosphate adenylyltransferase [155].

Two membrane-bound complexes are involved in *A. ambivalens* thiosulfate oxidation: (a) The terminal aa_3-type quinol oxidase which shuttles electrons from the caldariellaquinone pool to O_2 and consists of three subunits, which are encoded in a single operon in the *A. ambivalens* genome. (b) The membrane-bound tetrathionate-forming thiosulfate:quinone oxidoreductase which oxidizes thiosulfate to form tetrathionate with caldariellaquinone as electron acceptor [155, 157].

4.2 Eubacterial Inorganic Sulfur Compound Oxidation

The main enzymes or multienzyme complexes involved in sulfur compound oxidation are present both in CSB and phototrophic sulfur bacteria [49]. PSB and GSB use various combinations of sulfide, elemental sulfur, sulfite, and thiosulfate as electron donors in CO_2 fixation during anoxygenic photosynthetic growth [49]. Genetic and biochemical analyses show that the dissimilatory sulfur metabolism of the phototrophic organisms is very complex and still incompletely understood. We will only describe here the oxidative sulfur metabolism of anoxygenic phototrophic bacteria (GSB, PSB, and PNSB).

A variety of enzymes catalyzing inorganic sulfur oxidation reactions have been purified and biochemically and genetically characterized from PSB and GSB [49]. Complete genome sequence data are currently available for one strain of PSB and for ten strains of GSB. A number of genes potentially involved in the oxidative sulfur metabolism are present both in GSB and PSB: for example, genes for the sulfide:quinone oxidoreductase (SQR) and the sulfide-oxidizing enzyme flavocytochrome *c* [49]. On a molecular biochemical and genetic level, sulfur

compound oxidation is best characterized in the GSB *Chlorobaculum tepidum* and in the PSB *Alc. vinosum*.

4.2.1 Oxidation of Sulfide

Enzymes that oxidize sulfide are the membrane-bound SQR and the periplasmic flavocytochrome *c* sulfide dehydrogenase (FccAB, EC 1.8.2.3) [49].

4.2.1.1 Sulfide:Quinone Oxidoreductase

SQR (EC 1.8.5.4) catalyzing sulfide oxidation with an isoprenoid quinone as the electron acceptor is present in both phototrophic and chemotrophic bacteria [49]. SQR plays an important role for the sulfide oxidation in PSB and FccAB appears less widespread. SQR is the only known sulfide-oxidizing enzyme that is found in all GSB strains. SQR is a member of the flavin disulfide reductases family.

SQR of *Rhodobacter capsulatus* is a membrane-bound flavoprotein with its active site located in the periplasm. The first X-ray structure for an SQR has been recently determined to 2.6 Å resolution in *A. ambivalens* [158]. This membrane-bound flavoprotein has two redox active sites: a covalently bound FAD and an adjacent pair of cysteine residues bridged by a trisulfide bridge between the two cysteine residues [158].

4.2.1.2 Flavocytochrome *c* Sulfide Dehydrogenase

Flavocytochrome *c* is usually a periplasmic enzyme consisting of a small FccA cytochrome *c* subunit (20 kDa) and a larger sulfide-binding FccB flavoprotein subunit (44 kDa). *In vitro*, flavocytochromes can catalyze electron transfer from sulfide to a variety of small *c*-type cytochromes (such as *Alc. vinosum* cytochrome c_{550}) that may then donate electrons to the photosynthetic reaction center [49]. The *in vivo* role of flavocytochrome *c* is unclear and if indeed the FccAB oxidizes H_2S *in vivo*, both PSB and GSB have alternative sulfide-oxidizing enzyme systems, such as SQR, that may be quantitatively more important.

4.2.2 Oxidation of Polysulfides

Polysulfides appear to be the primary product of sulfide oxidation in a number of PSB and GSB. The oxidation of sulfur deposits is the least understood step of sulfur metabolism. It is currently unknown how polysulfides are converted into sulfur globules and it could be a purely chemical, spontaneous process as longer polysulfides are in equilibrium with S^0 [49].

Many PSB and GSB can oxidize externally supplied solid elemental sulfur. Utilization of solid S^0 must include binding and/or activation of the sulfur as well as transport inside the cells. In the case of cyclo-octasulfur, this activation process could be an opening of the S_8 ring by nucleophilic reagents, leading to the formation of linear organic or inorganic polysulfanes. It may be speculated that "sulfur chains" rather than the more stable "sulfur rings" are the microbiologically preferred form of S^0 for most SOB. It has been proposed in *Alc. vinosum* that the stored sulfur has to be reductively activated to the oxidation state of sulfide in order to serve as a substrate for sulfite reductase operating in reverse performing the six-electron oxidation of sulfide to sulfite [159].

4.2.3 Oxidation of Stored Sulfur to Sulfite

The mechanism by which the periplasmically stored sulfur is made available to the cytoplasmic dissimilatory sulfite reductase is still unclear. The only gene region known so far to be essential for the oxidation of stored sulfur is the *dsr* operon. The reverse DsrAB of *Alc. vinosum* is encoded together with 13 other proteins in the *dsr* operon, *dsr ABEFHCMKLJOPNRS*. A model of the *Alc. vinosum* sulfur oxidation pathway has been proposed [160]. It is suggested that the sulfur is reductively activated, transported to and further oxidized in the cytoplasm, since the proteins encoded at the dsr locus are either membrane-bound or cytoplasmic and cannot act directly on the extracytoplasmic sulfur globules [160].

4.2.4 Oxidation of Sulfite to Sulfate

Two different pathways for sulfite oxidation are known in phototrophic and chemotrophic SOB: (a) Indirect, AMP-dependent sulfite oxidation via APS. Sulfite is oxidized by APS reductase in a cytoplasmic reaction that consumes sulfite and AMP and produces APS and reducing equivalents. This oxidative pathway occurs exclusively in members of the *Chromatiaceae* and in some GSB. (b) Direct sulfite oxidation by sulfite dehydrogenase (EC 1.8.2.1), typically a molybdenum-containing protein belonging to the sulfite oxidase family of molybdoenzymes [161].

4.2.5 Oxidation of Thiosulfate

In phototrophic and chemotrophic SOB that do not form sulfur deposits a periplasmic thiosulfate-oxidizing multienzyme complex (Sox complex) is responsible for formation of sulfate from thiosulfate. *Alc. vinosum* can pursue two different thiosulfate oxidation pathways, first the complete thiosulfate oxidation to sulfate, and second formation of tetrathionate.

4.2.5.1 Oxidation of Thiosulfate to Tetrathionate

Alc. vinosum tetrathionate-forming thiosulfate dehydrogenase (TsdA) (thiosulfate:acceptor oxidoreductase EC 1.8.2.2) is a periplasmic, monomeric diheme 30 kDa cytochrome c_{554} with an isoelectric point of 4.2. UV-visible and EPR spectroscopies suggest methionine and cysteine as distal axial ligands of the two heme irons in TsdA.

4.2.5.2 Oxidation of Thiosulfate to Sulfate by the Sox Multienzyme System

Many GSB and PSB can oxidize thiosulfate completely to sulfate. The Sox complex, a periplasmic thiosulfate-oxidizing multienzyme complex was first found and characterized in *Paracoccus* (*Pc.*) *versutus* and *Pc. pantotrophus*. The Sox gene cluster of *Pc. pantotrophus* comprise 15 genes (*soxRSVWXYZABCDEFGH*). The so-called SoxAX cytochromes are heme-thiolate proteins playing a key role in bacterial thiosulfate oxidation [162]. They initiate the reaction cycle of a multienzyme complex in both photo- and chemotrophic SOB by catalyzing the attachment of sulfur substrates such as thiosulfate to a conserved cysteine present in a carrier protein.

Alc. vinosum possesses five *sox* genes in two independent loci (*soxBXA* and *soxYZ*) encoding proteins related to components of the *Pc. pantotrophus* Sox complex. Three sox-encoded proteins were purified and characterized from *Alc. vinosum*: the heterodimeric *c*-type cytochrome SoxXA, the monomeric SoxB containing a dimanganese center, and the heterodimeric thiosulfate-binding protein Sox YZ [160, 163].

In summary, Sox and Dsr proteins are absolutely essential in *Alc. vinosum* for the oxidation of thiosulfate and stored sulfur, respectively. Clusters of *dsr* and *sox* genes are also present in the only distantly related GSB as well as in the other sulfur-storing chemotrophic and phototrophic SOB. The mechanisms of thiosulfate oxidation via sulfur deposition and of the oxidation of deposited sulfur seem to be evolutionary highly conserved. Studies on *Alc. vinosum* can help to elucidate the sulfur oxidation pathway in other sulfur-storing bacteria. GSB oxidize sulfide and thiosulfate to sulfate, with extracellular sulfur globules as an intermediate and sulfur globule oxidation is strictly dependent on the dSiR system. In GSB, depending on the strain, sulfite is probably oxidized to sulfate by one or two mechanisms with different evolutionary origins, using either APS reductase or the polysulfide reductase-like complex 3 [164].

5 Conclusions

Anaerobic and aerobic microorganisms play a paramount role in biological cycling of inorganic sulfur compounds because they have developed enzyme systems to use inorganic sulfur compounds as electron donors or electron acceptors to facilitate growth.

Chemolithotrophic microorganisms, primarily SRB, shuttle electrons to sulfoxy compounds or S^0 with the accumulation of H_2S where sulfur is in the most reduced form. Aerobic SOB and CSB facilitate the removal of reduced sulfur compounds with the production of sulfate. Although there has been considerable advancement to understand the enzymology of enzymes for production and utilization of H_2S, further studies are needed to establish gene activities for these sulfur-metabolizing enzymes.

Such genomic studies should provide an insight into ancestry for horizontal gene transfer and to understand the energetic processes that drive the inorganic sulfur reactions. Since considerable similarity exists in the enzymology of H_2S production and H_2S utilization, future studies will be needed to determine which process evolved first.

Furthermore, investigations of the evolutionary links between the biological cycles of sulfur and nitrogen will be important to pursue since [4Fe-4S] clusters, iron porphyrins, and MGD cofactors are used in enzymes for both sulfite to hydrogen sulfide and nitrite to ammonia reduction [20].

Abbreviations and Definitions

A.	*Acidianus*
acetyl-CoA	acetyl-coenzyme A
ADP	adenosine 5'-diphosphate
Alc.	*Allochromatium*
AMP	adenosine 5'-monophosphate
APS	adenosine 5'-phosphosulfate
APSR	adenylylsulfate reductase
Ar.	*Archaeoglobus*
aSiR	assimilatory-type sulfite reductase
asr	anaerobic sulfite reduction
ATP	adenosine 5'-triphosphate
ATPS	ATP sulfurylase
CSB	colorless sulfur bacteria
D.	*Desulfovibrio*
Dba.	*Desulfobacter*
Dbu.	*Desulfobulbus*
DMSO	dimethylsulfoxide
Drm.	*Desulfuromonas*
Dsm.	*Desulfomicrobium*
dSiR	dissimilatory sulfite reductase
Dst.	*Desulfotomaculum*
D. vulgaris H	*Desulfovibrio vulgaris* Hildenborough
$\Delta G^{0'}$	standard free energy change
E.	*Escherichia*
$E^{0'}$	standard reduction potential

EPR	electron paramagnetic resonance
EXAFS	extended X-ray absorption fine structure
FAD	flavin adenine dinucleotide
GSB	green sulfur bacteria
MBX	membrane-bound oxidoreductase complex
MGD	molybdopterin guanine dinucleotide
MK	menaquinone
Ms.	*Methanosarcina*
NAD	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide, reduced
NADPH	nicotinamide adenine dinucleotide phosphate, reduced
NSR	coenzyme A-dependent NADPH sulfur oxidoreductase
P.	*Pyrococcus*
Pc.	*Paracoccus*
P_i	inorganic phosphate
PNSB	purple non-sulfur bacteria
PP_i	inorganic pyrophosphate
PSB	purple sulfur bacteria
PSR	polysulfide reductase
Py	*Pyrodictium*
Pyb.	*Pyrobaculum*
S.	*Sulfurospirillum*
Sal.	*Salmonella*
SOB	sulfide-oxidizing bacteria
SOR	sulfur oxygenase reductase
SQR	sulfide:quinone reductase
SR	sulfur reductase
SRB	sulfate-reducing bacteria
SRP	sulfate-reducing prokaryotes
T.	*Thermodesulfobacterium*
W.	*Wolinella*

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References

1. R. Wang, *Physiol. Rev.* **2012**, *92*, 791–896.
2. L. L. Barton, G. D. Fauque, *Adv. Appl. Microbiol.* **2009**, *68*, 41–98.
3. R. Rabus, T. A. Hansen, F. Widdel, in *The Prokaryotes,* Vol. 2, *Ecophysiology and Biochemistry,* Eds M. Dworkin, S. Falkow, E. Rosenberg, K.-K. Scheifer, E. Stackebrandt, Springer, Berlin, 2006, pp. 659–768.

4. G. D. Fauque, in *Biotechnology Handbooks*, Vol. 8, *Sulfate-Reducing Bacteria*, Ed L.L. Barton, Plenum Press, New York, 1995, pp. 217–241.
5. J. Loubinoux, J.-P. Bronowicki, I. A. C. Pereira, J.-L. Mougenel, A. LeFaou, *FEMS Microbiol. Ecol.* **2002**, *40,* 107–112.
6. G. Muyzer, A. J. M. Stams, *Nature Rev. Microbiol.* **2008**, *6*, 441–454.
7. B. Ollivier, J.-L. Cayol, G. Fauque, in *Sulphate-Reducing Bacteria: Environmental and Engineered Systems,* Eds L. L. Barton, W. A. Hamilton, Cambridge University Press, Cambridge, UK, 2007, pp. 305–328.
8. Y. A. Shen, R. Buick, D. E. Canfield, *Nature* **2001**, *410*, 77–81.
9. Y. Shen, R. Buick, *Earth-Sci. Rev.* **2004**, *64*, 243–272.
10. G. Fauque, B. Ollivier, in *Microbial Diversity and Bioprospecting*, Ed A.T. Bull, ASM Press, Washington, D.C., 2004, pp. 169–176.
11. H. Castro, N. Williams, A. Ogram, *FEMS Microbiol. Ecol.* **2000**, *31*, 1–9.
12. V. M. Gumerov, A Mardanov, A Beletsky, M. Prokofeva, E. A. Bonch-Osmolovskaya, N. Ravin, K. Skyrabin, *J. Bacteriol.* **2011**, *193*, 2355–2356.
13. L. Jabari, H. Gannoun, J.-L. Cayol, M. Hamdi, B. Ollivier, G. Fauque, M.-L. Fardeau, *Int. J. Syst. Evol. Microbiol.* **2013**, *63*, 2082–2087.
14. H. Cypionka, *Annu. Rev. Microbiol.* **2000,** *54*, 827–848.
15. G. Fauque, J. LeGall, L. L. Barton, in *Variations in Autotrophic Life*, Eds J. M. Shively, L. L. Barton, Academic Press, London, 1991, pp. 271–337.
16. J. LeGall, G. Fauque, in *Biology of Anaerobic Microorganisms*, Ed A. J. B. Zehnder, Wiley, New York, 1988, pp. 587–639.
17. J. J. G. Moura, P. Gonzalez, I. Moura, G. Fauque, in *Sulphate-Reducing Bacteria: Environmental and Engineered Systems,* Eds L. L. Barton, W. A. Hamilton, Cambridge University Press, Cambridge, UK, 2007, pp. 241–264.
18. H. D. Peck, Jr., *Proc. Natl. Acad. Sci. USA* **1959**, *45*, 701–708.
19. D. R. Kremer, M. Veenhuis, G. Fauque, H. D. Peck, Jr., J. LeGall, J. Lampreia, J. J. Moura, T. A. Hansen, *Arch. Microbiol.* **1988***, 150*, 296–301.
20. J. Simon, P. M. H. Kroneck, *Adv. Microbial Physiol.* **2013**, *62*, 45–117.
21. G. Fauque, H. D. Peck, Jr., J. J. G. Moura, B. H. Huynh, Y. Berlier, D. V. DerVartanian, M. Teixeira, A. E. Przybila, P. A. Lespinat, I. Moura, J. LeGall, *FEMS Microbiol. Rev.* **1988**, *54*, 299–344.
22. J. J. G. Moura, I. Moura, M. Teixeira, A. V. Xavier, G. Fauque, J. LeGall, in *Metal Ions in Biological Systems*, Vol. 23*, Nickel and Its Role in Biology*, Ed H. Sigel, Marcel Dekker Inc., New York, 1988, pp. 285–314.
23. I. A. C. Pereira, A. V. Xavier, in *Encyclopedia of Inorganic Chemistry*, Ed R. B. King, Vol. 5, 2nd edn, Wiley, New York, 2005, pp. 3360–3376.
24. I. A. C. Pereira, in *Microbial Sulfur Metabolism,* Eds C. Dahl, C. G. Friedrich, Springer, Berlin, 2008, pp. 24–35.
25. G. D. Fauque, L. L. Barton, *Adv. Microbial Physiol.* **2012**, *60*, 1–90.
26. G. D. Fauque, *Meth. Enzymol.* **1994**, *243*, 353–367.
27. G. Fauque, O. Klimmek, A. Kröger, *Meth. Enzymol.* **1994**, *243*, 367–383.
28. A. Kletzin, T. Urich, F. Muller, T. M. Bandeiras, C. M. Gomez, *J. Bioenerg. Biomem.* **2004**, *36*, 77–91.
29. A. Kletzin, in *Archaea: Evolution, Physiology, and Molecular Biology,* Eds R. A. Garrett, H.-P. Klenk, Blackwell Publishing Ltd., Madden, MA, USA, 2007, pp. 261–274.
30. A. LeFaou, B. S. Rajagopal, L. Daniels, G. Fauque, *FEMS Microbiol. Rev.* **1990**, *75*, 351–382.
31. J. Boulègue, *Phosphorus Sulfur Silicon Relat. Elem.* **1978**, *5*, 127–128.
32. R. Hedderich, O. Klimmek, A. Kröger, R. Dirmeier, M. Keller, K. O. Stetter, *FEMS Microbiol. Rev.* ***1999****, 22*, 353–381.
33. N. Pfennig, H. Biebl, *Arch. Microbiol.* **1976**, *110*, 3–12.
34. F. Widdel, N. Pfennig, in *The Prokaryotes*, Eds A. Balows, H. G. Trüper, M. Dworkin, W. Harder, K. H. Schleifer, 2nd edn, Vol. 4, New York, Springer, 1992, pp. 3379–3389.

35. A. Zöphel, M. C. Kennedy, H. Beinert, P. M. H. Kroneck, *Arch. Microbiol.* **1988***, 150*, 72–77.
36. A. Zöphel, M. C. Kennedy, H. Beinert, P. M. H. Kroneck, *Eur. J. Biochem.* **1991**, *195*, 849–856.
37. H. Biebl, N. Pfennig, *Arch. Microbiol.* **1977***, 112*, 115–117.
38. O. Ben Dhia Thabet, T. Wafa, K. Eltaief, J.-L. Cayol, M. Hamdi, G. Fauque, M.-L. Fardeau, *Curr. Microbiol.* **2011***, 62,* 486–491.
39. B. Ollivier, C. E. Hatchikian, G. Prensier, J. Guezennec, J.-L. Garcia, *Int. J. Syst. Bacteriol.* **1991**, *41*, 74–81.
40. R. Schauder, A. Kröger, *Arch. Microbiol.* **1993***, 159*, 491–497.
41. R. Thauer, K. Jungermann, K. Decker, *Bacteriol. Rev.* **1977**, *41*, 100–180.
42. P. Schönheit, T. Schäfer, *World J. Microbiol. Biotech.* **1995**, *11*, 26–57.
43. K. O. Stetter, G. Gaag, *Nature* **1983**, *305*, 309–311.
44. K. Finster, *J. Sulf. Chem.* **2008**, *29*, 281–292.
45. F. Bak, N. Pfennig, *Arch. Microbiol.* **1987***, 147*, 184–189.
46. K. Finster, W. Liesack, B. Thamdrup, *Appl. Environ. Microbiol.* **1998**, *64*, 119–125.
47. M. Kramer, H. Cypionka, *Arch. Microbiol.* **1989**, *151*, 232–237.
48. P. Philippot, M. Van Zuylen, K. Lepot, C. Fhomazzo, J. Farquhar, M. J. Van Kranendonk, *Science* **2007**, *317*, 1534–1537.
49. G. Muyzer, J. G. Kuenen, L. A. Robertson, in *The Prokaryotes – Prokaryotic Physiology and Biochemistry*, Eds E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, F. Thompson, Springer-Verlag, Berlin, Heidelberg, 2013, pp. 555–588.
50. N.-U. Frigaard, C. Dahl, *Adv. Microbial Physiol.* **2009**, *54*, 103–200.
51. J. F. Imhoff, in *Sulfur Metabolism in Phototrophic Organisms*, Eds R. Hell, C. Dahl, D. Knaff, T. Leustek, Springer, The Netherlands, 2008, pp. 269–287.
52. D.Y. Sorokin, H. Banciu, L. A. Robertson, J. G. Kuenen, M. S. Muntyan, G. Muyzer, in *The Prokaryotes – Prokaryotic Physiology and Biochemistry*, Eds E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, F. Thompson, Springer-Verlag, Berlin, Heidelberg, 2013, pp. 529–554.
53. M. S. Vandiver, S. H. Snyder, *J. Mol. Med.* **2012**, *90*, 255–263.
54. D. Julian, K. L April, S. Patel, J. R. Stein, S.E. Wohlgemuth, *J. Experiment. Biol.* **2005**, *208*, 4109–4122.
55. D. W. Kraus, J. E. Doeller, C.S. Powell, *J. Experiment. Biol.* **1996**, *199*, 1343–1352.
56. T. Bagarino, *Aquat. Toxicol.* **1992**, *24*, 21–62.
57. P. Nicholls, *Biochim. Biophys. Acta* **1975**, *396*, 24–35.
58. R. O. Beauchamp, Jr., J. S. Bus, J. A. Popp, C. J. Boreiko, D. A. Andjelkovich, *Crit. Rev. Toxicol.* **1984**, *13*, 25–97.
59. F. Widdel, in *Biology of Anaerobic Microorganisms*, Ed A. J. B. Zehnder, Wiley, New York, 1988, pp. 469–585.
60. S. M. Caffrey, G. Voordouw, *Antonie van Leeuwenhoek*. **2010**, *97*, 11–20.
61. J. M. Akagi, L. L. Campbell, *J. Bacteriol.* **1963**, *86*, 563–568.
62. H. Nakazawa, A. Arakaki, S. Narita-Yamada, I. Yashiro, K. Jinno, N. Aoki, A. Tsuruyama, Y. Okamura, S. Tanikawa, N. Fujita, H. Takeyama, *Genome Res.* **2009**, *19*, 1801–1808.
63. B. Suh, J. M. Akagi, *J. Bacteriol.* **1969**, *99,* 210–215.
64. J. P. Lee, H. D. Peck, Jr., *Biochem. Biophys. Res. Commun.* **1971**, *45*, 583–589.
65. J. M. Akagi, V. Adams, *J. Bacteriol.* **1973**, *116*, 392–396.
66. K. Kobayashi, S. Tachibana, M. Ishimoto, *J. Biochem.* **1969**, *65*, 155–157.
67. J.-P. Lee, J. LeGall, H. D. Peck, Jr., *J. Bacteriol.* **1973**, *115*, 529–542.
68. R. H. Haschke, L. L. Campbell, *J. Bacteriol.* **1971**, *106*, 603–607.
69. E. C. Hatchikian, J. G. Zeikus, *J. Bacteriol.* **1983**, *153*, 2111–1220.
70. W. Nakatsukasa, J. M. Akagi, *J. Bacteriol.* **1969,** *98*, 429–433.
71. E. C. Hatchikian, *Arch. Microbiol.* **1975**, *105*, 249–256.
72. R. M. Fitz, H. Cypionka, *Arch. Microbiol.* **1990**, *154*, 400–406.
73. M. Broco, M. Rousset, S. Oliveira, C. Rodrigues-Pousada, *FEBS Lett.* **2005**, *579*, 4803–4807.

74. H.D. Peck, Jr, J. LeGall, *Phil. Trans. R. Soc. B*. **1982,** *298*, 443–466.
75. K. Parey, U. Demmer, E. Warkentin, A. Wynen, U. Ermler, C. Dahl, *PLoS ONE* **2013**, 8: available on line, e74707. doi:10.1371/.
76. N. Sekulic, K. Dietrich, I. Paamann, S. Ort, M. Konrad, A. Lavie, *J. Mol. Biol.* **2007**, *367*, 488–500.
77. C. Dahl, H-G. Koch, O. Keuken, H. G. Trüper, *FEMS Microbiol. Lett*. **1990**, *67*, 27–32.
78. O. Y. Gavel, S. A. Bursakov, J. J. Calvete, G. N. George, J. J. Moura, I. Moura, *Biochemistry* **1998**, *37*, 16225–16232.
79. Y. Taguchi, M. Sugishima, K. Fukuyama, *Biochemistry* **2004**, *43*, 4111–4118.
80. O. Y. Gavel, A. V. Kladova, S. A. Bursakov, J. M. Dias, S. Texeira, V. L. Shnyrov, J. J. G. Moura, I. Moura, M. J. Romão, J. J. Trincão, *Acta Crystallogr. Sect . F, Struct. Biol. Cryst. Commun.* **2008**, *64*, 593–595.
81. Y.-L. Chiang, Y.-C. Hsieh, J-Y. Fang, E.-H. Liu, Y.-C. Huang, P. Chuankhayan, J. Jeyakanthan, M.-Y. Liu, S. I. Chan, C.-J. Chen, *J. Bacteriol.* **2009**, *191*, 7597–7608.
82. G. Fritz, T. Büchert, P. M. H. Kroneck, *J. Biol. Chem.* **2002**, *277*, 26066–26073.
83. B. Meyer, J. Kuever, *PLoSONE* **2008** 3, available online, e1514.doi10.1371/journal.pone.0001514.
84. J. Lampreia, I. Moura, M. Teixeira, H. D. Peck, Jr., J. LeGall, B. H. Huynh, J. J. G. Moura, *Eur. J. Biochem.* **1990**, *188*, 653–664.
85. A. R. Ramos, K. L. Keller, J. D. Wall, *Front. Microbiol.* **2012**, *3*, 137, available online, doi:10.3389/fmicb.2012.00137.
86. G. M. Zane, H-c. B. Yen, J. D. Wall, *Appl. Environ. Microbiol*. **2010**, *76*, 5500–5509.
87. I. Moura, A. R. Lino, *Meth. Enzymol.* **1994**, *243*, 296–303.
88. M. J. Murphy, L. M. Siegel, *J. Biol. Chem.* **1973**, *248*, 6911–6919.
89. A. Schiffer, K. Parey, E. Warkentin, K. Diederichs, H. Huber, K. O. Stetter, P. M. Kroneck, U. Ermler, *J. Mol. Biol.* **2008**, *379*, 1063–1074.
90. J. Steuber, P. M. H. Kroneck, *Inorg. Chim. Acta* **1998**, *276*, 52–57.
91. G. Fritz, A. Schiffer, A. Behrens, T. Buchert, U. Ermler, P. M. H. Kroneck, in *Microbial Sulfur Metabolism*, Eds C. Dahl, C. G. Friedrich, Berlin, Springer-Verlag, 2008, pp. 13–23.
92. Y.-C. Hsieh, M.-L. Liu, V. C.-C. Wang, Y.-L. Chiang, E.-H. Liu, W. G. Wu, S. I. Shan, C.-J. Chen, *Mol. Microbiol.* **2010**, *78*, 1101–1116.
93. T. F. Oliveira, E. Franklin, J. P. Afonso, A. R. Khan, N. J. Oldham, I. A. C. Pereira, M. Archer, *Front. Microbiol.* **2011**, *2*, 71 doi:10.3389.
94. F. Grein, I. A. C. Pereira, C. Dahl. *J. Bacteriol.* **2010**, *192*, 6369–6377.
95. T. F. Oliveira, C. Vornhein, P. M. Matias, S. S. Venceslau, I. A. C. Pereira, M. Archer, *J. Struct. Biol.* **2008**, *164*, 236–239.
96. T. F. Oliveira, C. Vonrhein, P. M. Matias, S. S. Venceslau, I. A. C. Pereira, M. Archer, *J. Biol. Chem.* **2008**, *283*, 34141–34149.
97. R. R. Karkhoff-Schweizer, M. Bruschi, G. Voordouw, *Eur. J. Biochem.* **1993**, *211*, 501–507.
98. J. Ostrowski, J.-Y. Wu, D. C. Rueger, B. E. Miller, L. M. Siegel, N. M. Kredich, *J. Biol. Chem.* **1989**, *264*, 15726–15737.
99. E. T. Adman, L. Sieker, L. Jensen, *J. Biol. Chem.* **1976**, *248*, 3987–3996.
100. M. Bruschi, F. Guerlesquin, *FEMS Microbiol. Rev.* **1988**, *54*, 155–176.
101. D. H. George, L. T. Hunt, L. S. L. Yeh, W. C. Barker, *J. Mol. Evol.* **1985**, *22*, 117–143.
102. I. Moura, J. LeGall, A. R. Lino, H. D. Peck, Jr., G. Fauque, A. V. Xavier, D. V. DerVartanian, J. J. G. Moura, B. H. Huynh, *J. Am. Chem. Soc.* **1988**, *110*, 1075–1082.
103. J. Steuber, H. Cypionka, P. M. H. Kroneck, *Arch. Microbiol.* **1994**, *162*, 255–260.
104. J. Steuber, A. F. Arendsen, W. R. Hagen, P. M. H. Kroneck, *Eur. J. Biochem.* **1995**, *233*, 873–879.
105. B. M. Wolfe, S. Lui, J. A. Cowan, *Eur. J. Biochem.* **1994**, *223*, 79–89.
106. A. J. Pierik, M. G. Duyvis, J. M. L. M. van Helvoort, R. B. J. Wolbert, W. R. Hagen, *Eur. J. Biochem.* **1992,** *205*, 111–115.

107. R. H. Pires, S. S. Venceslao, F. Morais, M. Teixeira, A. V. Xavier, I. A. Pereira, *Biochemistry* **2006**, *45*, 249–262.
108. R. R. Karkhoff-Schweizer, D. P. W. Huber, G. Voordouw, *Appl. Env. Microbiol.* **1995,** *61*, 290–296.
109. N. Mizuno, G. Voordouw, K. Miki, A. Sarai, Y. Higuchi, *Structure*, **2003**, *11*, 1133–1140.
110. J. C. Mathews, R. Timkovich, M.-Y. Lin, J. LeGall, *Biochemistry* **1995,** *34*, 5248–5251.
111. P. A. Trudinger, *J. Bacteriol.* **1970**, *104*, 158–170.
112. Ø. Larsen, T. Lien, N. K. Birkeland, *Extremophiles*, **1999**, *3*, 63–70.
113. D. V. DerVartanian, *Meth. Enzymol.* **1994***, 243*, 270–276.
114. N . Klouche, O. Basso, J.-F. Lascourrèges, J.-L. Cayol, P. Thomas, G. Fauque, M.-L. Fardeau, M. Magot, *Int. J. Syst. Evol. Microbiol.* **2009**, *59*, 3100–3104.
115. Ø. Larsen, T. Lien, N. K. Birkeland, *FEMS Microbiol. Lett.* **2000**, *186*, 41–46.
116. B. R. Crane, L. M. Siegel, E. D. Getzoff, *Science*, **1995**, *270*, 59–67.
117. C. Dahl, N. M. Kredich, R. Deutzmann, H. G. Trüper, *J. Gen. Microbiol.* **1993**, *139*, 1817–1828.
118. L. Debussche, D. Thibaut, B. Cameron, J. Crouzet, F. Blanche, *J. Bacteriol.* **1990**, *172*, 6239–6244.
119. G. Fauque, A. Lino, M. Czechowski, L. Kang, D. V. DerVartanian, J. J. G. Moura, J. LeGall, I. Moura, *Biochim. Biophys. Acta* **1990**, *1040*, 112–118.
120. E. C. Hatchikian, *Meth. Enzymol.* **1994***, 243*, 276–295.
121. O. Haouari, M.-L. Fardeau, J.-L. Cayol, G. Fauque, C. Casiot, F. Elbaz-Poulichet, M. Hamdi, B. Ollivier, *Syst. Appl. Microbiol.* **2008**, *31*, 38–42.
122. A. M. Stolzenberg, S. H. Strauss, R. H. Holm, *J. Am. Chem. Soc.* **1981**, *103*, 4763–4778.
123. C. Dahl, H. G. Trüper, *Meth. Enzymol.* **2001,** *331*, 427–441.
124. B. B. Jørgensen, *Science*, **1990**, *249*, 152–154.
125. L. Stoffels, M. Krehenbrink, B. C. Berks, G. Unden, *J. Bacteriol.* **2012**, *194*, 475–485.
126. R. Starkey, *Soil Sci.* **1950**, *70*, 55–66.
127. S. E. Winter, P. Thiennimitr, M. G. Winter, B. P. Butler, D. L. Huseby, R. W. Crawford, J. M. Russell, C. L. Bevins, L. G. Adams, R. M. Tsolis, J. R. Roth, A. J. Bäumler, *Nature* **2010**, *467*, 426–429.
128. E. F. Johnson, B. Mukhopadhyay, *J. Biol. Chem.* **2005**, *280*, 38776–38786.
129. E. F. Johnson, B. Mukhopadhyay, *Appl. Env. Microbiol.* **2008**, *74*, 3591–3595.
130. H. Laue, M. Friedrich, J. Ruff, A. M. Cook, *J. Bacteriol.* **2001**, *183*, 1727–11733.
131. G. Harrison, C. Curle, E. J. Laishley, *Arch. Microbiol.* **1984**, *138*, 72–78.
132. V. L. Barbosa-Jefferson, F. J. Zhao, S. P. McGrath, N. Morgan, *Soil Biol. Biochem.* **1998,** *30*, 553–559.
133. E. L. Barrett, M. A. Clark, *Microbiol. Rev.* **1987**, *51*, 195–205.
134. M. Hinojosa-Leon, M. Dubourdieu, J. A. Sanchez-Crispin, M. Chippaux*, Biochem. Biophys. Res. Commun.* **1986**, *136*, 577–581.
135. J. L. Burns and T. J. DiChristina, *Appl. Environ. Microbiol.* **2009**, *75*, 5209–5217.
136. A. P. Hinsley, B. C. Berks, *Microbiology* **2002**, *148*, 3631–3638.
137. P. Hallenbeck, M. A. Clark, E. L. Barrett, *J. Bacteriol.* **1989**, *171*, 3008–3015.
138. C. J. Huang, E. L. Barrett, *J. Bacteriol.* **1991**, *173*, 1544–1553.
139. L. M. Siegel, P.S. Davis, *J. Biol. Chem.* **1974**, *249*, 1587–1598.
140. M. Kern, M. G. Klotz, J. Simon, *Mol. Microbiol.* **2011**, *82*, 1515–1530.
141. S. Shirodkar, S. Reed, M. Romine, D. Saffarini, *Environ. Microbiol.* **2011**, *158*, 287–293.
142. H. L. Drake, J. M. Akagi*, Biochem. Biophys. Res. Commun.* **1976,** *71*, 1214–1219.
143. B. H. Huynh, L. Kang, D. V. DerVartanian, H. D. Peck, Jr., J. LeGall, *J. Biol. Chem.* **1984**, *259*, 15373–15376.
144. I. Moura, A. R. Lino, J. J. G. Moura, A. V. Xavier, G. Fauque, H. D. Peck, Jr., J. LeGall, *Biochem. Biophys. Res. Commun.* **1986**, *141*, 1032–1041.
145. J. J. G. Moura, I. Moura, H. Santos, A. V. Xavier, M. Scandellari, J. LeGall, *Biochem. Biophys. Res. Commun.* **1982**, *108*, 1002–1009.

146. G. Fauque, Doctorat d'Etat Thesis in Physical Sciences, University of Technology of Compiègne, France, 1985, 222 pages.
147. G. Fauque, D. Hervé, J. LeGall, *Arch. Microbiol.* **1979**, *121*, 261–264.
148. R. Cammack, G. Fauque, J. J. G. Moura, J. LeGall, *Biochim. Biophys. Acta* **1984**, *784*, 68–74.
149. G. D. Fauque, L. L. Barton, J. LeGall, *Sulphur in Biology: Ciba Foundation Symposium* **1980**, *72*, 71–86.
150. A. S. Alves, C. M. Paquete, B. M. Fonseca, R. O. Louro, *Metallomics* **2011**, *3*, 349–353.
151. I. A. C. Pereira, I. Pacheco, M.-Y. Liu, J. LeGall, A. V. Xavier, M. Teixeira, *Eur. J. Biochem.* **1997**, *248*, 323–328.
152. S. Laska, F. Lottspeich, A. Kletzin, *Microbiology* **2003**, *149*, 2357–2371.
153. M. Keller, R. Dirmeier, *Meth. Enzymol.* **2001**, *331*, 442–451.
154. S. L. Bridger, S. M. Clarkson, K. Stirrett, M. B. Debarry, G. L. Lipscomb, G. J. Schut, J. Westpheling, R. A. Scott, M. W. W. Adams, *J. Bacteriol.* **2011**, *193*, 6498–6504.
155. A. Kletzin, in *Microbial Sulfur Metabolism,* Eds C. Dahl, C. G. Friedrich, Springer, Berlin, 2008, pp. 184–201.
156. T. Urich, C. M. Gomes, A. Kletzin, C. Frazao, *Science* **2006**, *311*, 996–1000.
157. W. Purschke, C. L. Schmidt, A. Petersen, G. Schafer, *J. Bacteriol.* **1997,** *179*, 1344–1353.
158. J. A. Brito, F. L. Sousa, M. Stelter, T. M. Bandeiras, C. Vonrhein, M. Teixeira, M. M. Pereira, M. Archer, *Biochemistry* **2009**, *48*, 5613–5622.
159. M. Schedel, M. Vanselow, H. G. Trüper, *Arch. Microbiol.* **1979**, *121*, 29–36
160. F. Grimm, B. Franz, C. Dahl, in *Microbial Sulfur Metabolism,* Eds C. Dahl, C. G. Friedrich, Springer, Berlin, 2008, pp. 101–116.
161. R. Hille, *Chem. Rev.* **1996**, *96*, 2757–2816
162. U. Kappler, M. J. Maher, Cell. *Mol. Life Sci.* **2013**, *70*, 977–992.
163. H. Sakurai, T. Ogawa, M. Shiga, K. Inoue, *Photosynth. Res.* **2010**, *104*, 163–176.
164. L. H. Gregersen, D. A Bryant, N.-U. Frigaard, *Front. Microbiol.* **2011,** available online, doi: 10.3389/fmicb.2011.00116.
165. J. P. Lee, C. Yi, J. LeGall, H. Peck, Jr, *J. Bacteriol.* **1973**, *115*, 453–455.
166. A. F. Arendsen, M. F. Verhagen, R. B. Wolbert, A. J. Pierik, A. J. Stams, M. S. Jetten, W. R. Hagen, *Biochemistry* **1993**, *32*, 10323–10330.