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Microbiological disproportionation of inorganic sulfur compounds

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The disproportionation of inorganic sulfur intermediates at moderate temperatures (0–80 °C) is a microbiologically catalyzed chemolithotrophic process in which compounds like elemental sulfur, thiosulfate, and sulfite serve as both electron donor and acceptor, and generate hydrogen sulfide and sulfate. Thus the overall process is comparable to the fermentation of organic compounds such as glucose and is consequently often described as 'inorganic fermentation'. The process is primarily carried out by microorganisms with phylogenetic affiliation to the so called sulfate-reducing bacteria within the delta subclass of Proteobacteria. The organisms grow with sulfate as their external electron acceptor and low-molecular weight organic compounds or hydrogen as energy sources. Studies of the biochemistry of a few isolates indicate that the disproportionating microbes reverse the sulfate reduction pathway during disproportionation. However, investigations with elemental sulfur disproportionating bacteria present evidence for an alternative pathway involving the enzyme sulfite-oxidoreductase, an enzyme that has hitherto only been reported participating in the oxidation of sulfite in aerobic or phototrophic sulfide oxidizers.

Investigations bridging geology and microbiology have found strong evidence for disproportionating bacteria participating in and enhancing the rate at which pyrite forms and being partly responsible for the isotopic signatures of sulfidic minerals in recent and old sediments. New results indicate that elemental sulfur disproportionating microbes can be traced back in time as long as 3.5 billion years and elemental sulfur disproportionation would thus be one the oldest biological processes on Earth.

Keywords: disproportionation; sulfur metabolism; bacteria; fractionation; sulfate reduction

1. Introduction

Sulfate reduction is quantitatively the most important process in the oxidation of organic matter in anoxic marine environments and on a global scale about 7.2×10^9 tons of sulfate are reduced annually releasing an equimolar amount of H_2S (*1*). Only between 1% and 2% of the microbiologically generated hydrogen sulfide is permanently buried as pyrite (*2*) and thus by far the largest fraction is chemically or biologically oxidized by diverse groups of microorganisms with oxygen, nitrate, and/or metal oxides as electron acceptors. The chemical and biological oxidation of sulfide may not always proceed completely to sulfate and as a consequence oxidized inorganic sulfur intermediates such as elemental sulfur (S^0), thiosulfate, and sulfite are formed that can be degraded further (*3–5*).

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In the late 1980's a new chemolithotrophic process, termed sulfur disproportionation, was discovered involving the degradation of oxidized sulfur intermediates. The process was compared to the fermentation of organic matter where electrons are redistributed among the carbon atoms and ATP is synthesized without the involvement of electron transport and proton gradients.

Studying thiosulfate degradation under oxygen-free conditions, Bak and co-workers (*6*, *7*) discovered that thiosulfate was metabolized in a hitherto unrecognized manner:

$$\begin{gathered} S_2O_3^{2-} + H_2O \longrightarrow SO_4^{2-} + HS^- + H^+ \\ \Delta G^{0,} = -21.9\,\text{kJ mol}^{-1}\,S_2O_3^{2-} \end{gathered} \tag{1}$$

in which electrons were internally transferred from the oxidized sulfonate to the reduced sulfane sulfur. The first bacterium isolated able to carry out this process was identified as belonging to the genus *Desulfovibrio*, a group of genuine sulfate reducers and was designated *Desulfovibrio desulfodismutans*. Apart from thiosulfate *D. disulfodismutans* was also able to disproportionate sulfite according to the following equation:

$$\begin{gathered} 4\,SO_3^{2-} + H^+ \longrightarrow 3\,SO_4^{2-} + HS^- \\ \Delta G^{0,} = -58.9\,\text{kJ mol}^{-1}\,SO_3^{2-} \quad . \end{gathered} \tag{2}$$

In an extensive screening study Krämer and Cypionka (*8*) showed that thiosulfate and sulfite disproportionation was rather common among sulfate reducers and that it was not restricted to strains within the genus *Desulfovibrio* (Table 1). However, only a few strains could couple this metabolism to growth.

A few years after the discovery of microbial thiosulfate and sulfite disproportionation Thamdrup and coworkers (*9*) showed that elemental sulfur could also be metabolized in a similar way. However, in contrast to the disproportionation of thiosulfate and sulfite, sulfur disproportionation is endergonic under standard conditions (Equation 3) and requires the scavenging of the produced

Table 1. Organisms that disproportionate thiosulfate, sulfite and/or elemental sulfur.

Organism	Thiosulfate	Sulfite	Elemental sulfur
Desulfobacter curvatus[a]	D	–	n.d.
Desulfobacter hydrogenophilus[a]	D	–	n.d
Desulfococcus multivorans[a]	D	–	n.d
Desulfotomaculum nigrificans[a]	(D)	–	–
Desulfotomaculum thermobenzoicum[c]	G	n.d.	n.d.
Desulfovibrio desulfuricans CNS[a]	G	D	n.d.
Desufovibrio desulfodismutans[d]	G	G	
Desulfovibrio mexicanus[e]	D	D	n.d.
Desulfovibrio aminophilus[f]	D	D	n.d.
Desulfovibrio brasiliensis[g]	G	n.d.	n.d.
Desulfovibrio oxyclinae[h]	G	G	n.d.
Desulfomonile tiedje[i]	G	n.d.	n.d.
Desulfobulbus propionicus[ab]	(G)	–	(G)
Desulfofustis glycolicus (this paper)	n.d.	n.d.	G
Desulfocapsa thiozymogenes [j]	G	G	G
Desulfocapsa sulfoexigens[k]	G	G	G
Desulfocapsa Cad626[l]	G	G	G
Pantoea agglomerans[m]	n.d.	n.d.	G

G, Growth; (G), weak Growth; D, Disproportionated without Growth; (D), Weak disproportionation without Growth. – not disproportionated; [a]Krämer and Cypionka (*8*), [b]Lovley and Phillips (*49*), [c]Jacksen and McInerney (*50*), [d]Bak and Pfennig (*7*), [e]Hernandez-Eugenio *et al.* (*51*), [f]Baena *et al.* (*52*), [g]Warthmann *et al.* (*53*), [h]Krekeler *et al.* (*24*), [i]Mohn and Tiedje (*54*), [j]Janssen *et al.* (*12*), [k]Finster *et al.* (*18*), [l]Peduzzi *et al.* (*32*), [m]Obraztsova *et al.* (*15*).

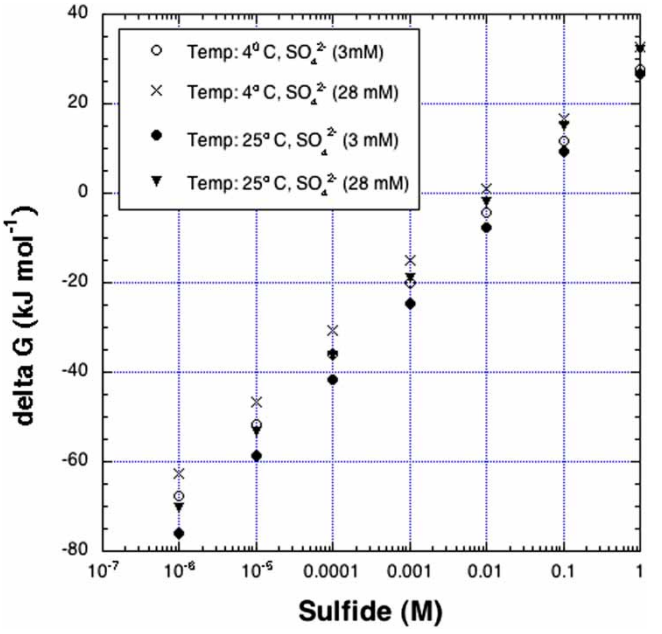

Figure 1. The free energy values as a function of hydrogen sulfide concentrations in cultures of elemental sulfur-disproportionating bacteria ($4\,S^0 + 4\,H_2O \rightarrow SO_4^{2-} + 3HS^- + 5H^+$). The reaction is not exergonic before sulfide concentrations are below 10 mM. The calculations were made assuming four different scenarios. The concentration of sulfate was set to 28 mM (marine conditions) or 3 mM (fresh water to brackish conditions), respectively and the temperature was set to 277 K or 298 K, respectively.

sulfide to be thermodynamically favorable (Equation 4):

$$4\,S^0 + 4\,H_2O \longrightarrow SO_4^{2-} + 3HS^- + 5H^+ \qquad \Delta G^{0,} = 10.2\,\mathrm{kJ\,mol^{-1}}\,S^0. \tag{3}$$

In the presence of ferric iron the following stoichiometry is obtained:

$$3\,S^0 + 2\,FeOOH \longrightarrow SO_4^{2-} + 2\,FeS + 2H^+. \tag{4}$$

The $\Delta G^{0,}$ of the latter reaction depends on the concentration of free hydrogen sulfide in solution (Figure 1) and is circa –30 kJ mol^{-1} S^0 if we assume a hydrogen sulfide concentration of 10^{-7} M and a sulfate concentration of 2.8×10^{-2} M, which are realistic concentrations in environments from which elemental sulfur disproportionating bacteria have been obtained.

Apart from interesting biochemical aspects, recent geochemical investigations have revealed that the microbial disproportionation of intermediately oxidized sulfur compounds has important implications for our understanding of sulfide oxidation in the environment as well as for the interpretation of sulfur isotope signatures in sulfidic minerals.

In the following review, I will summarize our current knowledge on the microbial disproportionation of inorganic sulfur compounds by summarizing results obtained in the following fields: microbiology, biochemistry, and biogeochemistry.

2. Diversity of sulfur compound disproportionating bacteria

After the isolation of *D. desulfodismutans* (*6*, *7*) a comprehensive screening of sulfate reducers as well sulfur reducers and oxidizers revealed that the capacity to disproportionate thiosulfate

and sulfite is relatively common among sulfate reducers, where 8 out of 19 strains were able to disproportionate thiosulfate, while it was absent among the tested sulfur reducers and oxidizers (*8*). Since the Krämer and Cypionka study several new isolates, all members of the delta Proteobacteria tested positive for the capacity to disproportionate thiosulfate and sulfite. However, this capacity may be more common than hitherto known, as only a minor fraction of the new isolates has been tested so far. Most of the thiosulfate and sulfite disproportionating bacteria that can couple disproportionation to growth belong to the genus *Desulfovibrio* with *Desulfomonile tiedje* and *Desulfotomaculum thermobenzoicum* being the only exceptions (Table 1). Microorganisms that in addition to thiosulfate and sulfite can couple growth to the disproportionation of elemental sulfur were found in the family *Desulfobulbaceae* within the genera *Desulfobulbus, Desulfofustis*, and *Desulfocapsa*. Among them, primarily species within the genus *Desulfocapsa* were studied in greater depth with respect to elemental sulfur disproportionation (*10*, *11*). Detailed studies with *Desulfocapsa sulfoexigens* (*11*) revealed that the strain can grow autotrophically using the reverse CO- dehydrogenase pathway for the fixation of CO_2 and that despite having the complete enzyme machinery required for sulfate reduction is not able to carry out this process. Interestingly, in the presence of molecular hydrogen reduction processes dominate over disproportionation in cultures supplemented with thiosulfate, while in cultures supplemented with elemental sulfur and sulfite–sulfate formation was suppressed completely and thus the reduction pathways outcompete disproportionation. In contrast to *D. sulfoexigens*, its closest relative, the fresh water strain *D. thiozymogenes* was able to grow as a sulfate reducer by oxidizing ethanol, propanol, and butanol to the corresponding fatty acids (*12*).

Clone libraries of 16S rDNA genes from elemental sulfur disproportionating enrichment cultures (*13*) revealed the predominance of 16S rDNA genes affiliated with sequences constituting the Desulfobulbaceae family (Figure 2). One of the clone sequences shows close affiliation to

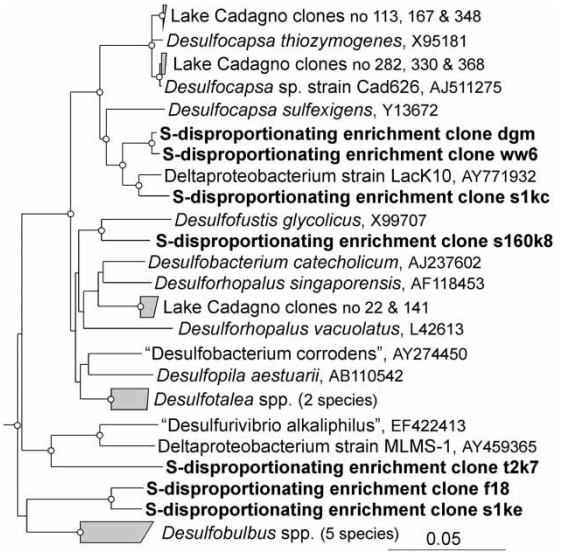

Figure 2. Phylogenetic positions of 16S rRNA gene sequences retrieved from sulfur disproportionating enrichment cultures by cloning (marked in bold) among members of the family *Desulfobulbaceae*. The tree was inferred from distance-matrix-based analysis of a dataset composed of 79 taxa using Jukes-Cantor distance correction with sampling of 1.297 sequence positions (PAUP*, *47*). A range of other deltaproteobacterial taxa, which were used to root the shown tree were subsequently removed. Open circles denote nodes receiving >70% neighbor-joining-based bootstrap support (100 replicates, PAUP*, *47*). Bar depicts 5% estimated sequence divergence. The clones were derived from the enrichment cultures studied by Canfield *et al.* (*48*). Clone dgm was obtained from station Dangast (Jadebusen, Germany), clone ww was obtained from tidal flat sediments of the Weser Estuary station Weddewarden (Germany), clones s1kc, s1ke, and s160k8 were obtained from two stations in the anoxic basin of the Golfo Dulce (Costa Rica), clone t2k7 was obtained from a freshwater pond in Bremen (Germany), and clone f18 was obtained from salt marsh sediment (Denmark).

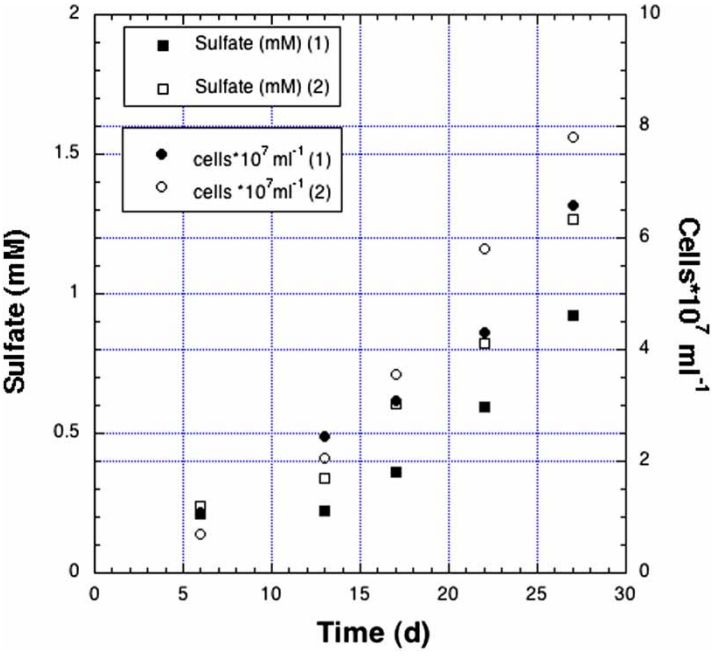

Figure 3. Time course experiments with two cultures of *D. glycolicus* grown with elemental sulfur in the presence of amorphous iron hydroxide and acetate (2 mM) as carbon source. ■ sulfate concentration in culture 1 (mM) , □ sulfate concentration in culture 2 mM, • cells ml^{-1} in culture 1, ∘ cells ml^{-1} in culture 2. Sulfate was determined by ion-chromatography. The cells were counted by epifluorescence microscopy after staining with the DNA staining dye Sybr Gold.

Desulfofustis glycolicus, a strain that was originally described as a heterotrophic sulfate reducer (*14*). However, recent growth experiments revealed that *D. glycolicus* can also be grown by the dispropotionation of elemental sulfur (Figure 3). All the isolates and also the 16S rDNA clone-tacked members of the enrichment cultures belong to the delta class of the Proteobacteria phylum, with the capacity to use sulfate, thiosulfate, or sulfite as electron acceptors in a respiratory type of metabolism. It was thus very surprising when Obraztsova *et al.* (*15*) reported that the facultative anaerobic bacterium *Pantoea agglomerans* was equally able to thrive on the disproportionation of elemental sulfur. The strain was originally isolated from a marine salt pond and studied for its capacity to reduce oxidized iron and chromium. Most surprisingly, close relatives of the Obraztsova strain are plant pathogens that can also cause disease in humans (*16*); features that have not been confirmed for this isolate. The mechanism by which *P. agglomerans* (strain Obraztsova) disproportionates elemental sulfur is not known and remains to be studied in future investigations. Apart from resolving this aspect of the physiology of *P. agglomerans* (strain Obraztsova) it would also be interesting to investigate (a) whether this trait is common among the other *P. agglomerans* strains, (b) whether the Obrazstova strain is able to disproportionate sulfite and thiosulfate in addition to elemental sulfur, and (c) whether *P. agglomerans* has the capacity to use the inorganic sulfur compounds as electron acceptor in anaerobic respiratory processes.

3. Biochemistry of sulfur compound disproportionation

3.1. *Thiosulfate and sulfite disproportionation by Desulfovibrio strains*

The metabolic pathways of sulfite and thiosulfate disproportionation have been studied in cultures of *D. desulfodismutans* and *Desulfovibrio desulfuricans* CNS and the enzymes involved in the

process were investigated in cell-free extracts of both cultures (*8*). Apart from disproportionating thiosulfate and sulfite the strains also thrive as typical sulfate reducers coupling the oxidation of hydrogen and ethanol to the reduction of sulfate, sulfite, and thiosulfate. Krämer and Cypionka (*8*) demonstrated that the capacities to reduce sulfate and to disproportionate sulfite or thiosulfate are present simultaneously and thus that the capacity to disproportionate was constitutive. Experimenting with the uncoupler carbonylcyanide m-chlorophenylhydrazone (CCCP) and the ATPase inhibitor *N*, *N*′–dicyclohexylcarbodiimide (DCCD) these authors demonstrated that in the presence of CCCP sulfite as well as thiosulfate could be reduced with hydrogen as electron donor while the disproportionation of both substrates as well as the reduction of sulfate were inhibited. DCCD exclusively inhibited sulfate reduction and did not affect the disproportionation of sulfite and thiosulfate. The CCCP results indicate that an energy-driven step is very likely involved in disproportionation while the DCCD results indicate that ATP synthesis during disproportionation is not dependent on a functional F_0F_1 ATP synthase but is dependent on substrate level phosphorylation. Thus, the authors concluded that the two *Desulfovibrio* strains used the enzymatic machinery that catalyze the reduction of sulfate when they disproportionate thiosulfate and sulfite but in reverse. They proposed that ATP was formed by substrate level phosphorylation via ATP sulfurylase. In addition, their results strongly indicated that a reversed electron transport step was involved in the reduction of thiosulfate to sulfide and that the oxidation of sulfite to APS was the source of these electrons. In a later study Cypionka *et al.* (*17*) investigated the pathway of thiosulfate disproportionation in *D. desulfuricans* CSN cultures using product sulfur isotope signatures by isotope mass spectrometry. They observed that the sulfate that was produced during disproportionation was isotopically heavier than the inner sulfonate sulfur of thiosulfate while the resulting sulfide was isotopically lighter than the outer sulfane sulfur of the disproportionated thiosulfate. As an explanation the authors proposed that thiosulfate was initially cleaved into sulfite and elemental sulfur and that both in later steps underwent disproportionation via a common pathway. This interpretation contradicts conclusions drawn from the previous demonstration of an active thiosulfate reductase in cell-free extracts that cleaves thiosulfate into hydrogen sulfide and sulfite in cell-free extracts of *D. desulfuricans* CNS (*8*). Thus, more studies are needed to resolve the mechanism by which thiosulfate is metabolized in this bacterium. This is also important with respect to establishing the reliability of isotopic data that are widely used in the interpretation of the contribution of different microbial pathways to the generation of isotopic signatures in sulfidic minerals.

3.2. *Disproportionation of thiosulfate and elemental sulfur by D. sulfoexigens*

Of the strains that have been reported to thrive on the disproportionation of elemental sulfur only *D. sulfoexigens* has been studied with respect to the biochemistry of this process. *D. sulfoexigens* was isolated from a marine sulfate-rich sediment and is related to sulfate reducers of the genera *Desulfobulbus* and *Desulfofustius* (*18*). Based on the outcome of studies with *D. sulfoexigens* cell-free extracts Frederiksen and Finster (*11*) proposed that thiosulfate disproportionation was initiated by the cleavage of thiosulfate into H_2S and sulfite by the enzyme thiosulfate reductase. This finding conflicts with the mechanisms proposed by Cypionka *et al.* (*17*) for *D. desulfuricans* (see paragraph 3.1). It would be interesting to carry out fractionation studies with *D. sulfoexigens* to determine the fractionation pattern generated by this organism and to compare it with the results obtained in the enzyme study.

The sulfite produced from thiosulfate can then be further oxidized to sulfate along two parallel pathways (Figure 4): pathway 1. reverses the reduction of sulfate and involves the enzymes APS reductase, ATP sulfurylase, and pyrophosphatase in the same way as has been proposed for *D. desulfodismutans* by Krämer and Cypionka (*8*) and a second pathway that exclusively depends on the enzyme sulfite oxidoreductase. Cultures of *D. sulfoexigens* that disproportionated

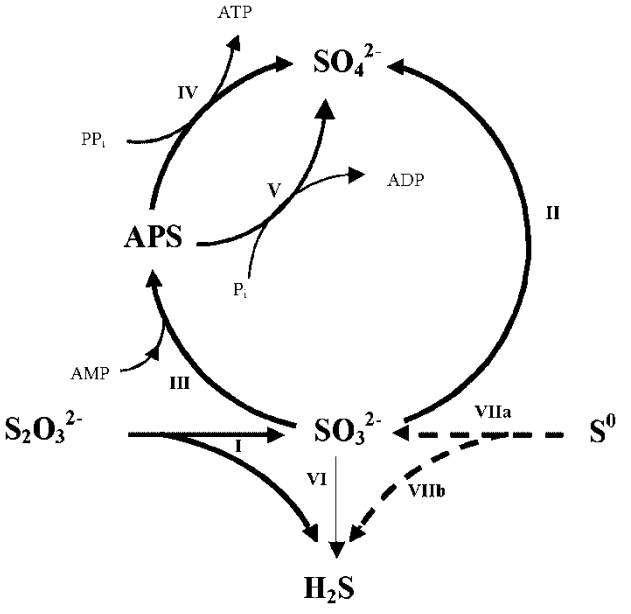

Figure 4. The figure depicts the proposed pathways of thiosufate and sulfur disproportion in cultures of *D. sulfoexigens* (*10*). The enzyme activities were measured in cell-free extract of cultures that were grown in a batch fermentor in the absence of a sulfide scavenger. Hydrogensulfide was removed by continuously flushing of the culture with a gas mixture of 10% CO_2 and 90 N_2. Activities of the following enzymes could be determined: I. Thiosulfate reductase, II. Sulfite oxidoreductase, III. APS reductase, IV. ATP sulfurylase, V. Adenylylsulfate:phosphate adenylyltransferase, VI. Sulfite reductase. VIIa and VIIb: unresolved reactions that may proceed via unidentified intermediates. Full bold lines indicate reactions that are involved in the disproportionation process. The rates that were measured in the enzyme assays were high enough to explain the disproportionation rates measured in the cultures used for cell extract preparation. Thin lines (*i.e.* reaction VI) indicate that the strain possess the enzyme but that the activity was to low to explain the measured overall disproportionation rates. Dotted bold lines indicate tentative pathways for which no enzyme activity could be measured.

elemental sulfur expressed the same enzymes as thiosulfate disproportionating cultures apart from thiosulfate reductase, which seems to be induced by the presence of thiosulfate. However, the enzymes involved in elemental sulfur reduction were not identified. The presence of sulfite reductase in elemental sulfur disproportionating cultures indicates that this enzyme may be involved in the oxidation of elemental sulfur to sulfite. In contrast to Krämer and Cypionka (*8*) Frederiksen and Finster (*11*) demonstrated the presence of sulfite oxidoreductase activity in cell-free extract from *D. sulfoexigens*. This enzyme is commonly found in phototrophic and chemotrophic sulfur-oxidizing bacteria many of which can also oxidize sulfite via the APS reductase pathway (*19*). One interesting feature of this enzyme is that it generates electrons that have a sufficiently negative reductive potential (−516 mV) to directly be used for the reduction of the sulfane sulfur in thiosulfate to hydrogen sulfide, a process that otherwise would depend upon energy requiring reverse electron transport as proposed by Krämer and Cypionka (*8*) for *D. desulfodismutans*. More detailed studies are needed to definitively resolve the expression, function and role of sulfite oxidoreductase in thiosulfate and sulfur disproportionating cells of *D. sulfoexigens* and related strains.

The role of sulfite as an intermediate in thiosulfate an elemental sulfur disproprotionation can also be addressed by studying the fractionation of the oxygen isotopes ^{18}O and ^{16}O (*20*). An enrichment of sulfate with heavy oxygen may be attributed to the intermediate formation of sulfite during disproportionation of thiosulfate or elemental sulfur as oxygen in sulfite exchanges very rapidly with water while sulfate does not (*21*). Such an enrichment of the sulfate pool in ^{18}O was demonstrated by Böttcher *et al.* (*20*) studying disproportionation with cultures of *Desulfobulbus propionicus*.

3.3. *The role of sulfur disproportionation in sulfur oxidation by sulfate reducers*

Although textbooks generally present sulfate reducers as obligate anaerobic oxygen sensitive microbes an increasing number of studies shows a more complex picture. Despite the fact that hitherto no sulfate reducer is known that can grow aerobically, it has been shown that many sulfate-reducing strains can reduce oxygen, some of them even at cell-specific rates that are higher than rates reported for aerobic bacteria (*e.g. 22–24*). Interestingly, sulfate reducers are also able to oxidize hydrogen sulfide, the exclusive end product of sulfate reduction (*25*). Detailed studies by Cypionka and colleagues (*25*, *26*) have revealed that sulfate reducers initiate the oxidation of hydrogen sulfide by the oxygen, nitrite or nitrate-dependent formation of elemental sulfur, which in a second external electron acceptor independent step, is disproportionated leading to the formation of hydrogen sulfide and sulfate (*25*, *26*). By oxidizing hydrogen sulfide to elemental sulfur with oxygen as electron acceptor sulfate reducers may be able to detoxify oxygen and in addition to obtain energy from the disproportionation of the produced elemental sulfur. This strategy may allow sulfate reducers not only to be present in oxic environments but also to compete successfully with regular facultatively aerobic sulfide oxidizers for their substrates.

4. Sulfur disproportionation and the sulfur cycle

4.1. *Thiosulfate disproportionation*

After thiosulfate disproportionation was demonstrated in pure cultures the obvious question to ask is whether this process has any significance in a natural environments and whether it contributes to the global sulfur cycle. Using inner and outer ^{35}S-labeled thiosulfate, Jørgensen (*3*, *4*) showed that thiosulfate disproportionation is a key process in the transformation of intermediately oxidized sulfur in both marine and fresh water sediments. He also provided experimental evidence for thiosulfate being a central intermediate in the oxidation of hydrogen sulfide *in situ*. This is in contrast to later findings by Fuseler and Cypionka (*25*) who showed that elemental sulfur but not thiosulfate is formed when *D. desulfuricans* oxidizes hydrogen sulfide. Assuming that the latter finding is the more typical route for sulfate reducers to oxidize hydrogen sulfide the observation made by Jørgensen indicates that microbes other than sulfate reducers are primarily responsible for sulfide oxidation in marine and fresh water sediments. The results obtained by Jørgensen were supported by a later study using ^{35}S-labeled hydrogen sulfide (*5*). These authors determined that hydrogen sulfide was oxidized to sulfate via thiosulfate and that thiosulfate was turned over by both oxidation and disproportionation. Using a combination of mass balance and radiotracer calculations these investigators were able to show that a maximum of 50% of the produced ^{35}S-labeled sulfate was produced from ^{35}S-thiosulfate turn over and they conclude that a direct pathway of hydrogen sulfide oxidation to sulfate may exist. This direct or better alternative pathway could either be the sulfide oxidation pathway proposed by Fuseler *et al.* (*25*) involving sulfate reducers that initially oxidize hydrogen sulfide to elemental sulfur which is then disproportionated thus by-passing thiosulfate. However, the results of Elsgaard and Jørgensen (*5*) are also consistent with the following scenario: ^{35}Sulfide-sulfur exchanges chemically into the sedimentary pool of elemental sulfur which in a biological mediated step is then disproportionated into hydrogen sulfide and sulfate. These authors also showed that nitrate stimulated the oxidation of sulfide and that ^{35}S-sulfur temporarily appeared in the pool of chromium reducible sulfur, which also includes elemental sulfur, a substrate for disproportionating microbes.

In order to determine the numerical abundance of thiosulfate disproportionating microbes in sediments Jørgensen and Bak (*27*) performed most probable number counts (MPN) using marine surface sediments. They found $>10^6$ cells of thiosulfate disproportionating bacteria per cm^3 of

sediment. These numbers are in the range of MPN counts for sulfate reducers in the same sediments and underline the significance of the disproportionation process in this marine sediment. However, these numbers do not prove that the enumerated microbes thrive on thiosulfate disproportionation under *in situ* conditions. They may, like *D. desulfodismutans*, have the potential to also live from sulfate reduction.

4.2. *Elemental sulfur disproportionation*

Canfield and Thamdrup (*28*) investigated the metabolism of elemental sulfur in homogenized tidal flat sediments. The sediment was amended with elemental sulfur and time course experiments were carried out following the concentration of elemental sulfur, sulfides, sulfate, and pH. Their results are consistent with ongoing sulfur disproportionation, as they observed an increase in the concentration of sulfides and sulfate and a concomitant decrease in pH due to sulfuric acid production. In addition, they observed an increase in organic carbon indicating the presence of autotrophic microbes assimilating inorganic carbon while they disproportionated elemental sulfur. The latter conclusion is consistent with results obtained with pure cultures and highly enriched cultures of sulfur-disproportionating bacteria. They can be consistently grown in minimal medium with bicarbonate as their sole source of carbon (*9, 12, 18*). The abundance of sulfur-disproportionating bacteria in sediment samples from several marine sites has been determined and was in the range of 10^5–10^6 cells cm^{-3} in coastal and inter tidal sediments while <100 cells cm^{-3} were recovered from off-shore sediments in the Skagerrak, a site that is characterized by high concentrations of MnO_2 (*9*, *29*). These data are consistent with the scenario proposed by Canfield and Thamdrup (*30*) that sulfur oxidation is predominant in sediments containing large amounts of oxidants such as MnO_2 whereas sulfur reduction is most significant in sulfide rich sediments and sulfur disproportionation thrives in between where an efficient sulfide scrubber is present (see Equations 3 and 4).

Molecular diversity studies using the 16S rDNA gene as a phylogenetic marker gene have identified the presence of strains that are closely related to *Desulfocapsa* species in habitats as diverse as the chemocline of a meromictic alpine lake (*31*, *32*), a mesophilic sulfide-rich spring (*33*), an epithermal gold mine (*34*), the scales of a hydrothermal vent gastropod (*35*), and Arctic permafrost soil (*36*).

5. Geological aspects of sulfur disproportionation

5.1. *History of sulfur disproportionation*

Recent studies by Philippot and co-workers (*37*) of ^{33}S-sulfur signatures in 3.5 billion year marine sulfate deposits provide indications for active elemental sulfur disproportionation. Their interpretation contradicts the conclusion drawn by Shen *et al.* (*38*), who claimed to have found evidence for early sulfate reduction from ^{34}S-sulfur in the same deposits. The two observations may, however, not be mutually exclusive as demonstrated by the physiological properties of modern sulfur-disproportionating bacteria which in addition to disproportioning elemental sulfur, thiosulfate, and sulfite can also reduce sulfate (see previous section). Assuming that the interpretation by Philippot *et al.* is correct disproportionation would be among the oldest metabolic systems in the history of life on Earth. If the bacteria that carried out disproportionation at that time are ancestors of modern disproportionating microbes it is also likely that they could thrive by an autotrophic life style and thus chemolithoautrophy would date back in time as long as photolithoautotrophy (*39*). Significant contributions of disproportionating bacteria to the isotopic record of sulfidic

minerals are not observed before 0.86 Gyr (*40*). The significant contribution of disproportionating bacteria to the heavy isotope depletions in the sulfides reflects according to Canfield and Teske (*40*) also the oxygen status of the oceans in which the processes occurred, as it goes hand in hand with sulfide concentrations. Despite the fact that the production of element sulfur is favored over sulfate formation by anoxygenic phototrophic bacteria at high sulfide concentration as they prevailed in the early oceans the same high concentrations of hydrogen sulfide hinder elemental sulfur disproportionation for thermodynamic reasons (see Equations 3 and 4). Thus, it is difficult to determine the role played by sulfur-disproportionating bacteria in the environment before the oxygen concentration in the atmosphere decreased the concentration of free sulfides in ocean waters and the sediments. Shallow lagoons may have been earlier favorable habitats for sulfur-disproportionating bacteria than the deeper water bodies.

5.2. *Sulfur disproportionation and the isotopic record of sulfidic minerals*

The isotopic composition of sulfur containing minerals is used to evaluate the sulfur cycle over time (*e.g. 40*) and to identify the appearance and quantitative significance of sulfur transforming processes in time (*e.g. 41*). It has for a long time been enigmatic why ^{34}S sulfide depletions of modern marine sedimentary sulfides are higher (24–71‰) than depletions measured in cultures of sulfate reducing bacteria (4–46‰). Until the discovery of sulfur-disproportionating bacteria sulfate reduction was the only known process by which difference in isotopic signatures were generated. An increasing number of investigations have now documented that the 'depletion puzzle' can be solved by including sulfur compound disproportionation (*30, 42, 43*).

An important question to be addressed is whether a chemical process could explain these observations or whether the pattern is due to biological processes. Recent investigations by Smith (*44*) have demonstrated that elemental sulfur is hydrolyzed into sulfate and sulfide at temperature ranging from 50 °C to 200 °C and that the process was enhanced by the presence of sulfide scavenging cations such as copper. Interestingly, Smith demonstrated that fractionation accompanying the involved chemical reactions was less the 3‰ and cannot explain the depletions observed in natural sulfides. Thus it is most likely that biological disproportionation is involved in the generation of the mineralogical signatures.

In a model proposed by Canfield and Thamdrup (*30*) fractionation of sulfur occurs via cycles of repeated disproportionation of elemental sulfur following reoxidation to elemental sulfur and precipitation with reduced iron. In an open system this leads to more and more depleted sulfides while sulfate becomes isotopically heavier. In a recent study, Böttcher and Thamdrup (*45*) demonstrated that this mechanism is largely dependent on the type of oxidant that oxidizes the sulfide, originally produced by sulfate reduction or disproportionation as well as on the rate at which sulfide precipitates. They could show that in the presence of MnO_2 the isotopic effects of elemental sulfur disproportionation were much smaller than in the presence of Fe (III) oxides. They attribute this difference to a significant re-oxidation of sulfide directly to sulfate instead to elemental sulfur, as it is the case with Fe (III) oxides. This has implications for the isotopic signatures in environments with high manganese concentrations or with rapid manganese turnover. Here isotopic signatures cannot be used to reflect the process by which they were produced.

Investigations of sulfur cycles process in cultures (*20*, see paragraph 3.2) and *in situ* (*46*) using stable isotopes may profit from a combined determination of sulfur ($^{34}S/^{32}S$) and oxygen ($^{18}O/^{16}O$) isotope ratios. Studying the sulfur cycle in the anoxic water column of Framvaren Fjord (Norway), Mandernack *et al.* (*46*) observed concomitant isotope enrichments in the heavy sulfur and oxygen isotopes of sulfate 3 m underneath the oxic-anoxic interface. These patterns were consistent with ongoing sulfur disproportionation, while the ratios determined at greater depth were consistent with the predominance of sulfate reduction.

5.3. *Elemental sulfur disporportionation and pyrite formation*

In the first publication on elemental disproportionation Thamdrup *et al.* (*9*) reported that not only FeS but also pyrite was formed while the organisms disproportionate elemental sulfur. This observation has later been confirmed in pure and enrichment cultures (*12, 13, 18*). By using sulfur isotopes Canfield *et al.* (*13*) could demonstrate that the pyrite was formed through two classical mechanisms, (1) the addition of elemental sulfur to FeS and (2) from the reaction of hydrogen sulfide with FeS. The presence of disproportionating bacteria increased the speed by which pyrite was formed 10^4–10^5 fold compared to what was expected from the reported kinetics of chemical pyrite-formation processes. The role of microbes in pyrite formation is still enigmatic and awaits more detailed investigation. During the formation of pyrite by the addition of hydrogen sulfide to FeS, hydrogen gas is formed which could be an energy source for the microbes involved in the process. *D. sulfoexigens* can grow on hydrogen and elemental sulfur (*11*) and may thus profit from pyrite formation and may have evolved mechanisms by which it can increase the rate of pyrite formation.

6. Concluding remarks

Microbiological disproportionation of intermediate sulfur compounds is an understudied area at the boundary of biology and geology that deserves more attention considering both its intriguing and hitherto unresolved biochemistry, the limited knowledge of organisms that can exploit the process and the implication it may have for our understanding of the geological sulfur record. Investigations of the biochemical pathway of sulfur intermediate disproportionation would largely profit from full-genome sequencing of selected microbes. This would provide insight into the toolbox that these organisms have at their disposition to disproportionate the different sulfur intermediates. In particular the mechanism by which elemental sulfur disproportionating microbes attack elemental sulfur in the presence of a sulfide scrubber is very interesting. On the basis of the genomic data, expression studies could be carried out that would allow to investigate among other things the pathways of sulfite and elemental sulfur oxidation, and the initial step of thiosulfate disproportionation, which are currently unresolved. These studies could also shed light on the unresolved questions: 1. Why do not all sulfate reducers possess the capacity to disproportionate, assuming that they all carry the required enzyme machinery? 2. Why do some strains disproportionate but do not grow? In addition, an identification of the enzymes that are involved in the metabolism of the intermediates would contribute to our understanding of the fractionation patterns that have been observed in cultures and *in situ*. Finally, I would like to encourage researchers within the fields of microbiology, geology, and chemistry to increase their joint efforts in order to understand the multiple aspects of the process, a collaboration that already has proven very fruitful.

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