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Isotope fractionation and sulfur metabolism by pure and enrichment cultures of elemental sulfur-disproportionating bacteria

D. E. Canfield,[1] *B. Thamdrup,*[1] *and S. Fleischer*
Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, 28359 Bremen, Germany

Abstract

We have explored the sulfur metabolism and accompanying fractionation of sulfur isotopes during the disproportionation of elemental sulfur by seven different enrichments and three pure bacterial cultures. Cultures were obtained from both marine and freshwater environments. In all cases appreciable fractionation accompanied elemental sulfur disproportionation, with two ranges of fractionation observed. All cultures except *Desulfobulbus propionicus* produced sulfide depleted in ^{34}S by between 5.5 and 6.9 per mil (avg of 6.3 per ml) and sulfate enriched in ^{34}S by between 17.1 and 20.2 per mil (avg of 18.8 per ml). The narrow range of fractionations suggests a conserved biochemistry for the disproportionation of elemental sulfur by many different marine and freshwater bacteria. Fractionations accompanying elemental sulfur disproportionation by *Db. propionicus* were nearly twice as great as the others, suggesting a different cellular level pathway of sulfur processing by this organism. In nearly every case pyrite formation accompanied the disproportionation of elemental sulfur. By using sulfur isotopes as a tracer of sulfur source, we could identify that pyrite formed both by the addition of elemental sulfur to FeS and from reaction between FeS and H_2S. Both processes were equally fast and up to 10^4–10^5 times faster than expected from the reported kinetics of inorganic pyrite-formation reactions. We speculate that bacteria may have enhanced rates of pyrite formation in our experimental systems. The organisms explored here have different strategies for growth and survival, and they may be active in environments ranging from dissolved sulfide-poor suboxic sediments to interfaces supporting steep opposing gradients of oxygen and sulfide. A large environmental range, combined with high bacterial numbers, significant isotope fractionations, and a possible role in pyrite formation, make elemental sulfur-disproportionating bacteria potentially significant actors in the sedimentary cycling of sulfur compounds.

Over the past 10 years newly discovered bacterial metabolisms of the sulfur cycle have considerably altered our view of sedimentary sulfur cycling. Bacteria conducting thiosulfate and sulfite disproportionation were first described in 1987 (Bak and Pfennig 1987), with high bacterial numbers of up to 2×10^6 cells ml^{-1} also reported (Jørgensen and Bak 1991). Both thiosulfate and sulfite disproportionation are completely anaerobic processes producing sulfate and sulfide as endproducts. Radiotracer experiments suggest a considerable role for thiosulfate disproportionation in marine and freshwater sediments (Jørgensen 1990*a*,*b*; Jørgensen and Bak 1991).

Thamdrup et al. (1993) discovered that marine sediments enriched with only elemental sulfur and iron oxides produced bacterial cultures whose livelihood is coupled to the disproportionation of elemental sulfur to sulfate and sulfide. In these experiments iron oxides acted as a sulfide sink, buffering sulfide to low levels where bacterial growth and the thermodynamics of the disproportionation process were most favorable. High numbers of sulfur-disproportionating bacteria of up to 10^6 cells ml^{-1} were reported in salt marsh sediments (Thamdrup et al. 1993). The first pure bacterial culture conducting elemental sulfur disproportionation, subsequently named *Desulfocapsa thiozymogenes,* was reported by Bak (1993). This organism is a freshwater bacterium originally isolated as a thiosulfate disproportionator. It grows chemolithoautotrophically by the disproportionation of either elemental sulfur, thiosulfate, or sulfite, or heterotrophically as a sulfate reducer on short-chain alcohols (Bak 1993; Janssen et al. 1996).

The disproportionation of elemental sulfur has also been reported for the sulfate reducer *Desulfobulbus propionicus* (Lovley and Phillips 1994) and for a number of other pure cultures of sulfate-reducing bacteria (Fuseler et al. 1996). Not all bacteria capable of elemental sulfur disproportionation, however, are sulfate reducers. Finster et al. (1988) have recently isolated a marine organism that actively grows while disproportionating elemental sulfur, but cannot grow by sulfate reduction.

Thus, numerous marine and freshwater bacteria from both pure and enrichment culture can disproportionate elemental sulfur to sulfate and sulfide. Furthermore, the role of elemental sulfur disproportionation in sediment sulfur transformations may be considerable. Elemental sulfur is a common and rather abundant sulfur intermediate in sediments (Troelsen and Jørgensen 1982; Smith and Klug 1981; Thode-Andersen and Jørgensen 1989; Thamdrup and Canfield 1996)

[1] Present address: Institute of Biology, Odense University, Campusvej 55, 5230 Odense M, Denmark.

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and was actively disproportionated when added to surficial, intertidal, marine sediment from the Weser Estuary in Germany (Canfield and Thamdrup 1996). During this disproportionation appreciable isotope fractionation occurred with the sulfide 7–8 per mil depleted in ^{34}S relative to the elemental sulfur and the sulfate 14–16 per mil enriched in ^{34}S (Canfield and Thamdrup 1994). This observation was used to argue that the generally large ^{34}S depletions of up to 70 per mil in marine sedimentary pyrites could be generated from an initial fractionation by sulfate reducing bacteria of ~20–30 per mil, followed by multiple cycles of sulfide oxidation to elemental sulfur with subsequent disproportionation (Canfield and Thamdrup 1994; Canfield and Teske 1996). A role has also been suggested for thiosulfate disproportionation in influencing sediment sulfur isotope dynamics (Jørgensen 1990*b*).

Taken together, the disproportionation of sulfur compounds may play a significant role in sulfur cycling and sulfur isotope systematics in aquatic systems. Despite this, only limited information is available on the extent to which organisms conduct geochemically relevant processes such as isotope fractionation during disproportionation. In this study we consider isotope fractionation during the disproportionation of elemental sulfur. We explore most known pure bacterial cultures capable of this metabolism, as well as enrichments from widely separated parts of the ocean and from several freshwater sediments from northern Germany. We also consider rates and pathways of pyrite formation during the disproportionation of elemental sulfur. Pyrite has previously been identified as an important product during this metabolism (Thamdrup et al. 1993). In a related manuscript we explore isotope fractionations during the bacterial metabolism of sulfite and thiosulfate (Habicht et al. unpubl.).

Methods

Cultures—Desulfobulbus propionicus (DSM 2032) was obtained from Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany. Active cultures of *Desulfocapsa thiozymogenes* (strain Bra2; DSM 7269; Janssen et al. 1996) and elemental sulfur-disproportionating strain SB164P1 *Desulfocapsa sulfoexigens* (DSM 10523; Finster et al. 1998) were kindly provided by Peter Janssen and Kai Finster, respectively.

Inocula for freshwater enrichment cultures were obtained from surface sediments from a canal (Kuhgraben) and two ponds (Teich1 and Teich2) situated near the MPI for Marine Microbiology, Bremen. Marine inocula were obtained from the tidal flats of the Weser Estuary (Weddewarden) and Jadebusen (Dangast), German Waddensea, and from two stations in the anoxic basin of the Golfo Dulce on the Pacific coast of Costa Rica (S1 and S160).

For all pure and enrichment cultures, either a fresh- or saltwater version, as appropriate, of anoxic bicarbonate-buffered medium was prepared with the addition of vitamins and nonchelated trace metals according to Widdel and Bak (1991), but with the omission of sulfate. The bacteria were cultured in this medium in 50-ml screw-cap bottles with no headspace, and were supplemented with ~15 mmol flowers of sulfur (Fluka) and 1.5 mmol ferrihydrite (S/Fe medium; Thamdrup et al. 1993). As we were unable to maintain growth of *Db. propionicus* under these conditions, this organism was maintained on 20 mM propionate/20 mM sulfate and only transferred to S/Fe medium for the disproportionation experiments. During the reduction of sulfate with propionate by *Db. propionicus,* acetate is formed in stoichiometric amounts (Widdel and Pfennig 1982). Thus, some residual sulfate, propionate and acetate may have been transferred with these cultures to the S/Fe medium used for the disproportionation experiments.

Enrichment cultures were initially inoculated with 2% vol/vol of homogenized sediment. Both enrichment and pure cultures were transferred by inoculating fresh media with 5 ml of grown culture. Enrichment cultures were transferred at least 15 times before experiments began. Pure cultures were kept at their normal temperatures of cultivation which are 35°C for *Db. propionicus* and 30°C for *Dc. thiozymogenes* and *Dc. sulfoexigens*. The enrichments were kept at 28°C, except for the strains from Golfo Dulce, which were kept at 20°C. For the disproportionation experiments, 8 to 10 bottles with S/Fe medium were inoculated simultaneously and harvested as described below every 1 to 5 d depending on the rate of disproportionation. A duplicate incubation was performed on the enrichment from Dangast, and the two incubations are termed Dangast1 and Dangast2. This duplicate experiment is to be contrasted with Teich1 and Teich2, which represent different enrichments established from two ponds in same area.

Sampling—Incubations were terminated by rapidly mixing the contents of one screw-cap bottle with 20 ml of 10% wt/vol Zn acetate after first collecting 2 ml of sample for pH determination (against NBS buffers) and 2 ml for bacterial enumeration after fixing in 2.5% (final concn) glutaraldehyde. Bacterial numbers were counted by epifluorescence microscopy after dissolution of ferrihydrite and FeS (Thamdrup et al. 1993). The Zn acetate solution both poisoned the sample, inhibiting further bacterial metabolism, and fixed the free and acid-volatile solid-phase sulfides (mostly FeS) as ZnS. Fixed samples were stored frozen and later filtered through GF/F glass-fiber filters. Sulfate was precipitated from the filtrate solution as $BaSO_4$, which was collected by filtration and weighed for mass determination. This $BaSO_4$ was saved for isotope analysis. The solid collected by filtering the Zn acetate solution was dried, weighed, and a portion (usually about half) digested in 5 N HCl to liberate the acid-volatile sulfides, which were trapped as Ag_2S. The Ag_2S was dried, weighed, and used for isotope determination.

The solids remaining after acid digestion, mainly S° and pyrite, were collected on a glass-fiber filter and dried. The filter was cut in half, with one-half archived and the other half soxhlet extracted with acetone for 48 h to remove elemental sulfur, which was precipitated as Cu_2S on pure Cu shavings in the boiling flask of the soxhlet apparatus (Berner 1964). A portion of Cu_2S was digested in a hot acidic Cr^{2+} solution (Canfield et al. 1986) to release the sulfide, which was collected as Ag_2S and used for isotope determination. The sediment remaining after soxhlet extraction was washed free of any remaining elemental sulfur in CS_2 and rinsed

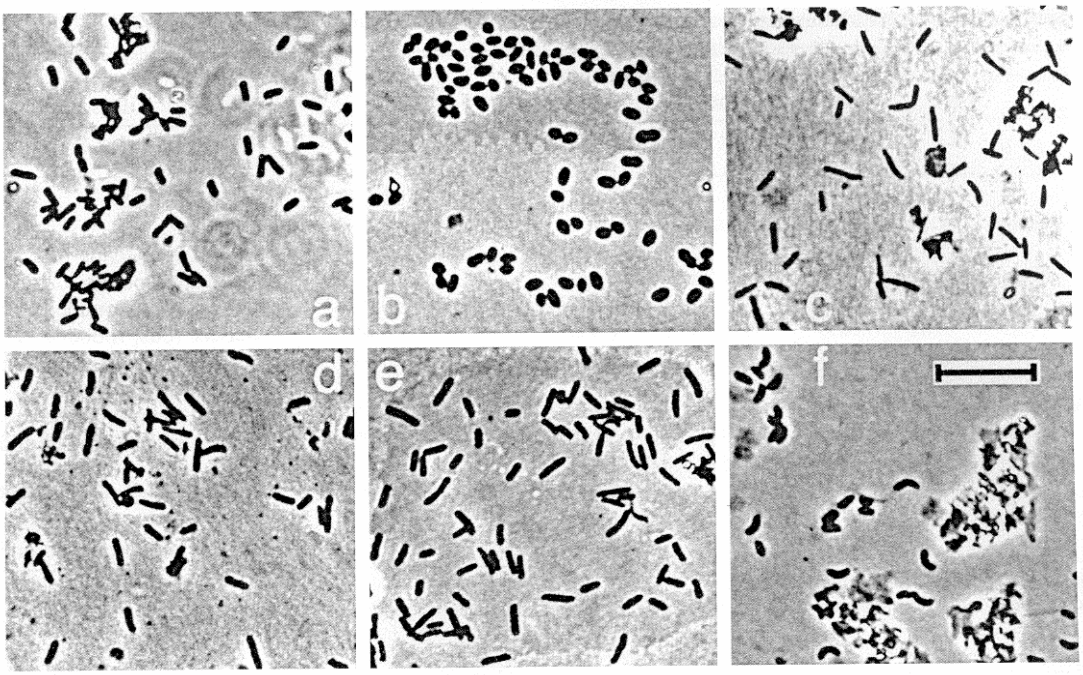

Fig. 1. Phase-contrast photomicrographs of cells from pure cultures (upper row) and dominating types from enrichment cultures (lower row), all from incubations with elemental sulfur and ferrihydrite. (a) *Dc. thiozymogenes,* (b) *Db. propionicus,* (c) *Dc. sulfoexigens,* (d) Teich2 (freshwater, also similar to Teich1 and Kuhgraben), (e) Wedderwarden (marine, also similar to Dangast and Golfo Dulce S1), (f) Golfo Dulce S160 (marine). Amorphous particles, particularly prominent in frames c and f, are sulfur and iron precipitates. Cell suspensions were prepared as for counting. Scale bar is 10 μm.

twice with acetone. This sediment was then digested in Cr^{2+} as above, with the liberated sulfide coming from pyrite formed during the incubation. This sulfide was collected as Ag_2S for mass and isotope determinations. The presence of pyrite was confirmed by visual observation (characteristic brassy color) and SEM observations. Pyrite formation in enrichment cultures conducting elemental sulfur disproportionation has previously been confirmed by X-ray diffraction (Thamdrup et al. 1993).

Samples of $BaSO_4$ and Ag_2S for stable-isotope analysis were ground with freshly prepared Cu_2O and converted to SO_2 at 1,150°C and 1,050°C, respectively. SO_2 gas was purified on a high-vacuum extraction line and collected in glass break-seal ampules for isotope analysis. Replicate samples were reproducible to $<\pm 0.3$ per mil. All isotope values are reported relative to CDT (Canyon Diablo Troilite). As a check on our chemical separation procedure and extraction-line performance, seawater sulfate has been routinely precipitated and analyzed with an average isotopic composition of 20.1 ± 0.2 per mil. No such isotope standard has been carried through the sulfide extraction procedure, although >30 determinations of the isotopic composition of elemental sulfur have yielded an average value of 16.1 ± 0.2 per mil, with no long-term trends.

Results

Bacterial cultures—From all sediment inoculations, enrichment cultures were established that grew by the disproportionation of elemental sulfur similar to the earlier description (Thamdrup et al. 1993). After numerous transfers, all of the enrichments grew rather homogeneous bacterial populations, although small amounts of morphologically distinct bacterial contamination were generally also found. The enrichments and the pure cultures *Dc. thiozymogenes* and *Dc. sulfoexigens* all developed similarly, whereas disproportionation by *Db. propionicus* was slow and was only observed after the first transfer from the sulfate/propionate medium. Sulfur-disproportionating bacteria formed a wide range of morphotypes (Fig. 1), with basic morphologic information, as well as freshwater or marine affinity for the pure and enrichment cultures, summarized in Table 1.

Experimental results—In all experiments the disproportionation of elemental sulfur was evidenced by the accumulation of both sulfide and sulfate. The sum of sulfur formed into pyrite and AVS gave sulfide : sulfate production ratios in the range of 1.2 : 1 to 2 : 1 (Table 2), consistent with previous results on elemental sulfur disproportionation in the presence of iron oxides (Thamdrup et al. 1993; Canfield and Thamdrup 1996). Pyrite generally began to form after 5–15 d of incubation, which was equivalent to the point where ~200 μmol of sulfate had formed (Fig. 2a). Sulfate concentration rather than time is chosen here as the progress variable owing to different rates of disproportionation in the different cultures. In some cases pyrite formation was quite significant, and dominated the reduced sulfur pool by the end of the incubation. A pH drop always accompanied elemental sulfur disproportionation (Fig. 2b), consistent with reaction stoichiometry and previous observations (Thamdrup et al. 1993; Canfield and Thamdrup 1996).

Bacterial growth was evident during the initial 2–10 d of

Table 1. Description of elemental sulfur-disproportionating bacteria from pure cultures and enrichments.

Culture*	Pure	Fresh	Marine	Length (μM)	Width (μM)	Description
Desulfocapsa thiozymogenes	X	X		17.3±0.28	0.63±0.1	Fat rod, rounded end
Desulfobulbus propionicus	X	X	†	1.64±0.23	1.08±0.05	Lemon-shaped
Desulfocapsa sulfoexigens	X		X	3.07±0.87	0.5±0.0	Slender rods, ends like truncated points
Dangast			X	2.45±0.42	0.55±0.05	Slender rods, rounded ends
Weddewarden			X	2.70±0.38	0.63±0.06	Slender rods, rounded ends
Golfo Dulce S1			X	2.78±0.28	0.62±0.07	Irregular slender rods, rounded ends, some vibroid
Golfo Dulce S160			X	1.95±0.26	0.88±0.11	Vibroid, length is line measurement
Teich 1st enrichment		X		2.3±0.46	0.61±0.08	Slender rods, rounded ends
Teich 2nd enrichment		X		2.3±0.64	0.64±0.06	Slender rods, rounded ends
Kuhgraben		X		2.60±0.27	0.58±0.04	Slender rods, rounded ends

* The dominant type is described for enrichment cultures.
† Marine pure cultures are in cultivation (Widdel and Pfennig 1982), but have not been tested here.

incubation, with population sizes generally increasing about one order of magnitude from ~1–3 × 10^6 to 1–2 × 10^7 cells ml^{-1} (Table 2). Cell doubling times were on the order of 1 d, although they were somewhat slower for S1 and S160, which can be attributed to their lower incubation temperature of 20°C. We observed slow growth during the initial 5 d of incubation with *Db. propionicus,* although we could not maintain the culture through successive transfers with elemental sulfur. Very slow growth of this organism during elemental sulfur disproportionation was previously reported by Lovely and Phillips (1994). We speculate that in our experiments the initial growth of *Db. propionicus* need not have been coupled to the autotrophic disproportionation of elemental sulfur as for the other cultures. Thus, some residual sulfate and propionate may have been transferred from the growth medium for this organism to the experimental incubation bottles, potentially fueling some sulfate reduction. Also, acetate, which was certainly transferred from the propionate/sulfate medium to the experimental bottles, is utilized for growth by *Db. propionicus* in some circumstances (Widdel and Pfennig 1982), although it is not known if this occurs during the disproportionation of elemental sulfur.

Table 2. Production ratios of sulfate to sulfide, specific rates of elemental sulfur disproportionation, and final cell counts.

Culture	Prod. ratio sulfate : sulfide	Spec. rate (μmol S $cell^{-1}$ d^{-1})	Final cell count* (cells ml^{-1})
Desulfocapsa thiozymogenes	1.5	2.9×10^{-7}	0.7×10^{7}
Desulfobulbus propionicus	2.0	2.2×10^{-8}	1.3×10^{7}
Desulfocapsa sulfoexigens	1.5	9.6×10^{-8}	1.3×10^{7}
Dangast1	2.0	8.7×10^{-8}	2.0×10^{7}
Dangast2	1.9	1.0×10^{-7}	1.5×10^{7}
Weddewarden	1.4	9.1×10^{-8}	2.0×10^{7}
Golfo Dulce S1	2.0	4.1×10^{-8}	2.2×10^{7}
Golfo Dulce S160	1.2	5.2×10^{-8}	1.2×10^{7}
Teich 1st enrichment	1.7	9.1×10^{-8}	1.2×10^{7}
Teich 2nd enrichment	1.8	1.0×10^{-7}	1.5×10^{7}
Kuhgraben	2.1	1.3×10^{-7}	1.2×10^{7}

* Initial counts were ~2 × 10^6 cells ml^{-1}.

In most cases considerable sulfur disproportionation continued after growth had ceased, with rates of disproportionation showing no appreciable differences during and after active cell growth. Our added Fe oxides exceeded by at least a factor of three the maximum amounts of sulfide produced during the experiments, and thus precluded the inhibitory effect of sulfide accumulation on disproportionation rate as previously reported (Thamdrup et al. 1993). This is because reactive Fe oxides as ferrihydrite actively scavenge sulfide to very low concentrations (Canfield et al. 1992). Specific rates of elemental sulfur disproportionation of between 2.2 × 10^{-8} and 1.3 × 10^{-7} μmol $cell^{-1}$ d^{-1} are calculated (Table 2) for the stationary phase of growth by combining bacterial population sizes (Table 2) with the average production rates of sulfate plus sulfide (including pyrite-S).

Stable sulfur isotopes—In all cases isotope fractionation accompanied elemental sulfur disproportionation. For sulfate, fractionations frequently increased during the course of the experiment, whereas for sulfide this was not observed (Table 3, Fig. 3). It is possible that isotopically distinct sulfate was delivered with the original sediment inoculum. We measured the isotopic composition of sulfate carried with the inoculum for the experiments with *Db. propionicus.* From this result we are able to explain the temporal trend in sulfate isotopic composition by the dilution of the rather ^{34}S-depleted sulfate ($\delta^{34}S$ of 4.5 per mil), carried with the inoculum, with much more ^{34}S-enriched sulfate produced during

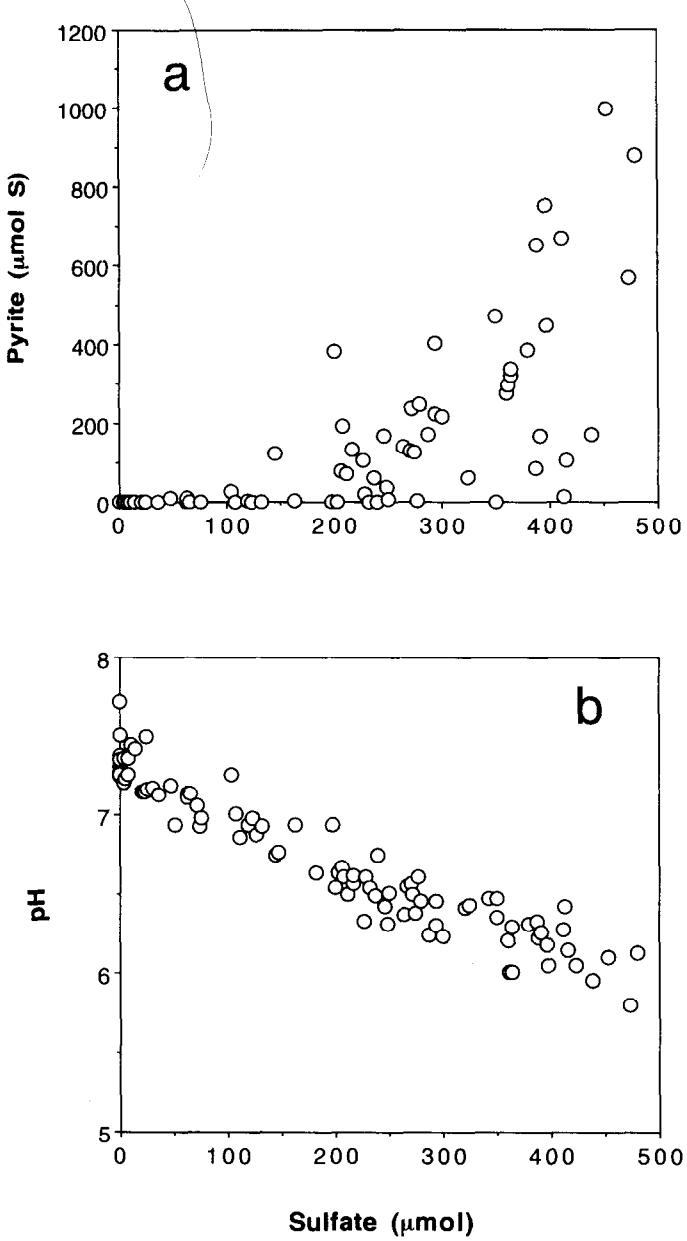

Fig. 2. (a) Amount of pyrite sulfur accumulated into the experimental bottles vs. the amount of sulfate accumulated. Sulfate is used here as a master progress variable. Results from all of the incubations are combined. All pyrite and sulfate values have been corrected for the amount transferred in the initial inoculation of the bottles. (b) Solution pH is shown vs. sulfate accumulation. Results from all of the experiments are combined.

the disproportionation experiment (Fig. 3). The case for *Db. propionicus*, however, is probably extreme, as this organism was transferred from a media amended with sulfate and not from flasks where sulfate had accumulated naturally from the disproportionation of elemental sulfur, as for the other cultures. Unfortunately, we have not measured the isotopic composition of sulfate delivered with the inoculum in any of the other experiments.

The temporal trends in the isotopic composition of sulfate could also reflect real changes in fractionation during the

Table 3. Isotope fractionations (per mil) during elemental sulfur disproportionation.

Culture	$\Delta_{AVS\text{-}S^{=}}$ measured	$\Delta_{SO_4\text{-}S^{=}}$ measured	R_{frac}*	$\Delta_{AVS\text{-}S^{=}}$ cellular†	$\Delta_{SO_4\text{-}S^{=}}$ cellular
Desulfocapsa thiozymogenes	−5.9‡	17.3	−2.93	−5.8	17.4
Desulfobulbus propionicus	−15.5	30.9	−1.99	−11.3	33.9
Desulfocapsa sulfoexigens	−5.8	16.0	−2.76	−5.5	16.4
Dangast1	−6.6	18.2	−2.76	−6.2	18.7
Dangast2	−6.5	16.7	−2.57	−5.8	17.4
Weddewarden	−6.2	17.9	−2.89	−6.0	18.1
Golfo Dulce S1	−7.9	19.7	−2.49	−6.9	20.6
Golfo Dulce S160	−8.0	17.1	−2.14	−6.2	18.5
Teich 1st enrichment	−6.4	16.4	−2.56	−5.7	17.1
Teich 2nd enrichment	−6.2	22.0	−3.55	−6.2	18.6§
Kuhgraben	−7.0	19.9	−2.84	−6.7	20.2
Average¶	−6.6	18.1	−2.78	−6.3	18.8

* Fractionations calculated at end of experiment. For *Db. propionicus*, a correction was made based on the measured initial sulfate concentration and isotopic composition.

† $R_{frac} = \Delta^{34}S_{SO_4\text{-}S^{=}}/\Delta^{34}S_{AVS\text{-}S^{=}}$.

‡ Cellular-level fractionations are those in the absence of any oxidation by reaction of sulfide with Fe-oxides (*see text*).

§ Calculated from $\Delta^{34}S_{AVS\text{-}S^{=}}$ cellular, assuming R_{frac} of 3. Note that a larger R_{frac} is indicated by the data.

¶ Calculated without *Db. propionicus*.

experiment. One would expect, however, that similar trends in the isotopic composition of acid-volatile sulfur (AVS) would also be observed. This was not the case (Fig. 3). In our opinion isotope values measured at the end of the experiment, particularly for sulfate, give the best representation of the fractionations during the experiment. This is because dilution of any sulfate delivered during the initial transfer of the bacterial culture to the experimental flasks is at a maximum. We cannot comment further on the possibility of fractionation changes during the experiment. In Table 3 we have listed fractionations calculated from the isotopic compositions of sulfate and AVS measured at the end of the experiments relative to an average $\delta^{34}S$ for elemental sulfur of 16.1 per mil, which did not vary appreciably during or between experiments. For *Db. propionicus* we have corrected the fractionation value into sulfate for the contribution of sulfate delivered during the initial culture transfer.

For all of the enrichment cultures, as well as the pure cultures *Dc. sulfoexigens* and *Dc. thiozymogenes*, sulfide was formed 5–8 per mil depleted in ^{34}S compared to the elemental sulfur, while the sulfate was 14–22 per mil enriched in ^{34}S (Fig. 3, Table 3). These fractionations are similar to those reported earlier by Canfield and Thamdrup (1994) for marine enrichments from Weddewarden, Germany, and Århus Bay, Denmark, as well as for sulfur-amended Weddewarden sediment (Canfield and Thamdrup 1994). Results for *Db. propionicus* were exceptional in that fractionations were more than twice as great as in any of the other experiments or in any earlier reported values (Fig. 3, Table 3).

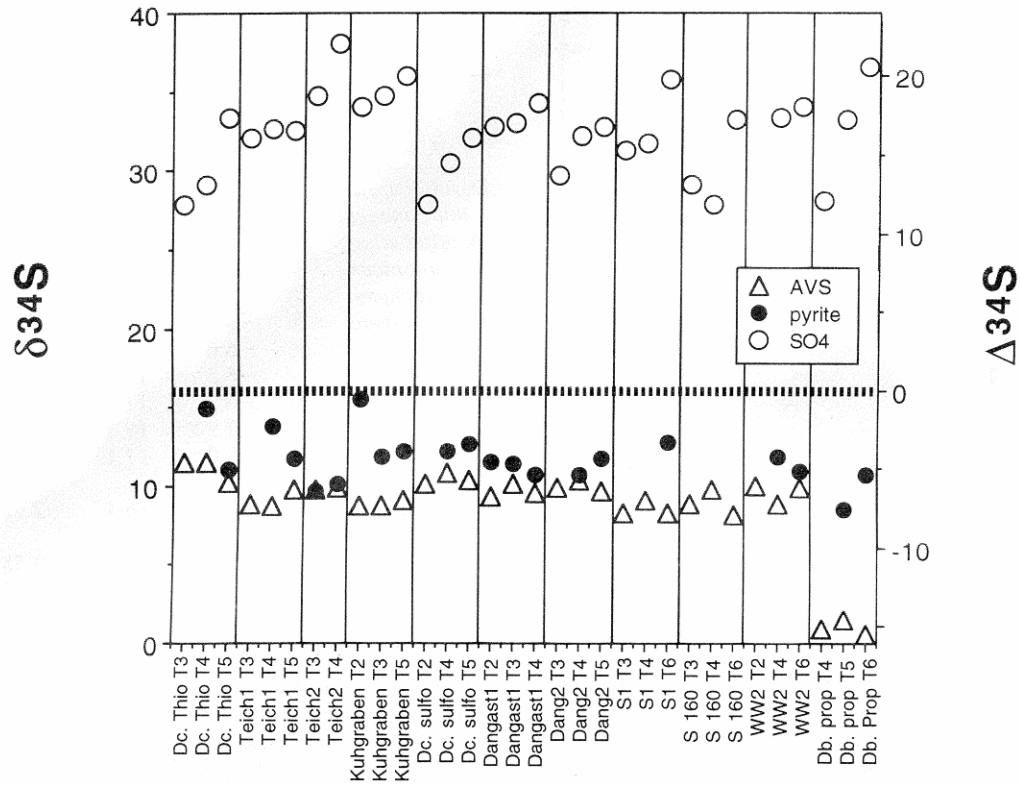

Fig. 3. Isotopic compositions of sulfate, acid-volatile sulfur, and pyrite within the experimental bottles. Analyses are arranged consecutively with time; numbers refer to the sampling order; for example, T3 was the third sampling of the bottles. The bold dashed line represents the average isotopic composition of elemental sulfur added to the bottles. Also provided is a secondary scale expressing isotopic compositions relative to the isotopic composition of elemental sulfur.

The isotopic composition of pyrite was variable, with values ranging between those measured for AVS and about halfway between the AVS values and those for elemental sulfur (Fig. 3). In a few cases the isotopic composition of pyrite was nearly the same as for the elemental sulfur, which could represent contamination by elemental sulfur due to incomplete removal during our extraction procedure.

Discussion

Bacterial classification—At least three types of organisms found in nature conduct the disproportionation of elemental sulfur. These are distinguished by either inhabiting unique environmental niches or by using unique strategies for employing elemental sulfur disproportionation. The first type of organism is represented by *Dc. sulfoexigens*, which was isolated using elemental sulfur, the same as we have done to obtain our enrichment cultures. This organism grows autotrophically by disproportionating the inorganic sulfur intermediates sulfite, thiosulfate, and elemental sulfur (Finster et al. 1998). Furthermore, the organism has only a limited ability to reduce sulfate heterotrophically and cannot grow by this process—it is also an obligate anaerobe.

The second type of elemental sulfur-disproportionating bacteria is represented by *Dc. thiozymogenes* (strain Bra2; Bak 1993; Janssen et al. 1996). This organism grows both by the autotrophic disproportionation of the sulfur intermediates thiosulfate, sulfite, and elemental sulfur, as well as by coupling sulfate reduction to the oxidation of ethanol, propanol, and butanol to the corresponding fatty acid (Janssen et al. 1996). *Dc. thiozymogenes* may thus survive by both heterotrophic and autotrophic metabolisms—it is also a strict anaerobe.

The third type of organism is represented by *Db. propionicus* that was isolated originally, and is known primarily, as a sulfate reducer (Widdel and Pfennig 1982). This organism conducts elemental sulfur disproportionation as well as thiosulfate disproportionation (Lovley and Phillips 1994; Krämer and Cypionka 1989). In an interesting new result, *Db. propionicus* and four other strains of oxygen-tolerant sulfate-reducing bacteria have been found to disproportionate the elemental sulfur they produce during the oxidation of sulfide with oxygen and nitrate (Fuseler and Cypionka 1995; Fuseler et al. 1996). In fact, the disproportionation of elemental sulfur is argued to be the terminal sulfate-forming step during sulfide oxidation by these bacteria (Fuseler et al. 1996). Some of the strains, although probably not *Db. propionicus,* may actually disproportionate elemental sulfur under microaerobic conditions (Fuseler et al. 1996). By contrast with the type 1 and type 2 organisms described above, these sulfate reducers grow only poorly, if at all, by the disproportionation of elemental sulfur (Lovley and Phillips

Table 4. Types of elemental sulfur-disproportionating bacteria.

	Representatives	Growth?	Sulfate reducer?	Oxygen tolerance
Type 1	*Desulfocapsa sulfoexigens*	Y	N	Strict anaerobe
Type 2	*Desulfocapsa thiozymogenes*	Y	Y	Strict anaerobe
Type 3	*Desulfobulbus propionicus*	N*	Y	Microaerobe†
Type 3	Alkalphilic strain Z-7935	N	Y	Microaerobe
Type 3	Strain P1B	N	Y	Microaerobe
Type 3	*Desulfovibrio desulfuricans* CSN	N	Y	Microaerobe
Type 3	*Desulfovibrio desulfuricans* Essex 6	N	Y	Microaerobe

* Poor growth by *Db. propionicus* and no growth by other microaerophilic sulfate reducers (*see text*).

† *Db. propionicus* and strain Z-7935 can withstand oxygen but seem to disproportionate elemental sulfur in the absence of oxygen, whereas strain P1B and *D. desulfuricans* CSN and *D. desulfuricans* Essex 6 begin to disproportionate under microaerobic conditions (Fuseler et al. 1996).

1994; Janssen et al. 1996; H. Cypionka pers. comm.; this study). The disproportionation of elemental sulfur cannot therefore be their primary metabolism. Tentatively, *Db. propionicus* and the oxygen-tolerant strains of sulfate reducers explored by Fuseler et al. (1996) are classified together into type 3 elemental sulfur disproportionators.

The main distinguishing characteristics of the three types of elemental sulfur-disproportionating bacteria are summarized in Table 4. Overall, the strictly anaerobic bacteria, represented by types 1 and 2, are best suited to utilize the disproportionation of elemental sulfur as a primary metabolism, due to their growth during the process. Although not coupled to growth, at least some of the type 3 bacteria could metabolize the elemental sulfur produced under microaerobic conditions at sulfide–oxygen interfaces. Hence, although type 3 organisms cannot conduct elemental sulfur disproportionation as a primary metabolism, they expand to very geochemically and biologically active areas the possible environments where the bacterial disproportionation of elemental sulfur can occur.

Enrichment cultures—We enriched bacteria from a large number of environments, and from geographically distant areas not knowing whether we would obtain known organisms or possibly find new bacteria capable of elemental sulfur disproportionation. Because our marine enrichment medium was the same as that used to obtain *Dc. sulfoexigens* (type 1; Table 4), and because all of our enrichments grew by elemental sulfur disproportionation, we expected the best chance of morphotypic and phenotypic similarity between our enrichments and *Dc. sulfoexigens* and *Dc. thiozymogenes* (types 1 and 2; Table 4).

In general, our enrichments can be divided into a few different morphotypes. The most common is a slender rod 2–2.8 μm long and ~0.6 μm wide, with hemispherically rounded ends. These were found in the freshwater enrichments Teich1, Teich2, and Kuhgraben, and in the marine enrichments from Weddewarden, Dangast, and Golfo Dulce S1. All of these were morphologically similar, although cells in the Teich1 enrichment were a bit shorter than the others (Table 1). The shortest cells in Teich1 were similar to the longest ones of *Dc. thiozymogenes,* but average lengths were significantly different ($P < 0.05$, *t*-test). Thus, the freshwater enrichments appear to hold organisms different from the pure cultures. The marine enrichments Dangast, Weddewarden, and Golfo Dulce S1 may further be distinguished from *Dc. thiozymogenes* as the latter is saltwater-sensitive (Janssen et al. 1996). The rod ends of the marine enrichments were also different from those of the marine isolate *Dc. sulfoexigens*, which is more like a truncated point than a rounded end. Thus, these enrichments probably represent new organisms.

The vibrioid from Golfo Dulce S160 is unlike any of the other enrichments or pure cultures, and surely represents a unique organism. None of the enrichments bear any resemblance to *Db. propionicus,* so that this sulfate reducer is not enriched with our protocol. In summary, none of the enrichments are likely candidates for any of the pure cultures explored in our study. Thus, from these enrichments there is likely represented several new organisms capable of elemental sulfur disproportionation. Owing to their active growth during elemental sulfur disproportionation, each of these enrichments likely represents either a type 1 or type 2 bacterium as outlined in Table 4. We have yet to isolate any of these enrichments into pure culture and to conduct a broader survey of their physiological capabilities.

Sulfur metabolism—The disproportionation of elemental sulfur was the main sulfur metabolism for each of the pure and enrichment cultures in our S/Fe medium. This is evidenced by an increase in both sulfate and sulfide during the experiments (Table 2). Furthermore, except possibly for *Db. propionicus,* there were no organic substrates in the cultures to fuel heterotrophic metabolism such as sulfate reduction or elemental sulfur reduction. Thamdrup et al. (1993) have shown that the theoretical sulfide-to-sulfate ratio produced during elemental sulfur disproportionation in the presence of reactive iron oxides is 2 : 1 (Eq. 4). This stoichiometry arises from an initial 3 : 1 sulfide-to-sulfate production ratio (Eq. 1), followed by the reoxidation of some sulfide back to elemental sulfur during the reduction of iron oxides (Eq. 2) and precipitation of sulfide as FeS (Eq. 3):

$$4H_2O + 4S^\circ \rightarrow 3H_2S + SO_4^{2-} + 2H^+ \quad (1)$$

$$4H^+ + H_2S + 2FeOOH \rightarrow 2Fe^{2+} + S^\circ + 4H_2O \quad (2)$$

$$2H_2S + 2Fe^{2+} \rightarrow 2FeS + 4H^+ \quad (3)$$

$$3S^\circ + 2FeOOH \rightarrow SO_4^{2-} + 2FeS + 2H^+ \quad (4)$$

Somewhat lower stoichiometries of between 1.75 : 1 and 1.5 : 1 were found by Canfield and Thamdrup (1996) for the disproportionation of sulfur added to Weddewarden sediment, as well as for cultures enriched from this sediment. These lower-than-expected sulfide : sulfate production ratios were argued to result from the incorporation of reducing equivalents into

carbon as growing biomass. It is also possible that some AVS was oxidized in the presence of iron oxides during our acid distillation (Berner 1964), thereby lowering our measured sulfide : sulfate production ratios in some cases.

In the present study, sulfide : sulfate production ratios ranged from 1.2 : 1 to 2.1 : 1, with an average of 1.74 : 1 (Table 2). Both bacterial growth and experimental artifacts are possible explanations for our production ratios that fall below 2 : 1, though our results do not let us distinguish between these two possibilities. An experimental artifact is the only explanation we can offer for the exceptionally low 1.2 : 1 production ratio for Golfo Dulce S160, as there was no evidence for higher rates of growth compared to the other experiments.

Stable isotopes—Except for *Db. propionicus,* the fractionations accompanying elemental sulfur disproportionation were remarkably similar for all marine and freshwater enrichments, as well as for the pure cultures *Dc. thiozymogenes* and *Dc. sulfoexigens* (Fig. 3, Table 3). Fractionations during the disproportionation of elemental sulfur by *Db. propionicus* are clearly of a different magnitude than the others and must be considered unique. The pathways and biochemistries of sulfur metabolism during elemental sulfur disproportionation are not known, and hence neither are the cellular-level processes leading to the fractionations observed here. Krämer and Cypionka (1989), however, have concluded from enzyme identification and inhibitor studies that many of the same enzymes involved in sulfate reduction are also active in the disproportionation of the sulfur intermediates thiosulfate and sulfite. All of the pure cultures explored here can disproportionate thiosulfate, while *Dc. thiozymogenes and Dc. sulfoexigens* can also disproportionate sulfite (Krämer and Cypionka 1989; Bak 1993; Janssen et al. 1996; Finster et al. 1998). Also, two of the pure cultures can reduce sulfate (Table 3). Thus, the pure culture bacteria explored here, as well as the process of elemental sulfur disproportionation, may also share common enzymes with sulfate reduction. However, both a true model of isotope fractionation during elemental sulfur disproportionation and an explanation for the large fractionations from *Db. propionicus* await investigations into the biochemistry of the process.

One aspect of isotope fractionation during the disproportionation of elemental sulfur operates independently of bacterial enzymatics. With no fractionation during the cellular uptake of elemental sulfur, the 3 : 1 production stoichiometry (Eq. 1) of sulfide to sulfate should, by isotope mass balance, produce a 3 : 1 isotopic difference in the measured fractionations between sulfate and elemental sulfur and between sulfide and elemental sulfur. We define this isotope difference as R_{frac}, and note that isotope differences are relative to the initial isotopic composition of the added elemental sulfur. During the time-course experiments conducted here the elemental sulfur pool maintained this initial value. Owing to the opposite direction of the fractionations into sulfate and sulfide, R_{frac} carries a negative sign, which will be dropped in subsequent discussion:

$$R_{frac} = \Delta^{34}S_{SO_4\text{-}S^0}/\Delta^{34}S_{AVS\text{-}S^0} \quad (5)$$

In the presence of reactive iron oxides, as in these experiments, approximately one-third of the sulfide formed during elemental sulfur disproportionation is converted back to elemental sulfur (Eq. 2). Thus, the production ratio of sulfide to sulfate becomes lower than 3 : 1, and should fall to ~2 : 1.

The consequences of this for isotope fractionation depend on the immediate fate of the elemental sulfur re-formed by the reaction of H_2S with the Fe-oxides (ferrihydrite) (Eq. 2). This re-formed sulfur will be isotopically depleted in ^{34}S compared to the original starting elemental sulfur, and if it is preferentially disproportionated, the product sulfide will become further depleted in ^{34}S and the sulfate will become less ^{34}S-enriched. Preferential disproportionation might be expected if the re-formed elemental sulfur is more "reactive" than the original sulfur. This could be the case in some of our experiments as re-formed sulfur is likely of a finer grain size than the rather coarse-grained laboratory-grade flowers of sulfur we utilized. If the re-formed elemental sulfur is completely disproportionated during the course of the experiment, then R_{frac} will be reduced to the net sulfide : sulfate production ratio of ~2 : 1. This ratio could be somewhat lower if the initial production ratio of sulfide to sulfate was <3 : 1 due to, for example, the channeling of reducing equivalents into cell biomass as discussed in Canfield and Thamdrup (1996).

By contrast, if the iron-oxide-produced elemental sulfur is disproportionated at a rate equivalent to or slower than the rate for the original flowers of sulfur, then the re-formed sulfur will accumulate. The impact of this accumulating, re-formed elemental sulfur on measured isotope fractionations depends on how much of it actually accumulates and how much is re-disproportionated. In the present experiments only a small proportion of the originally added flowers of sulfur was disproportionated (<10%). If re-formed sulfur is utilized only slowly, then it will become diluted into a large preexisting sulfur pool. Only a small amount of re-formed sulfur will therefore be disproportionated, producing a minimal impact on the isotopic composition of the AVS and the sulfate that accumulate. Re-formed elemental sulfur will only affect the measured isotopic compositions of sulfate and AVS if a large portion of it is re-disproportionated. Thus, with minimal disproportionation of re-formed sulfur, R_{frac} (Eq. 5) will remain close to 3 : 1, even though the production ratio of sulfate to sulfate may be closer to 2 : 1.

In the present series of experiments R_{frac} varied between 2 : 1 to 3 : 1 (Table 3). The error associated with any given R_{frac} determination is about ±0.15, considering the precision of individual isotope determinations (±0.3 per mil). The measured range of R_{frac} values encompasses the range of possibilities outlined above (Table 3) and the range that is likely to be encountered in nature. There is a tendency for lower ratios to correspond with cultures metabolizing at low specific rates of elemental sulfur disproportionation, and for the ratio to increase at higher rates (Fig. 4). This implies that at high specific rates of disproportionation the organisms are less discriminate of their sulfur source than at lower rates. Although this explanation seems reasonable and is consistent with the isotope results, it is difficult to prove or otherwise substantiate with the present experiments.

If it is assumed that deviations of R_{frac} from 3 : 1 result from the extent to which re-formed elemental sulfur is utilized during continued disproportionation, and not from deviations in the original production stoichiometry, then the

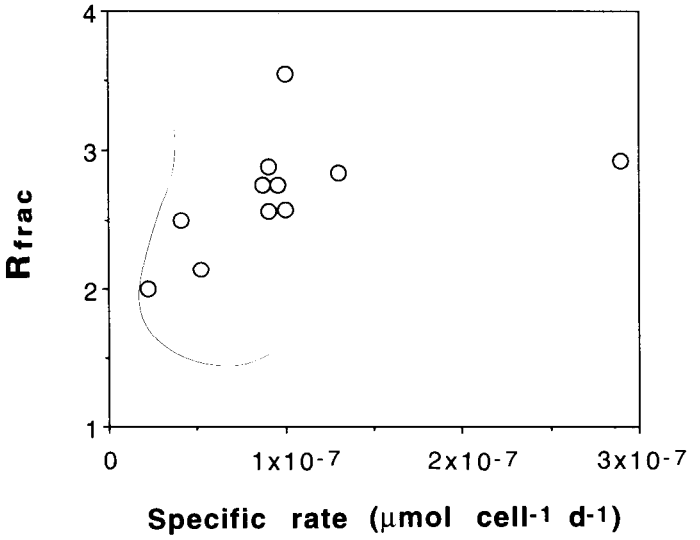

Fig. 4. Ratio of isotope fractionations into sulfate and acid-volatile sulfur (R_{frac}) are compared to the specific rate of elemental sulfur disproportionation during the stationary phase of cell growth. *See* text for details.

cellular-level fractionations can be calculated. Cellular-level fractionations are those encountered in the absence of any H_2S reoxidation by Fe oxides; they are also the fractionations that enzyme models should seek to reproduce. To determine cellular-level fractionations, numerical experiments were performed where repeated cycles of disproportionation (Eq. 1) were followed by sulfide reaction with Fe oxides (Eq. 2), producing re-formed elemental sulfur that was further disproportionated. We assumed that the original production stoichiometry of sulfide to sulfate was 3 : 1. By varying the amount of re-formed elemental sulfur that was further disproportionated, a full range of R_{frac} values of between 2 : 1 and 3 : 1 was reproduced. These numerical results provided relationships between R_{frac} and the ratio of measured to cellular-level fractionations for both sulfate and sulfide, R_{SO_4} and R_{AVS}:

$$R_{SO_4} = \Delta^{34}S_{SO_4\text{-}S°m}/\Delta^{34}S_{SO_4\text{-}S°c} \quad (6)$$

$$R_{AVS} = \Delta^{34}S_{AVS\text{-}S°m}/\Delta^{34}S_{AVS\text{-}S°c}, \quad (7)$$

with the subscript m denoting measured fractionations and c representing fractionations at the cellular level. The following function was fit to the model results to obtain a relationship between R_{frac} and R_{AVS} ($R^2 = 1.000$):

$$R_{AVS} = 2.58 + 0.62R_{frac} - 0.0451(R_{frac})^2 - 0.0251(R_{frac})^3. \quad (8)$$

To determine cellular level fractionations, Eq. 8 was supplied with R_{frac} values from Table 3. This produced R_{AVS} from which $\Delta^{34}S_{AVS\text{-}S°c}$ could be calculated with the measured values of $\Delta^{34}S_{AVS\text{-}S°m}$ (Eq. 6), also taken from Table 3. The cellular-level fractionation for sulfate, $\Delta^{34}S_{SO_4\text{-}S°c}$, was taken as 3 times the $\Delta^{34}S_{AVS\text{-}S°c}$ value. Calculation results are summarized in Table 3 and demonstrate a sizable correction compared to measured fractionations in some cases.

Overall, fractionations at the cellular level show a remarkably small range (in the absence of *Db. propionicus*), with values from −5.5 to −6.9 per mil for $\Delta^{34}S_{AVS\text{-}S°c}$ and 16.4 to 20.6 per mil for $\Delta^{34}S_{SO_4\text{-}S°c}$. This consistency holds for different organisms from widely different environments, geographic areas, and with different overall physiological capabilities (Table 4). It may be assumed that the cellular level fractionations reported here will generally hold for bacteria of types 1 and 2 as outlined in Table 4. It is also reasonable that such similarity in fractionations underlies a conserved biochemistry for the disproportionation process among these organisms, although nothing is yet known of this biochemistry.

By contrast, a constancy in isotope fractionation is not observed among sulfate reducers, where fractionation is highly dependent on sulfate concentration and cell metabolic rate (e.g. Kaplan and Rittenberg 1964). Both of these factors influence the extent to which sulfate may freely exchange across the cell membrane, and ultimately the extent to which the enzyme-level fractionations that occur during the sulfate reduction process may be expressed (e.g. Rees 1973). We observed no large range in fractionations during elemental sulfur disproportionation by bacteria of types 1 and 2 and no relationship between fractionation and specific rates of disproportionation. Thus, fractionation is apparently not influenced by transport of sulfur species across the cell membrane as for sulfate reducers.

There is also no preferential uptake of either ^{34}S or ^{32}S elemental sulfur during the disproportionation process. This is apparent from the results of Canfield and Thamdrup (1994), where the isotopic composition of elemental sulfur remained the same despite the utilization of over one-half of the original sulfur. Thus, Rayleigh distillation effects need not influence fractionations during the disproportionation of elemental sulfur. The accumulation of re-formed sulfur could cause changes in the isotopic composition of elemental sulfur during a time-course experiment (although this was not observed in the present experiments). Still, all scenarios of re-formed sulfur accumulation explored here produce R_{frac} values somewhere between 2 : 1 and 3 : 1, as discussed above.

The fractionations accompanying elemental sulfur disproportionation by *Db. propionicus* are nearly double those of the others. No systematics between specific disproportionation rate and fractionation, or any other obvious variable such as temperature, can explain these high fractionations. We must conclude that different cellular-level processes, perhaps biochemical pathways, account for these differences. It will be interesting to see if any of the other sulfate reducers grouped as type 3 with *Db. propionicus* also produce large fractionations during elemental sulfur disproportionation.

Formation of pyrite—Pyrite was an important product in nearly all of the pure and enrichment cultures explored (Fig. 2). In general, pyrite formation did not occur from the beginning of the experiments, but only after some initial amount of elemental sulfur disproportionation. The formation of pyrite was in some cases quite rapid at rates of up to 2.0×10^{-3} mol liter^{-1} d^{-1} (Kuhgraben, Teich2), with more typical rates in the range of $0.5–1.0 \times 10^{-3}$ mol liter^{-1} d^{-1} (Table 5).

Two pathways of pyrite formation have been advocated for marine sediments, both of which may potentially operate

Table 5. Pyrite formation mechanisms and rates.

Culture	Apparent form. mech.	Form. rate, meas. (mol liter^{-1} d^{-1})	FeS (mol liter^{-1})	Form. rate, calc. (mol liter^{-1} d^{-1})	Meas./ calc.
Desulfocapsa thiozymogenes	H_2S*?	6.7×10^{-4}	4×10^{-3}	3.6×10^{-8}	1.9×10^{4}
Desulfobulbus propionicus	S°†	4.0×10^{-4}	2×10^{-3}	1.8×10^{-8}	2.2×10^{4}
Desulfocapsa sulfoexigens	H_2S/S°‡	5.8×10^{-4}	4×10^{-3}	3.6×10^{-8}	1.6×10^{4}
Dangast1	H_2S/S°	1.0×10^{-3}	5×10^{-3}	4.5×10^{-8}	2.2×10^{4}
Dangast2	H_2S/S°	6.7×10^{-4}	3×10^{-3}	2.7×10^{-8}	2.5×10^{4}
Weddewarden	H_2S/S°	6.3×10^{-4}	3×10^{-3}	2.7×10^{-8}	2.4×10^{4}
Golfo Dulce S1	S°	2.0×10^{-4}	6×10^{-3}	5.3×10^{-8}	3.7×10^{3}
Golfo Dulce S160	N.P.§	N.P.	N.P.	N.P.	N.P.
Teich 1st enrichment	S°?	1.2×10^{-3}	4×10^{-3}	3.6×10^{-8}	3.3×10^{4}
Teich 2nd enrichment	H_2S	2.0×10^{-3}	3×10^{-3}	2.7×10^{-8}	7.5×10^{4}
Kuhgraben	S°	1.6×10^{-3}	3×10^{-3}	2.7×10^{-8}	6.0×10^{4}

* Pyrite formation by H_2S addition (Eq. 8); ? implies lack of certainty.
† Pyrite formation by S° addition (Eq. 7).
‡ Pyrite formation simultaneously by both mechanisms.
§ No measurable pyrite was formed.

here. In the first, elemental sulfur is added, possibly through polysulfide intermediates, to FeS in the presence of H_2S (Eq. 9; Berner 1970; Rickard 1975; Luther 1991). In the second, H_2S acts as an oxidizing agent, oxidizing the sulfur in FeS to the oxidation level of sulfur in pyrite, and producing H_2 as the reduced product (Rickard 1997; Eq. 10).

$$FeS + S^\circ \rightarrow FeS_2 \quad (9)$$

$$FeS + H_2S \rightarrow FeS_2 + H_2. \quad (10)$$

Our isotope results allow us to explore the relative importance of these processes in the different experiments. To do this, we assume that during pyrite formation elemental sulfur was added to FeS with its bulk average isotopic composition, and that H_2S was added to FeS to form pyrite with the same isotopic composition as AVS. Thus, for Eq. 9 to dominate, pyrite should form from 1 AVS and 1 S°, with a final isotopic composition halfway between the two. If the reaction in Eq. 10 is most important, pyrite should have the same isotopic composition as AVS. To use isotopes to deduce reaction pathways, as outlined here, we must assume that minimal fractionation occurs during the formation of pyrite by reactions expressed in both Eq. 9 and 10. This problem has not been explored in detail, although Wilkin and Barnes (1996) observed minimal isotope difference between H_2S and pyrite when pyrite formed, apparently by a pathway analogous to Eq. 10. Also, when Sweeney and Kaplan (1973) aged FeS with elemental sulfur (Eq. 9), they formed pyrite with an isotopic composition intermediate between the two reacting sulfur species.

A potential complication in using the above scheme to deduce reaction pathways is isotope exchange that is known to occur between H_2S, elemental sulfur, and AVS. Rapid exchange occurs when dissolved sulfide accumulates into solution (Fossing and Jørgensen 1990); however, without dissolved sulfide, exchange between AVS and elemental sulfur is suppressed. As discussed below, dissolved sulfide levels in our experiments were likely very low. Furthermore, in none of our experiments was there any indication that the AVS pool approached the isotopic composition of elemental sulfur, as would be expected with active isotopic exchange (Fig. 3).

From the summary of isotope results in Fig. 3 it appears that for Teich2 and possibly *Dc. thiozymogenes,* pyrite was formed by H_2S addition (Eq. 10). For *Db. propionicus,* Golfo Dulce S1, Kuhgraben, and possibly Teich1, S° addition (Eq. 9) is indicated as the formation pathway; for the others, both processes apparently operated simultaneously. Thus, our results indicate that during the disproportionation of elemental sulfur, pyrite formation occurs by two quite different, competing pathways (Table 5). The circumstances by which one pathway outcompetes the other are not clear.

Both of the pathways expressed by Eq. 9 and 10 have been explored kinetically (Rickard 1975; Rickard 1997). For the reaction between elemental sulfur and FeS, rates of pyrite formation vary with the surface area of FeS squared, with the surface area of elemental sulfur, and with the partial pressure of H_2S. Unfortunately, the strong dependence on FeS surface area makes this rate law difficult to apply to natural systems and systems such as ours, where FeS surface area is extremely difficult to quantify.

Recently, Rickard (1997) proposed the following rate law for the reaction expressed by Eq. 10:

$$dFeS_2/dt = k(FeS)(H_2S_{aq}), \quad (11)$$

where k is a second-order rate constant with a value of 1.03×10^{-4} liters mol^{-1} s^{-1} at 25°C, and FeS and H_2S_{aq} are concentrations in moles liter^{-1} of reacting solution. This rate law is much easier to apply to natural systems, and Rickard (1997) viewed this formation pathway as the fastest yet identified.

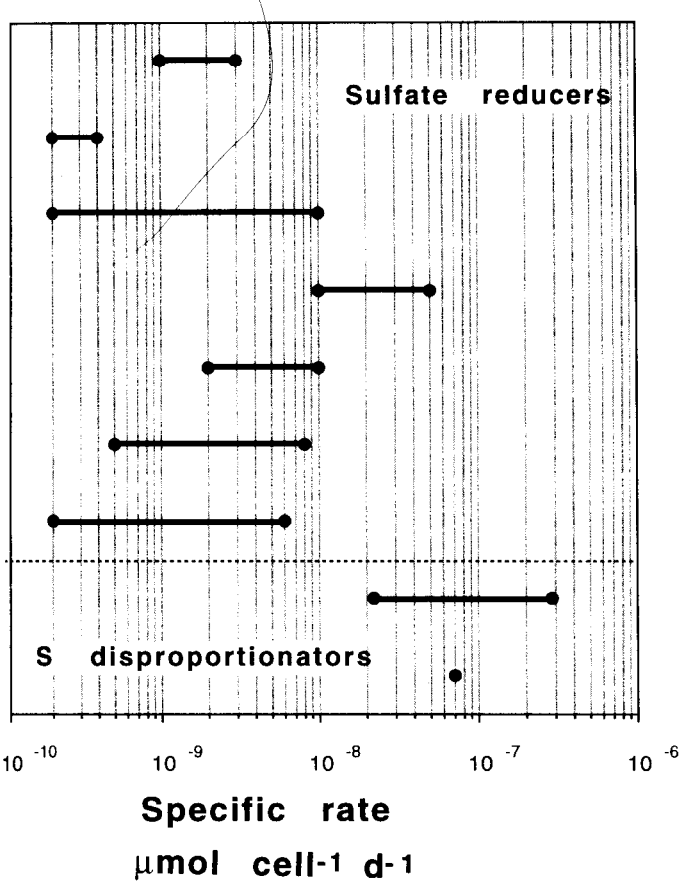

Fig. 5. Literature reports of specific rates of sulfate reduction from pure cultures of sulfate-reducing bacteria compared to specific rates of elemental sulfur disproportionation from the present study (*see Fig. 4*) and the study of Thamdrup et al. (1993). Data for sulfate-reducing bacteria are from the compilation in Jørgensen (1978), with additional data from Chambers et al. (1975). Numerous different sulfate-reducing bacteria grown with different electron donors are represented. Units are μmol cell^{-1} d^{-1} of sulfate reduced or elemental sulfur disproportionated.

To compare our measured pyrite formation rates to those of the inorganic reaction system of Rickard (1997), we calculated the anticipated rates of pyrite formation using our experimental conditions and the rate law in Eq. 11. FeS concentrations were obtained from our experimental results, whereas H_2S_{aq} was not measured in our sampling procedure. We note, however, that excess reactive Fe oxides were added as ferrihydrite to all of our experimental bottles and these should, even with active sulfide formation as encountered here, buffer the concentration of dissolved sulfide to low levels (Canfield et al. 1992). Furthermore, in experiments with strain *Dc. sulfoexigens* similar to those conducted here, H_2S was measured, but it never accumulated to the detection limit of 1 μM, even during rapid pyrite formation (Finster et al. 1998). Finally, we were never able to smell H_2S during the sampling of any of the experimental bottles. For calculation purposes we use 1 μM as an upper limit to the concentration of dissolved H_2S in our experiments. Combining this value for H_2S_{aq} with the average concentration of FeS during the course of pyrite formation, rates of pyrite formation from between 2 and 5 $\times$ 10^{-8} mol liter^{-1} d^{-1} were calculated (Table 5). These values are 10^4–10^5 times lower than the measured rates of pyrite formation (Table 5).

We conclude that rates of pyrite formation in our bacterial cultures are considerably faster than expected from inorganic reactions. Also, our measured rates were equally fast whether the reaction pathway expressed in Eq. 9 or Eq. 10 seemed to dominate. The extent to which pyrite formation was actually mediated by bacteria in our experiments is not known, but our results suggest that bacteria may enhance rates of pyrite formation in natural systems. We note, however, that appreciable pyrite formation did not occur during the disproportionation of elemental sulfur added to Weddewarden sediments (Canfield and Thamdrup 1996). The reasons for this, as well as the possible role of bacteria in promoting pyrite formation in sediments, require further investigation.

Ecological considerations—Earlier reports have indicated a substantial role for elemental sulfur disproportionation in the sedimentary cycling of sulfur (e.g. Canfield and Thamdrup 1996; Canfield and Teske 1996). It has also been proposed that the isotope fractionations occurring during elemental sulfur disproportionation can explain the long-standing dilemma of large ^{34}S depletions in sedimentary sulfides in the face of only modest ^{34}S depletions in the production of sulfide by sulfate-reducing bacteria (Canfield and Thamdrup 1994; Canfield and Teske 1996). We have demonstrated here that a substantial fractionation occurs during the disproportionation of elemental sulfur by pure cultures conducting this metabolism and by enrichment cultures from widely separated marine and freshwater environments. We thus expand considerably our earlier findings on isotope fractionation during the disproportionation of elemental sulfur (Canfield and Thamdrup 1994). Furthermore, for *Db. propionicus*, fractionations stand out as much larger than for the other bacteria.

Db. propionicus can disproportionate an exogenous source of elemental sulfur and, along with a number of other sulfate reducing bacteria, it can also disproportionate elemental sulfur as the terminal step of sulfide oxidation with oxygen and nitrate. These sulfate-reducing bacteria may therefore be active in disproportionating elemental sulfur at the interface between oxygen and sulfide where the elemental sulfur is supplied either though their own metabolism or from other sulfide-oxidation pathways. It is not clear, however, whether sulfate-reducing bacteria can compete with colorless sulfur bacteria for oxygen and sulfide at oxic–anoxic interfaces.

A possible insight into the ecological significance of elemental sulfur-disproportionating bacteria is gained by compiling the specific rates of sulfur disproportionation from this and other experiments (Thamdrup et al. 1993) and comparing these with specific rates of sulfate reduction obtained from various pure culture investigations (Fig. 5). It is obvious from this comparison that specific rates of elemental sulfur disproportionation are high—some 2 orders of magnitude higher than for sulfate-reducing bacteria. This implies that the process of elemental sulfur acquisition into the cell is quite efficient. Furthermore, these specific rates ($\sim 10^{-7}$ μmol S cell^{-1} d^{-1}) can be combined with the most probable number population size estimates (10^5–10^6 cells ml^{-1}; Thamdrup et al.

1993) to yield elemental sulfur disproportionation rates of 10^{-5}–10^{-4} mol S liter^{-1} d^{-1}. These rates are comparable to rates of sulfate reduction in surficial continental margin sediments (e.g. Jørgensen 1977; Westrich 1983). It seems that elemental sulfur-disproportionating bacteria are of abundant population size to metabolize a sizable percentage of the sulfide produced by sulfate reduction in sediments. Previous estimates demonstrate that most of the sulfide produced by sulfate reduction in sediments is reoxidized by some pathway (e.g. Jørgensen 1977, 1982; Canfield and Teske 1996).

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