Pergamon

Geochimica et Cosmochimica Acta, Vol. 65, No. 10, pp. 1573–1581, 2001

0016-7037/01 $20.00 + .00

PII S0016-7037(00)00622-0

Anaerobic sulfide oxidation and stable isotope fractionation associated with bacterial sulfur disproportionation in the presence of MnO_2

MICHAEL E. BÖTTCHER[1,*] and BO THAMDRUP[2]

[1]Department of Biogeochemistry, Max Planck Institute for Marine Microbiology, D-28359 Bremen, Germany
[2]Danish Center for Earth System Science, Institute of Biology, SDU Odense University, DK-5230 Odense, Denmark

(*Received April* 10, 2000; *accepted in revised form November* 14, 2000)

Abstract—The sulfur and oxygen isotope effects associated with anaerobic bacterial disproportionation of elemental sulfur by a pure culture (*Desulfocapsa thiozymogenes*) and an enrichment culture were investigated experimentally in the presence of synthetic Mn(IV)oxides. During bacterial disproportionation, $^{34}S/^{32}S$ were fractionated in dissolved sulfate compared to elemental sulfur by −0.6 to +2.0‰ (*D. thiozymogenes*) and −0.2 to +1.1‰ (enrichment culture) at cellular sulfur disproportionation rates of 10^{-16} mol S°/cell/h and 10^{-17} mol S°/cell/h, respectively. The measured sulfur isotope effects are much smaller than those observed previously for the same cultures in the presence of Fe(III) and Fe(II) compounds, indicating that microbial isotope fractionation was superimposed by the chemical re-oxidation of hydrogen sulfide by MnO_2 to sulfate. Significant re-oxidation of H_2S to sulfate was additionally confirmed by the oxygen isotopic composition of sulfate, which was enriched in ^{18}O compared to water by +8 to +12‰. These new experimental results imply that the overall influence of bacterial disproportionation on stable isotope partitioning in natural surface sediments depends on the proportion and relative recycling rates of reactive Fe(III) to Mn(IV)(oxyhydr)oxides. *Copyright © 2001 Elsevier Science Ltd*

1. INTRODUCTION

The bacterial disproportionation of sulfur compounds is a class of processes during which sulfur species of intermediate oxidation state are transformed to sulfide and sulfate with no external electron donor or acceptor, somewhat similar to the fermentation of organic matter (Bak and Cypionka, 1987; Thamdrup et al., 1993). Sulfur species with intermediate oxidation states as elemental sulfur (S°), thiosulfate ($S_2O_3^{2-}$), and sulfite (SO_3^{2-}) are metabolized. Whereas transformations of thiosulfate and sulfite are exergonic under standard conditions, the disproportionation of S°, according to:

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

is endergonic (Bak and Cypionka, 1987), but may become energetically favorable when H_2S is removed from the solution, e.g., by the reaction with Mn or Fe (oxyhydr)oxides (Thamdrup et al., 1993). Disproportionating bacteria are widespread and abundant in anoxic sediments (Thamdrup et al., 1993; Finster et al., 1998) and disproportionation reactions have been found to be important in the sulfur cycle of both limnic and marine sediments, where they may play a key role in the oxidation of sulfide produced by bacterial sulfate reduction (Jørgensen, 1990a,b; Jørgensen and Bak, 1991; Thamdrup et al., 1993; Canfield and Thamdrup, 1996). Intermediate sulfur species have been found as products of abiotic sulfide oxidation (Cline and Richards, 1965; Chen and Morris, 1972; Yao and Millero, 1993, 1995, 1996). In natural sediments, high turnover rates lead to typically low concentrations of SO_3^{2-} and $S_2O_3^{2-}$, but elemental sulfur is a rather common compound (Troelsen and Jørgensen, 1982; Thode–Andersen and Jørgensen, 1989; Canfield and Thamdrup, 1996).

Significant sulfur isotope fractionation effects have been found associated with the disproportionation reactions (Canfield and Thamdrup, 1994; Canfield et al., 1998; Cypionka et al., 1998; Habicht et al., 1998; Böttcher et al., 2001), and are believed to contribute to the strong overall enrichment of ^{32}S in sedimentary sulfides (Jørgensen, 1990a; Canfield and Thamdrup, 1994). Most recently, a large isotopic fractionation effect has also been observed for oxygen during the formation of SO_4^{2-} by S° disproportionation (Böttcher et al., 2001), with possible implications for the observed variations in the oxygen isotope composition of SO_4^{2-} in coastal waters (Böttcher et al., 1998b). Thus, investigations of the regulation of the disproportionation processes and their associated isotopic fractionations are important for our understanding of the modern sulfur cycle and for interpretations of the geological isotope record (Canfield and Teske, 1996).

Iron is an important scavenger of sulfide from sediment pore waters, and elemental sulfur disproportionation and associated isotope effects have been investigated experimentally in the presence of Fe(III)oxyhydroxides or Fe(II) carbonate (Canfield and Thamdrup, 1994; Canfield et al., 1998; Böttcher et al., 2001). Manganese oxides have, however, also been found to be important in the oxidation of reduced sulfur compounds in sediments (Aller and Rude, 1988; Canfield et al., 1993; Aller, 1994; Schippers and Jørgensen, 2001), and disproportionating bacteria are potentially significant in this oxidation (Thamdrup et al., 1993). In contrast to Fe-containing systems, sulfide does not precipitate during S° disproportionation in the presence of Mn(IV)oxides but is rapidly reoxidized. Both S° and SO_4^{2-} are products of abiotic sulfide oxidation with MnO_2 (Fig. 1; Yao and Millero, 1993, 1995).

In the present study, we present the first results on the

* Author to whom correspondence should be addressed (mboettch@mpi-bremen.de).

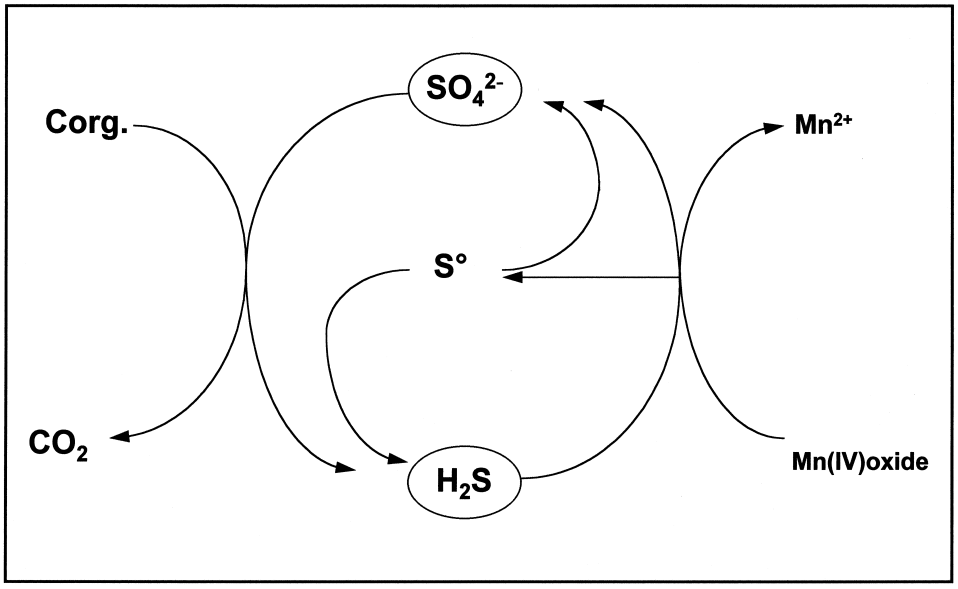

Fig. 1. Simplified sedimentary sulfur cycle illustrating the role of bacterial sulfur disproportionation and the influence of Mn(IV)oxides.

influence of Mn(IV)oxides on sulfur and oxygen isotope fractionation during bacterial disproportionation of elemental sulfur. Experiments were conducted with a pure culture (*Desulfocapsa thiozymogenes*) and an enrichment culture. We use stable oxygen isotope partitioning to interpret the pathways of sulfur transformation. It is found that the superimposition of sulfur disproportionation and spontaneous partial re-oxidation of hydrogen sulfide to sulfate leads to a negligible overall sulfur isotope fractionation between elemental sulfur and dissolved sulfate. The results indicate that the overall influence of bacterial disproportionation on sulfur isotope partitioning in natural surface sediments depends on the available proportions of reactive Fe(III) to Mn(IV)(oxyhydr)oxides.

2. EXPERIMENTAL TECHNIQUES

A pure culture of *D. thiozymogenes* (strain Bra2, DSM 7269; Janssen et al., 1996) and the enrichment culture "Kuhgraben" (Canfield et al., 1998), both freshwater cultures, were used in the present study. Both *D. thiozymogenes* as well as the organism that dominates the Kuhgraben enrichment are rod-shaped with lengths of about 1.7 and 2.6 μm, respectively, and a width of 0.6 μm (Canfield et al., 1998). *D. thiozymogenes* is a strictly anaerobic strain and is capable of reducing sulfate, as well as disproportionating thiosulfate and sulfite (Janssen et al., 1996). The dominating organism from the Kuhgraben enrichment has not yet been isolated for further physiological characterization. Both cultures have been shown in a previous study (Canfield et al., 1998) to fractionate sulfur isotopes to the same magnitude as marine strains.

An anoxic bicarbonate-buffered medium without sulfate was prepared with the addition of vitamins and nonchelated trace metals according to Widdel and Bak (1991). Flowers of synthetic sulfur (Fluka, Buchs, Switzerland; purum quality) were ground in a mortar with distilled water, and the suspension was autoclaved at 114°C for 30 min. Elemental sulfur was added in excess (~5 mmol) to the medium (54 cm^3) with a sterile spatula, and 2.2-mmol synthetic X-ray amorphous manganese dioxide (MnO_2) with an average oxidation state of 3.98 $\pm$ 0.05 (Thamdrup et al., 1993) was also added under anoxic conditions. MnO_2 was used as scavenger for hydrogen sulfide to keep the disproportionation reaction exergonic (Thamdrup et al., 1993; Lovley and Phillips, 1994).

Six completely filled screw-capped glass bottles (54 cm^3) for each culture were inoculated simultaneously and harvested after different time intervals. Experiments were carried out at 28°C in a dark thermoconstant room. At the end of the experiment, the bottles were vigorously shaken and aliquots were taken for measurements of pH, cell numbers, and total and dissolved manganese. H_2S was determined spectrophotometrically on selected filtered samples (0.2 μm; N_2-flushed cellulose acetate filters from Sartorius, Göttingen, Germany) (Cline, 1969). The pH was determined on unfiltered samples immediately after sampling by using an Orion combination electrode (Boston, MA, USA). Another aliquot was filtered through cellulose acetate filter (0.45 μm). The Mn(II) in the precipitate remaining on the filter, which is believed to consist of adsorbed Mn(II) and precipitated $Mn(II)CO_3$, was measured after dissolution. After the filter was washed with distilled water, Mn(II) was leached with 2 mL of 0.5 M HCl and 2 mL of distilled water. Pure MnO_2 is not dissolved by this procedure. The combined acid leachate and water from the last wash were analyzed for Mn(II). The filtrate was acidified with HCl and analyzed for manganese by standard procedures by using an ICP-OES (Perkin–Elmer Optima 3000XL). Total manganese was completely dissolved in dithionite-citrate-acetic acid and measured by atomic absorption spectroscopy (Perkin–Elmer). Samples for the determination of cell numbers were fixed with glutaraldehyde (2.5% final concentration) and stored at 4°C in the dark. After dissolution of MnO_2 by a dithionite-citrate-acetic acid solution, cells were stained with DAPI and counted with a Zeiss Axiolab epifluorescence microscope. Initial cell counts were about 3×10^7 cells/mL for *D. thiozymogenes* and 6×10^7 cells/mL for Kuhgraben.

The remaining experimental solution was rapidly mixed with 20 mL of 10% wt./vol. zinc acetate. The solution was filtered (0.45 μm, polynitrate filters) and sulfate was precipitated from the filtrate as $BaSO_4$ for stable isotope and gravimetric sulfate determination. Purity of selected $BaSO_4$ samples was checked by Fourier transform infrared spectroscopy.

Elemental sulfur was extracted from the final dried solids via Soxlet extraction with acetone for 48 h. S° was precipitated on activated Cu as CuS, which was further converted into Ag_2S via HCl distillation (Allen and Parkes, 1995). Additionally, an aliquot of the dried samples was treated with hydroxylamin hydrochloride solution (Thamdrup et al., 1993) to reduce residual Mn(IV) and then distilled with HCl (Allen and

Table 1. Experiments conducted to investigated stable isotope fractionation during bacterial sulfur disproportionation in the presence of MnO_2.

Time (h)	pH (28°C)	Log cell number/mL	SO_4 (mM)	$\delta^{34}S(SO_4)$ (‰)	$\delta^{18}O(SO_4)$ (‰)	Mn(II),aq (μM)	Mn(II), leach (mM)
B-Mn							
2	7.29	7.51	0	n.a.[a]	n.a.	0	1.1
94	7.49	8.15	0.8	15.0	n.d.	45	4.2
145	7.64	8.04	1.3	15.1	3.7[b]	122	5.7
290	7.93	8.22	2.5	17.4	3.7[b]	200	6.6
338	8.25	8.49	3.2	17.6	4.9[b]	161	10.5
408	8.26	8.28	3.0	17.4	4.9[b]	47	7.8
K-Mn							
2	7.30	7.82	0	n.a.	n.a.	0	1.0
169	7.56	8.18	1.0	15.5	0.8[b]	116	2.9
219	7.65	8.29	1.3	15.4	0.8[b]	123	3.6
364	7.69	8.18	1.4	16.5	1.4[b]	171	4.3
412	7.73	8.43	1.5	16.7	1.4[b]	188	4.4

Cellular sulfur disproportionation rates (cSDR) as estimated from the sulfate concentrations (Eqn. 7; see text) were $10^{-9.8\pm0.1}$ (μmol S°/cell/h) in experiment B-Mn, and $10^{-10.5\pm0.5}$ (μmol S°/cell/h) in experiment K-Mn.

[a] n.d., not determined; n.a., not applicable.

[b] Oxygen isotopic composition was measured on pooled barium sulfate of two experiments each (B-Mn: 145 + 290 h, 338 + 408 h; K-Mn: 169 + 219 h, 364 + 412 h).

Parkes, 1995) to convert potential ZnS resulting from the fixation of hydrogen sulfide into Ag_2S. No hydrogen sulfide, however, was found in any of the samples.

$BaSO_4$ precipitates were carefully washed, dried, and analyzed for the sulfur isotopic composition by combustion isotope-ratio-monitoring mass spectrometry (C-irmMS) as described by Böttcher et al. (1998a) by using a Carlo Erba EA 1108 elemental analyzer connected to a Finnigan-MAT 252 mass spectrometer via a Finnigan Conflo II open split interface (Bremen, Germany). International silver sulfide standards IAEA-S-1 and IAEA-S-2 were used to calibrate the mass spectrometer for sulfur isotope measurements. $\delta^{34}S$ values of +20.6‰ and +16.3‰ were obtained for the international reference materials NBS-127 (barium sulfate) and IAEA-S-4 (elemental sulfur). Replicate measurements agreed within ±0.15‰. The oxygen isotope composition of $BaSO_4$ was analyzed at the University of Tübingen by fluorination with BrF_5 (Clayton and Mayeda, 1963; Pickthorn and O'Neil, 1985). Of the possible volatile reaction products: O_2, SO_2, BrF_3, and excess BrF_5, only O_2 passes through the liquid nitrogen cold trap and, hence, only 50% of the sulfate oxygen is extracted. The O_2 is quantitatively converted to CO_2 by passing it over a graphite rod heated by a Pt-coil. The $^{18}O/^{16}O$ of CO_2 was measured on a Finnigan-MAT 252 mass spectrometer. It had been determined by Pickthorn and O'Neil (1985) and also confirmed in the laboratory at Tübingen University that the fractionation between extracted O_2 and residual products is independent of reaction temperature between 400°C and 600°C and of reaction time (commonly 12 h). The present results were thus corrected for systematic isotope fractionation resulting from the extraction of only 50% of the sulfate oxygen by using NBS-127 ($\delta^{18}O$ = +9.34‰ vs. V-SMOW). Replicate measurements agreed to within ±0.3‰. The oxygen isotope composition of a filtered aliquot (1–2 mL) of the experimental solution was measured by equilibration with CO_2 in a closed system at 25°C. The fractionation factor between CO_2 and H_2O at 25°C was taken from O'Neil et al. (1975) and replicates reproduce better than 0.1‰. Oxygen isotope measurements were carried out on a Finnigan MAT 252 (University of Tübingen) or a Fisons Optima mass spectrometer (ETH Zürich, Switzerland). $^{18}O/^{16}O$ and $^{34}S/^{32}S$ ratios are reported in the δ-notation relative to the V-SMOW and V-CDT standards, respectively.

Elemental sulfur used in the experiments had a sulfur isotope composition of +15.6 ± 0.2‰ vs. V-CDT (n = 13), and the experimental solutions had an oxygen isotope composition of −7.4 ± 0.1‰ vs. V-SMOW. Concentrations and sulfur isotopic compositions of dissolved sulfate (Table 1) were corrected for the small amounts initially derived from the pre-cultures.

3. RESULTS AND DISCUSSION

3.1. Manganese and Sulfate

During the course of the experiments with *D. thiozymogenes* and Kuhgraben, cell growth and a continuous increase in the concentrations of dissolved SO_4^{2-}, and OH^-, and the amounts of extractable manganese were observed (Fig. 2). Extracted manganese is assumed to consist of adsorbed Mn(II) and precipitated $MnCO_3$ (rhodochrosite). Both cultures showed the same general trends for the concentrations of the dissolved species, although the reaction progress with time for the Kuhgraben enrichment was slower than for *D. thiozymogenes*. After an initial increase in dissolved $Mn(II)_{,aq}$, a drop at higher pH values (Table 1) indicates a further limitation of $Mn(II)_{,aq}$ accumulation by the enhanced precipitation of $MnCO_3$ due to thermodynamic supersaturation. Precipitation of MnS can be neglected under the present experimental conditions because very high sulfide concentrations are required to make MnS to become more stable in bicarbonate-containing aqueous solution than $MnCO_3$ (Böttcher and Huckriede, 1997). From a comparison of the data in Table 1 we conclude that essentially all reduced manganese was bound to the solid phase. The precipitation of $Mn(II)CO_3$ was additionally confirmed by a FT infrared spectroscopic investigation of selected final solids, which showed the typical infrared absorption bands of $Mn(II)CO_3$ with very limited incorporation of Ca^{2+} into the carbonate lattice (White, 1974; Böttcher et al., 1992; Böttcher, 1998).

No hydrogen sulfide was found in any of the samples. The observed compositional variations are consistent with the overall process of hydrogen sulfide and sulfate production during bacterial disproportionation of elemental sulfur followed by the spontaneous oxidation of hydrogen sulfide (Thamdrup et al., 1993) according to a combination of Eqns. 1 and 2:

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

$$3H_2S + 3MnO_2 \rightarrow 3Mn^{2+} + 3S° + 6OH^- \quad (2)$$

$$S° + 3MnO_2 + 4H^+ \rightarrow SO_4^{2-} + 3Mn^{2+} + 2H_2O \quad (3)$$

In contrast to the disproportionation of elemental sulfur in iron-containing systems (Canfield et al., 1998; Böttcher et al., 2001), the superimposition of Eqns. 1 and 2 leads to a continuous increase in pH (Eqn. 3), in general agreement with the experimental results (Table 1). In Eqn. 2 it was assumed, that the reaction of H_2S with MnO_2 yields elemental sulfur as the only re-oxidation-product (Thamdrup et al., 1993). The observed stoichiometry between sulfate and accumulated Mn(II) is slightly lower than the theoretical value of 3 which is expected from Eqn. 3. This is most likely due to a nonquantitative extraction of $Mn(II)CO_3$ from the solids by HCl, because the dissolution velocity of synthetic rhodochrosite in aqueous solution is slow (Böttcher, unpublished experimental data). By using sulfuric acid as extracting agent, Thamdrup et al. (1993) found a stoichiometry close to 3 during disproportionation of elemental sulfur by an enrichment culture in the presence of Mn(IV).

3.2. Stable Isotopes

Under the conditions described by equations (1) and (2), the stable isotopic composition of dissolved sulfate should reflect the same enrichments in ^{34}S and ^{18}O caused by the disproportionation process (Eqn. 1) as found in experiments with Fe(III) and Fe(II) compounds (Böttcher et al., 2001; Canfield et al., 1998). For the cultures under investigation, a range between +11 and +18‰ has been found for $^{34}S/^{32}S$ fractionation between sulfate and elemental sulfur and between +16 and +17‰ for $^{18}O/^{16}O$ partitioning between sulfate and water under experimental conditions similar to the present experiments, but using iron compounds as scavengers (Fig. 3; Böttcher et al., 2001). In the presence of Mn(IV)oxides, however, the magnitude of overall isotope fractionation is significantly decreased for both sulfur and oxygen isotopes (Fig. 3; Table 1). Dissolved sulfate, for instance was only enriched in ^{34}S compared to elemental sulfur by −0.6 to +2.0‰ (*D. thiozymogenes*) and −0.2 to +1.1‰ (Kuhgraben). This indicates that not all reactions taking place in our experiments are described by Eqns. 1 and 2, and dissolved sulfate must have been produced by additional oxidative reactions, besides S° disproportionation.

Sulfate formation in agreement with the observed concentration shifts during the time courses can be explained by two different mechanisms:

A) Bacterial oxidation of elemental sulfur to sulfate reducing by Mn(IV)oxide, or
B) Bacterial disproportionation of elemental sulfur to hydrogen sulfide and sulfate followed by the reoxidation of hydrogen sulfide to elemental sulfur and sulfate.

Both a one-step bacterial oxidation of elemental sulfur by Mn(IV)oxides (A) and the combined pathway according to process B would lead to the same stoichiometry between Mn(II) and sulfate of 3 : 1 (Eqn. 3). A combination of Eqn. 1

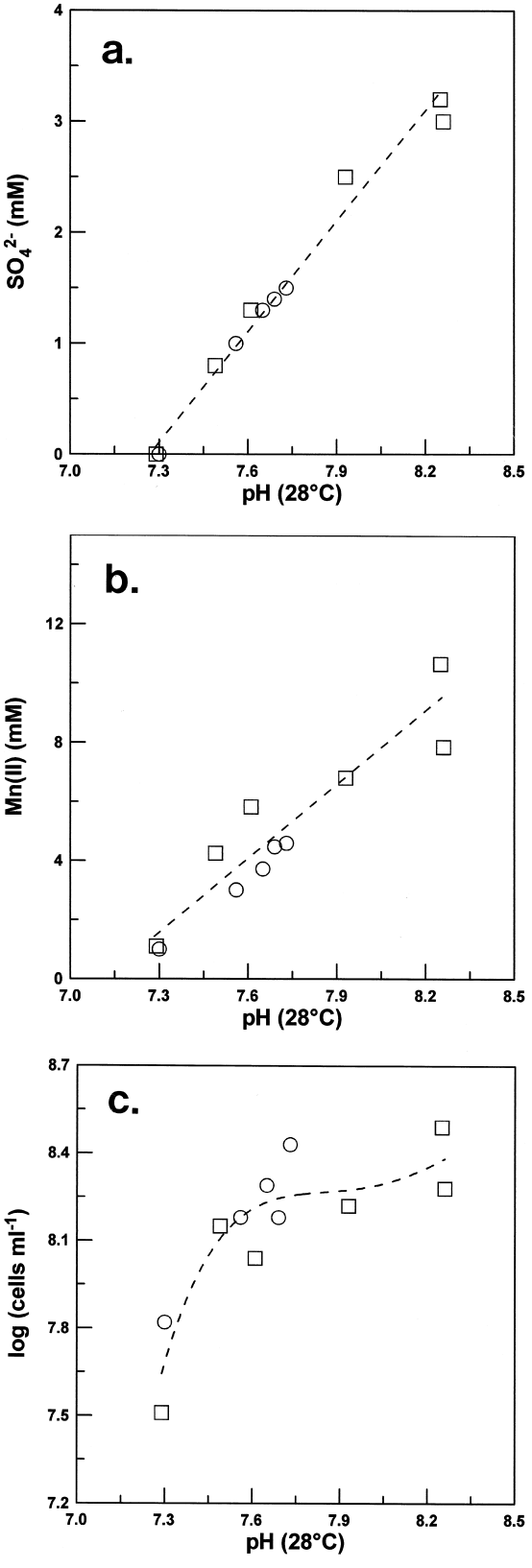

Fig. 2. Reaction progress of dissolved sulfate (a), total Mn(II) (b), and cell numbers (c) during bacterial disproportionation of elemental sulfur in the presence of Mn(IV)oxides. Squares: *Desulfocapsa thiozymogenes*; circles: Kuhgraben enrichment. The pH was used as the reaction progress variable.

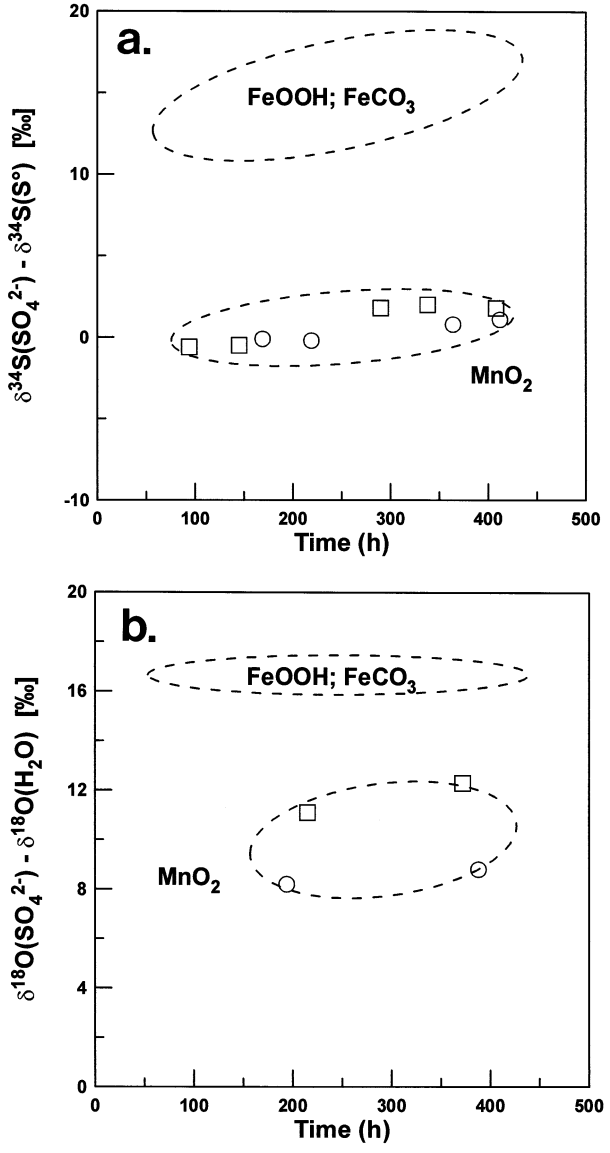

Fig. 3. Sulfur (a) and oxygen isotope (b) fractionation between elemental sulfur and dissolved sulfate in the presence of different metal(oxyhydr)oxides. Data for FeOOH and $FeCO_3$ are from Böttcher et al. (2001). Squares: *Desulfocapsa thiozymogenes*; circles: Kuhgraben enrichment. Note: Average run times were used to present oxygen isotope fractionation (Table 1).

with the complete oxidation of hydrogen sulfide to sulfate (Eqn. 4), for instance, yields the overall Eqn. 3:

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

$$H_2S + 6H^+ + 4MnO_2 \rightarrow 4Mn^{2+} + SO_4^{2-} + 4H_2O \quad (4)$$

$$S^\circ + 3MnO_2 + 4H^+ \rightarrow SO_4^{2-} + 3Mn^{2+} + 2H_2O \quad (3)$$

Thamdrup et al. (1993) have experimentally shown that microbial disproportionation of elemental sulfur occured in the absence of Mn(IV)oxide by a bacterial culture that was enriched on Mn(IV) and elemental sulfur, arguing that Eqns. 1 and 2 took place in the presence of Mn(IV)oxide. Additionally, under Mn(IV) limited conditions they observed the final accumulation of hydrogen sulfide after all manganese was reduced; the same was found in longterm experiments with the Kuhgraben enrichment in the presence of a limited amount of Mn(IV)oxide (Böttcher and Thamdrup, unpublished). The formation of sulfate besides elemental sulfur during the chemical oxidation of hydrogen sulfide by Mn(IV)oxides has previously been observed experimentally (Yao and Millero, 1993, 1995, 1996) and is also in agreement with theoretical considerations based on the frontier-orbital theory approach (Luther, 1990). Additional support comes from experiments where only ~50% of hydrogen sulfide oxidized experimentally by Mn(IV)oxides was recovered as elemental sulfur (Burdige and Nealson, 1986). Yao and Millero (1993, 1995, 1996) found up to 30% of H_2S chemically oxidized to sulfate by MnO_2 (pH 7.5; 25°C). In the latter experiments, the importance of sulfate as an oxidation product increased with increasing $[MnO_2]/[H_2S]$ ratios. In the experiments conducted in the present study, no measurable accumulation of hydrogen sulfide above levels of 5 μM was found, indicating that a very high $[MnO_2]/[H_2S^-]$ ratio was maintained. From the experimental results of Yao and Millero (1996) derived in seawater, it is expected that the relative importance of sulfate as an oxidation product exceeded 30% in the experimental solutions of the present study.

A more detailed and quantitative estimate for the sources of sulfate can be done following oxygen isotope partitioning.

3.2.1. Oxygen isotopes

An important and quantitative argument that Eqns. 1, 2, and 4 took place during the course of our experiments comes from oxygen isotope fractionation between water and dissolved sulfate: under anoxic conditions, the oxygen atoms of newly formed sulfate are completely derived from water. Nonbiological oxygen isotope exchange between sulfate and water is extremely slow at a neutral pH and low temperatures (e.g., Lloyd, 1968; Mizutani and Rafter, 1969; Chiba and Sakai, 1985) and can be neglected under the experimental conditions of the present study. Therefore, the oxygen isotopic compositions of both species are related by (Taylor et al., 1984):

$$\delta^{18}O(SO_4) = \delta^{18}O(H_2O) + \epsilon_{H2O} \quad (5)$$

The magnitude of the isotope enrichment factor, ε_{H2O}, depends on the bacterial and chemical processes involved in sulfate formation (e.g., Taylor et al., 1984; van Stempvoort and Krouse, 1994). For the present study, ε_{H2O} values between +8.2 and +12.3‰ are calculated for the data in Table 1. On the other hand, microbial disproportionation of elemental sulfur without reoxidation of H_2S was accompanied by a ε_{H2O} value of about +17‰ (Böttcher et al., 2001). During bacterial oxidation of elemental sulfur, Mizutani and Rafter (1969) found no oxygen isotope fractionation between sulfate and water and, therefore, ε_{H2O} was close to zero. Isotope effects of different magnitudes were reported for the oxidation of hydrogen sulfide (van Stempvoort and Krouse, 1994), and Taylor et al. (1984) reported a mean value of about +4‰. These previous studies show that the experimental results of the present study cannot be described by an one-step oxidation of elemental sulfur to sulfate (mechanism A). Actually, the oxygen isotopic composition of dissolved sulfate additionally confirms its dual origin

from the bacterial disproportionation of elemental sulfur and the reoxidation of H_2S (mechanism B). The overall isotopic composition of sulfate observed in the present study (Table 1) is consistent with a binary mixture of sulfate originating from Eqns. 1 and 4 according to:

$$\delta^{18}O(SO_4) = X\{\delta^{18}O(H_2O) + \epsilon_{H2O-1}\} + (1 - X)\{\delta^{18}O(H_2O) + \epsilon_{H_2O-2}\} \quad (6)$$

where X is the fraction of sulfate derived from the disproportionation of elemental sulfur and ε_{H2O-1} is the associated isotope effect. ε_{H2O-2} is the isotope enrichment factor related to reoxidation of hydrogen sulfide to sulfate. By using the experimental results from Table 1 and average values of +17‰ and +4‰ for ε_{H2O-1} and ε_{H2O-2}, respectively, the contribution from the reoxidation of H_2S into the sulfate pool is estimated from Eqn. 6 to range between 31 and 69%. The relative proportion of the latter reaction seems to decrease with the reaction progress (Table 1), probably indicating the importance of kinetic factors influencing the processes at the interface between oxidizing metal oxide and the solution.

Considering a contribution of 50% to the total dissolved sulfate pool derived directly from disproportionation of elemental sulfur, cellular rates for the disproportionation (cSDR) of elemental sulfur in the presence of MnO_2 can be estimated from

$$\text{cSDR}[\mu\text{mol S°/cell/h}] = 2(S_i - S_{i-1})((C_i + C_{i-1})/2)^{-1}(t_i - t_{i-1})^{-1} \quad (7)$$

S, C, and t refer to the amounts of sulfate [μmol], the total cell number, and reaction time, respectively, at time intervals i and $i - 1$. cSDR values varied between $10^{-9.7}$ to $10^{-9.9}$ μmol S°/cell/h for *D. thiozymogenes*, and $10^{-10.0}$ and $10^{-11.0}$ μmol S°/cell/h for Kuhgraben. Cell growth actually stopped during the final stages of the experiments. This could have been caused by an increase in pH during the disproportionation process in the batch experiments (Table 1) as indicated by the overall Eqn. 3. Janssen et al. (1996) reported for *D. thiozymogenes* growth within a range of pH 6.8 and 8.0, in agreement with our observations. cSDRs in the presence of Mn(IV) are smaller than those observed with the same cultures in the presence of Fe(II) and Fe(III) compounds (Canfield et al., 1998; Böttcher et al., 2001). The difference is more pronounced for the Kuhgraben enrichment than for *D. thiozymogenes* (Table 1).

3.2.2. Sulfur isotopes

$^{34}S/^{32}S$ ratios in dissolved sulfate were fractionated compared to elemental sulfur by −0.6 to +2.0‰ (*D. thiozymogenes*) and −0.2 to +1.1‰ (Kuhgraben). Because the H_2S that is produced upon disproportionation has a lower $^{34}S/^{32}S$ ratio than the consumed elemental sulfur (Canfield and Thamdrup, 1994; Canfield et al., 1998), these results are consistent with significant reoxidation of hydrogen sulfide to sulfate (Eqn. 4) and the oxygen isotope results. A kinetic isotope effect (ε_{Oxi}) of about −5‰ upon H_2S oxidation has been observed experimentally (Fry et al., 1988) and the overall sulfur isotope fractionation into sulfate can, therefore, be described according to:

$$\delta^{34}S(SO_4) = X\{\delta^{34}S(S°) + \epsilon_{Dis}\} + (1 - X)\{\delta^{34}S(H_2S) + \epsilon_{Oxi}\} \quad (8)$$

where X is the fraction of sulfate derived from the disproportionation of elemental sulfur and ε_{Dis} is the associated isotope effect. However, the approach used for oxygen isotopes (Eqn. 6) is not quantitatively applicable to the sulfur isotopic composition of sulfate, because the effective magnitude of sulfur isotope partitioning between the educt H_2S and the product SO_4^{2-} (and S°) depends not only on the kinetic isotope effect, ε_{Oxi}, but also on the reservoir sizes. The size of the H_2S pool from which the oxidation took place is not known but should be rather small. Additionally, both S° and SO_4^{2-} are the products of the oxidation reaction (Eqns. 2 and 4). Whereas no preferential isotope consumption with respect to elemental sulfur was observed during disproportionation (Canfield and Thamdrup, 1994), effective and fast H_2S reoxidation may decrease the overall isotope fractionation.

Thiosulfate was observed as a byproduct of H_2S oxidation by Mn(IV)oxides in seawater (Yao and Millero, 1995, 1996), and the formation of this sulfur intermediate cannot be excluded for the present experiments. However, thiosulfate is also rapidly disproportionated by *D. thiozymogenes* (Janssen et al., 1996) and this process is associated by a pronounced sulfur isotope fractionation between the products sulfate and hydrogen sulfide (Cypionka et al., 1998; Habicht et al., 1998). The small overall enrichment of ^{34}S in dissolved sulfate indicates that this process was of minor importance in the present experiments.

By using an average observed ^{34}S enrichment of about 1‰ in the experiments of the present study (Table 1), an enrichment of +14‰ for sulfate formation through the disproportionation process (Böttcher et al., 2001), and an average contribution of 50% to the total sulfate pool derived from disproportionation (Section 3.2.1), then the average sulfur isotopic composition of sulfate derived from reoxidation of H_2S ($\{\delta^{34}S(H_2S) + \varepsilon_{Oxi}\}$ in Eqn. 8) is estimated to be about 4‰. This is isotopically somewhat lighter than the actual measurements on H_2S (AVS) in cultures with Fe (Canfield et al., 1998; Böttcher et al., 2001) indicating that isotope fractionation during H_2S oxidation to sulfate took place. This finding is in general agreement with the experimental results of Fry et al. (1988). Sulfur isotope fractionation between H_2S and the sulfate produced during oxidation may have occurred, because two oxidation products were formed and isotopes were discriminated between these two pools. Therefore, isotope fractionation was possible although the H_2S pool was generally very small.

It should be noted that in a recent study, Smith (2000) reported small sulfur isotope fractionation effects during pure abiotic hydrolysis of elemental sulfur at 50°C. The experiments, however, have been carried out in highly concentrated Cu acetate solutions and cannot be directly compared to the present study. Bacterial disproportionation of elemental sulfur leads to significant isotope discrimination (Canfield et al., 1998; Böttcher et al., 2001) and only an additional abiotic (oxidation) reaction yields the small isotope effects observed in the present study.

3.3. Geochemical Implications

The present experimental findings have strong implication for the influence of sulfur disproportionation on the sedimentary stable isotope isotope record. Canfield and Thamdrup (1994) suggested that the strong depletion of ^{34}S observed in sedimentary sulfides may be related to cycles of reoxidation of hydrogen sulfide to elemental sulfur followed by the bacterial disproportionation to sulfate and hydrogen sulfide. This sulfur isotope effect has actually been confirmed experimentally using Fe(III) or Fe(II) compounds as scavengers for H_2S (Canfield and Thamdrup, 1994; Canfield et al., 1998; Böttcher et al., 2001). However, besides Fe(III)oxyhydroxides, Mn(IV)oxides may also contribute to the reoxidation of hydrogen sulfide and FeS in the chemocline of modern anoxic basins (Jacobs et al., 1985; Millero, 1991a,b,c) and near-surface sediments (Canfield et al., 1993; Aller, 1994; Moeslund et al., 1994). The experimental results of the present study imply that the overall isotope effect associated with elemental sulfur disproportionation in natural surface sediments depends on the ratio and relative recycling rates of reactive Fe(III)oxyhydroxides to Mn(IV)oxides. Although reactive manganese is typically present in smaller amounts than iron, the much higher turnover rates of manganese (Thamdrup et al., 1994) may also lead to a more pronounced importance for the interaction with the biogeochemical sulfur cycle. The relative rates may additionally be influenced by the reactivity of the sedimentary oxides, which is related to their mineralogy (Thamdrup, 2000, and references therein). The overall sulfur isotope signature preserved in the sediment will additionally be influenced by the relative rates of iron sulfide formation and H_2S reoxidation. The indirect influence of different metal(oxyhydr)oxides on the oxygen isotopic composition of dissolved sulfate in pore and surface waters may be of special significance in marine surface sediments, where high turnover rates in the biogeochemical sulfur cycle and intense bioturbation may occur and variable $^{18}O/^{16}O$ ratios in sulfate have been observed (Böttcher et al., 1998b). It is, therefore, expected that in coastal and estuarine sediments with high biological activity and very dynamic sulfur and manganese cycles (Sundby et al., 1981; Thamdrup et al., 1994; Canfield and Thamdrup, 1996; Huettel et al., 1998; Kristensen et al., 2000) the influence of Mn(IV)oxides may be increased. One likely effect of Mn(IV) is a decrease of the overall sulfur isotope effect, which would otherwise be observed if only Fe(III) would act as a scavenger for H_2S. In the case of the oxygen isotopic composition of sulfate, manganese dioxide may lead to a lowering of the isotope signal compared to a system dominated by sulfate reduction and disproportionation in the presence of Fe(III) compounds (Böttcher et al., 2001).

In the sedimentary system, dissolved Mn(II) is much more mobile than Fe(II), leading to a flux of Mn(II) from reduced sediments into the bottom waters, whereas iron is easily precipitated in the anoxic part as iron sulfide (Calvert and Petersen, 1996). However, even in anoxic systems, Mn(IV) may have an impact on metabolism of sulfur intermediates and associated isotope effects. The relative contribution of Mn(IV) on isotope partitioning in surface sediments may increase in environmental systems that are enriched in manganese as the anoxic basins of the Baltic Sea (Huckriede and Meischner, 1996). Huckriede and Meischner (1996) proposed a scheme in which reoxidation of dissolved Mn(II) and H_2S in the anoxic water column of the deeps takes place during "flushing-events" when specific metereological conditions force water from the North Sea to enter the Baltic Sea. This oxygenated dense water mixes with the anoxic waters and Mn(IV)oxides are precipitated, which form discrete layers at the sediment water interface. The reestablishment of suboxic/anoxic conditions in the bottom waters leads to the reduction of Mn(IV)oxide and the formation of $Mn(II)CO_3$. Although the exact biogeochemical mechanisms associated with the formation of the discrete carbonate layers are not yet known, a complex interaction between the manganese, sulfur, iron, and carbon cycles is suggested, including the formation of sulfur intermediates. Under these conditions, the reactions investigated experimentally in the present study may have an enhanced impact. This biogeochemical environment has been suggested to be a model for the enrichment of manganese in manganiferous black shales (Huckriede and Meischner, 1996), and the influence of Mn(IV) on stable isotope partitioning in sedimentary sulfur phases may have to be considered here as well.

From the changes in the oxygen concentration of the Earth's atmosphere (Canfield and Teske, 1996) and the accumulation of manganese ores of sedimentary origin (Laznicka, 1992), with time it can be inferred that the biogeochemical role of Mn(IV)oxides in water may have changed during geological time. Additionally, changes in the seafloor hydrothermal fluxes (e.g., Carpenter and Lohmann, 1997) may have influenced the availability of Mn(VI) in marine sediments influenced by metal input originating from hydrothermal activity.

More studies in recent and sub-recent sediments are needed to characterize and quantify the relations between biogeochemical reactions, microbial community structure, with special regard to disproportionating bacteria, and the resulting stable isotope signals to proof the importance of the different parallel reactions and derive further interpretation tools for the fossil sedimentary record. Future studies should consider both oxygen and sulfur isotope fractionation.

Acknowledgments—The authors thank Prof. J. Rullkötter and Prof. H.-J. Brumsack for access to the analytical facilities at the Institute of Chemistry and Biology of the Marine Environment (ICBM), University of Oldenburg, and S. Fleischer (MPI Bremen) and Dr. B. Schnetger (ICBM) for technical assistance. The oxygen isotopic composition of sulfate was measured by Dr. T. Vennemann (University of Tübingen), and the water samples were kindly analyzed for ^{18}O contents by Drs. T. Vennemann and S. Bernasconi (ETH Zürich). Dr. P.-L. Gehlken (Ebergötzen, Germany) kindly carried out the FTIR spectroscopic measurements. Comments by Drs. J. Kuever, O. Larsen, and A. Schippers improved an early version of the manuscript. The study was supported by Max Planck Society, Munich. The manuscript was improved by the constructive comments of three anonymous journal reviewers and the associate editor Prof. K. Kyser.

Associate editor: K. Kyser

REFERENCES

Allen R. E. and Parkes R. J. (1995) Digestion procedures for determining reduced sulfur species in bacterial cultures and in ancient and recent sediments. *ACS Symp. Ser.* **612,** 243–257.

Aller R. C. (1994) The sedimentary Mn cycle of Long Island Sound: Its role as intermediate oxidant and the influence of bioturbation, O_2, Corg flux on diagenetic reaction balances. *J. Mar. Res.* **52,** 259–295.

Aller R. C. and Rude P. D. (1988) Complete oxidation of solid phase sulfides by manganese and bacteria in anoxic marine sediments. *Geochim. Cosmochim. Acta* **52,** 751–765.

Bak F. and Cypionka H. (1987) A novel type of energy metabolism involving fermentation of inorganic sulphur compounds. *Nature* **326,** 891–892.

Böttcher M. E. (1998) Manganese(II) partitioning during experimental precipitation of rhodochrosite-calcite solid-solutions from aqueous solutions. *Mar. Chem.* **62,** 287–297.

Böttcher M. E., Gehlken P.-L., and Usdowski E. (1992) Infrared spectroscopic investigations of the calcite-rhodochrosite and parts of the calcite-magnesite mineral series. *Contrib. Mineral. Petrol.* **109,** 304–306.

Böttcher M. E. and Huckriede H. (1997) First occurrence and stable isotope composition of authigenic γ-MnS in the central Gotland Deep (Baltic Sea). *Mar. Geol.* **137,** 201–205.

Böttcher M. E., Brumsack H.-J., and de Lange G. J. (1998a) Sulfate reduction and related stable isotope (^{34}S, ^{18}O) variations in interstitial waters from the eastern Mediterranean. In *Proceedings of the Ocean Drilling Program, Scientific Results* (eds. A. H. F. Robertson, et al.), ODP College Station, TX, Vol. 160, pp. 365–373.

Böttcher M. E., Oelschläger B., Höpner T., Brumsack H.-J., and Rullkötter J. (1998b) Sulfate reduction related to the early diagenetic degradation of organic matter and "black spot" formation in tidal sandflats of the German Wadden Sea: Stable isotope (^{13}C, ^{34}S, ^{18}O) and other geochemical results. *Org. Geochem.* **29,** 1517–1530.

Böttcher M. E., Thamdrup B., and Vennemann T. W. (2001) Oxygen and sulfur isotope fractionation during anaerobic bacterial disproportionation of elemental sulfur. *Geochim. Cosmochim. Acta* **65,** 1601–1609.

Burdige D. J. and Nealson K. H. (1986) Chemical and microbiological studies of sulfide-mediated manganese reduction. *Geomicrobiol. J.* **4,** 361–387.

Calvert S. E. and Petersen T. F. (1996) Sedimentary geochemistry of manganese: Implications for the environment of formation of manganiferous Black Shales. *Econ. Geol.* **91,** 36–47.

Canfield D. E. and Teske A. (1996) Late Proterozoic rise in atmospheric oxygen concentration inferred from phylogenetic and sulfur-isotope studies. *Nature* **328,** 127–132.

Canfield D. E. and Thamdrup B. (1994) The production of ^{34}S-depleted sulfide during bacterial disproportionation of elemental sulfur. *Science* **266,** 1973–1975.

Canfield D. E. and Thamdrup B. (1996) Fate of elemental sulfur in an intertidal sediment. *FEMS Microbiol. Ecol.* **19,** 95–103.

Canfield D. E., Thamdrup B., and Fleischer S. (1998) Isotope fractionation and sulfur metabolism by pure and enrichment cultures of elemental sulfur-disproportionating bacteria. *Limnol. Oceanogr.* **43,** 253–264.

Canfield D. E., Thamdrup B., and Hansen J. W. (1993) The anaerobic degradation of organic matter in Danish coastal sediments: Fe reduction, Mn reduction and sulfate reduction. *Geochim. Cosmochim. Acta* **57,** 2563–2570.

Carpenter S. J. and Lohmann K. C. (1997) Carbon isotope ratios of Phanerozoic marine sediments: Re-evaluating the global carbon and sulfur systems. *Geochim. Cosmochim. Acta* **61,** 4831–4846.

Chen K. Y. and Morris J. C. (1972) Kinetics of oxidation of aqueous sulfide by O_2. *Environ. Sci. Technol.* **6,** 529–537.

Chiba H. and Sakai H. (1985) Oxygen isotope exchange rate between dissolved sulfate and water at hydrothermal temperatures. *Geochim. Cosmochim. Acta* **49,** 993–1000.

Clayton R. N. and Mayeda T. K. (1963) The use of bromine pentafluoride in the extraction of oxygen from oxides and silicates for isotopic analysis. *Geochim. Cosmochim. Acta* **27,** 43–52.

Cline J. D. (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol. Oceanogr.* **14,** 454–458.

Cline J. D. and Richards F. A. (1969) Oxygenation of hydrogen sulfide in seawater at constant salinity, temperature, and pH. *Environ. Sci. Technol.* **3,** 838–843.

Cypionka H., Smock A., and Böttcher M. E. (1998) A combined pathway of sulfur compound disproportionation in *Desulfovibrio desulfuricans*. *FEMS Microbiol. Lett.* **166,** 181–186.

Finster K., Liesack W., and Thamdrup B. (1998) Elemental sulfur and thiosulfate disproportionation by *Desulfocapsa sulfoexigens* sp. nov., a new anaerobic bacterium isolated from marine surface sediment. *Appl. Environ. Microbiol.* **64,** 119–125.

Fry B., Ruf W., Gest H., and Hayes J. M. (1988) Sulfur isotope effects associated with oxidation of sulfide by O_2 in aqueous solution. *Chem. Geol. (Isot. Geosc. Sec.)* **73,** 205–210.

Habicht K., Canfield D. E., and Rethemeier J. (1998) Sulfur isotope fractionation during bacterial reduction and disproportionation of thiosulfate and sulfite. *Geochim. Cosmochim. Acta* **62,** 2585–2595.

Huckriede H. and Meischner D. (1996) Origin and environment of manganese-rich sediments within black-shale basins. *Geochim. Cosmochim Acta* **60,** 1399–1413.

Huettel M., Ziebis S., Forster S., and Luther G. W. III (1998) Advective transport affecting metal and nutrient distribution and interfacial fluxes in permeable sediments. *Geochim. Cosmochim Acta* **62,** 613–631.

Jacobs L., Emerson S., and Skei J. (1985) Partitioning and transport of metals across the O_2/H_2S interface in a permanently anoxic basin: Framvaren Fjord, Norway. *Geochim. Cosmochim. Acta* **49,** 1433–1444.

Janssen P. H., Schuhmann A., Bak F., and Liesack W. (1996) Disproportionation of inorganic sulfur compounds by the sulfate-reducing bacterium *Desulfocapsa thiozymogenes* gen. nov., sp. nov. *Arch. Microbiol.* **166,** 184–192.

Jørgensen B. B. (1990a) A thiosulfate shunt in the sulfur cycle of marine sediments. *Science* **249,** 152–154.

Jørgensen B. B. (1990b) The sulfur cycle of freshwater sediments: Role of thiosulfate. *Limnol. Oceanogr.* **35,** 1329–1343.

Jørgensen B. B. and Bak F. (1991) Pathways and microbiology of thiosulfate transformations and sulfate reduction in a marine sediment (Kattegat, Denmark). *Appl. Environ. Microbiol.* **57,** 847–856.

Kristensen E., Bodenbender J., Jensen M. H., Rennenberg H., and Jensen K. M. (2000) Sulfur cycling of intertidal Wadden Sea sediments (Konigshafen, Island of Sylt, Germany): Sulfate reduction and sulfur gas emission. *J. Sea Res.* **43,** 93–104.

Laznicka P. (1992) Manganese deposits in the global lithogenic system: Quantitative approach. *Ore Geol. Rev.* **7,** 279–356.

Lloyd R. M. (1968) Oxygen isotope behavior in the sulfate-water system. *J. Geophys. Res.* **73,** 6099–6110.

Lovley D. R. and Phillips E. J. P. (1994) Novel processes for anaerobic sulfate production from elemental sulfur by sulfate-reducing bacteria. *Appl. Environ. Micobiol.* **60,** 2394–2399.

Luther G. W. III (1990) The frontier-molecular-orbital theory approach in geochemical processes. In *Aquatic Chemical Kinetics*. (ed. W. Stumm), pp. 173–198. Wiley.

Millero F. J. (1991a) The oxidation of H_2S in Framvaren Fjord. *Limnol. Oceanogr.* **36,** 1007–1014.

Millero F. J. (1991b) The oxidation of H_2S in Chesapeake Bay. *Estuarine Coastal Shelf Sci.* **33,** 521–527.

Millero F. J. (1991c) The oxidation of H_2S in Black Sea waters. *Deep-Sea Res.* **38,** S1139–S1150.

Mizutani Y. and Rafter T. A. (1969a) Oxygen isotopic composition of sulphates - part 3. *New Zealand J. Sci.* **12,** 54–59.

Mizutani Y. and Rafter T. A. (1969b) Oxygen isotopic composition of sulphates - part 4. *New Zealand J. Sci.* **12**, 60–68.

Moeslund L., Thamdrup B., and Jørgensen B. B. (1994) Sulfur and iron cycling in a coastal sediment: Radiotracer studies and seasonal dynamics. *Biogeochemistry* **27**, 129–152.

O'Neil J. R., Adami L. H., and Epstein S. (1975) Revised value for the O^{18} fractionation between CO_2 and water at 25°C. *U.S. Geol. Surv. J. Res.* **3,** 623–624.

Pickthorn W. J. and O'Neil J. R. (1985) ^{18}O relations in alunite minerals: Potential single-mineral thermometer. *GSA Abstr. Progr.* **17,** 689.

Schippers A. and Jørgensen B. B. (2001) Oxidation of pyrite and iron sulfide by manganese dioxide in marine sediments. *Geochim. Cosmochim. Acta* **65,** 915–922.

Smith J. W. (2000) Isotopic fractionation accompanying sulfur hydrolysis. *Geochem. J.* **34**, 95–99.

Sundby B., Christensen S., and Silverberg N. (1981) Pathways of manganese in an open estuary. *Geochim. Cosmochim Acta* **45,** 293–297.

Taylor B. E., Wheeler M. C., and Nordstrom D. K. (1984) Stable isotope geochemistry of acid mine drainage: Experimental oxidation of pyrite. *Geochim. Cosmochim. Acta* **48,** 2669–2678.

Thamdrup B. (2000) Bacterial manganese and iron reduction in aquatic sediments. In *Adv. Microbial. Ecol*. (ed. Schink, B.), Vol. 16, pp. 41–84. Kluwer Academic/Plenum Publishers.

Thamdrup B., Finster K., Hansen W., and Bak F. (1993) Bacterial disproportionation of elemental sulfur coupled to chemical reduction of iron and manganese. *Appl. Environ. Microbiol.* **59,** 101–108.

Thamdrup B., Fossing H., and Jørgensen B. B. (1994) Manganese, iron, and sulfur cycling in a coastal marine sediment, Aarhus Bay, Denmark. *Geochim. Cosmochim. Acta* **58,** 5115–5129.

Thode–Andersen S. and Jørgensen B. B. (1989) Sulfate reduction and the formation of ^{35}S-labeled FeS, FeS_2, and S° in coastal marine sediments. *Limnol. Oceanogr.* **34,** 793–806.

Troelsen H. and Jørgensen B. B. (1982) Seasonal dynamics of elemental sulfur in two coastal sediments. *Estuar. Coast. Shelf Sci.* **15,** 255–266.

Van Stempvoort D. R. and Krouse H. R. (1994) Controls of $\delta^{18}O$ in sulfate: Review of experimental data and application to specific environments. *ACS Symp Ser*. **550,** 466–480.

White W. B. (1974) The carbonate minerals. In (Farmer V. C., ed.) Infrared spectra of the minerals. *Min. Soc. Monogr.* **4,** 227–284.

Widdel F. and Bak F. (1991) Gram-negative mesophilic sulfate-reducing bacteria. In *The Procaryotes*. (eds. A. Balows et al.), pp. 3352–3378. Springer.

Yao W. and Millero F. J. (1993) The rate of sulfide oxidation by δ-MnO_2 in seawater. *Geochim. Cosmochim. Acta* **57,** 3359–3365.

Yao W. and Millero F. J. (1995) Oxidation of hydrogen sulfide by Mn(IV) and Fe(III) (hydr)oxides in seawater. *ACS Symp. Ser.* **612,** 260–279.

Yao W. and Millero F. J. (1996) Oxidation of hydrogen sulfide by hydrous Fe(III) oxides in seawater. *Mar. Chem.* **52,** 1–16.