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Oxygen and sulfur isotope fractionation during anaerobic bacterial disproportionation of elemental sulfur

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Abstract—Bacterial disproportionation of elemental sulfur is an important process in the sulfur cycle of natural sediments and leads to the formation of hydrogen sulfide and sulfate. The oxygen atoms in sulfate during this anaerobic process are completely derived from water according to the overall reaction:

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

In the present study, stable oxygen isotope fractionation during formation of sulfate via this reaction was experimentally investigated for a pure culture (Desulfocapsa thiozymogenes) and an enrichment culture ("Kuhgraben") at 28°C. Synthetic $FeCO_3$ and FeOOH were used as scavengers for hydrogen sulfide to keep the disproportionation reaction exergonic and to suppress polysulfide formation and isotope exchange between elemental sulfur and hydrogen sulfide. Compared to water, dissolved sulfate was enriched in ^{18}O by $+17.4 \pm 0.1$‰ (*Desulfocapsa thiozymogenes*) and $+16.6 \pm 0.5$‰ (Kuhgraben) at cell specific sulfur disproportionation rates of $10^{-15.4 \pm 0.4}$ mol S° $cell^{-1}$ h^{-1} and $10^{-14.4 \pm 0.9}$ mol S° $cell^{-1}$ h^{-1}, respectively. Oxygen isotope fractionation was not influenced by the type of iron-bearing scavenger used, corroborating earlier findings that H_2S oxidation by FeOOH yields elemental sulfur as the dominant oxidation product. Sulfite is suggested to be formed as a metabolic intermediate to facilitate isotope exchange with water. Due to bacterial disproportionation, dissolved sulfate was also enriched in ^{34}S compared to elemental sulfur by $+11.0$ to $+18.4$‰ (*D. thiozymogenes*) and $+12.7$ to $+17.9$‰ (Kuhgraben). FeS was depleted in ^{32}S compared to elemental sulfur by -3.7 to -5.3‰ (*D. thiozymogenes*).

1. INTRODUCTION

Mineralization of organic matter in coastal sediments proceeds to a significant amount via bacterial activity with sulfate as the final electron acceptor (Jørgensen, 1982). Most of the hydrogen sulfide which is produced during dissimilatory sulfate reduction is not buried in the sediment but oxidized by microbial or chemical reactions (Jørgensen, 1978; Thamdrup et al., 1994). In addition to sulfate, sulfur species with intermediate oxidation states such as elemental sulfur (S°) and thiosulfate ($S_2O_3^{2-}$) have been found as oxidation products (Cline and Richards, 1969; Chen and Morris, 1972; Yao and Millero, 1995; 1996). Elemental sulfur, for example, is a common compound in natural surface sediments (Troelsen and Jørgensen, 1982; Thode-Andersen and Jørgensen, 1989). The intermediate sulfur species may further be oxidized, reduced or disproportionated (Bak and Cypionka, 1987; Jørgensen, 1990a,b; Jørgensen and Bak, 1991; Fry et al., 1986a; Smock et al., 1998; Thamdrup et al., 1993) leading to the formation of hydrogen sulfide and/or sulfate. Bacterial disproportionation reactions of elemental sulfur and thiosulfate have been found to contribute significantly to the sedimentary cycle of sulfur in limnic and marine sediments (Jørgensen, 1990a,b; Jørgensen and Bak, 1991; Canfield and Thamdrup, 1996).

It was demonstrated experimentally that the disproportionation of these sulfur intermediates is accompanied by significant fractionation of the stable sulfur isotopes ^{34}S and ^{32}S (Canfield and Thamdrup, 1994; Canfield et al., 1998; Cypionka et al., 1998; Habicht et al., 1998), which is believed to contribute to the strong overall enrichment of ^{32}S in natural sedimentary sulfides (e.g., Canfield and Thamdrup, 1994; Jørgensen, 1990a). In addition to sulfur isotope fractionation, the stable oxygen isotopes ^{18}O and ^{16}O have also been found to be extremely useful for characterization of biogeochemical reactions (e.g., Mizutani and Rafter, 1973; Taylor et al., 1984; van Stempvoort and Krouse, 1994).

Dissolved sulfate has the advantage of being naturally labeled with two biogeochemically useful isotopic systems. Nonbiologic oxygen isotope exchange between sulfate and water is extremely slow at neutral pH and low temperatures (e.g., Lloyd, 1968; Mizutani and Rafter, 1969a; Chiba and Sakai, 1985), which leads to an isotope exchange disequilibrium between SO_4^{2-} and H_2O in seawater. However, the oxygen isotope composition may be strongly influenced by bacterial processes (e.g., van Stempvoort and Krouse, 1994). Significant isotope discrimination of both sulfur and oxygen isotopes in residual sulfate modified by bacterial dissimilatory sulfate reduction (BSR) has been observed in experiments (Lloyd, 1968; Mizutani and Rafter, 1973; Fritz et al., 1989; Böttcher, Benecke and Cypionka, unpublished data) and in naturally occurring marine sediments (Zak et al., 1980; Böttcher et al., 1998a,b; Ku et al., 1999; Aharon and Fu, 2000). Figure 1 provides an example of the covariation of sulfur and oxygen isotope compositions that is observed in many natural and experimental settings. In this example, the data are from interstitial waters of

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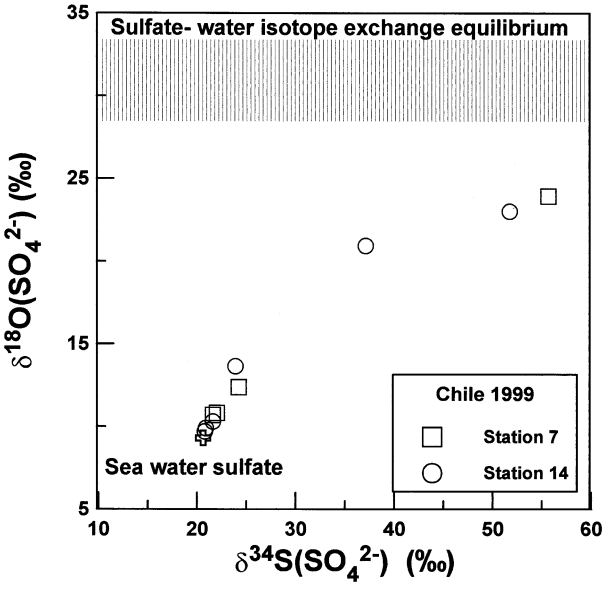


Fig. 1. Covariation of the sulfur and oxygen isotope composition of pore water sulfate in sediments from the upwelling area off central Chile (Böttcher; unpublished data). The isotope exchange equilibrium between sulfate and water under in situ conditions was extrapolated from hydrothermal experiments of Mizutani and Rafter (1969a) and Lloyd (1968). The shaded area indicates the range between these two extremes. The isotopic composition of sea water sulfate was taken to be $\delta^{34}S(SO_4) = +20.5‰$ (Böttcher et al., 2000) and $\delta^{18}O(SO_4) = +9.3‰$.

sediments dominated by bacterial sulfate reduction in the upwelling region off central Chile. The relative enrichment of sulfur and oxygen isotopes as a function of the extent of the reaction in sediments was related previously to rates of bacterial sulfate reduction (Böttcher et al., 1998a,b; Aharon and Fu, 2000) or a superimposition of BSR by the reoxidation of H_2S (Ku et al., 1999). The impact of anaerobic bacterial disproportionation processes on oxygen isotope partitioning in the sedimentary sulfur cycle of surface sediments has, however, not been considered.

The present study summarizes the first reported results for oxygen isotope fractionation during bacterial disproportionation of elemental sulfur. These new findings have implications for the biochemistry of the disproportionation process and must be considered in biogeochemical interpretations of stable isotope fractionations in natural sediments.

2. EXPERIMENTAL TECHNIQUES

A pure culture of *Desulfocapsa 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 *Desulfocapsa 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 widths of 0.6 μm (Canfield et al., 1998). *Desulfocapsa thiozymogenes* is a strictly anaerobic strain and is capable of reducing sulfate with ethanol, propanol, or butanol, as well as disproportionating thiosulfate and sulfite (Janssen et al., 1996). The dominant bacterium from the Kuhgraben enrichment has not yet been isolated for further physiologic characterization. Both cultures were shown in a previous study to fractionate sulfur isotopes to the same extent as marine strains (Canfield et al., 1998).

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, 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 (about 5 mmol) to the medium with a sterile spatula together with synthetic X-ray amorphous ferric oxyhydroxide (FeOOH) or ferrous carbonate, both prepared as described by Thamdrup et al. (1993). Solids were added under anoxic conditions. Synthetic $FeCO_3$ or FeOOH were used as scavengers for hydrogen sulfide to keep the disproportionation reaction exergonic (Thamdrup et al., 1993; Lovley and Phillips, 1994) and to suppress polysulfide formation and isotope exchange between elemental sulfur and hydrogen sulfide.

Completely filled screw-capped glass bottles (54 cm^3) were inoculated simultanously 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 collected with a syringe for measurements of pH, cell numbers, and dissolved iron. H_2S was determined spectrophotometrically on selected filtered samples (0.2 μm; N_2-flushed cellulose acetate filters from Sartorius) (Cline, 1969). The pH was measured on unfiltered samples using an Orion combination electrode. The filtrate of a separate aliqout was acidified with HCl and analyzed for iron by standard procedures using ICP-OES (Perkin Elmer Optima 3000XL) or AAS (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 complete dissolution of FeOOH and FeS (acid-volatile sulfide; AVS) by a dithionite-citrate-acetic acid solution (Thamdrup et al., 1993), cells were stained with DAPI and counted with a Zeiss Axiolab epifluorescence microscope. Initial cell counts were about 1×10^7 cells ml^{-1} for *Desulfocapsa thiozymogenes* and 3 to 4×10^6 cells ml^{-1} 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 (precision 2%). Selected samples where checked to consist of pure $BaSO_4$ using FT infrared spectroscopy. The AVS and pyrite fractions were also separated from the samples fixed with Zn-acetate and the AVS isotopically characterized as described earlier (Canfield et al., 1998).

$BaSO_4$ precipitates were carefully washed, dried and analysed for the sulfur isotopic composition by combustion isotope-ratio-monitoring mass spectrometry (C-irmMS) as described by Böttcher et al. (1998a) using a Carlo Erba EA 1108 elemental analyzer connected to a Finnigan MAT 252 mass spectrometer via a Finnigan Conflo II open split interface. International silver sulfide standards IAEA-*S*-1 and IAEA-*S*-2 were used to calibrate the mass spectrometer for sulfur isotope measurements. A $\delta^{34}S$ value of +20.6‰ was obtained for the international barium sulfate standard NBS-127. Replicate measurements agreed within ±0.15‰. The oxygen isotope composition of $BaSO_4$ was analyzed 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 is 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 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 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 reproduced at better than 0.1‰. $^{18}O/^{16}O$ 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

Table 1. Chemical and isotopic composition of experimental solutions during disproportionation of elemental sulfur by the pure culture *Desulfocapsa thiozymogenes* and the enrichment culture Kuhgraben in the presence of $FeCO_3$ (B-II; K-II) and FeOOH (B-III, K-III) at 28°C.

Time (h)	pH	log cells/ml	SO_4 (mM)	$\delta^{34}S(SO_4)$	$\delta^{18}O(SO_4)$	$\delta^{34}S(AVS)$	Fe,aq (μM)	AVS (mM)	logSDR
B-II									
2	7.32	7.11	0.0	n.a.	n.a.	n.d.	29	0.4″	n.a.
94	7.09	7.69	0.4	27.4	n.d.	n.d.	36	n.d.	−9.2
145	7.01	7.92	0.7	28.5	n.d.	n.d.	49	n.d.	−9.5
290	6.65	7.93	2.1	31.2	9.9	11.9	n.d.	6.7	−9.4
338	6.35	8.07	3.8	33.0	n.d.	11.8	294	8.4	−8.9
408	6.34	7.95	4.1	34.0	9.8	11.8	307	9.2	−9.8
B-III									
2	7.28	7.10	0.0	n.a.	n.a.	n.d.	0	0.4*	n.a.
94	6.98	8.04	1.4	26.5	n.d.	n.d.	260	3.7*	−9.0
145	6.76	8.05	2.8	27.2	n.d.	n.d.	461	5.3*	−9.0
290	6.19	8.41	7.4	28.8	n.d.	11.5	864	4.4	−9.2
338	6.14	8.47	7.6	30.2	n.d.	11.0	788	3.8	−10.2
408	5.85	8.52	9.3	32.4	9.7	10.3	287	4.0	−9.5
K-II									
2	7.31	6.58	0.0	n.a.	n.a.	n.d.	51	0.5″	n.a.
72	6.92	6.79	1.2	29.1	n.d.	n.d.	67	n.d.	−7.8
169	6.46	6.78	3.1	32.6	9.7	n.d.	309	n.d.	−7.9
219	6.26	6.99	4.5	32.5	8.8	n.d.	491	3.8	−7.9
364	5.93	7.08	7.6	32.0	8.4	n.d.	1134	2.5	−8.1
412	5.91	6.90	7.8	32.8	n.d.	n.d.	1251	n.d.	−8.6
K-III									
2	7.23	6.41	0.0	n.a.	n.a.	n.d.	15	0.5*	n.a.
72	6.78	6.47	3.0	28.3	8.7	n.d.	652	4.8*	−7.2
169	6.29	7.52	7.3	30.4	n.d.	n.d.	1701	4.8	−8.0
219	6.28	7.81	7.7	31.5	8.6	n.d.	1519	3.6	−9.2
364	5.99	7.54	9.5	33.2	n.d.	n.d.	566	0.5	−9.6
412	5.98	7.77	9.6	33.5	9.3	n.d.	759	0.4	−9.9

n.a.: not applicable; n.d., not determined.

Concentrations and sulfur isotopic compositions of dissolved sulfate were corrected for small amounts of sulfate initially present in the pre-cultures. Initial solutions of experiments B-II and -III contained 0.32 ± 0.01 mM SO_4^{2-} ($\delta^{34}S$ = 23‰); experiments K-II and -III contained 0.32 ± 0.03 mM SO_4^{2-} ($\delta^{34}S$ = 29‰). Sulfur disproportionation rate (SDR) are given in [μmol S° $cell^{-1}h^{-}$].

* Based on the Fe(II) content of the solid leached with HCl as described by Thamdrup et al. (1993). ″Based on analogy to experiments B-III and K-III.

solution had an oxygen isotope composition of −7.5 ± 0.1‰ vs. V-SMOW (n = 4). The oxygen isotope composition of the solution did not change during the course of the experiments, beyond analytical error. Concentrations and sulfur isotopic compositions of dissolved sulfate were corrected for small amounts of sulfate initially present in the precultures (Table 1).

3. RESULTS AND DISCUSSION

During the course of the experiments with *Desulfocapsa thiozymogenes* and Kuhgraben, cell growth and a continuous increase in the concentrations of SO_4^{2-} and H^+ were observed (Fig. 2a,b). No other dissolved sulfur species were previously found in experiments conducted with these cultures in the presence of FeOOH (Thamdrup, unpublished results). The observed compositional variations are consistent with the overall process of hydrogen sulfide and sulfate production during sulfur disproportionation, followed by the fast reaction of hydrogen sulfide with FeOOH or $FeCO_3$ (Thamdrup et al., 1993) according to:

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

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

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

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

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

$$3H^+ + 3FeCO_3 \rightarrow 3Fe^{2+} + 3HCO_3^- \quad (5)$$

$$3H_2S + 3Fe^{2+} \rightarrow 3FeS + 6H^+ \quad (6)$$

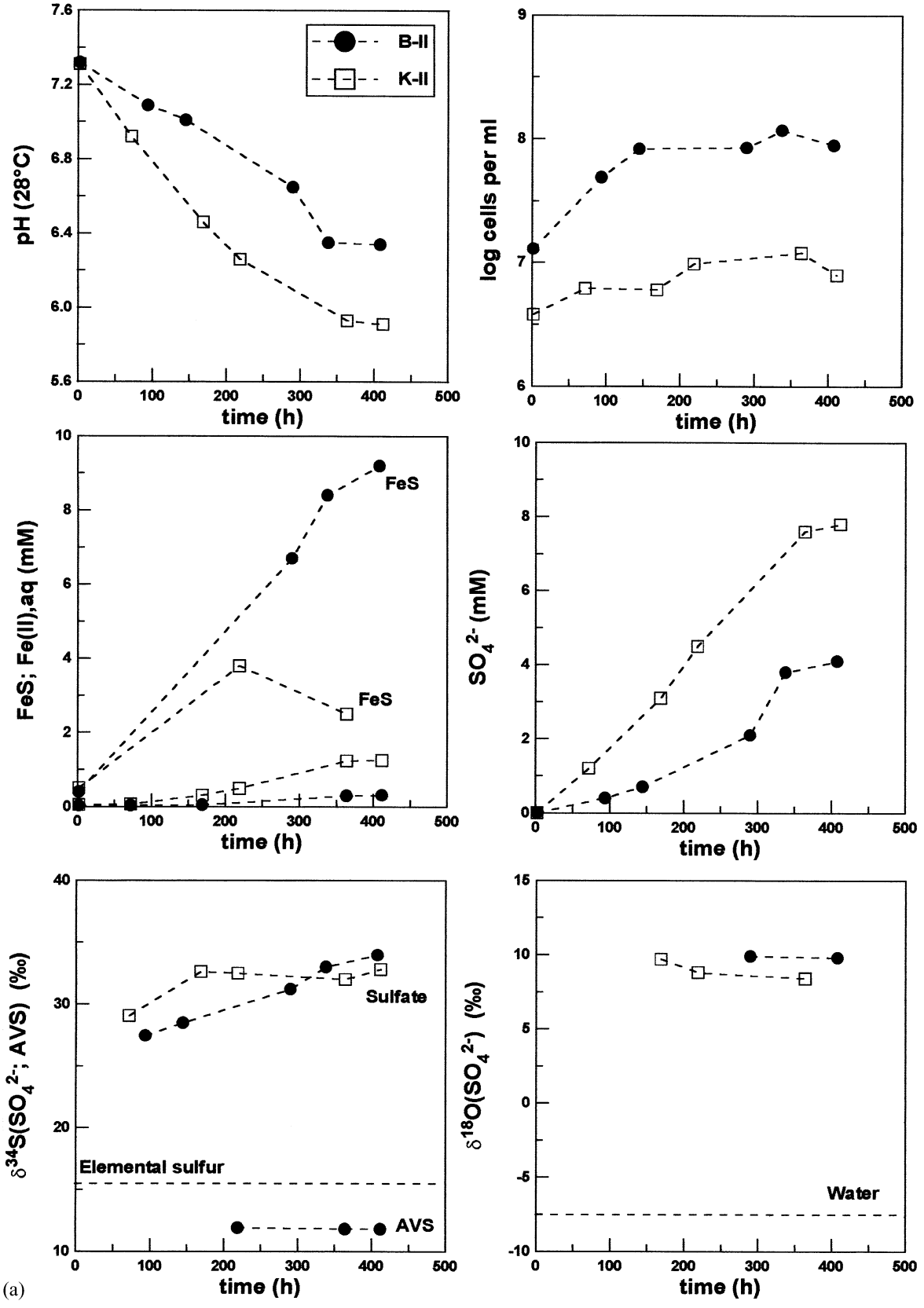
$$4S° + 4H_2O + 3FeCO_3 \rightarrow SO_4^{2-} + 3FeS + 2H^+ + 3H_2CO_3 \quad (7)$$

Dissolved Fe^{2+} showed a maximum due to the dissolution of FeOOH or $FeCO_3$ followed by the incorporation into FeS (Eqn. 3 and 6). High concentrations of dissolved iron are compatible with the absence of hydrogen sulfide in the experimental solutions.

As additional reactions, processes leading to the formation of pyrite have to be considered. In previous experiments with the same strains used in the present study, pyrite formation was observed at pH values below about 6.8 (Canfield et al., 1998). Pyritization can take place via the reaction of FeS precursors with either elemental sulfur (Berner, 1970; Rickard, 1975; Schoonen and Barnes, 1991; Wilkin and Barnes, 1996) or hydrogen sulfide (Rickard, 1997):

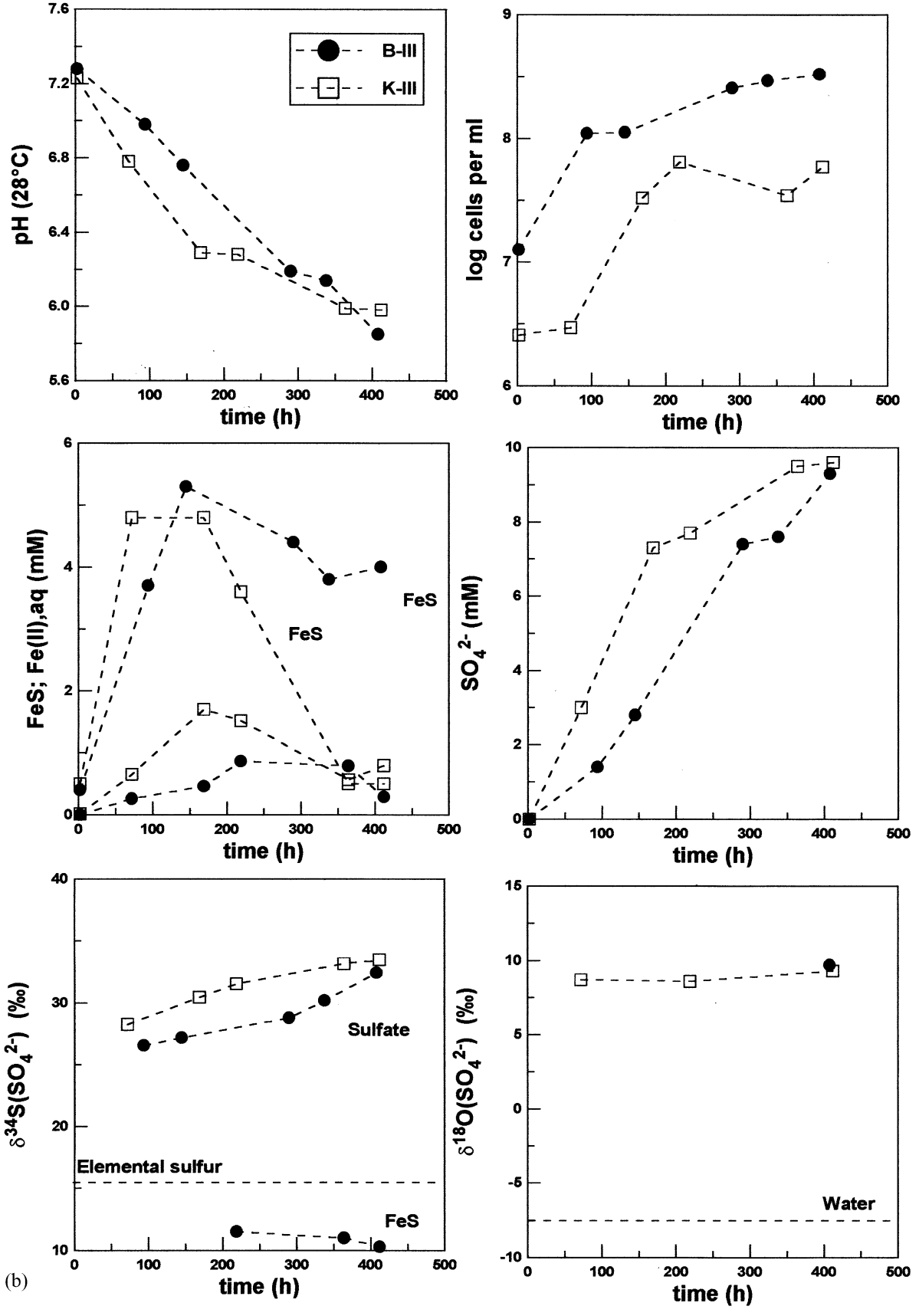
$$FeS + S° \rightarrow FeS_2 \quad (8)$$

and



(a)

Fig. 2. a) Variation of selected parameters during experimental disproportionation of elemental sulfur by strains Bra2 (B-II) and Kuhgraben (K-II) in the presence of $FeCO_3$. b) Variation of selected parameters during experimental disproportionation of elemental sulfur by strains Bra2 (B-III) and Kuhgraben (K-III) in the presence of FeOOH.



(b)

Fig. 2. (Continued)

$$FeS + H_2S \rightarrow FeS_2 + H_2. \quad (9)$$

Pyritization via Eqn. 8 may involve polysulfide formation by reaction of H_2S with elemental sulfur (Luther, 1991).

FeS contents were measured as acid volatile sulfide (AVS) in the experimental solids of selected experiments (Table 1). In experiments B-III (94 h) and B-II (290 h) the molar FeS/SO_4^{2-} ratios were 2.4 and 3.0, respectively, which agree with the theoretical values of 2 and 3 (Eqn. 4 and 7). Accordingly, no pyrite formation was observed in these experiments. With increasing reaction time, however, the FeS/SO_4^{2-} ratios decreased (Table 1) due to the parallel formation of pyrite according to Eqn. 8 and/or (9). Pyrite formation seems to be restricted to lower pH values in the aqueous solution, in agreement with previous results on sulfur disproportionation (Canfield et al., 1998), and the evaluation of pyritization rates as a function of pH (Rickard, 1997). All experiments analysed for AVS with the enrichment culture where influenced by pyrite precipitation as indicated by the FeS/SO_4^{2-} ratios. This difference was due to a faster reaction rate and decrease in pH of the Kuhgraben enrichment compared to *Desulfocapsa thiozymogenes* (Fig. 2; Table 1). In experiments with these cultures in the presence of FeOOH, Canfield et al. (1998) measured $(FeS + FeS_2)/SO_4^{2-}$ ratios of 2.1 and 1.5, close to the theoretical value of 2. The pyritization mechanism during S° metabolism in the presence of FeOOH was also discussed by Canfield et al. (1998). Based on sulfur isotope partitioning the authors tentatively related the pyrite formation in experiments with the enrichment and pure culture to mechanisms (8) and (9), respectively. Detailed kinetic results on pyrite formation from the present study and additional experiments with iron(II) compounds will be discussed in a later publication.

Both cultures showed the same general concentration trends (Fig. 2) and our results for the incubations with FeOOH are similar to those reported by Canfield et al. (1998) using the same cultures. The changes in concentration of dissolved iron with time reflect the reductive and/or acidic dissolution of FeOOH and $FeCO_3$. These concentrations are further influenced by precipitation of FeS and, in later stages of the experiments, precipitation of FeS_2. Additionally, the aqueous concentration of Fe(II) is determined by the pH of the solution (Fig. 2), solubility of iron carbonate, and availability of H_2S.

Cellular sulfur disproportionation rates (SDR) were estimated based on the change in cell numbers and sulfate concentrations with time:

$$SDR\ [\mu mol\ S°\ cell^{-1}\ h^{-1}] = 4(S_i - S_{i-1})\ ((C_i + C_{i-1})/2)^{-1}\ (t_i - t_{i-1})^{-1} \quad (10)$$

where S, C and t refer to the amount of sulfate [μmol], the total cell number, and the reaction time, respectively, at time intervals i and i-1. SDR values varied between $10^{-9.5}$ and $10^{-10.8}$ μmol S° $cell^{-1}$ h^{-1} for *Desulfocapsa thiozymogenes* and $10^{-7.8}$ and $10^{-10.5}$ μmol S° $cell^{-1}$ h^{-1} for Kuhgraben. These rates appear to decrease with progress of the reaction. Cell growth actually stopped during the final stages of the experiments. This could reflect a decrease in pH during the disproportionation process in batch experiments (Table 1) as indicated by the overall reactions (4) and (7). Janssen et al. (1996) reported growth of *Desulfocapsa thiozymogenes* within a pH range of 6.8 and 8.0. Our results indicate that the lower limit for growth during disproportionation of elemental sulfur at 28°C is a slightly lower pH range of around 6.2 to 6.4. The same limit applies to the Kuhgraben enrichment (Table 1).

3.1. Stable Sulfur Isotopes

Given the conditions described by Eqn. 1 to (7), the isotopic composition of dissolved sulfate reflects the isotope effects caused by the bacterial disproportionation process. Compared to the starting elemental sulfur ($\delta^{34}S = 15.6 \pm 0.2$‰), dissolved sulfate was enriched in ^{34}S by 10.9 to 18.4‰ (*Desulfocapsa thiozymogenes*) and 12.7 to 17.9‰ (Kuhgraben). The isotope effects observed for *Desulfocapsa thiozymogenes* in this study are similar to those presented in a figure by Canfield et al. (1998), but the range obtained for the Kuhgraben enrichment is extended in the present study. The enrichment of ^{34}S in the sulfate produced with respect to initial S° increased during the course of the experiments by up to 7‰ (Table 1).

In the present study, all $\delta^{34}S$ values of dissolved sulfate were corrected for the contribution from the initial small amounts of sulfate. The data presented in Table 1 are, therefore, not affected by the small amounts of sulfate transferred from the precultures. Additionally, no reservoir effect on sulfur isotope fractionation (closed system with respect to elemental sulfur; e.g., Hartmann and Nielsen, 1969) is expected because elemental sulfur was added in significant excess, and no change in its isotopic composition was observed in experiments limited with respect to S° (Canfield and Thamdrup, 1994). Comparison of experiments with (K-III, B-III) and without (K-II, B-II) oxidation of H_2S by FeOOH shows no significant difference in the $\delta^{34}S$ values of sulfate (Table 1). This relationship indicates that little or no sulfate was formed during the reaction with FeOOH, and S° was by far the dominant oxidation product, as described by Eqn. 2. This is also in agreement with earlier experimental findings (Yao and Millero, 1996). The trend in sulfur isotope fractionation is similar to the data (uncorrected for initial sulfate) given by Canfield et al. (1998) for experiments with FeOOH. The authors argued that the variation in sulfur isotope fractionation during reaction progress can be explained by the preferred metabolism of elemental sulfur which is newly formed during the oxidation reaction (2) and has a different $\delta^{34}S$ value compared to the initial S°. However, since we observed the same trend in incubations with $FeCO_3$, wherein S° is not produced, reoxidation of H_2S can not be the explanation for the change in the sulfur isotope composition of sulfate with time.

As an additional possibility we have to consider that hydrogen, which is produced during pyrite formation (Eqn. 9), may be used by the bacteria for the reduction of sulfate (Postgate, 1984). This process could explain the observed shift to more enriched sulfate with time (Kaplan and Rittenberg, 1964; Chambers and Trudinger, 1979). However, it has been shown experimentally, that H_2 is not used by *Desulfocapsa thiozymogenes* as an electron donor during sulfate reduction (Janssen et al., 1996). Additionally, bacterial sulfate reduction should lead to a shift in the oxygen isotope composition of residual sulfate (section 3.2) towards higher $\delta^{18}O$ values (e.g., Fritz et al., 1989), and sulfate concentrations should be reduced. Such trends are actually not found in our experiments (Table 1), indicating that sulfate reduction did not take place.

We conclude that the change in sulfur isotope fractionation between initial S° and total SO_4^{2-} with ongoing reaction is probably related to the change in the cell-specific sulfur disproportionation rates (SDR). Such a relationship was also proposed by Canfield et al. (1998) to explain variations in fractionation observed between different cultures. Further experimental investigations aimed at determining the influence of SDR on the isotope composition of sulfate is in progress.

The AVS fraction, that should reflect the isotopic composition of H_2S (Price and Shieh, 1979; Böttcher et al., 1998c) was found to be depleted with respect to elemental sulfur by -3.8 ± 0.1‰ (B-II) and -4.7 ± 0.6‰ (B-III). This is in general agreement with measurements by Canfield et al. (1998) in the presence of FeOOH. Whereas constant isotope compositions were observed in the presence of ferrous carbonate, the presence of FeOOH led to a slight shift to decreasing ^{34}S contents with reaction progress. This indicates a minor sulfur isotope effect during reoxidation of H_2S to S° in the presence of FeOOH. The change in the isotope effect is probably due to the decrease in pH of the experimental solution, because the relative proportions of the aqueous reduced sulfur species formed during disproportionation, H_2S_{aq} and HS^-, will change. In aqueous solution, both species are expected to be in isotope equilibrium due to very fast exchange reactions (Eigen and Kustin, 1960), and have been found to differ isotopically from each other (Fry et al., 1986b). Therefore, the overall isotope effect associated with the parallel oxidation and iron sulfide precipitation (Eqn. 2 and 3) may be influenced by pH and may be an explanation for the slight changes in the isotopic composition of FeS in experiment B-III (Table 1). The isotopic composition of FeS in runs of B-II, on the other hand, reflect the isotope effect between elemental sulfur and H_2S associated with reaction (1).

3.2. Stable Oxygen Isotopes

Dissolved sulfate formed during microbial disproportionation of elemental sulfur was significantly enriched in ^{18}O when compared to water in the experimental solution ($\delta^{18}O = -7.5 \pm 0.1$‰ vs. V-SMOW), and the results for both strains were independent of the type of Fe scavenger used (Table 1; Fig. 2).

It is shown by the sulfur isotope composition of sulfate observed in experiments with iron(II) and iron(III) compounds (section 3.1) that reoxidation of H_2S in the presence of FeOOH led to the formation of elemental sulfur but not sulfate, as described by Eqn. 1 to 4, and in agreement with earlier experimental studies (Yao and Millero, 1995; 1996).

Under the anoxic conditions of the present experiments, the oxygen atoms of newly formed sulfate are, therefore, completely derived from water. Water was present in far excess and isotopically not influenced by the bacterial disproportionation process. Hence, the oxygen isotope composition of both species are related by (e.g., Taylor et al., 1984):

$$\delta^{18}O(SO_4) = \delta^{18}O(H_2O) + \varepsilon_{H2O} \quad (11)$$

The magnitude of the kinetic isotope enrichment factor, ε_{H2O}, depends on the bacterial and chemical processes involved in sulfate formation, and isotope effects of different magnitudes were reported accordingly (e.g., van Stempvoort and Krouse, 1994 and references therein). Taylor et al. (1984) for instance, reported a mean ε_{H2O} value of +4‰ for the experimental oxidation of sulfides.

In the present study, anaerobic bacterial disproportionation of elemental sulfur was accompanied by an oxygen isotope effect of $+17.4 \pm 0.1$‰ (*Desulfocapsa thiozymogenes*) and $+16.6 \pm 0.5$‰ (Kuhgraben). No significant differences were observed between experiments using the two strains of this study, and $FeCO_3$ or FeOOH. Since no oxidation of H_2S took place in experiments B-II and K-II, the similarity between these results and experiments B-III and K-III further substantiates the conclusion from the sulfur isotope results that no oxidation of H_2S to sulfate occurred in the latter experiments. Additionally, no significant change in oxygen isotope fractionation was observed during the course of the experiments indicating that cell specific disproportionation rates in the range under investigation did not significantly affect the reactions responsible for oxygen isotope discrimination.

The oxygen isotope fractionation between water and sulfate during the oxidative part of elemental sulfur disproportionation is much higher than isotope effects observed during bacterial oxidation of elemental sulfur (Mizutani and Rafter, 1969b) and chemical and microbial oxidation of sulfides (Taylor et al., 1984). Non-biologic isotope exchange between water and sulfate can be neglected under the conditions of pH, temperature, and time of our experiments (e.g., Mizutani and Rafter, 1969a; Lloyd, 1968; Chiba and Sakai, 1985). On the other hand, oxygen isotope exchange is fast between sulfite and water (van Stempvoort and Krouse, 1994). Under equilibrium conditions, sulfite should be significantly enriched in ^{18}O compared to water. Our experimental results, therefore, suggest that oxygen isotope exchange took place with water, likely via sulfite as an intermediate sulfur species. Consequently, our results confirm earlier experimental findings for sulfide oxidation by sulfate-reducing bacteria, where sulfite was found as an intermediate during elemental sulfur disproportionation (Fuseler and Cypionka, 1995; Fuseler et al., 1996). Cell-internal isotope exchange between sulfite and water was also suggested as a possibility to explain oxygen isotope fractionation during bacterial dissimilatory sulfate reduction (Mizutani and Rafter, 1973; Fritz et al., 1989). Sulfite is also thought to be an intermediate during metabolism of many sulfide-oxidizing but non-sulfate-reducing bacteria (Nelson and Hagen, 1995). Oxygen isotope effects observed for selected bacteria, however, seem to be rather small (Taylor et al., 1984), and we suggest as an explanation for the difference to our experimental results that the lifetime of intracellular sulfite during disproportionation of elemental sulfur may be longer than in aerobic oxidizers.

3.3. Geochemical Implications

Based on the present study of bacterial sulfur disproportionation and earlier investigations of bacterial sulfate reduction (Mizutani and Rafter, 1973; Fritz et al., 1989) it can be concluded that these anaerobic processes lead to a significant enrichment in ^{18}O in dissolved sulfate compared to the aqueous solution. Direct chemical or microbial oxidation of H_2S to sulfate, by contrast, is accompanied by a much smaller oxygen isotope effect. The oxygen and sulfur isotope compositions of

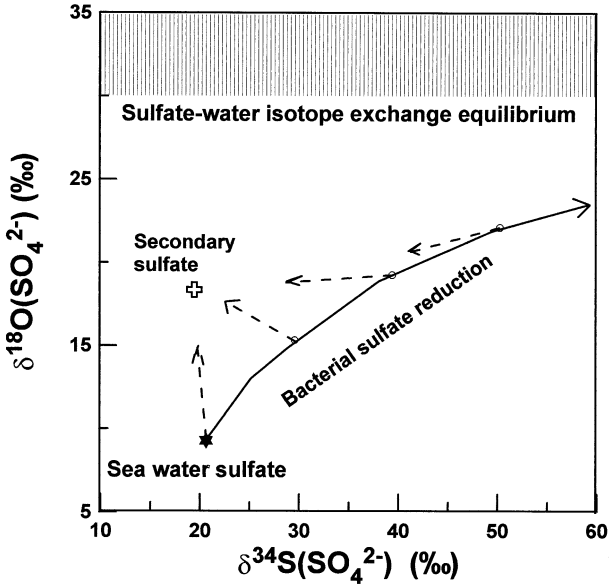


Fig. 3. Schematic presentation of the covariation of sulfur and oxygen isotope compositions of pore water sulfate during sulfate reduction and the possible influence by sulfur disproportionation. The sulfate reduction trend is based on data from the Gulf of Mexico (Aharon and Fu, 2000). The isotope exchange equilibrium between sulfate and water under in situ conditions was extrapolated from hydrothermal experiments of Mizutani and Rafter (1969a) and Lloyd (1968). The shaded area indicates the range between these two extremes. $\delta^{18}O(H_2O) = 2‰$ was taken from Aharon and Fu (2000). It was assumed that elemental sulfur ($\delta^{34}S = 0‰$) disproportionation by bacteria was accompanied by oxygen and sulfur isotope fractionations of +17‰ and +18‰, respectively (this study). The newly produced sulfate was added to the residual sulfate at different extents of sulfate reduction according to a binary mixing model.

sulfate under anaerobic conditions are determined by the microbial processes, the isotopic compositions of water and sulfur sources, respectively, and the extent of reaction.

The combined oxygen and sulfur isotope composition of dissolved sulfate can be used to trace different microbial processes in the biogeochemical cycle of marine surface sediments. Figure 3 shows possible evolution pathways in a plot of $\delta^{34}S$ versus $\delta^{18}O$ values, which illustrates isotope fractionation during bacterial sulfate reduction (field data taken from Aharon and Fu, 2000) and possible changes in the isotope compositions by S° disproportionation pathways. The isotope exchange equilibrium between sulfate and water under in situ conditions was extrapolated from hydrothermal experiments of both Mizutani and Rafter (1969a) and Lloyd (1968). The shaded area in Figure 3 indicates the range between these two extremes. $\delta^{34}S$ values of elemental sulfur in marine sediments have been found to range between about −20 and +20‰ (Anderson and Pratt, 1995). Therefore, elemental sulfur with an intermediate value of 0‰ was assumed to be disproportionated by bacteria, and that this process was accompanied by oxygen and sulfur isotope fractionation effects with respect to sulfate of +17‰ and +18‰, respectively (this study). The newly produced (secondary) sulfate was added to the sulfate which remained from different extents of microbial sulfate reduction according to a binary mixing model. The evolution of each different pathway for a superimposition of sulfate reduction by disproportionation is given by a separate arrow. It is clear, particulary at small extents of the reaction, that elemental sulfur disproportionation shifts the oxygen isotope composition of sulfate to higher values compared to the proposed BSR trend. On the basis of models presented by Böttcher et al. (1998a) and Aharon and Fu (2000), sulfate reduction rates should influence the isotope fractionation factor, and Böttcher et al. (1998a) proposed a relative enrichment of ^{18}O at lower sulfate reduction rates. Based on the boundary conditions chosen for the example in Figure 3, analogous trends in ^{18}O enrichment would be caused by the effects of disproportionation of elemental sulfur superimposed on sulfate reduction. In nature a continuous process of superimposing metabolic reactions are expected. From the results of the present study it is clear that anaerobic bacterial disproportionation can contribute significantly to the oxygen isotope composition of dissolved sulfate. The most intense impact of disproportionation, however, is expected at small extents of net sulfate reduction (Fig. 3) which should prevail close to the sediment-water interface. In the near-surface sediments the number of sulfur disproportionating bacteria has been found to be high (Thamdrup et al., 1993) and bioturbation provides an effective mechanims for generating intermediate sulfur species via the reoxidation of hydrogen sulfide.

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