Sulfur isotope fractionation during bacterial sulfate reduction in organic-rich sediments

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Abstract—Isotope fractionation during sulfate reduction by natural populations of sulfate-reducing bacteria was investigated in the cyanobacterial microbial mats of Solar Lake, Sinai and the sediments of Løgten Lagoon sulfuretum, Denmark. Fractionation was measured at different sediment depths, sulfate concentrations, and incubation temperatures. Rates of sulfate reduction varied between 0.1 and 37 μmol cm⁻² d⁻¹, with the highest rates among the highest ever reported from natural sediments. The depletion of ³⁴S during dissimilatory sulfate reduction ranged from 16‰ to 42‰, with the largest ³⁴S-depletions associated with the lowest rates of sulfate reduction and the lowest ³⁴S-depletions with the highest rates. However, at high sulfate reduction rates (> 10 μmol cm⁻² d⁻¹) the lowest fractionation was 20‰ independent of the rates. Overall, there was a similarity between the fractionation obtained by the natural populations of sulfate reducers and previous measurements from pure cultures. This was somewhat surprising given the extremely high rates of sulfate reduction in the experiments. Our results are explained if we conclude that the fractionation was mainly controlled by the specific rate of sulfate reduction (mass cell⁻¹ time⁻¹) and not by the absolute rate (mass volume⁻¹ time⁻¹).

Sedimentary sulfides (mainly FeS₂) were on average 40‰ depleted in ³⁴S compared to seawater sulfate. This amount of depletion was more than could be explained by the isotopic fractionations that we measured during bacterial sulfate reduction. Therefore, additional processes contributing to the fractionation of sulfur isotopes in the sediments are indicated. From both Solar Lake and Løgten Lagoon we were able to enrich cultures of elemental sulfur-disproportionating bacteria. We suggest that isotope fractionation accompanying elemental sulfur disproportionation contributes to the ³⁴S depletion of sedimentary sulfides at our study sites. Copyright © 1997 Elsevier Science Ltd

1. INTRODUCTION

Isotope fractionation during bacterial sulfate reduction is of great importance for the interpretation of δ³⁴S values from both modern and ancient sedimentary sulfides (Cameron, 1982; Chambers, 1982; Schidlowski et al., 1983). Studies of isotope fractionation during dissimilatory reduction of sulfate by pure cultures have shown that the kinetic isotope effect produces sulfide depleted in ³⁴S by 5‰ to 46‰ compared to the isotopic composition of sulfate (Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; Chambers et al., 1975). Specific rates of sulfate reduction (mass cell⁻¹ time⁻¹) exert an important influence on fractionation at seawater sulfate levels. However, other factors such as sulfate concentration, substrates, temperature, pH, bacterial species, and growth conditions also have an impact on the ³⁴S depletion of sulfide.

The extent of isotope fractionation by pure cultures of sulfate-reducing bacteria is insufficient to explain the much larger depletions of sedimentary sulfides in ³⁴S of up to 70‰ compared to seawater sulfate (e.g., Canfield and Teske, 1996). This difference has been attributed to additional fractionations during the oxidative part of the sulfur cycle (Jørgensen, 1990; Canfield and Thamdrup, 1994). This conclusion, however, is drawn in the absence of fractionation measurements during sulfate reduction by natural populations of sulfate-reducing bacteria; natural populations have not yet been demonstrated to produce fractionations of a comparable magnitude to the pure cultures. To begin to provide this context we report on the ability of sulfate-reducing bacteria to fractionate during sulfate reduction under natural conditions. We have chosen to work in two benthic photosynthetic sediments: the microbial mats of Solar Lake, Egypt and the sediments of the Løgten Lagoon sulfuretum, Denmark. We have also compared our fractionation measurements during sulfate reduction with the isotopic composition of the sulfides fixed in the sediment. This comparison allows us to comment on the possibility of sulfur isotope fractionations by processes other than sulfate reduction within the sediments. These results are complemented by fractionation measurements during the disproportionation of elemental sulfur by bacterial enrichments from both sediments. In an earlier report we discussed the extent of isotope fractionation by natural populations of sulfate reducing bacteria from Solar Lake within the context of the evolution of the sulfur cycle (Habicht and Canfield, 1996).

2. MATERIALS AND METHODS

2.1. Study Site

Microbial mats were collected from the hypersaline Solar Lake, Sinai, Egypt. The lake is separated from the Gulf of Aqaba by a 60 m wide gravel bar through which seawater seeps into the lake and evaporates to a salinity of 70–180‰ (Cohen et al., 1977; Jørgensen and Cohen, 1977; Revsbech et al., 1983). Mat pieces of 70 × 50 cm and 10 cm thickness were sampled by hand in November 1994 from the east side of the lake, where the salinity was 85‰ and the...
temperature 20°C. The mat pieces were transported to the nearby H. Steinitz Marine Biology Laboratory of Eilat, Israel and stored in a manmade pond filled with seawater from Agaba Bay, which had evaporated to a salinity of 90‰. The water depth in the pond was 0.5 m.

Lagten Lagoon is located near the head of Aarhus Bay, Denmark. It has a sulfuretum-type sediment consisting of a 10 cm layer of decomposed plant material mixed with silt and sand. At the time of sampling, July 1995, less than 10 cm of water was covering the sediment and the temperature of the surface sediment was 15°C. The sediment was largely covered by growing green macroalga (Ulva sp. and Enteromorpha sp.), cyanobacteria, and purple sulfur bacteria. Sediment cores were collected in Plexiglas tubes by hand and stored at Aarhus University at situ temperature. Initial sediment handling was done within 24 h after sampling.

2.2. Chemical and Stable Isotope Analysis

Sediment cores were placed in an O2-free glove bag and sectioned in intervals of 1 cm (top of core) to 3 cm (bottom of core). Porewater was extracted from the sediments under N2 pressure in a pneumatic squeezer equipped with 0.45 μm filters (Reebergh, 1967) and fixed in 1 mL of 2% ZnCl2 to preserve dissolved porewater sulfate as ZnS. After porewater separation, the remaining sediment was fixed in 20 mL 20% Zn-acetate and immediately homogenized (for the mats an Ultra-Turax was used).

Hydrogen sulfide concentration was determined spectrophotometrically at 670 nm by the methylene blue method (det. limit 1 μM, S.D. 5%; Clinch, 1990). Sulfate was analyzed by nonsuppressed anion chromatography using an anion exchange column and a conductivity detector (Waters), with 1 mM isopipelic acid (in 10% methanol, pH 4.7) used as the eluent (S.D. 1%). For stable sulfur isotope measurements of porewater sulfide, ZnS was converted to Ag2S as described below, and porewater sulfate was precipitated as BaSO4.

Solid inorganic sulfur compounds were extracted from the sediments as acid volatile sulfur, AVS (≡ HS− + FeS2) and chromium reducible sulfur, CRS (≡ SO4− + FeS2) by the two step chromium distillation method (Canfield et al., 1986; Fossing and Jorgensen, 1989). During the first step, AVS was volatilized as H2S after conc. HCl had been added to the sample to a final acid concentration of ca. 4N. In the second step, reduced chromium, Cr3+, was added and the samples which were boiled to evolve H2S from the reduction of SO4−. The evolved H2S was removed by a stream of N2 to a trap with AgNO3 which precipitated H2S as Ag2S. Elemental sulfur was obtained from separate portions of the Zn-acid fixed sediment by Soxhlet extraction in acetone and collected as CuS onto metallic Cu (Berner, 1964), which was subsequently distilled by chromium reduction (S.D. 5%). In some cases elemental sulfur was extracted from the Zn-acid fixed sediment with methanol and subsequently analyzed by HPLC using a C18 column, a UV-detector (254 nm, Waters), and methanol as eluent (S.D. 1%; Ferdelman et al., 1997). The Ag2S produced during the Cr reduction and AVS distillations was weighed for concentration determination. Concentrations of Fe2+ were calculated as the difference between AVS and porewater H2S concentration, while the difference between CRS and SO4− concentrations yielded the sulfur in Fe2+.

Samples of Ag2S and BaSO4 were converted to SO2 by combustion with CuO in a high-vacuum extraction line. Sulfur dioxide gas was analyzed for stable isotopic composition on a mass spectrometer with the results calculated as per mil deviation relative to the Canyon Diablo Troilite (CDT) standard. Standard compounds of pyrite and sulfate carried through the analytical procedures had an error of ±0.5‰.

Mat and sediment density was calculated from fresh sample weight and volume, the water content by weight loss of fresh sample after drying for 24 h at 105°C, and the organic matter content was measured as the weight lost on dried samples after combustion for 4 h at 540°C.

2.3. Sulfate Reduction Rate

To measure sulfate reduction rate (SRR) in the microbial mats, fresh cores were withdrawn from the experimental pond in Eilat in clear Plexiglas tubes, injected vertically in the top 0–20 mm in triplicate with 25 μL radiolabeled sulfate diluted in seawater, (35S, 40 K Bq/μL). Amerithins (tightly sealed, and returned to the pond) to maintain in situ temperature (20°C) and light conditions. Tracer (4 μL, 40 K Bq/μL) was injected horizontally in 1 cm intervals in sediment collected from Lagten Lagoon through small silicone-stoppered holes in the side of 26 mm cores and incubated in the lab at in situ temperature (15°C). After 30 min incubation time, the microbial mat cores from Solar Lake were sectioned into 2 mm intervals from 0 to 4 mm, and 3 mm sections from 4 to 13 mm, while the Lagten Lagoon sediment cores were sectioned into intervals of 5 cm from 0 to 2 cm and 1 cm from 2 to 10 cm. All samples were fixed with 10 mL ice-cold 20% ZnAc, homogenized and frozen. For analysis, the samples were centrifuged, and a subsample from the supernatant solution was taken for measuring 35SO4− radioactivity and SO4− concentration. The remaining sediment pellet was analyzed for reduced sulfur radioactivity by the single step chromium reduction distillation method (Canfield et al., 1986). Sulfide liberated during distillation was trapped into 10 mL 5% ZnAc and a 5 mL subsample was measured for radioactivity by liquid scintillation counting.

2.4. Diel Cycle of S0

In the microbial mats of Solar Lake a diel cycle of S0 was measured in a thermostated water-bath containing constantly aerated water from Solar Lake, a 30 × 20 cm piece of mat was incubated under in situ light conditions at 20°C. At intervals of 3 h, cores were collected in duplicate with a 10 mL syringe and immediately stored at −80°C to stop all biological activity. The frozen cores were then sectioned with a razor blade in intervals of 0.5 mm over the top 2 mm and 1 mm from 2 to 6 mm. Mat pieces were immediately fixed into 5 mL 20% ZnAc and frozen. Elemental sulfur was extracted from the sediment with methanol, and its concentration was measured as described above.

2.5. Bag Incubations

Isotope fractionation accompanying sulfate reduction was measured in core sections from the surface five adjacent sediment intervals using the same sampling strategy as described above for sulfate reduction. Each slice was carefully placed into a piece of dialysis tubing that was sealed with plastic clamps at both ends. The dialysis tubes were placed in gas-tight plastic bags (RiT-O-Ten; Hansen, 1992; Kruse, 1993) that were heat-sealed. Through a glass inlet each bag was filled with approximately 150 mL filtered, N2-purged, Solar Lake water (65 mM SO4−) or Aarhus Bay water (13 mM SO4−) as appropriate. One series of samples from Solar Lake was incubated with diluted Solar Lake water (Solar Lake water + deionised water, 1:2; producing a final sulfate concentration of approx. 20 mM SO4−). To explore for a temperature dependency on fractionation, perhaps resulting from changes in sulfate reduction rates, bags from parallel cores were incubated at temperatures ranging from 10°C to 30°C. For all experiments bags were incubated in the dark. The hydrogen sulfide produced by sulfate reduction diffused from the mat or sediment into the water in the bag. From an outlet in the bag, water samples were frequently withdrawn for H2S and SO4− concentration measurements. When enough H2S was produced for stable isotope analysis, the bag was removed from the water sample, filtered, and fixed into 10 mL 20% ZnAc, and replaced with new N2-purged water from the sample site. This procedure was repeated from between one to four times during 7–11 days depending on the rate of sulfate reduction. To limit the influence of preexisting sedimentary H2S on the isotopic composition of the sulfide produced during bacterial sulfate reduction, water from the first sampling was discarded. The rate of sulfate reduction in the bag was calculated from the H2S production rate. To check that the produced H2S was from sulfate reduction occurring in the sediment and not in the water added to the bag, 5 mL of the water from each bag was injected with 10 μL 35SO4− (40 K Bq/μL). After 10 h of anoxic incubation the water sample was fixed in 20% ZnAc and analyzed for sulfate reduction rate as described above. The test
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Table 1. Concentrations of the inorganic sulfur compounds in the microbial mats of Solar Lake.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>SO$_2^-$ (mM)</th>
<th>H$_2$S (mM)</th>
<th>S$^0$ (µmol cm$^{-3}$)</th>
<th>FeS (µmol cm$^{-3}$)</th>
<th>FeS$_2$ (µmol cm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>65.2</td>
<td>0.2</td>
<td>2.4</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>1.5</td>
<td>69.1</td>
<td>0.8</td>
<td>4.2</td>
<td>27.8</td>
<td>11.5</td>
</tr>
<tr>
<td>2.5</td>
<td>76.8</td>
<td>1.3</td>
<td>1.1</td>
<td>10.3</td>
<td>8.7</td>
</tr>
<tr>
<td>3.5</td>
<td>81.3</td>
<td>1.8</td>
<td>1.8</td>
<td>8.5</td>
<td>14.1</td>
</tr>
<tr>
<td>4.5</td>
<td>84.0</td>
<td>1.9</td>
<td>1.5</td>
<td>5.3</td>
<td>8.5</td>
</tr>
<tr>
<td>5.5</td>
<td>90.3</td>
<td>1.8</td>
<td>0.5</td>
<td>5.4</td>
<td>5.4</td>
</tr>
<tr>
<td>6.5</td>
<td>88.0</td>
<td>2.0</td>
<td>1.1</td>
<td>6.9</td>
<td>12.1</td>
</tr>
<tr>
<td>8.5</td>
<td>93.0</td>
<td>0.9</td>
<td>0.9</td>
<td>4.4</td>
<td>17.5</td>
</tr>
</tbody>
</table>

showed that no sulfate reduction occurred in the water phase of the bags.

2.6. Enrichment Cultures

Cultures of elemental sulfur-disproportionating bacteria from both Solar Lake and Løgten Lagoon were enriched from 2% v/v surface sediment added to 50 mL screw-cap bottles filled without headspace with anoxic carbonate-buffered, sulfate free, saltwater medium (see for details Thamdrup et al., 1993). The initial pH of the medium was 7.3, and it contained no organic energy source besides a small amount of vitamins. The bottles contained about 16 mmol sterile flowers of S$^0$ and 1.5 mmol ferrihydrite (approx. Fe(OH)$_3$). The incubation temperature was 30°C. Each culture was grown and transferred (10% volume transferred to fresh medium) between eight to ten times before the fractionations accompanying elemental sulfur disproportionation were measured. To measure fractionations a series of 50 mL screw-cap bottles were inoculated from each culture and regularly sampled over a period of about 4 weeks, where the whole content of a bottle was added to 20 mL 20% ZnAc and frozen. Concentrations and the isotopic composition of the sulfur compounds were then analyzed as described above.

3. RESULTS

3.1. Sediment Analysis

The microbial mats of Solar Lake and the sediments of the Løgten Lagoon sulfuretum contained high amounts of organic matter, averaging 39 ± 13% and 33 ± 10% of dry weight, respectively. In both sediments dissolved sulfide was measured within the surface 0–1 cm and reached maximum concentrations of up to 2 mM (Tables 1 and 2). For both sediments maximum concentrations of solid phase sulfides were rather similar, with the concentration of FeS ranging up to 28 µmol cm$^{-3}$ and CRS attaining concentrations of up to 34 µmol cm$^{-3}$.

The diel cycle of elemental sulfur was measured in the top 6 mm of the microbial mats from Solar Lake (Fig. 1). From sunrise at 0630, the integrated concentration of S$^0$ over the whole 6 mm depth increased from 68 µmol cm$^{-2}$ to a maximum concentration of 104 µmol cm$^{-2}$ in the afternoon. Hereafter, the amount of S$^0$ decreased to a value of 69 µmol cm$^{-2}$ at midnight. The daytime increase in S$^0$ was particularly evident in the surface 0–3 mm of the mat. In the early morning, before sunrise, a secondary maximum in elemental sulfur concentration was measured at 2–3 mm depth.

At the time of our study, the salinity of Solar Lake was 85% and, thus, low relative to its summertime maximum of 180% (Cohen et al., 1977). Rain during fall months and a lower evaporation rate at this time contribute to a reduction of the salinity in the lake. In the surface sediment the sulfate concentration was 65 mM and increased to 93 mM by 8.5 cm depth (Table 1). The general increase in sulfate concentration probably reflects variations in lake water salinity as mentioned above. There was little variation in the isotopic composition of either sulfate, at 22 ± 1‰, or of the reduced sulfur compounds which averaged −18 ± 2‰ (Fig. 2a). In the sediments of Løgten Lagoon the sulfate concentration decreased from 13 mM in the surface sediment to 2 mM at 7–9 cm due to sulfate consumption by sulfate reduction (Table 2). Due to discrimination against $^{34}$S during the reduction of sulfate, the isotopic composition of sulfate increased from 25‰ in the surface sediment interval to 50‰ by 6 cm depth (Fig. 2b). The isotopic composition of dissolved sulfide increased from −1‰ to 4‰ from the sediment surface to 7 cm depth. The isotopic composition of AVS (mainly FeS) was rather steady at −11 ± 1‰ through the whole sediment core while the isotopic composition of CRS dropped from −7‰ to −18‰ over the surface 2 cm, with no further changes with sediment depth (Fig. 2b). These results show that the formation of, and the associated sulfur fractionation into, FeS and CRS mainly occurred in the sur-
During sulfate reduction, sulfide was depleted in $^{34}\text{S}$ by between 16% and 42% compared to seawater sulfate (Fig. 4). Isotope fractionation seemed to depend on the incubation temperature, with the highest fractionations occurring at low temperature which, however, also correlated with the lowest rates of sulfate reduction. At a given temperature the fractionation changed only little with sediment depth (except for surface samples). Also, in the Solar Lake mat, fractionations were similar for sediment incubated with both 20 mM and 65 mM sulfate (Fig. 4 and 5a).

In Fig. 5a we have compiled all fractionation data for the two sites and show the $^{34}\text{S}$ depletion during sulfate reduction vs. the rate of sulfate reduction. At low sulfate reduction rates (<10 μmoles cm$^{-3}$ d$^{-1}$) isotope fractionation showed the greatest dependence on rate. By contrast, at high rates of sulfate reduction and over a range of sulfate concentrations from 13 mM to 65 mM, the depletion of $^{34}\text{S}$ into sulfide showed little dependence on rate, with fractionations ranging from 20 to 25%. These results are consistent with the observations of Habicht and Canfield (1996) on Solar Lake mat alone. Fractionation data are compared to literature results from pure culture studies in Fig. 5b.

The isotopic difference between seawater sulfate and CRS was on average 40 ± 2% for Solar Lake sediment and 37 ± 4% for sediments from Lögten Lagoon. These isotopic differences are generally greater than the fractionations measured during bacterial sulfate reduction, particularly for fractionation determinations at or near to in situ temperatures (20°C for Solar Lake, and 15°C for Lögten Lagoon; Fig. 4).

3.2. Isotope Fractionation During Bacterial Sulfate Reduction

The in situ rates of sulfate reduction ranged from 0.6 to 3.1 μmol cm$^{-3}$ d$^{-1}$ in the microbial mats of Solar Lake with the highest rates occurring between 2 and 4 mm depth (Fig. 3a). In the sulfuretum of Lögten Lagoon a maximum SRR of 7 μmol cm$^{-3}$ d$^{-1}$ was measured in the surface 0.5 cm, and rates decreased exponentially with depth to 0.1 μmol cm$^{-3}$ d$^{-1}$ at 7–9 cm (Fig. 3b). For the sediment incubated within bags, the rates of sulfate reduction were highly dependent on the incubation temperature, with the highest rates occurring at high temperature (Fig. 3). Overall, rates of sulfate reduction ranged from 0.2 to 37 μmol cm$^{-3}$ d$^{-1}$ and include some of the highest rates ever reported from natural samples. Rates of sulfate reduction were constant through the whole incubation period (7–11 days) except in the bags stored at 30°C; in these bags the rates increased with time. The rates of sulfate reduction in the bags incubated at in situ temperatures were similar to those measured in the sediment cores, except for the surface sediment interval where rates of sulfate reduction were higher in the bag incubations. During the bag incubations the concentration of sulfate was reduced by less than 1%. Sulfate concentrations of both 20 and 65 mM were used in the incubation of Solar Lake mat with no effect on the rates of sulfate reduction (data not shown).
4.1. Sulfate Reduction

(Canfield et al., 1994; Canfield et al., 1997). These results are consistent with previous observations while sulfate was enriched in $^{34}$S by 14.0% to 16.7% relative to elemental sulfur during disproportionation, with FeS formation in the presence of Fe-oxides (Thamdrup et al., 1993). In the enrichment cultures from Solar Lake, AVS and sulfate formed in a ratio of about 2:1 during $S^{0}$-disproportionation. This ratio was in accordance with the expected overall stoichiometry for $S^{0}$-disproportionation with FeS formation in the presence of Fe-oxides (Thamdrup et al., 1993). In the Logten Lagoon enrichments the ratio was much lower at 1.4:1. For both enrichment cultures the concentration of AVS decreased and pyrite formed as pre-

3.3. Isotope Fractionation During Elemental Sulfur Disproportionation

It was possible to enrich $S^{0}$-disproportionating bacteria from both the microbial mats of Solar Lake and from the sulfate rich at Logten Lagoon. Enrichments grew and metabolized similarly to those described by Thamdrup et al. (1993). In the enrichment cultures from Solar Lake, AVS (FeS) and sulfate formed in a ratio of about 2:1 during $S^{0}$-disproportionation. This ratio was in accordance with the expected overall stoichiometry for $S^{0}$-disproportionation with FeS formation in the presence of Fe-oxides (Thamdrup et al., 1993). In the Logten Lagoon enrichments the ratio was much lower at 1.4:1. For both enrichment cultures the concentration of AVS decreased and pyrite formed as previously described for a wide variety of $S^{0}$-disproportionating cultures (Thamdrup et al., 1993; Canfield et al., 1997). Also for both enrichments sulfide was depleted in $^{34}$S by 7.0%o to 8.9%o relative to elemental sulfur during disproportionation, while sulfate was enriched in $^{34}$S by 14.0%o to 16.7%o (Table 3). These results are consistent with previous observations (Canfield et al., 1994; Canfield et al., 1997).

4. DISCUSSION

4.1. Sulfate Reduction

The rate of bacterial sulfate reduction in sediments depends, among other things, on the quality and quantity of the organic matter available for oxidation (Westrich and Berner, 1984). In highly productive areas such as benthic microbial mats or sulfate rich sediments, extremely high rates of sulfate reduction are obtained as sulfate reducing bacteria live in close association with photosynthesizing cyanobacteria and algae or secondary producers such as chemolitho-

Table 3. Sulfur fractionation during elemental sulfur disproportionation measured in enrichment cultures from Solar Lake and Logten Lagoon.

<table>
<thead>
<tr>
<th>Enrichment cultures</th>
<th>$\Delta_{AVS-S^{0}}$ (%)</th>
<th>$\Delta_{SO_{4}^{2-}-S^{0}}$ (%)</th>
<th>Production AVS/SO_{4}^{2-}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solar Lake (I)</td>
<td>-8.9</td>
<td>16.7</td>
<td>2.1</td>
</tr>
<tr>
<td>Solar Lake (II)</td>
<td>-8.2</td>
<td>16.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Logten Lagoon</td>
<td>-7.0</td>
<td>14.0</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*) Per mill fractionation of AVS and sulfate relative to $S^{0}$. 

Fig. 4. The fractionations during sulfate reduction by natural populations of sulfate-reducing bacteria, at different temperatures, and with sediment depth, compared to the isotopic composition of CRS.

(a) Solar Lake mats incubated at 10°C, 20°C, and 30°C and (b) Logten Lagoon sediment incubated at 15°C and 20°C. Note the different depth scales for the different sites. The error bars indicate either the standard deviation (three to eight samples) or the difference between duplicate measurements.
4.2. Isotope Fractionation During Bacterial Sulfate Reduction

The isotopic fractionation accompanying sulfate reduction did not vary significantly with sediment depth, with the exception of the surface sediment interval where lower fractionations were found at both sites. Other than this, the main factor influencing fractionation was the incubation temperature, where the average fractionation in the mats of Solar Lake at 10°C was 32 ± 4%o compared to 28 ± 3%o and 26 ± 3%o at 20°C and 30°C, respectively. In the Løgten Lagoon sediments the average fractionation was 31 ± 4%o at 14°C and 29 ± 4%o at 20°C. The rate of sulfate reduction was correlated with incubation temperature (Fig. 3), and hence, a correlation was, therefore, also observed between the rate of sulfate reduction and fractionation, particularly at rates ≤10 μmol cm⁻³ d⁻¹ (Fig. 5). At high sulfate reduction rates of >10 μmol cm⁻³ d⁻¹, isotope fractionation was 20–25%o independent on the rates (Fig. 5a).

The relationship between the extent of isotope fractionation and the rate of sulfate reduction as determined here for natural populations of sulfate reducing bacteria (Fig. 5a) can be compared to the relationship observed in pure bacterial cultures (Fig. 5b). When pure cultures are supplied with the organic electron donors lactate and ethanol, the correlation between fractionation and the rate of sulfate reduction is actually quite similar to the relationship observed for the natural populations of sulfate reducing bacteria. In both cases the extent of isotope fractionation is an inverse function of rate (Fig. 5b) although it is important that volume-based rates are reported for the natural populations (rate per volume of sediment) whereas specific rates of sulfate reduction (rate per cell) are correlated with fractionation for the pure bacterial cultures. When H₂ is the electron donor, the fractionations observed in pure cultures are commonly small and do not correlate with the specific rate of reduction (Fig. 5b; Kaplan and Rittenberg, 1964; Kemp and Thode, 1968). Such low fractionations were not found in the sediments of Solar Lake and Løgten Lagoon; neither did we observe the same trend between the rate of sulfate reduction and extent of fractionation as has been reported for pure cultures utilizing H₂. Thus, our stable isotope results would seem to indicate the utilization of H₂ by sulfate reducers is not of great importance in the sediments of Solar Lake and Løgten Lagoon.

The similarity between the fractionations observed in natural populations of sulfate reducing bacteria and pure cultures using organic compounds as substrate requires further consideration (Fig. 5). It is important, however, to reemphasize the difference in the rate units utilized; in the present natural population experiments we have expressed rates of sulfate reduction per volume sediment, with units of mass volume⁻¹ time⁻¹. In the pure culture studies specific rates of sulfate reduction are used with units of mass cell⁻¹ time⁻¹. These two units of sulfate reduction are related to each other by the cell density (cell volume⁻¹), such that

\[ \text{specific SRR} = \frac{\text{SRR}}{\text{cell density}} \]

Assuming that the cell densities of the sulfate reducing bacterial populations in the sediments of Solar Lake and Løgten Lagoon were not widely variable over the depth ranges explored, we can divide the volume-based rates of sulfate reduction in Fig. 5a by assumed cell densities to yield specific rates of sulfate reduction. The resulting trends between isotope fractionation and specific rates of sulfate reduction are then compared to the trends from the pure culture studies (Fig. 6). We have performed this analysis separately for ourSolar Lake and Løgten Lagoon results using a wide range
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Fig. 6. Isotope fractionation by natural populations of sulfate reducing bacteria in (a) Solar Lake and (b) Løgten Lagoon at different specific SRR (mole cell⁻¹ d⁻¹) predicted from assumed population densities of 10⁹ and 10¹⁰ cells cm⁻³. The gray zone indicates the complete range from pure culture studies using organic electron donors. See text for details.

of cell densities. For Løgten Lagoon in particular (Fig. 6b), but even for Solar Lake, our calculated trends between isotope fractionation and specific rates of sulfate reduction converge with the results from the pure culture studies with population sizes of <10¹⁰ cells cm⁻³, but ≥10⁹ cells cm⁻³.

These population sizes are much greater than those previously measured in the mats of Solar Lake by most probable number techniques (MPN) where up to 10⁷ cells cm⁻³ were counted (Jørgensen and Cohen, 1977; Teske et al., 1998). Bacterial numbers estimated with the MPN technique, however, are likely to be too low as it is difficult to find the right mix of culture conditions to grow all sulfate reducing bacteria within a sediment (e.g., Jørgensen, 1978). Thus, using MPN counts, Teske et al. (1996) found 1000-fold lower numbers for the population size of sulfate reducing bacteria in the anoxic water-column of Mariager Fjord, Denmark, compared to the population size obtained by direct counting of sulfate reducing bacteria after the fluorescent hybridization of bacteria collected by filtration. Furthermore, direct counts of sulfate-reducing bacteria from an organic-rich, biologically active, biofilm, yielded populations of up to 2 × 10⁹ cells cm⁻³ using molecular probe techniques (Ramsing et al., 1993). These high numbers lend some support to our results.

Isotope fractionation by natural populations of sulfate reducers might, therefore, prove to be independent of absolute rates of sulfate reduction and to vary more consistently with specific rates. Thus, sediments with lower availability of substrate should be expected to support both lower volume-based rates of sulfate reduction (e.g., Westrich and Berner,
In this way, a large variation in specific rates of sulfate reduction might not be expected as populations should adjust to substrate availability. By contrast absolute rates of sulfate reduction could vary over a large range. In less active sediments with lower, but rather constant, populations of sulfate-reducing bacteria, we might find similar trends between fractionation and SRR as in Fig. 5a, but with lower rates.

While our results from natural populations of sulfate-reducing bacteria are consistent with those from pure culture studies, we wish to understand in more detail the actual magnitude of fractionation values. To do this, we take a closer look at the kinetics of the sulfate reduction pathway. The pathway of dissimilatory sulfate reduction can be generalized in the following four steps, although the actual pathway may be more complicated and might vary between bacterial species (Widdel and Hansen, 1992):

\[
\begin{align*}
\text{SO}_4^{2-} & \xrightarrow{1} \text{SO}_3^{2-} \\
\text{H}_2\text{S} & \xleftarrow{3} \text{SO}_3^{2-} \\
\text{APS} & \xleftarrow{2} \text{SO}_3^{2-} \\
\text{ATP} & \xrightarrow{4} \text{H}_2\text{S}
\end{align*}
\]

There are several steps in this scheme where fractionation is possible (Harrison and Thode, 1957; Rees, 1973; Thode 1991). Low or even positive fractionation values (3\%\text{e}) have been assigned Reaction 1, the uptake of sulfate by the bacterium. Step 2, the reaction of sulfate with ATP (adenosine triphosphate) to form APS (adenosine-5'-phosphosulfate) is accompanied with no fractionation. A fractionation of 25\%\text{e} has been proposed for each of reactions 3 and 4 due to the splitting of S-O bounds in the reduction of APS to sulfitre and the reduction of sulfate to sulfide. No fractionation is assumed during the backward reactions of steps 1, 2, and 3.

In general, isotope fractionation depends on which step is rate-determining in the sulfate reduction pathway, and the overall isotope effect is the sum of the kinetic isotope effects from each reaction step until the rate-limiting reaction is reached (Rees, 1973). At high specific rates of sulfate reduction with excess sulfate, reaction 3, the reduction of APS to SO$_3^{2-}$, might be the rate-limiting step as the rate of sulfite reduction is believed to be faster than APS reduction (Rees, 1973). A minimum fractionation of about (25\%\text{e} - 3\%\text{e} = 22\%\text{e}) is, therefore, expected. However, if the reduction of SO$_3^{2-}$ to H$_2$S is rate-limiting, and this could be the case at low specific rates of sulfate reduction, fractionation values over 22\%\text{e} can be obtained as a sum of the individual isotope fractionations associated with Reactions 1-4 (Rees, 1973).

At limiting sulfate concentrations or at high specific rates of sulfate reduction where fractionation only depends on the sulfate supply into the cell, low fractionation values (<22\%\text{e}) will occur. In our experiments the depletion of $^{34}$S in sulfide during sulfate reduction was generally higher than about 22\%\text{e}, consistent with a nonlimiting sulfate supply. This was confirmed for sulfate reducers in Solar Lake and Løgten Lagoon, as varied concentrations of sulfate did not limit sulfate reduction, or substantially affect isotopic fractionation to sulfate levels as low as 13 mM (Fig. 5a).

Thus, the extent of isotope fractionation during sulfate reduction in sediments from Solar Lake and Løgten Lagoon nicely fits with theoretical considerations and demonstrates that fractionation is not limited by sulfate supply at these sites. This may be counterintuitive, given the high rates of sulfate reduction in these sediments. However, a lack of sulfate limitation on fractionation is consistent with the above discussion showing that large population sizes likely reduce the specific rates of sulfate reduction to values that may be generally encountered in marine sediments. We re-emphasize that it is the specific rate of sulfate reduction and not the absolute rate that controls fractionation.

4.3. Sulfur Fractionation in the Sediment

In the microbial mats of Solar Lake and in the sediments of the Løgten Lagoon sulphuretum, the isotopic differences between seawater sulfate and CRS (mainly FeS$_2$) where on average 40 ± 2\%\text{e} and 37 ± 4\%\text{e}, respectively. At in situ temperature we measured fractionations during sulfate reduction of 28 ± 3\%\text{e} in the Solar Lake mats (20°C) and 31 ± 4\%\text{e} in the sediments of Løgten Lagoon (14°C). When combined these results show that sedimentary sulfides (CRS) in Solar Lake mats were 12\% more depleted in $^{34}$S than could be accounted for by sulfate reduction alone; in Løgten Lagoon sedimentary sulfides (CRS) were more depleted in $^{34}$S by 7\%\text{e}. The fractionations during bacterial sulfate reduction that we measured under natural conditions cannot, therefore, explain the larger fractionations preserved in these sediments as pyrite. This point has previously been emphasized by Jørgensen (1990), Canfield and Thamdrup (1994), and Canfield and Teske (1996).

The present results demonstrate directly that the isotopic composition of sedimentary sulfides can record fractionations in addition to those associated with the bacterial reduction of sulfate. Additional fractionations probably result during oxidative processes within the sulfur cycle (Jørgensen, 1990; Canfield and Thamdrup, 1994). Thus, the reduced sulfur compounds FeS and CRS become most depleted in $^{34}$S in both Solar Lake and Løgten Lagoon sediments in then upper 1-2 cm where most of the sulfide oxidation is expected (Fig. 2).

In the mats of Solar Lake the sulfide loss by reoxidation, calculated as the percentage difference between the depth-integrated rate of sulfate reduction and the rate of sulfur burial (as pyrite and AVS) in the sediment, was 99\%\text{e} using the accretion rate of Jørgensen and Cohen (1977). Similar values are expected in the sediment of Løgten Lagoon sulphuretum, as the SRR and the concentration of the reduced sulfur compounds were similar to the Solar Lake mats. Also, in coastal marine sediments typically 90\% of the sulfide produced by sulfate reduction is reoxidized, and only a small fraction is buried in the sediment, mainly as FeS$_2$ (Jørgensen, 1982). Pathways of sulfide oxidation in sediments are poorly understood, as sulfide may be either chemically or bacterially oxidized to sulfate through a variety of intermediate sulfur compounds, with different oxidation states, such as S$_2$O$_4^{2-}$, S$_2$O$_3^{2-}$, S,O$_2^{2-}$, and SO$_4^{2-}$. Each of these compounds can
then be further oxidized, reduced, or disproportionated. The fractionations during chemical and bacterial oxidation of sulfide with O₂ as an electron acceptor to S⁰, S₂O₇⁻, and SO₄⁻ are small (0–5%). The oxidation of S₂O₇⁻ and SO₄⁻ to sulfate is also associated with small fractionations (Fry et al., 1985, 1986). Canfield and Thamdrup (1994), however, found rather large fractionations into both sulfide and sulfate during the bacterial disproportionation of S⁰. The formation and disproportionation of other sulfur intermediates such as thiosulfate and sulfite could also provide additional fractionations leading to depletion of sulfide in ³⁴S, and these isotope systematics are currently under investigation. A repeated sulfide oxidation to S⁰ and maybe other sulfur intermediates, followed by disproportionation can generate sulfide more depleted in ³⁴S than the original sulfide produced from sulfate reduction (Canfield and Thamdrup, 1994).

We observed significant isotope fractionations during the disproportionation of elemental sulfur by bacterial cultures enriched from both Solar Lake and Løgten Lagoon (Table 3), consistent with previous observations of elemental sulfur disproportionating bacteria (Canfield and Thamdrup, 1994; Canfield et al., 1997). Furthermore, rather large concentrations of S⁰ were measured in the mats of Solar Lake, varying between 2 and 30 μmol cm⁻² in the surface 0–6 mm (Fig. 1). Thus, the process of elemental sulfur disproportionation likely contributes to the large ³⁴S depletions into sedimentary sulfides from Solar Lake and Løgten Lagoon.

Diel measurements showed that the pool size of elemental sulfur was very dynamic (Fig. 1). We interpret the high near-surface concentrations of elemental sulfur during the daytime to result from sulfide oxidation. In the daytime O₂ accumulates in the mat surface to concentrations up to 500% air saturation due to high rates photosynthesis (7–10 nmol O₂ cm⁻²s⁻¹; M. Kühn pers. commun.). Oxygen can react with sulfide and form S⁰. Also, during anoxygenic photosynthesis by green and purple sulfur bacteria, and during chemosynthetic oxidation of sulfide by colorless sulfur bacteria (e.g., Beggiatoa sp.), S⁰ is produced which may accumulate as granules in the cell or be excreted to the surrounding sediment. At night we observed that S⁰ was consumed which could be accounted for by oxidation, reduction, and disproportionation processes, although we cannot be certain about which processes dominate. We measured a curious accumulation of S⁰ at 2–4 mm depth in the dark (Fig. 1). The reasons for the apparent production of elemental sulfur under reducing conditions, and without light are, however, unclear.

The rather modest depletions of ³⁴S into the CRS fraction of the sediments from Solar Lake and Løgten Lagoon are lower than might be expected from the arguments of Canfield and Thamdrup (1994) who have noted a pronounced positive correlation between the extent of sulfide oxidation (which is very high in these sediments) and the depletion of sedimentary sulfides in ³⁴S. Canfield and Teske (1996) have previously noted that benthic photosynthetic systems produce less fractionation into sulfide than their large degree of sulfide oxidation might suggest. They argued that reduced ³⁴S-depletion could result from a relatively larger importance of direct sulfide oxidation to sulfate by anoxygenic photosyn-

![Fig. 7. Comparisons of the fractionation during sulfate reduction by pure cultures and natural populations of sulfate-reducing bacteria and the fractionation between sedimentary sulfides (mainly as FeS₂) and seawater sulfate. Sediment and pure culture data are from Canfield and Teske (1996) and natural population results are from this study.](image)

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**4.4. Comparison of Natural Population, Pure Culture, and Sediment Fractionation Results**

We have summarized in histogram form all of our natural population fractionation results (Fig. 7), and we compare these with previous measurements of isotope fractionation by pure cultures of sulfate-reducing bacteria and the isotopic composition of sedimentary sulfides (see Canfield and Teske, 1996). Isotope fractionation by the natural populations of sulfate reducing bacteria was on average 28%, which is higher than the average previously determined for pure cultures (with seawater sulfate levels) with an average value of 18%. The main difference between the natural population and pure culture results is that we did not observe small isotope fractionations in the natural populations. They did not apparently metabolize with an insufficient supply of SO₄²⁻ nor did they apparently use H₂ as electron donor; these two factors normally leading to low fractionations. The upper range of fractionations in our natural populations are within a few mil of the pure cultures results, but are not high enough to explain the much larger fractionations frequently preserved into marine sulfides. As pointed earlier, we do not anticipate higher fractionations than those measured here to be found in natural populations from other sedimentary environments.
5. CONCLUSIONS

The depletion of $^{34}$S in sulfide during sulfate reduction depends on the rate of sulfate reduction, with the highest fractionations at low rates and the lowest fractionations at high rates. This trend is similar to previous reports from pure cultures of sulfate reducing bacteria where the fractionations have been correlated with the specific rate of sulfate reduction (rate cell⁻¹ time⁻¹). We believe that isotope fractionations accompanying sulfate reduction in marine sediments also depend on the specific rate of sulfate reduction and not absolute rates (rate volume⁻¹ time⁻¹). We anticipate that similar high fractionations of up to 42%, as we observed in these very active sediments, will also be encountered in normal coastal marine sediments metabolizing at similar specific rates of sulfate reduction. The isotope fractionation that we measured during bacterial sulfate reduction was less than the depletion of $^{34}$S into sediment sulfides (FeS₂) at both Solar Lake and Løgten Lagoon. Fractionations in addition to those produced by sulfate reduction must, therefore, occur in the sediments, and these additional fractionations might be related to the oxidative part of the sulfur cycle. Isotope fractionation during elemental sulfur disproportionation could explain the additional depletion of $^{34}$S into sedimentary sulfides and elemental sulfur-disproportionating bacteria where isolated from the two investigated stations.

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