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Controls on stable sulfur isotope fractionation during bacterial sulfate reduction in Arctic sediments

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Abstract—Sulfur isotope fractionation experiments during bacterial sulfate reduction were performed with recently isolated strains of cold-adapted sulfate-reducing bacteria from Arctic marine sediments with year-round temperatures below 2°C. The bacteria represent quantitatively important members of a high-latitude anaerobic microbial community. In the experiments, cell-specific sulfate reduction rates decreased with decreasing temperature and were only slightly higher than the inferred cell-specific sulfate reduction rates in their natural habitat. The experimentally determined isotopic fractionations varied by less than 5.8‰ with respect to temperature and sulfate reduction rate, whereas the difference in sulfur isotopic fractionation between bacteria with different carbon oxidation pathways was as large as 17.4‰. Incubation of sediment slurries from two Arctic localities across an experimental temperature gradient from −4°C to 39°C yielded an isotopic fractionation of 30‰ below 7.6°C, a fractionation of 14‰ and 15.5‰ between 7.6°C and 25°C, and fractionations of 5‰ and 8‰ above 25°C, respectively. In absence of significant differences in sulfate reduction rates in the high and low temperature range, respectively, we infer that different genera of sulfate-reducing bacteria dominate the sulfate-reducing bacterial community at different temperatures. In the Arctic sediments where these bacteria are abundant the isotopic differences between dissolved sulfate, pyrite, and acid-volatile sulfide are at least twice as large as the experimentally determined isotopic fractionations. On the basis of bacterial abundance and cell-specific sulfate reduction rates, these greater isotopic differences cannot be accounted for by significantly lower in situ bacterial sulfate reduction rates. Therefore, the remaining isotopic difference between sulfate and sulfide must derive from additional isotope effects that exist in the oxidative part of the sedimentary sulfur cycle. *Copyright © 2001 Elsevier Science Ltd*

1. INTRODUCTION

The dissimilatory reduction of sulfate to sulfide by many sulfate-reducing bacteria is coupled to a pronounced enrichment in the stable isotope ^{32}S in sulfide (Harrison and Thode, 1958). ^{32}S enrichment within sulfide minerals in marine sediments as far back as the early Precambrian has been taken as evidence for the presence and activity of sulfate-reducing bacteria and thus helps to constrain the redox conditions of the ancient ocean-atmosphere system (Canfield and Teske, 1996). A more detailed biogeochemical interpretation of the isotopic difference between reduced and oxidized sulfur species is complicated by several factors. Pure cultures of sulfate-reducing bacteria show considerable variation in their isotopic fractionation ranging from 2‰ to 46‰ (e.g., Kaplan and Rittenberg, 1964; Kemp and Thode, 1968). The biochemical mechanisms behind this large variation remain poorly understood. Most experimental studies exploring the controls on isotopic fractionation were performed with *Desulfovibrio desulfuricans* strains (Chambers et al., 1975; Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; McCready, 1975). These experiments suggested that sulfate reduction rates are coupled to temperature and yield increasing fractionations as temperature and/or rates of sulfate reduction decrease (Kaplan and Rittenberg, 1964; Kemp, 1968).

More than 90% of the ocean sea floor is below 4°C (Levitus and Boyer, 1994), and only shallow-water habitats exceed temperatures of 15°C. *Desulfovibrio desulfuricans* is a mesophilic sulfate reducer that does not grow at temperatures of 4°C, and therefore cannot be a dominant species in cold marine sediments. Extrapolation of experimental results obtained with mesophilic sulfate reducers to normal marine sediments is therefore questionable. It is important that the dominant genera of sulfate-reducing bacteria for the investigated sediments are known, and that the fractionation studies include realistic growth conditions. Here we determined sulfur isotopic fractionation factors with recently isolated sulfate-reducing bacteria from Arctic sediments that have growth yield optima near the *in situ* temperatures and represent quantitatively important genera in sediments of the Arctic (Knoblauch and Jørgensen, 1999). Our experimental results are compared with field data using molecular genetic information on distributions and abundances of these sulfate-reducing bacteria in their natural habitats. Finally, we compare our experimental results with field data on abundance and isotopic composition of sedimentary sulfides in surface sediments from localities close to the original habitat of the above sulfate-reducing bacteria (Knoblauch and Jørgensen, 1999). This approach allows us to relate our experimental results to the geochemistry of the associated sediment and to explore the potential of stable sulfur isotopes as markers for specific sulfate reducers and their metabolic activity.

2. MATERIAL AND METHODS

2.1. Pure Cultures

2.1.1. Cultivation and experimental setup

Pure cultures of the sulfate reducers *Desulfotalea psychrophila* (LSv54), *Desulfotalea arctica* (LSv514), *Desulfofrigus oceanense*

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(ASv26), and the mesophilic strain ASv20 were grown in sealed vials at temperatures of −1.8°C, 2°C, 4°C, 9°C, 16°C, and 20°C. LSv54 and ASv26 have temperature optima for growth at 10°C, LSv514 has a growth optimum at 18°C, and ASv20 is a mesophilic sulfate reducer with a temperature optimum above 20°C (Knoblauch et al., 1999). The highest temperatures chosen for each culture were just below the optimum temperature for growth, except for LSv514 where the highest temperature was above the optimum growth temperature. LSv54, LSv514, and ASv26 are described by Knoblauch et al. (1999) and are available from the German Collection of Microorganisms (DSMZ). LSv54 and LSv514 are incomplete oxidizers and oxidize lactate according to the following reaction:

$$2CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2CH_3COO^- + 2HCO_3^- + HS^- + H^+. \quad (1)$$

Acetate is released as the incomplete oxidation product. By contrast, ASv26 and ASv20 oxidize acetate and other substrates completely:

$$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-. \quad (2)$$

All cultures were grown with headspace containing 90:10 (*v*/*v*) N_2/CO_2 in the anaerobic saltwater medium described by Widdel and Bak (1992) using cultivation methods described in Knoblauch et al. (1999). Organic substrates were lactate (20 mM final concentration) for LSv54 and LSv514 and acetate (20 mM final concentration) for ASv26 and ASv20. The cultures were grown to the stationary phase of growth. Measurements were made after inoculation (t_0), in the lag phase (t_1), the early exponential (t_2), the late exponential (t_3), and the stationary phase (t_4). Cultures were grown in individual 100 ml bottles to each time point. At each time point, subsamples were taken to determine concentrations of dissolved sulfate and sulfide, $\delta^{34}S_{sulfate}$, $\delta^{34}S_{sulfide}$, cell numbers, and sulfate reduction rates using ^{35}S labelled sulfate.

2.1.2. Determination of cell-specific sulfate reduction rates

At each of the above time points, a 500 μl aliquot of the culture was used for cell counting using a modified Neubauer grid (0.0025 mm^2 × 0.02 mm). At each time point, 10 ml of each culture were incubated in triplicate for 3 h with $^{35}SO_4$ (100 kBq total activity). After this time, 1 ml 20% zinc acetate solution was added to terminate bacterial activity and to fix dissolved sulfide as zinc sulfide. A 100 μl subsample of the fixed cultures was collected to determine the total ^{35}S radioactivity. Precipitated zinc sulfide was redissolved under N_2 in cold 6N HCl, distilled, and trapped in 7 ml 5% zinc acetate. The entire content of the trap was used for scintillation counting. A Canberra-Packard 2400 TR liquid scintillation counter and Packard Ultima Gold scintillation fluid were used to determine Zn ^{35}S and $^{35}SO_4^{2-}$ radioactivities. Cell-specific sulfate reduction rates were calculated according to Fossing (1995) from the fraction of radioactive reduced sulfide produced during the incubation and the concentration of sulfate at that time point:

$$SRR = \frac{a}{A}[SO_4^{2-}]\frac{24}{h}\left(\frac{1.5\varepsilon}{1000}+1\right)\frac{1}{z}\ \text{moles cell}^{-1}\ \text{day}^{-1}, \quad (3)$$

where a is the radioactivity in the trap, A is the total radioactivity in 10 ml of culture, h is hours of incubation, z is cell numbers per ml culture, and the term $[(1.5\varepsilon/1000) + 1]$ takes into account the isotopic discrimination during bacterial sulfate reduction between ^{32}S and ^{35}S. We used the experimentally determined stable isotopic fractionation factor (ε) for each culture and used the factor 1.5 to take into account the increase in mass difference between ^{32}S and ^{35}S relative to ^{32}S and ^{34}S assuming that the heavier isotope ^{35}S reacts 1.5 times more slowly than ^{34}S (Jørgensen, 1978). Sulfate reduction rates are reported as moles of sulfate reduced per bacterial cell per day. Precision of cell-specific sulfate reduction rates based on triplicate measurements was better than 5%.

2.1.3. Determination of sulfate and sulfide concentrations

At each time point, duplicates of 50 μl aliquots were added to 300 μl 20% zinc acetate. Sulfate was determined after further dilution by nonsuppressed anion chromatography and conductivity detection. The eluent was 1 mM isophtalic acid in 10% methanol adjusted to a pH of 4.7 with sodium tetraborate. Detection limit for the method is 10 μM with an analytical precision better than 5 μM. Sulfide was determined spectrophotometrically (Cline, 1969). Analytical precision based on triplicate analyses was 10 μM.

2.1.4. Stable sulfur isotope analysis

For stable sulfur isotope determination 10 ml 20% zinc acetate were added to the remaining aliquot to terminate bacterial activity and to precipitate dissolved sulfide. Dissolved sulfate and precipitated zinc sulfide were separated by acid distillation with cold 6N HCl under N_2. H_2S was trapped in 0.25 mM $AgNO_3$. Dissolved sulfate was recovered as $BaSO_4$. The isotopic composition of dissolved sulfate and sulfide were determined by continuous flow isotope ratio monitoring gas chromatography mass spectrometry according to methods described in Giesemann et al. (1994). The sulfur isotopic composition is expressed in the standard δ notation given by $\delta^{34}S = (R_{sample}/R_{standard} - 1) \times 1000$, where $R = {}^{34}S/{}^{32}S$. Values are expressed on a permil (‰) basis using the VCDT scale (Robinson, 1995). The precision for isotopic analysis is based on duplicate analyses from the same culture bottles and is 0.5‰ or better. Mean and standard deviation for the international reference standard NBS 127 (20.0‰) were 20.0 ± 0.2‰ vs. VCDT, respectively.

2.1.5. Determination of isotopic fractionation factors

Bacterial reduction of sulfate by the culture occurred in sealed serum vials without loss of product. These conditions are analogous to closed systems, allowing calculation of the isotopic fractionation according to a Rayleigh fractionation model (Mariotti et al., 1981). Isotopic fractionation factors (ε) with

$$\varepsilon = \left(\frac{(\delta^{34}S_{sulfide} + 1000)}{(\delta^{34}S_{sulfate} + 1000)} - 1\right) \times 1000 \quad (4)$$

were calculated by nonlinear regression in three ways.

(1) Using a nonlinear regression model to determine a function best reflecting the isotopic composition of dissolved sulfate ($\delta^{34}S$) at each time point (t) on the basis of the isotopic composition of sulfate and the fraction of remaining sulfate (f_{SO_4}) according to

$$\delta^{34}S^{t_i}_{SO_4} = \varepsilon \ln f_{SO_4} + \delta^{34}S^{t_0}_{SO_4}. \quad (5)$$

(2) Using a nonlinear regression model to determine a function best reflecting the isotopic composition of dissolved sulfide at each time point on the basis of the isotopic composition of sulfide and the fraction of dissolved sulfide formed according to

$$\delta^{34}S^{t_1}_{HS^-} = -\varepsilon(1 - f_{HS^-}) \ln (1 - f_{HS^-})/f_{HS^-} + \delta^{34}S^{t_0}_{SO_4}. \quad (6)$$

A detailed discussion for the derivation of Eqns. (5) and (6) is given in Mariotti et al. (1981). The concentration and isotopic composition of sulfide was corrected for residual sulfide transferred during inoculation of the culture vials.

(3) Using the isotopic difference between dissolved sulfate and dissolved sulfide when the fraction of sulfate remaining was greater than 95%.

Thus, two independent sets of data were used to determine the isotopic fractionation factor. Methods (1) and (2) were used as controls to test the assumption of the Rayleigh model that mass conservation exists between consumed sulfate and formed sulfide. The temperature experiments with LSv54 were repeated to assess the reproducibility of the determined isotopic fractionation factors. The isotopic fractionation factors in the second experiment determined with method (1) agreed with the first experiment within 0.1‰ (Table 1). The differences in ε between the two experiments when using methods (2) and (3) were sometimes larger than 1‰. Since greater internal consistency was achieved with method (1), we relied for the interpretation of experimental results on method (1). Tabulated raw data of the fractionation experiments are available from the first author upon request.

Table 1. Isotopic fractionation factors (ε) as a function of temperature and substrate determined by regression on $\delta^{34}S_{sulfate}$, $\delta^{34}S_{sulfide}$, and from the isotopic difference (Δ) between sulfate and sulfide at time t_1 (see methods).

	Analyte used	ASv20 acetate	ASv20 lactate	ASv26 acetate	ASv26 lactate	LSv54 lactate	LSv514 lactate	
−1.8°C	Sulfate			22.0	4.6	4.5	7.5	
	Sulfide			22.0		10.0	7.0	8.5
	Δ			21.3		7.4	6.0	8.7
2°C	Sulfate			22.0				
	Sulfide			22.0				
	Δ			20.1				
4°C	Sulfate	13.0		22.0	10.6	4.6	4.5	5.0
	Sulfide	13.5		22.0	9.2	8.0	7.0	6.4
	Δ	13.8		20.9		9.6	5.8	6.3
9°C	Sulfate	17.5	6.8	22.0		4.6	4.5	4.6
	Sulfide	14.0	8.5	22.0		6.3	7.0	7.0
	Δ	14.3		19.7		5.6		8.0
16°C	Sulfate	15.0						6.5
	Sulfide	16.5						7.5
	Δ	17.3						9.5
20°C	Sulfate	18.8						6.1
	Sulfide	16.0						9.0
	Δ	21.0						

2.2. Sediments

2.2.1. Sampling

Sediments were collected with a Haps corer on board the RV Farm in August 1998 from four stations (D, F, G, J) off the coast of western Svalbard, Norway (Fig. 1). Bottom water temperatures at all stations are permanently below 1°C. The Haps core was subsampled with two 36 mm acrylic cores, plugged with butyl rubber stoppers, and stored at ambient seawater temperatures until further processing. One core was used to determine the abundance and isotopic compositions of acid-volatile sulfides (AVS), chromium-reducible sulfide interpreted as pyrite (CRS), and dissolved sulfate. The second subcore was used to determine sediment porosity. On land, the first core was sectioned into 1 cm intervals and squeezed to extract porewater. Porewater from the squeezed sections was directly fixed in 10 ml of a 5% zinc acetate solution. The squeezed cakes were stored in 20 ml of a 20% zinc acetate solution for later determination of AVS and CRS. Additional sediment was taken from stations F and J for slurry preparation. The sediment was collected in multiple corer deployments to obtain enough material from the 0–3 cm depth interval.

2.2.2. Slurry preparation and incubation

A 500 ml sediment sample was mixed with an equal amount of deoxygenated seawater under N_2 to which 1.5 g of *Spirulina* cyanobacterial powder was added to stimulate bacterial activity. Portions of the slurry (10 ml) were placed in culture tubes under N_2, covered with a butyl rubber stopper, and sealed. The culture tubes were incubated in a temperature gradient block consisting of an aluminum block with 30 predrilled slots to hold the culture tubes (Sagemann et al., 1998). The block was heated at one end with a thermostat to 39°C and cooled at the other end with a Julabo cooling unit to −4°C establishing a continuous temperature gradient with a temperature difference of 1.3°C between each slot. The slurries were incubated over the full temperature range for 12 days (Station F) and 14 days (Station J), respectively. After this time, 1 ml of slurry in each culture tube was withdrawn and centrifuged at 14,000 rpm. The supernatant was filtered through a 0.45 μm filter, fixed in 500 μl 2% zinc chloride solution, and retained for the determination of dissolved sulfate. The remaining slurry in each culture tube was transferred into Corning polyethylene centrifuge tubes filled with 20 ml of 20% zinc acetate to stop bacterial activity and retain dissolved sulfide and labile iron sulfide as zinc sulfide.

2.2.3. Laboratory analyses of sediment cores and slurries

Concentrations of sulfate were determined for an aliquot of the squeezed porewater by nonsuppressed anion chromatography. Remaining sulfate was precipitated from the supernatant with $BaCl_2$ at pH 4. Sediments were centrifuged and washed twice with double deionized water. Approximately 2 g wet sediment was ultrasonified with 20 ml MeOH to remove elemental sulfur. Extraction of acid-volatile sulfides (AVS) and pyrite (CRS) was performed using methods described in Fossing and Jørgensen (1989). AVS was extracted from the MeOH-extracted sediment residue under N_2 with cold 6N HCl for 1 h. Evolved H_2S was trapped in 0.1 M $AgNO_3$. Pyrite was extracted subsequently in a boiling, acidic (3N HCl) 1 M $CrCl_2$ solution for 1 h, and liberated H_2S was trapped as described above. Concentrations of distilled AVS and CRS were determined gravimetrically. Triplicate extractions of selected samples indicated that the determined concentrations varied by less than 10% of the average concentration. For sulfur isotopic determination, 300 to 400 μg of Ag_2S derived from AVS and CRS and 400 μg of precipitated $BaSO_4$ from dissolved sulfate were weighed into tin cups with a tenfold excess of V_2O_5. The sulfur isotopic composition was determined by GC-IRMS as described above.

Slurries in the Corning centrifuge tubes were spun at 5000 rpm to obtain enough porewater for isotopic analysis of the dissolved sulfate. The supernatant was filtered through 0.45 μm millipore filters and acidified to pH 4. Concentrations and sulfur isotope compositions of dissolved sulfate in the filtrates were determined as described above. The isotopic fractionation factors (ε) were determined from the Rayleigh equations using concentration and isotopic composition of sulfate at time point t_0 (beginning of incubation) and time point t (end of incubation) according to Eqn. (2).

3. RESULTS

3.1. Pure Cultures

3.1.1. Sulfate reduction rates

^{35}Sulfate reduction rates for the four different strains are presented in Figure 2 as a function of temperature. For each culture, sulfate reduction rates varied by nearly an order of magnitude over the experimental temperature range chosen. All analyzed strains showed a characteristic decrease in sulfate reduction rates as a function of temperature. The temperature range of all experiments spans the range commonly encoun-

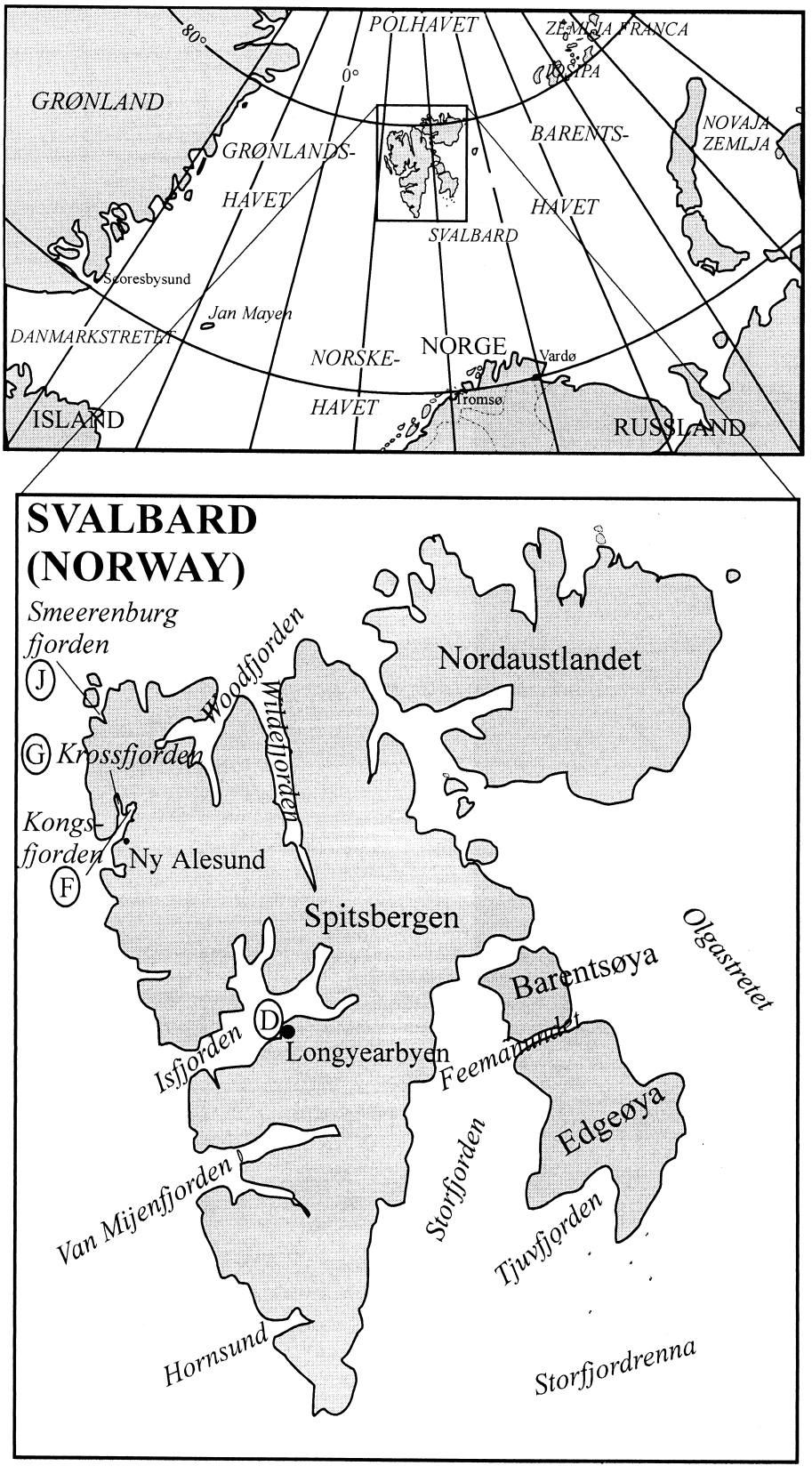

Fig. 1. Map of Svalbard showing the locations of the stations.

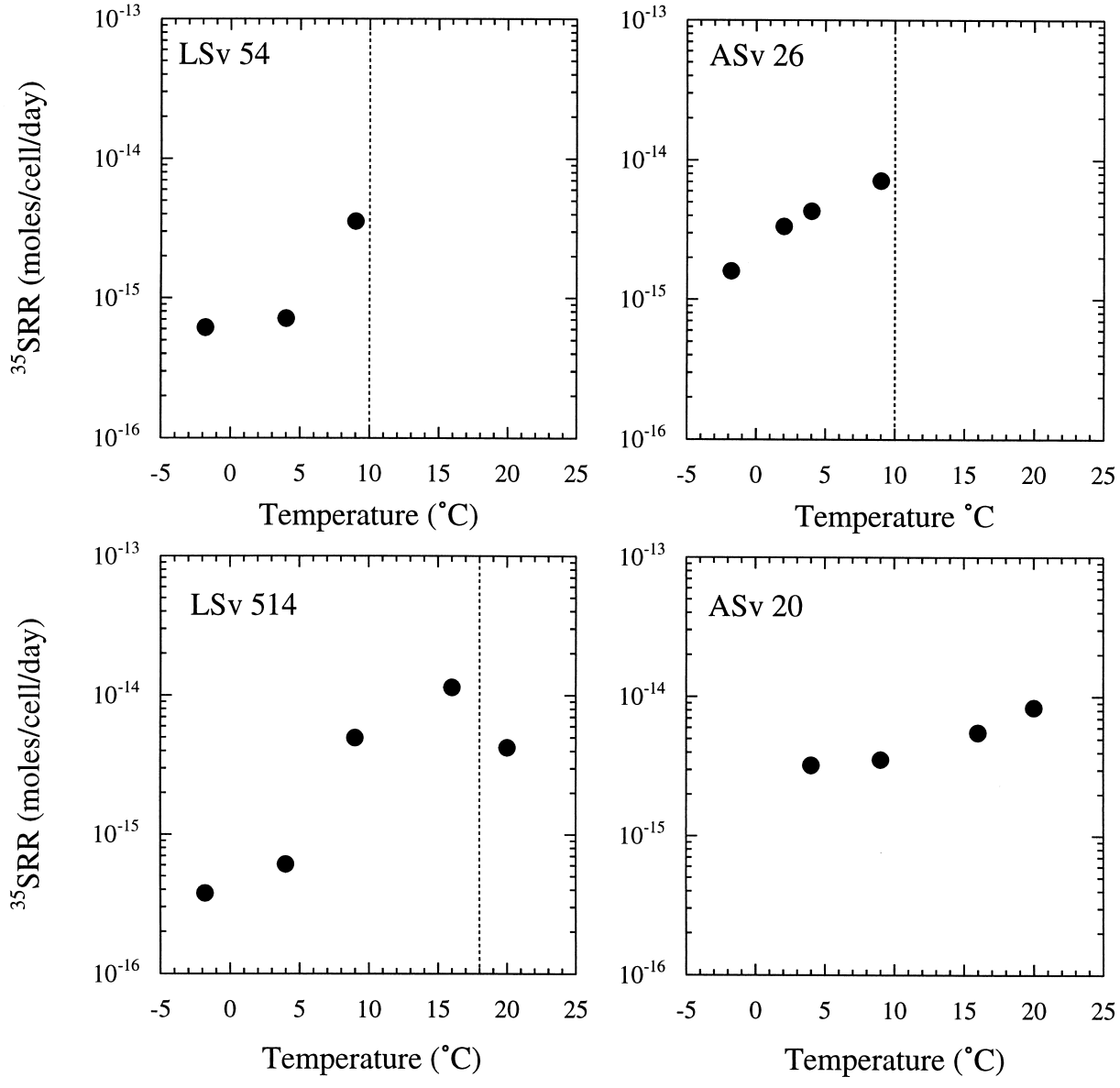

Fig. 2. Temperature dependence of cell-specific sulfate reduction rates measured in discrete time intervals with the ^{35}S-radiotracer method for the psychrophilic sulfate reducers LSv54, LSv514, ASv26, and the mesophilic sulfate reducer ASv20. The cultures on the right side are complete oxidizers, the cultures on the left side are incomplete oxidizers. The vertical dashed lines indicate the temperatures for optimum growth of each culture. Strain ASv20 had a temperature optimum above 25°C.

tered in cold and temperate marine sediments. For LSv514, sulfate reduction rates decreased above the temperature for optimum growth suggesting damaged cell membranes and/or enzymes.

3.1.2. Sulfur isotopic fractionation

The stable isotopic fractionation factors (ε) derived by regression using the Rayleigh equations for dissolved sulfate and sulfide were either constant at the different growth temperatures or showed no consistent variation with temperature (Fig. 3). The regressions on sulfate yielded isotopic fractionations that varied between 4.6‰ and 22‰. Incomplete-oxidizing cultures LSv54 and LSv514 fractionated sulfate between 4.6‰ and 10‰ when growing on lactate whereas the complete-oxidizing cultures ASv26 and ASv20 fractionated sulfate between 13‰ and 22‰ when growing on acetate (Table 1). Isotopic fractionation factors derived from the isotopic evolution of residual sulfate were often different from those derived for sulfide (Fig. 3). For LSv54, ε increased for sulfide as temperature decreased while the ε derived from sulfate was constant. Calculation of the sum of dissolved sulfate and dissolved sulfide for this strain also showed that 25% to 30% of the consumed sulfate remained unaccounted for. The proportion of missing sulfate increased as temperatures decreased. We

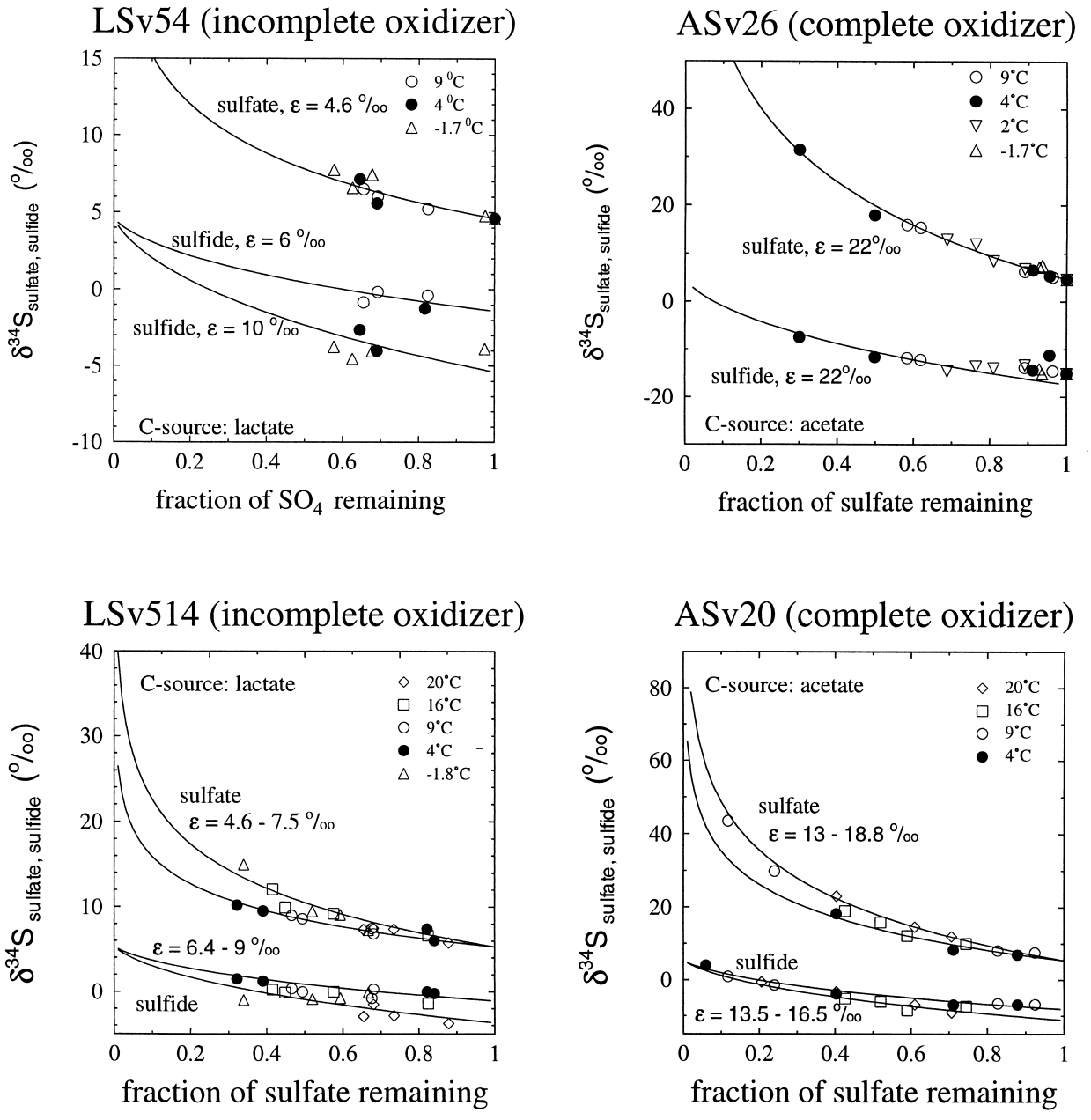

Fig. 3. Rayleigh plots for the determination of isotopic fractionation factors for the four isolates. The regressions and isotopic fractionation factors are shown for each temperature for sulfate (upper lines) and sulfide (lower lines). The cultures on the right side are complete oxidizers, the cultures on the left side are incomplete oxidizers.

infer that the remaining sulfur resided in intermediate sulfur species such as trithionate, thiosulfate, or sulfite. In ASv26, ε was the same for dissolved sulfide and sulfate, which is consistent with the observation that the sum of dissolved sulfate and dissolved sulfide was nearly constant during the experiment and accounted for all of the starting amount of dissolved sulfate. A smaller ε value resulted when ASv26 was grown on lactate instead of acetate (Table 1). The calculated fractionation factor for ASv26 when grown on lactate was only slightly greater than for the incomplete-oxidizing cultures LSv54 and LSv514, also grown on lactate. The other complete-oxidizing bacterium ASv20, when grown on lactate, also yielded small isotopic fractionations of 6.8‰ and 8.5‰ that were similar to that of the incomplete-oxidizing strains (Table 1).

The effective isotopic difference between dissolved sulfate and sulfide at time point t_1 (less than 5% of electron acceptor consumed) showed no overall correlation with sulfate reduction rates (Fig. 4). A negative correlation ($r^2 = 0.83$) exists for the incomplete-oxidizing LSv54 and LSv514, but the change in the isotopic difference is <2‰ between the highest and the lowest sulfate reduction rate. No such relationship exists between sulfate reduction rates and the isotopic difference for the com-

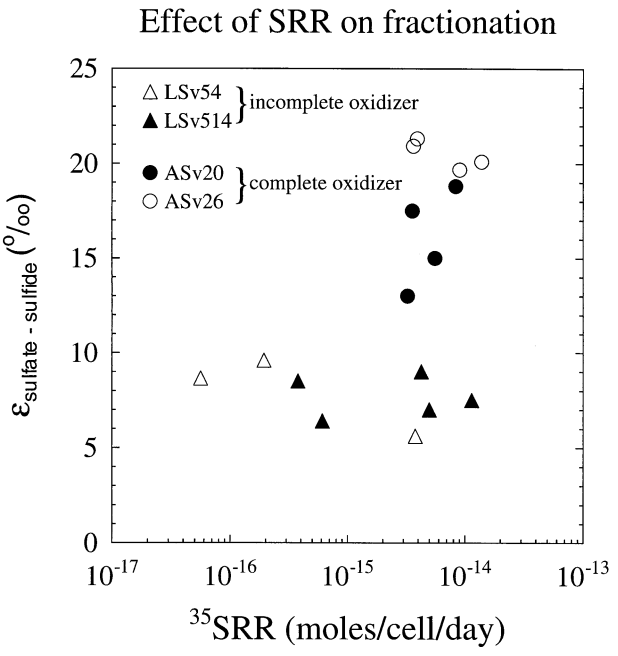

Fig. 4. Plot showing the relationship between 35-sulfate reduction rates in the early exponential phase and the stable isotopic difference between sulfate and sulfide for all experimental temperatures. The data suggest that species-specific effects on the isotopic fractionation are much greater than variations induced by changing sulfate reduction rates.

plete-oxidizing strains. It is important to note that the experimental temperature-induced variation in sulfate reduction rates is by far greater than variations in sulfate reduction rates commonly observed over seasonal cycles in temperate coastal sediments with comparable temperatures (Jørgensen, 1977; Chanton et al., 1987). A comparison of sulfate reduction rates for the different cultures indicates a variation over two orders of magnitude (Fig. 4). Yet, the variation in isotopic fractionation for a given bacterial species is relatively minor. The interspecies variations appear to be of much greater importance.

3.2. Sediments

Concentrations of dissolved sulfate were close to normal marine values of 28 mM and showed negligible gradients in concentration changing less than 0.5 mM over the analyzed depth range of 0–30 cm. Sulfate reduction rates determined with the $^{35}SO_4$ radiotracer method (Fossing and Jørgensen, 1989) were highest within the 0–10 cm depth interval (Jørgensen and Knoblauch, in preparation). The isotopic composition of dissolved sulfate was close to the normal marine values and varied between 20.0‰ and 20.8‰ vs. VCDT.

Concentrations of AVS and CRS gradually increased with depth in all profiles indicating net burial of dissolved sulfide by precipitation of iron sulfides (Fig. 5). The isotopic composition of AVS showed little change with depth and varied between −25.0‰ and −35.0‰ at the three stations (Fig. 5). AVS could not be analyzed isotopically near the sediment surface at stations F and G because of low concentration. CRS was consistently enriched in ^{34}S relative to AVS, but a gradual convergence in isotopic composition with depth towards that of AVS was observed at stations D and G (Fig. 5). This change may reflect the gradual conversion of ^{32}S-enriched AVS to pyrite with increasing burial and suggests continuous formation of pyrite with burial.

3.3. Temperature Gradient Block Experiments

Sediment slurries from station F and J showed a distinct relationship between sulfate reduction rates and temperature. In both slurries, the sulfate reduction rate was high between 10°C and 23°C (Figs. 6A and 6B). For the station J slurry, the greatest depletion of sulfate occurred above 34°C. The optimum temperatures for sulfate reduction were thus considerably higher than the *in situ* temperatures of 0.7°C and −0.4°C for stations F and J, respectively. Sulfur isotope values of dissolved sulfate closely follow sulfate concentrations, which is consistent with closed system conditions during the incubation (Figs. 7A and 7B). The sedimentary isotopic fractionation factors fall into three distinct temperature groups at station F: (1) −1°C to 8°C, ε = 30‰; (2) 8°C to 25°C, ε = 15.5‰; (3) 25°C to 39°C, ε = 5‰. For station J, the calculated isotopic fractionation factors are 14‰ between −4.4°C and 25°C and 8‰ between 25°C and 39°C. Given the uncertainty for the calculation of ε when less than 90% of the sulfate was consumed, we calculated the precision of ε values on the basis of the analytical uncertainty for the determination of sulfate concentration and the isotopic composition of sulfate. The resulting ε values differed by less than 1‰ from the values presented in Figure 7.

4. DISCUSSION

4.1. Temperature and Rate Dependence of Isotopic Fractionation in Pure Cultures

The incubation experiments with LSv54 and LSv514 suggest only a weak relationship between sulfur isotope fractionation, temperature, and sulfate reduction rate. These results contrast with experimental results using *Desulfovibrio desulfuricans* by Kemp and Thode (1968) and Kaplan and Rittenberg (1964) who concluded that the sulfur isotope fractionation is inversely proportional to sulfate reduction rates. According to their model, organic substrate and temperature should influence the fractionation in so far as they influence sulfate reduction rates. However, the lowest temperatures chosen in their experiments (0°C) were below the growth limit for *Desulfovibrio desulfuricans*. Furthermore, the sulfate reduction rates reported for growth on ethanol by Kaplan and Rittenberg (1964) were so low that it appears reasonable to assume that the cells experienced physiologically stressed conditions. The value of 46‰, which is the highest fractionation value ever reported in pure culture fractionation experiments, may thus not be a representative maximum value for this species. A high fractionation value of 34.8‰ was also reported in a continuous culture experiment with *Desulfovibrio desulfuricans*. This value was achieved at a sulfate reduction rate of 6.7 femtomole/cell/day (Chambers et al., 1975). This sulfate reduction rate is in the same range as the rates achieved in our experiments at the lowest temperatures. Since LSv54 and ASv26 are specifically adapted to low temperatures, we are certain that the bacteria in

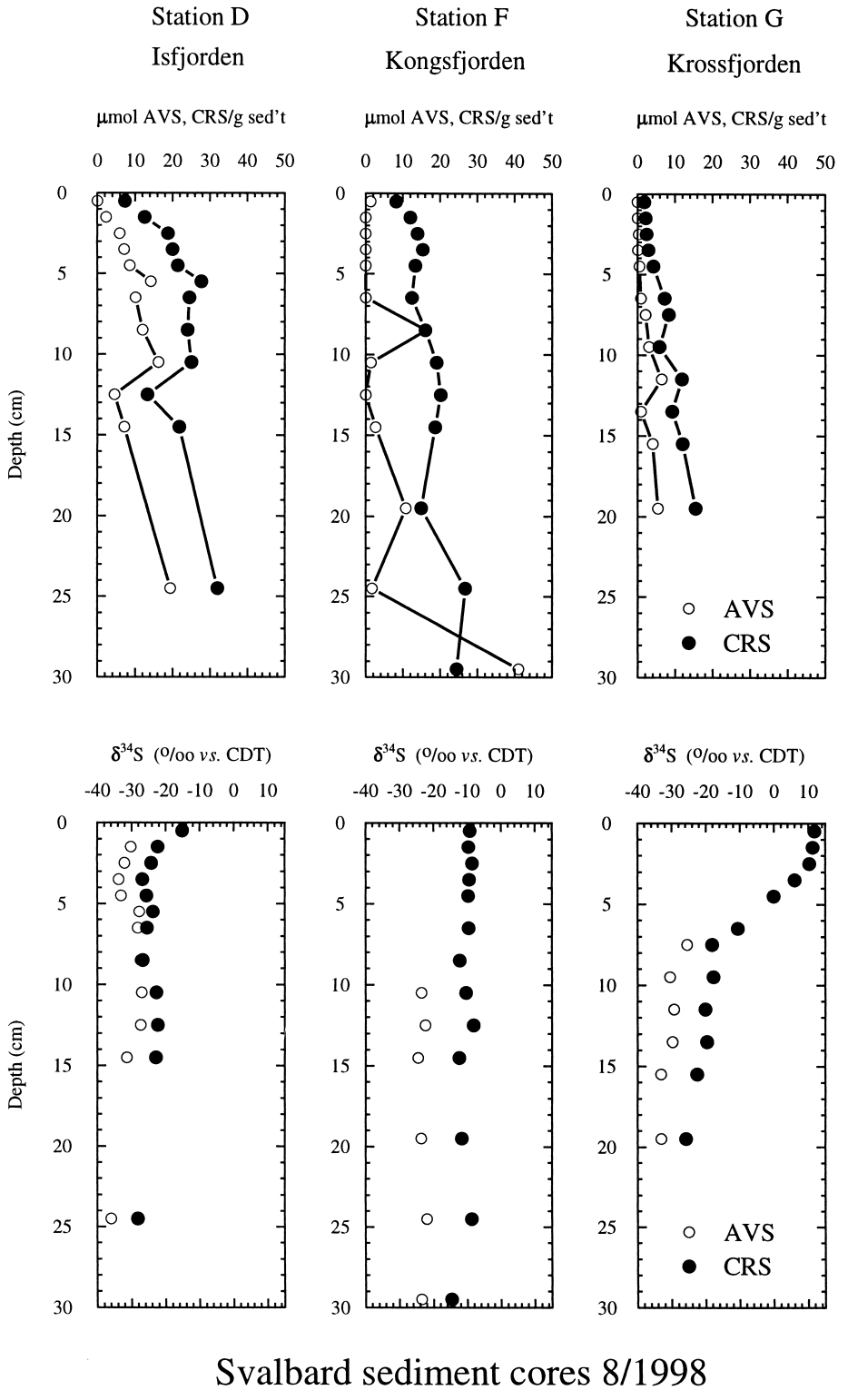

Fig. 5. Top row: Depth distribution of acid-volatile sulfide (AVS) and chromium-reducible sulfide (CRS), commonly interpreted as pyrite for three sediment stations from Svalbard. Bottom row: Depth distribution of the stable sulfur isotopic composition of AVS and CRS at the three sediment stations.

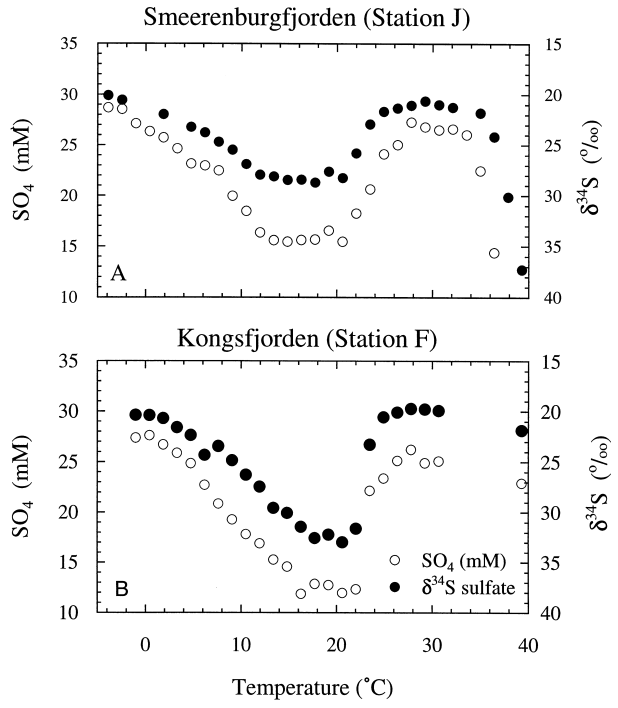

Fig. 6. Concentration and stable sulfur isotopic composition of sulfate in sediment slurries of a temperature gradient block experiment. The incubation times for stations F and J sediment were 12 and 14 days, respectively.

our experiments did not experience physiologically stressed conditions. However, even at the lowest sulfate reduction rates the fractionation of LSv54 and LSv514 was below 10‰. Moreover, ASv26 and ASv20 produced fractionations between 18‰ and 22‰ at rates of sulfate reduction higher than those of LSv54 and LSv514 that yielded smaller fractionations. This suggests that factors other than reduction rate and temperature influence the isotopic fractionation. It appears that the difference in fractionation between different genera of sulfate-reducing bacteria is greater than the differences induced by variation in environmental conditions such as temperature. Possibly, other sulfate-reducing bacteria can also achieve high fractionations such as the ones reported by Chambers et al. (1975) and Kaplan and Rittenberg (1964), but it is critical to ensure that these fractionations are determined under physiologically healthy conditions.

4.2. Metabolic and Phylogenetic Effects on the Isotopic Fractionation by Sulfate-Reducing Bacteria

On the basis of experiments with four cultures and two different substrate oxidation pathways, it would be premature to generalize our results. However, it is worthwhile to consider the different factors involved in the regulation of the isotopic fractionation during sulfate reduction (Rees, 1973). There are certain physiological differences between sulfate-reducing bacteria that can affect isotopic fractionation (Widdel and Hansen, 1992). These include differences in cell membranes, structurally different APS and dissimilatory sulfite reductases, different

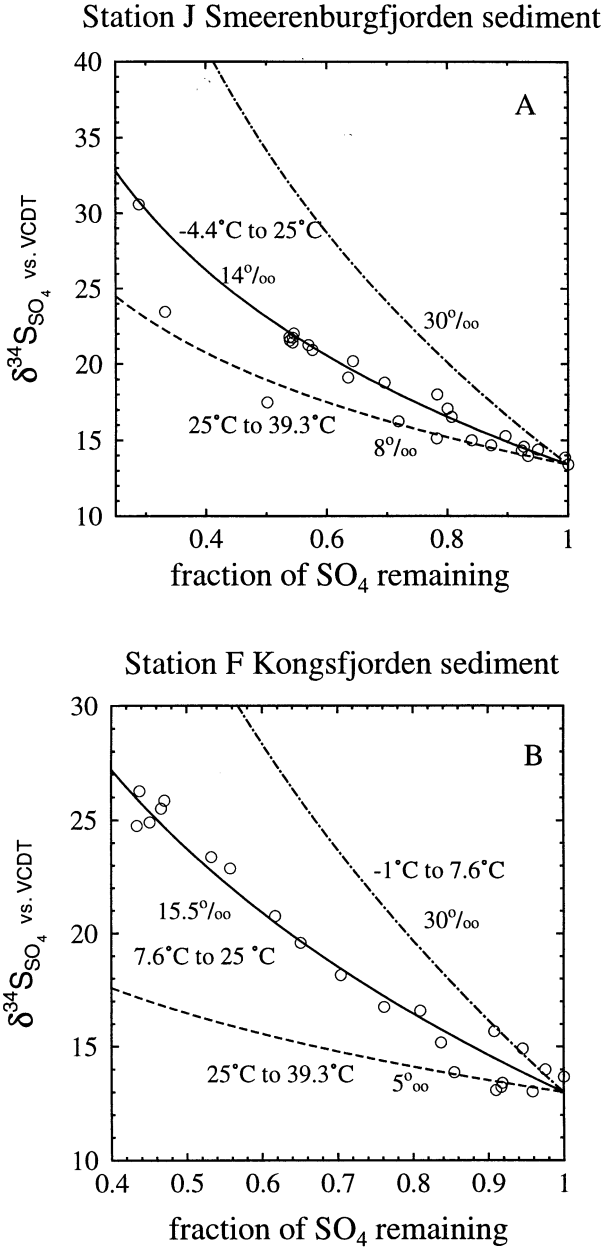

Fig. 7. Rayleigh plots of $\delta^{34}S_{sulfate}$ to determine bulk isotopic fractionation factors in the sediment slurries. The data points fall on distinct lines in different temperature ranges suggesting that distinct communities of sulfate reducers with characteristic fractionations are active in the different temperature ranges.

enzymes in the electron transport chain, and the different substrate spectra of sulfate-reducing bacteria (Hansen, 1994).

The pronounced isotopic difference between the complete- and incomplete-oxidizing cultures is evidence for the importance of different metabolic pathways in the isotopic fractionation. The free energy change ($\Delta G^{\circ\prime}$) and the resulting difference in redox potential ($\Delta E^{\circ\prime}$) for the incomplete oxidation of lactate to acetate with sulfate as electron acceptor is more than three times greater than for the oxidation of acetate to bicarbonate. Using data from Thauer et al. (1977) and Hanselmann

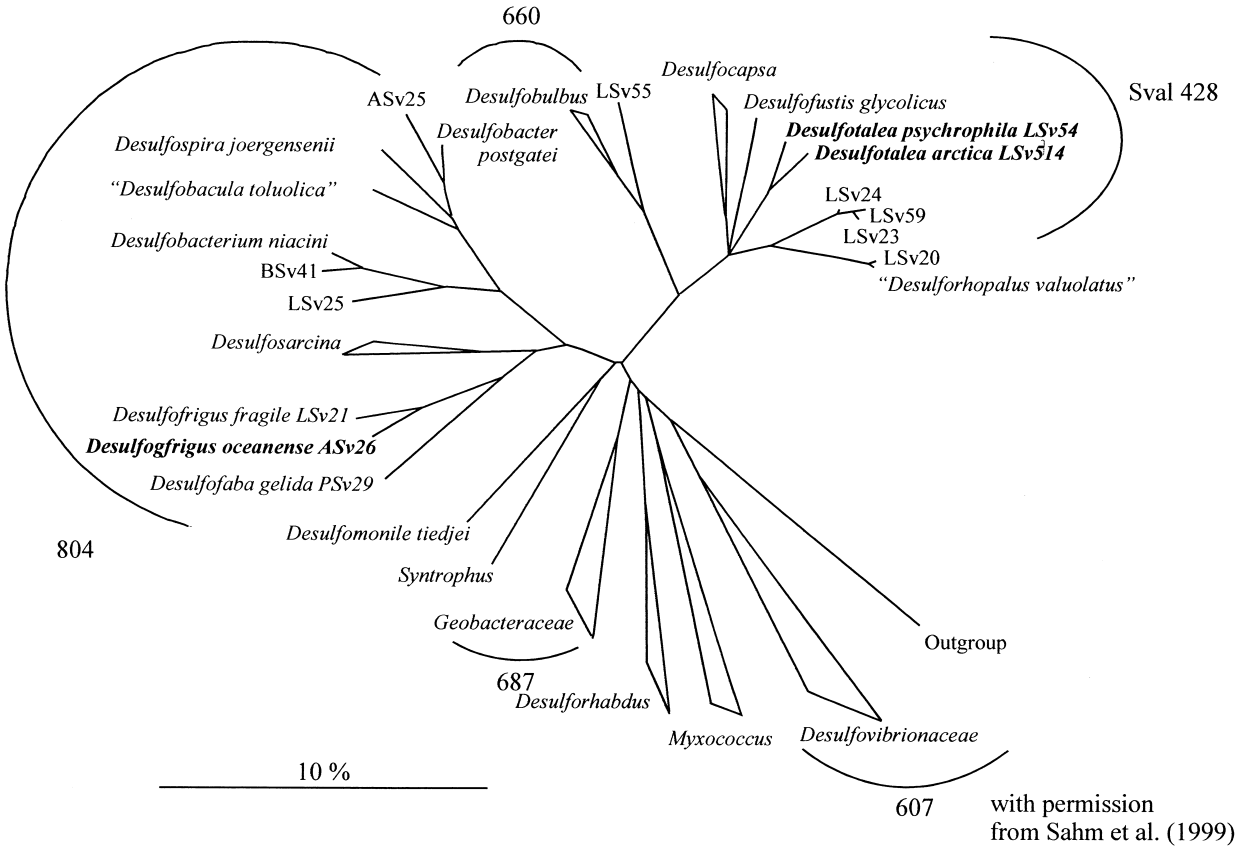

Fig. 8. Phylogenetic tree based on 16S rRNA showing the affiliations of the new isolates in the δ-group of the proteobacteria (Sahm et al., 1999a). The similarly fractionating strains LSv54 and LSv514 are phylogenetically closely related, whereas strain ASv26 is genetically distinct from these two strains. The brackets span the strains of different sulfate reducers that were targeted with different oligonucleotide probes for *in situ* detection and quantification (with permission after Sahm et al., 1999b).

(1991), the resulting values for $\Delta G^{\circ\prime}$ and $\Delta E_0'$ are -160.1 kJ/mole SO_4^{2-} and -207 mV, respectively, for the incomplete oxidizers and -47.6 kJ/mole SO_4^{2-} and -64 mV for the complete-oxidizing sulfate reducers. Conceptually, these different redox potentials can be regarded as a measure of the electron flow on the APS reductase and the dissimilatory sulfite reductase. For a reaction yielding a high, i.e., more positive $\Delta E_0'$, electrons are forced on the APS reductase and the dissimilatory sulfite reductase thus shifting the overall reaction equilibrium towards the production of bisulfide, minimizing the buildup of intermediates, and allowing greater expression of isotopic fractionation. Therefore, if the transport of electrons to a reducing enzyme is either slow or inhibited due to a lack of electron donor, the isotopic fractionation is expressed more strongly. The extent to which isotopic fractionation is expressed thus depends on the availability of electrons that are transported via the electron-transport chain to the APS reductase and the dissimilatory sulfite reductase.

There is also genetic evidence for different APS reductases and dissimilatory sulfite reductases in sulfate-reducing bacteria (Minz et al., 1999; Wagner et al., 1998). Furthermore, sulfate-reducing bacteria possess different cytochromes for the regulation of the electron flow from the organic substrate (Hansen, 1994). The differently structured enzymes probably require different activation energies, which may in turn influence the isotopic fractionation. At the present time, however, the critical biochemical and genetic information necessary to understand the regulation of the isotopic fractionation at the biochemical level is lacking.

As a first approach, however, we related the phylogenetic similarity between our sulfate reducers to their isotopic fractionation. Figure 8 shows a phylogenetic tree that was constructed based on the sequence similarity of the 16S rDNA in several genera of sulfate-reducing bacteria including those investigated here (Sahm et al., 1999a,b). The length of the lines connecting the different species shows the dissimilarity of their 16S DNA sequences in %. Comparison of the isotopic fractionation factors to the relative distance in the phylogenetic tree reveals that the genetically similar strains LSv54 and LSv514 also show similar fractionations whereas ASv26, which is more distant to LSv54 and LSv514, also fractionated differently. The effects of phylogenetic and metabolic diversity of sulfate reducers are currently being explored with other sulfate-reducing bacteria and will be reported in more detail elsewhere. In general, we have found that sulfate-reducing bacteria that oxidize their substrates completely fractionate stronger. Phylogeny appears to play a role in so far as it reflects different

Table 2. Percentages of sulfate and organic substrate remaining at the end of each experiment.

Temperature	ASv20	ASv26	LSv54	LSv514
A. Sulfate				
20°C	20.6			32.1
16°C	42.6			20.2
9°C	11.8	61.8	65.5	24.8
4°C	5.9	30.1	64.5	19.2
2°C		68.8		
−1.8°C		91.6	57.7	18.8
B. Substrate				
20°C	17.2			26.8
16°C	35.5			16.8
9°C	9.8	51.5	5.7	20.7
4°C	4.9	25.1	12.1	16.0
2°C		57.3		
−1.8°C		76.3	8.7	15.7

metabolic characteristics such as complete and incomplete substrate oxidation.

4.3. Comparison with natural conditions

In the natural environment, bacterial activity is generally limited by the availability of organic substrate (Morita, 1987). Conceivably, different fractionation factors could result when the rate of sulfate reduction is limited by the available carbon source. This condition may be more comparable to the stationary phase of growth in batch culture experiments or to continuously grown cultures. Substrate-limited conditions were approached in the experiment at 4°C with ASv20 and in the experiment at 9°C with LSv54 (Table 2). However, the constant slopes of the regression lines in the Rayleigh plots in Figure 3 indicate that the isotopic composition of sulfate in these experiments reflected constant isotopic fractionation over the course of the experiments. Therefore, the isotopic fractionation in the stationary phase is similar to the isotopic fractionation for data points in the lag and exponential phase. Furthermore, the specific sulfate reduction rates at the lowest temperatures (−1.8°C) of our experiments are within the range reported for continuous culture experiments at substrate-limited conditions (Chambers et al., 1975). Since the sulfate reduction rates in these different experimental setups are comparable, isotopic fractionations similar to our results may also be expected under substrate-limited conditions in continuous culture.

The best current estimates for the abundance of specific genera of sulfate-reducing bacteria in Arctic sediments are based on in situ fluorescence hybridization counts (FISH). More specifically, the estimates from fjords of Svalbard are between 10^7 and 10^8 cells/cm^3 of sulfate reducers in near-surface sediment (Ravenschlag et al., 2000). We have used these cell numbers and the experimental sulfate reduction rates from our batch culture experiments that were performed between −1.8°C and 2°C in the early exponential growth phase to calculate potential sulfate reduction rates for sediments (Table 3). The potential sulfate reduction rates were then compared with in situ sulfate reduction rates in Arctic sediments from Hornsund, Storfjorden, and station J (Fig. 1) (Knoblauch and Jørgensen, 1999). If we assume that a hypothetical population size of 10^8 cells/cm^3 would consist exclusively of the strain

Table 3. Theoretical sediment sulfate reduction rates in nmol/cm^3/day) calculated from the rates in pure culture as a function of population size.

	Population size (1×10^7)	Population size (1×10^8)
LSv514, −1.8°C	3.8	37.6
LSv54, −1.8°C	2.1	21.3
ASv26, 2°C	221.2	2212.0
ASv26, −1.8°C	333.2	3331.9
ASv20, 4°C	32.4	324.1
35-Sulfate reduction rates, measured in situ	0.5–45.4	

ASv26, the potential sulfate reduction rates would exceed the measured sulfate reduction rates by two orders of magnitude. By contrast, if we assume a population of 10^7 cells/cm^3 for LSv54, LSv514, and ASv20, we obtain potential sulfate reduction rates that are within the range of the measured rates. This comparison reflects the common problem of batch culture experiments, namely that bacteria commonly grow significantly faster than in natural environments. It also highlights the need for accurate determinations of the actual numbers of sulfate-reducing bacteria in marine sediments. Nevertheless, the calculations suggest that some of the sulfate reduction rates at the lowest temperatures in the batch culture experiments were comparable to or only slightly higher than in the sediment. Based on this similarity, it is conceivable that at the lowest temperatures the bacteria yield isotopic fractionations that are comparable in sediments and in batch cultures.

Another factor to be considered for naturally occurring fractionations is an assessment of whether our investigated bacteria are abundant in Arctic sediments. Sahm et al. (1999a) applied a radioactively labeled genetic probe (Sval 428) specifically targeting the cluster of sulfate reducers containing the *Desulfotalea* strains LSv54 and LSv514 and quantified the 16S rRNA abundance by slot-blot hybridization. This cluster represents between 1.4% and 21% of prokaryotic RNA in Storfjorden sediment and between 0.6% and 2.6% in Hornsund sediment (Sahm et al., 1999a). At station J in Smeerenburgfjorden, Ravenschlag et al. (2000) found that *Desulfotalea* accounts for 5.6% of the prokaryotic RNA, but that *Desulfofrigus* was below detection. These molecular studies suggest that the *Desulfotalea* strains are abundant genera within the sulfate-reducing bacterial community in Arctic sediments. Thus, the group of incomplete-oxidizing sulfate-reducing bacteria from this group must influence the total sediment isotopic fractionation during sulfate reduction in the Svalbard sediments. Unfortunately, we do not have comparable information on the most abundant complete-oxidizing bacteria because there has been no successful attempt to isolate them to date.

The temperature gradient block experiments with the sediment slurries clearly distinguish temperature ranges with characteristic fractionations (Fig. 7). The fractionation values are either between those of the incomplete- and complete-oxidizing bacteria or only slightly higher. This consistency suggests that a comparable bacterial community was active in the slurries. Ecological comparisons between temperature gradient block experiments with substrate-amended sediment slurries and natural settings are difficult. Temperature may favor sulfate re-

ducers with optimal adaptations in the different temperature ranges. However, the rate of substrate release during the hydrolysis of the cyanobacterial biomass from the added *Spirulina* may also favor different sulfate reducers at different temperatures because the rate of hydrolysis is in itself a temperature-dependent process (Arnosti et al., 1998). Different fermenting bacteria may be active in the different temperature ranges, and the release of their specific fermentation products may also favor different sulfate reducers (Rosselló-Mora et al., 1999). The calculated fractionation factors are independent of sediment sulfate reduction rates, but we cannot infer that the cell-specific sulfate reduction rates changed because we do not have information on the abundance of specific sulfate reducers. For the station F slurry, low sulfate reduction rates were measured both at the very cold and the very high temperature end, but the fractionation factors were very different −5‰ and 30‰, respectively. Since sulfate reduction rates cannot explain the different fractionations, the relative proportions of sulfate-reducing bacteria with different characteristic isotopic fractionations must have changed as a function of temperature. Similar fractionation factors for the two slurries at the intermediate temperatures between 7.6°C and 25°C suggest similar communities of sulfate-reducing bacteria in this temperature interval.

Of particular interest is the community of sulfate reducers that was active above 34°C in the slurry from station J. After the 14 day incubation, this community had consumed more than 70% of the available sulfate (Fig. 6A). It is likely that the long incubation period over two weeks allowed growth of a bacterial community that was initially not active, but became predominant at the highest temperatures. The presence of thermophilic sulfate-reducing bacteria in temperate marine sediments was also demonstrated in long-term tracer experiments in sediment from Aarhus Bay, Denmark, and other Arctic sediments (Isaksen et al., 1994; Jørgensen and Knoblauch, unpublished results). Currently, we have no genetic information on the bacteria active in the high temperature range of our experiments. Possibly, these bacteria represent sporulating species, but it remains unclear which environmental conditions other than high temperature favor their growth or inactivity. Nevertheless, our results demonstrate that the temperature gradient block experiments and the isotopic results are important tools to probe the sulfate reducer community present in marine sediments. These results suggest that under different environmental conditions, the community of sulfate-reducing bacteria changes, and that distinct isotopic signatures characterize these communities.

4.4. Comparison with the Geochemical Record

Acid-volatile sulfides are often considered the initial precipitates in the formation of pyrite. The isotopic difference between AVS and dissolved sulfate in the analyzed sediments ranges from 42‰ to 56‰, which considerably exceeds the fractionation achieved in our pure cultures. This observation highlights the importance of additional fractionation effects resulting from redox recycling of sulfide in marine porewaters (Canfield et al., 1998). Significant isotope effects have not been demonstrated for the chemoautotrophic oxidation of sulfide to elemental sulfur and sulfate (Fry et al., 1988). However, disproportionation of sulfur intermediates (elemental sulfur, thiosulfate, and sulfite) can significantly increase the total isotopic difference between sulfide and sulfate (Canfield and Thamdrup, 1994; Cypionka et al., 1998; Habicht et al., 1998).

Our results allow us to determine how much of the total isotopic difference is produced during sulfate reduction. While we are unable to account for the isotopic fractionation produced by all active sulfate-reducing bacteria in our sediments, we have determined the fractionation produced by the abundant species. Based on the foregoing discussion, it is reasonable to assume that the experimental cellular rates exceeded in situ cellular sulfate reduction rates by less than an order of magnitude. Furthermore, the temperature gradient block experiments produced fractionations of 30‰ at station F and only 14‰ at station J at in situ temperatures. These values can serve as baselines to calculate the relative contributions of isotope effects during recycling of sulfide. Using the fractionation values from the temperature gradient block experiments, the collective fractionations that arise during the disproportionation of elemental sulfur, thiosulfate, and sulfite contribute between 26% and 71% of the overall isotopic difference. Using published values for the fractionations during disproportionation of these three sulfur intermediates (Canfield and Thamdrup, 1994; Cypionka et al., 1998; Habicht et al., 1998) we calculated that the probability of a sulfide molecule being recycled is between 60% and 95%. These results are in agreement with radiotracer studies of sulfur cycling in marine sediments (e.g., Jørgensen, 1977; Ferdelman et al., 1997).

The sulfur isotope values of pyrite in the uppermost 2 cm at stations F and G are unusually enriched in ^{34}S compared to AVS and to published values for pyrite in recent and ancient marine sediments (e.g., Zaback and Pratt, 1992; Habicht and Canfield, 1997; Brüchert, 1998; Hurtgen et al., 1999; Brüchert et al., 2000). The following hypotheses may explain these values: (1) near-surface pyrite represents an instantaneous precipitate of sulfide without recycling of sulfide; (2) the heavy isotopic values are the result of partial oxidation of pyrite that leaves residual pyrite enriched in ^{34}S; (3) the pyrite is recent, but from a different source. This source may be puddles of glacial runoff that were observed downstream of glaciers discharging into the fjords. These puddles smelled of hydrogen sulfide, and the bottoms were covered with black precipitates, possibly sulfides (A. Hodson, personal communication). Additional sampling of surrounding detrital source rocks and glacial runoff will further clarify these questions. Unfortunately, at present we are not able to constrain any of the above hypotheses further. Interpretations of AVS and CRS isotope values are also complicated by transition processes in the course of pyrite formation and the occasional stable coexistence of AVS and CRS in marine sediments (Hurtgen et al., 1999). While detailed interpretations of the processes involved in the formation of AVS and CRS are beyond the scope of the study, our results highlight the discrepancy in isotopic fractionation between isotopic fractionation during sulfate reduction alone and the complex integrated signals recorded in sedimentary sulfides.

5. CONCLUSIONS

This is the first report of isotopic fractionations by populations of sulfate-reducing bacteria that are capable of growing at the temperatures of very cold marine habitats and at tempera-

tures that are common to most temperate marine environments worldwide. This is a significant step beyond earlier studies that reported experimental isotopic results with *Desulfovibrio* strains at temperatures at which the cultures were not adapted or that were too high to be considered representative for most marine environments. These new results clearly indicate that variations in the isotopic fractionation cannot exclusively be attributed to variations in cellular rates or to variations in bulk sedimentary rates of bacterial sulfate reduction. The slurry experiments in temperature gradient blocks and the pure culture experiments indicate that temperature is an important variable only in so far as it regulates community composition. Ultimately, genetic and physiological differences between genera of sulfate-reducing bacteria are decisive in determining isotopic fractionation. Thus, the characteristic populations in a particular marine habitat control isotopic fractionations during bacterial sulfate reduction.

Which sulfate-reducing bacteria are present in a particular environment and which specific substrates are utilized become relevant controlling parameters for the isotopic fractionation. There is no general agreement about the dominant substrates for sulfate-reducing bacteria in marine environments. Acetate and lactate are generally regarded as important terminal substrates in marine environments (Sørensen et al., 1981; Parkes et al., 1989), but hydrogen can be an important substrate in syntrophic bacterial communities and is particularly relevant at very low temperatures (Conrad et al., 1986). Since the composition of the organic matter varies from place to place, it is likely that the anaerobic food chain and the microbial community of sulfate reducers in different habitats varies as well. There is now growing molecular genetic evidence for the presence of different sulfate-reducing bacteria in different marine habitats (Llobet-Brossa et al., 1998; Ravenschlag et al., 1999; Sahm et al., 1999b). Consequently, it would be expected that the overall isotopic fractionations by sulfate-reducing communities in different environments vary because different sulfate reducers are present.

For geologic applications, these results help to constrain how much of a total isotopic difference measured between oxidized and reduced sulfur species in sediments can be attributed to sulfate reduction and how much of the fractionation occurs in the oxidative part of the sedimentary sulfur cycle due to oxidation and disproportionation. Based on earlier studies it was speculated that fractionations higher than the maximum experimentally determined fractionation of 46‰ could be achieved if sulfate reduction rates were kept lower than the ones reported by Kaplan and Rittenberg (1964) and Chambers et al. (1975). However, technical problems with continuous cultures at very low dilution rates have precluded proof of this assertion. In the Svalbard sediments, it appears that reoxidation processes play a significant role because we could constrain the actual sulfate reducer community and quantified sulfate reduction rates. In order to make similar assessments for other sediments, comparable information on the type and abundance of sulfate reducing bacteria is required. This poses a serious problem for reconstructing the sulfur cycle in ancient sediments. To advance toward the quantitative use of the sulfur isotopic composition in ancient marine sediments, complementary information on preserved biomarkers of different sulfate-reducing and sulfide-oxidizing bacteria is necessary.

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