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CONTENTS Page

VORWORT

Die vorliegende Arbeit wurde an der Universität Bremen eingereicht zu Erlangenung des Grades eines Doktor der Naturwissenschaften, Dr. rer. nat. Die Arbeit wurde am Max-Planck Institut für Marine Mikrobiologie in Bremen unter Leitung von Prof. Dr. Bo B. Jørgensen, in der Abteilung für Biogeochemie mit Prof. Dr. Donald Canfield als Betreuer, angefertigt. Das Projekt wurde von der Deutschen Forschungs-Gemeinschaft im Rahmen des Graduierten Kolleg "Stoff-Flüsse in Marinen Geosystemen" und von der Max-Planck Gesellschaft finanziert. In der vorliegenden Dissertationsschrift habe ich die Schwefelfraktionierung in marinen Standorten näher untersucht. Auf Grund von experimentellen Untersuchungen von natürlichen Proben und in Bakterienkulturen, habe ich die Ergebnisse in 4 englischsprachliche Manuskripte zusammengefaßt, die in internationalen Zeitschriften veröffentlicht werden sollen. Von den 4 Manuskripten ist eines publiziert, eines ist eingereicht, und zwei werden vorbereitet für eine mögliche Veröffentlichung. Ich möchte alle Autoren für eine gute und fruchtbare Zusammenarbeit danken.

Einen besonderen Dank möchte ich Prof. Dr. Donald Canfield, für die Betreuung dieser Arbeit aussprechen. Ich danke Prof. Dr. Bo B. Jørgensen dafür, daß er meine Doktorarbeit ermöglicht hat und für nete Unterstützung. Außerdem möchte ich Dr. Bo Thamdrup herzlich für die große Hilfe danken, die er während der ganzen Doktorarbeites gewährleistet hat. Ich möchte auch allen ehemaligen und jetzigen Mitarbeiter des Max-Planck Institutes für die große Hilfsbereitschaft und eine freundliche Zusammenarbeit danken. Speziell gilt mein Dank der Abteilung für Biogeochemie für viele hilfreiche Diskussionen. Frau Swantje Fleischer und Frau Kirsten Neumann danke ich für die technische Hilfe.

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Zuletzt möchte ich herzlichst Bo, meinen Bruder Andreas und meinen Eltern, für die große Unterstützung danken, die Sie mir immer gegeben haben.

INTRODUCTION

In coastal marine environments, an important part of the organic matter produced by primary production is remineralized in the sediments to the original compounds CO_2, H_2O and nutrients (Jørgensen and Revsbech 1989). The O_2-containing zone of the sediments is, however, only few millimeters thick. Below this aerobic zone the mineralization is carried out by a combination of fermentation and anaerobic mineralization pathways. In continental magin sediments sulfate reduction is the most important anaerobic metabolism, and about 50% of the total mineralized carbon is oxidized by this process (Jørgensen 1982). Sulfide produced during sulfate reduction can in sediments react with iron and precipitate as iron sulfides. However, about 90% of the reduced sulfur is transported up to the oxidized sediment layers, were it is reoxidized to sulfate. Thus, in coastal marine environments typically 50% of the sediment O_2 consumption is used either directly or indirectly for the reoxidation of sulfide (Jørgensen 1982). The bacterial sulfate reduction and the sulfide oxidation are therefore two of the quantitatively most important redox processes in the sediments. Studies of sulfur in ancient sediments have indicated that this also was true through most of the Earth's history (Berner and Canfield 1989). An understanding the sulfur cycle over geological time is therefore an integral part of the understanding of the oxidation state of the Earth's oceans and atmosphere.

The interpretation of the evolution of the sulfur cycle has mainly been based on stable sulfur isotope measurements from sulfur deposits available through the geological record. The stable sulfur isotope composition of a sample is reported as $\delta^{34}S$ which is the deviation of the ratio of the two most common sulfur isotopes, ^{34}S (~ 4%) and ^{32}S (~ 95%), in the sample from the ratio in the Canyon Diablo Troilite (CDT):

$$\delta^{34}S = [(^{34}S/^{32}S)_{sample} - (^{34}S/^{32}S)_{CDT}] \times (^{34}S/^{32}S)_{CDT}^{-1} \times 1000$$

The sulfur isotope composition of the CDT is believed to have the initial value of the Earth's crust and mantel. An isotope ratio different from the CDT is therefore an indication of either biological or chemical transformations of the original sulfur compounds.

Fractionation during sulfate reduction

Dissimilatory sulfate reduction is a main contributor to changes in the original sulfur isotope values, as sulfate-reducing bacteria discriminate between the two stable sulfur isotopes during their metabolism. Because of the kinetic isotope effect, $^{32}SO_4^{2-}$ is reduced faster than $^{34}SO_4^{2-}$, and the produced H_2S therefore becomes depleted in ^{34}S compared to the remaining SO_4^{2-}. In pure cultures of sulfate-reducing bacteria the depletion of ^{34}S in sulfides during sulfate reduction has been found to range from 5‰ to 46‰. This range of ^{34}S depletions was mainly dependent on the specific rate of sulfate reduction (rate per cell) and on the electron donor (Harrison and Thode 1958; Kaplan and Rittenberg 1964; Chambers et al. 1975). With the organic electron donors ethanol and lactate, the ^{34}S depletion correlated negatively with the rate of sulfate reduction, such that low isotope fractionations were associated with high rates of sulfate reduction. With H_2 as electron donor however, the isotope fractionations were directly correlated with the sulfate reduction rates and were generally lower than the values found with organic donors. Low ^{34}S depletions in H_2S were also measured at sulfate concentrations below 1 mM (Harrison and Thode 1957). Other physical and chemical parameters like the temperature, bacterial species, substrate and pH might also influence the isotope fractionation but was believed to be of minor importance (Chambers and Trudinger 1979). However, since these early studies (all before 1980), where only few sulfate-reducing bacteria were known, many new species have been discovered, some of which can even live under extreme conditions such as in the surface layers of microbial mats at high concentrations of O_2 (Canfield and Des Marais 1991; Widdel and Bak 1992; see also Manuscript 3). Also, it is not known to what extent the isolates represent the dominating species *in situ*. To overcome these

uncertainties, I have measured the isotope fractionation by natural populations of sulfate reducers at *in situ* conditions (Manuscript 1 and 2). Furthermore, a quantification of the natural population and a description of its species composition was carried out (Manuscript 3). By knowing the population size, the fractionation by the natural population could be related to the specific rate of sulfate reduction (rate per cell), and these values could be correlated with the pure culture studies mentioned above (Manuscript 2).

Interpreting the geological record

In the interpretation of $\delta^{34}S$ values of sulfate and sulfide in sediments it is important to consider whether the sulfur compounds were formed in a closed or open sediment system with respect to sulfate (e. g. Ohmoto 1992). In a totally closed system with no external supply of sulfate, the concentration of sulfate will decrease when consumed by sulfate reduction. For the isotopes, a Rayleigh distillation will occur, where the residual sulfate becomes increasingly enriched in the heavy ^{34}S, and consequently heavier and heavier sulfide is produced. When all the sulfate is consumed no isotope fractionation can be measured as the isotope composition of sulfide will be the same as the initial $\delta^{34}S$ value of sulfate. In a sediment there will always be some supply of sulfate because of the diffusion of sulfate into the sediment. Still, a sediment is considered closed with respect to sulfate, when the supply rate of sulfate equals the rate of sulfate reduction such that all sulfate is consumed within the sediment. Diminished isotope fractionation values of sedimentary sulfides can be found in a sediment closed with respect to sulfate. In an open sediment, pore-water sulfate can exchange with the overlying water column at a rate which is much higher than the sulfate reduction rate. Maximum depletion of ^{34}S in sedimentary sulfides can be measured in open sediments as the $\delta^{34}S$ of sulfate does not change with time.

In the sediment record the stable isotope values have changed for both sulfides (mainly measured as pyrite) and sulfate through Earth's history (Fig. 1). In the oldest sedimentary rocks from the Archean (3.8 - 2.5 billion years ago) and early Proterozoic

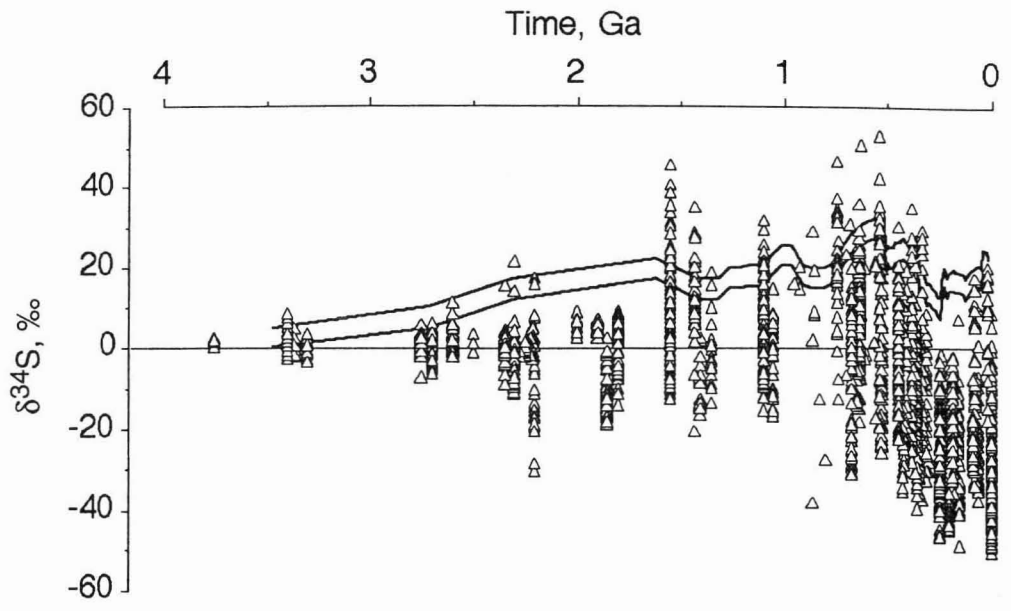

Figure 1. The isotopic composition of sedimentary sulfides over the Earth's history. The double line represents the estimated isotopic composition of sea-water sulfate. After Canfield (1996).

times, the $\delta^{34}S$ values of both sulfide and sulfate were about 0‰. At around 2.2 to 2.3 billion years the first marked isotope shift was measured, and from 2.2 - 0.8 billion years, the isotope record showed a consistent pattern with a maximum ^{34}S depletion in sedimentary sulfides of 35‰ - 40‰ compared to contemporaneous sulfate. At around 0.8 billion years, sulfide became depleted in ^{34}S by 55‰ or more compared to seawater sulfate, and this pattern has persisted into modern time.

The change in the sulfur isotope values through geological time is probably related with changes of the ocean chemistry over the course of the Earth's history. The earliest Archean ocean was reduced and contained no sulfate (Cameron 1982). Based on stable sulfur isotope data it has been argued that dissimilatory sulfate reduction evolved in response to a rise of sulfate in the oceans (Schidlowsky 1983). The first marked isotope shift at about 2.2 to 2.3 billion years ago has been related to a biogenic origin (Cameron 1982). It correlates with the large early Proterozoic burial of carbon, which liberated O_2 and, as a consequence of oxidative weathering of the reduced sulfur compounds, also sulfate (Des Marais et al. 1992). However, the oldest bacteria known from microbial stromatolites date back to 3.5 billion years ago (Des Marais 1990). It has therefor being suggested that dissimilatory sulfate reduction already

occurred long before 2.3 billion years ago, and that the first appearance of presumably bacteriogenic sulfides only gave a minimum age for this biological event (Cameron 1982; Schidlowski et al. 1983; Ohmoto et al. 1993). Three different hypotheses have been proposed to explain the clustering of sulfur isotopic values around 0‰ for sediments older than about 2.3 billion years.

1) The sulfides with 0‰ isotope values result from sulfide-rich volcanic emanations or from high temperature sulfate reduction, and are therefore not of biogenic origin (Cameron 1982).

2) Dissimilatory sulfate reduction existed but in an Archean ocean with low sulfate concentrations (below 1 mM; Cameron 1982). At low concentrations of sulfate the isotope fractionation during sulfate reduction is small (Harrison and Thode 1957). Further, a Rayleigh distillation effect is likely to occur, and no depletion in ^{34}S of the sedimentary sulfide can be detected.

3) The 0‰ sulfur isotope values in the Archean rocks have further been explained by bacterial sulfate reduction in an Archean ocean containing sulfate concentrations similar to the values of today (> 10 mM; Ohmoto et al. 1993). The diminished isotope fractionation was rather a result of extremely high rates of sulfate reduction (> 27 $\mu mol\ cm^{-3}\ d^{-1}$). This hypothesis was mainly based on the pure culture studies of sulfate-reducing bacteria, which showed that the lowest depletion of ^{34}S in sulfide was measured at high rates of sulfate reduction.

Isotope values of about 0‰ in the Archean sulfur compounds have thus both been argued to result from bacterial sulfate reduction in an Archean ocean with low or high concentrations of sulfate. The fact that two such very different scenarios have been introduced to explain the geological observations, is to a large extent due to the lack of fractionation data from sulfate reduction of natural populations. There exists, for example, no determination of the ability of a natural population to fractionate sulfur at high rates of sulfate reduction. To provide such information, the fractionation during sulfate reduction by the natural population of sulfate-reducing bacteria from the microbial mats of Solar Lake, Egypt was measured as part of this study

(Manuscript 1). The microbial mats were chosen as they support some of the highest rates of sulfate reduction known on Earth, and are believed to be reasonable homologues to ancient microbial mat systems like stromatolites. Such a study would support one of the existing hypotheses on whether or not there had been an accumulation of sulfate already in the Archean ocean (3.5 billion years ago).

Isotope fractionation in recent sediments

In modern sediments, the isotope fractionation between seawater sulfate and sedimentary sulfides ranges up to 70‰ (Canfield and Thamdrup 1994). However, during sulfate reduction by pure cultures of sulfate reducers, the isotope fractionation was maximally 46‰, and can therefore not explain the large depletions of ^{34}S measured in the sediments. It has been proposed that an additional sulfur fractionation to that of bacterial sulfate reduction might occur in sediments. Such an isotope fractionation has been related to the oxidative part of the sulfur cycle (Jørgensen 1990; Canfield and Thamdrup 1994). A study by Canfield and Thamdrup (1994) has supported this suggestion, as a good correlation between the observed isotope fractionation and the amount of H_2S oxidized in marine sediments from different areas of the World was found. The pathways for H_2S oxidation are, however, poorly understood, as H_2S can be reoxidized to intermediates sulfur compounds such as elemental sulfur (S^0), thiosulfate ($S_2O_3^{2-}$), or sulfite (SO_3^{2-}) either bacterially or chemically. The intermediate sulfur compounds are after production actively further transformed by either reduction, disproportionation or oxidation, leaving only small concentrations (mainly below 10 µM for $S_2O_3^{2-}$ and SO_3^{2-} and below 10 µmol cm^{-3} for S^0) in the sediments (Thode-Andersen and Jørgensen 1989; Jørgensen and Bak 1991; Thamdrup et al. 1994).

Isotope fractionations associated with the oxidative part of the sulfur cycle have been measured in various chemical and bacterially catalyzed reactions. The chemical or bacterial, anaerobic or aerobic oxidation of, respectively, H_2S, S^0, SO_3^{2-} and $S_2O_3^{2-}$ to either S^0 or SO_4^{2-} were only associated with small fractionations (±

5‰; e. g. Fry et al. 1986). Also, no isotope fractionation has been found during the chemical reactions of H_2S with iron producing FeS or FeS_2 (Mossmann et al. 1991). However, Canfield and Thamdrup (1994) measured a marked isotope effect associated with the bacterial disproportionation of S^0. During the disproportionation of S^0, sulfide was depleted in ^{34}S by about 8‰, and sulfate enriched in ^{34}S by about 13‰ compared to the isotope composition of S^0. The isotope fractionation associated with S^0 disproportionation could therefore explain the additional depletion of ^{34}S in sedimentary sulfides. Sulfide first depleted in ^{34}S by sulfate reduction can be additionally depleted in ^{34}S by a repeated cycle of oxidation to S^0 by, e. g., Fe (III) followed by disproportionation. Similar roles have been suggested for transformations through $S_2O_3^{2-}$ and SO_3^{2-}, although the isotope systematics of these pathways had not yet been explored (Jørgensen 1990; Canfield and Thamdrup 1994). To fill out the gaps in the knowledge needed for the consideration of isotope fractionations in the oxidative part of the sulfur cycle, the isotope fractionation during metabolisms of $S_2O_3^{2-}$ and SO_3^{2-}, including both reduction and disproportionation, was measured as a part of the present study (Manuscript 4).

Overview of the publications

To obtain a better understanding of the stable sulfur isotope data from both modern and ancient sediments, the following experimental work has been carried out. In the first manuscript the sulfur fractionation during sulfate reduction by a natural population of sulfate-reducing bacteria from the microbial mats of Solar Lake, Egypt has been studied. The sulfur fractionation was measured at some of the highest rates of sulfate reduction ever reported from a natural environment. In the second manuscript the studies of the sulfur fractionation by natural populations were extended with measurements from the sediments of the Løgten Lagoon sulfuretum, Denmark. The isotope fractionation during sulfate reduction was compared to the depletions of ^{34}S measured in the sedimentary sulfides. As a larger depletion was measured in the sedimentary sulfides than could be explained by sulfate reduction alone, the potential

for a additional fractionation due to bacterial disproportionation of S^0 in Solar Lake and Løgten Lagoon sediments was tested. In the third manuscript the sulfate-reducing bacteria in the microbial mats of Solar Lake were quantified with most-probable-number counts, and these measurements were correlated with the rate of sulfate reduction (rate per volume sediment) to obtain an estimate of the specific rate of sulfate reduction (rate per cell). Also individual species of sulfate-reducing bacteria were identified. Finally, in Manuscript 4 the isotope fractionation during transformation of $S_2O_3^{2-}$ and SO_3^{2-} was measured in bacterial cultures.

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Andreas Prip Canfield, Don Canfield, and Bo Thamdrup at Løgten Lagoon, Denmark.

1

Sulphur Isotopic Fractionation in Modern Microbial Mats and the Evolution of the Sulphur Cycle.

Kirsten S. Habicht and Donald E. Canfield

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Contents Page

INTRODUCTION

The sulphur cycle has evolved over the course of the Earth's history[1,2]. The early Earth's surface environment was reducing, containing little atmospheric oxygen[3], and with seawater sulphate concentrations estimated at less than a few percent of those found today. The accumulation of sulphate to much higher concentrations was probably coincident with the initial accumulation of oxygen in the atmosphere and the consequent oxidative weathering of sulphide minerals on land[4,5]. Past changes in sulphate concentrations in ancient oceans have previously been assessed by comparing the systematics of isotope fractionation by sulphate-reducing bacteria[6-9] with the isotopic composition of sedimentary sulphides[1,2,5,10,11]. But such interpretations have proven equivocal: the generally small ^{34}S depletions in Archean sulphides (deposited ~2.5 - 3.8 billion years ago) have been separately argued to result from both rapid sulphate reduction in a sulphate-rich ocean[5,12], and from sulphide formation in a sulphate-poor ocean[1,2,11]. We report large ^{34}S depletions of 20-25‰ during rapid sulphate reduction by sulphate-reducing bacteria in modern photosynthetic cyanobacterial mats from Solar Lake, Sinai. We conclude that high sulphate concentrations give rise to highly ^{34}S-depleted sulphides, and thus that appreciable concentrations of seawater sulphate did not accumulate until the initial accumulation of oxygen into the atmosphere in post-Archean times.

RESULTS AND DISCUSSION

Sulphate-reducing bacteria catalyze the oxidation of organic carbon using sulphate as an electron acceptor, producing sulphide isotopically depleted in the heavy isotope, ^{34}S (refs. 6-8). With sulphate levels >1mM, pure cultures of sulphate-reducing bacteria produce sulphides depleted in ^{34}S by 4 - 46‰ (refs. 6-8,13), with an average of 18‰ (ref. 14). Only limited ^{34}S depletion ($\leqq$4‰), is observed however,

when sulphate reaches low levels <1.0 mM (ref. 15). Sedimentary sulphides from the Archean and lower Proterozoic have relatively consistent $\delta^{34}S$ values of ~0 ± 5‰, similar to meteorites and mantle-derived igneous rocks[2,11,16-20], and that for contemporaneous seawater[1,16]. These sulphides have therefore been attributed to either bacterial sulphate reduction in low concentrations of seawater sulphate, or to a mantle origin[2,17-19,21].

Alternatively, with abundant sulphate, pure cultures of sulphate-reducing bacteria produce the most ^{34}S-depleted sulphides at low specific rates of sulphate reduction (rate per unit cell), with ^{34}S depletion decreasing as the rate increases[6,8]. Also, a tendency has been noted for the depletion of modern sedimentary sulphides in ^{34}S to decrease as the sulphate-reduction rate increases (rate per volume)[5,9] (but see refs 14, 22). These observations have been extrapolated, and used to argue that the small isotopic differences between sedimentary sulphides and coeval sulphate in Archean oceans have resulted from small ^{34}S depletions during rapid sulphate reduction in a sulphate-rich ocean[5,12]. Sulphate reduction rates of between 27 to 270 μmoles cm^{-3} d^{-1} have been suggested[5]. Thus, two very different scenarios for ocean chemistry have been forwarded from essentially the same isotopic results.

The choice between these two scenarios, and subsequently high or low levels of sulphate in Archean oceans, rests on the ability of natural populations of sulphate-reducing bacteria to fractionate at high rates of sulphate reduction, for which no determinations have previously been reported. To provide this information we measured the fractionation of sulphur isotopes during sulphate reduction in natural populations of sulphate-reducing bacteria from the microbial mats of Solar Lake, Sinai. Our choice of a mat environment is driven by two considerations. First, modern microbial mats are viewed as reasonable homologs to ancient benthic, photosynthetic, microbial communities, including stromatolites[23]. Second, microbial mats support the highest rates of sulphate reduction (per volume) yet reported on Earth[24,25]. Extreme rates of sulphate reduction are driven by high rates of phototrophic carbon fixation, which, when depth-integrated, are similar to those measured in coastal marine

waters[26], but are compressed into a zone extending over only 0.5-3 millimeters depth[25,27]. Most of the photosynthetically-fixed carbon in cyanobacterial mats, like those of Solar Lake, is oxidized by sulphate-reducing bacteria[25,27]. Thus, the direct transfer of large amounts of fresh photosynthetically-produced organic carbon to sulphate reducers, over small vertical dimensions, is responsible for the high rates of sulphate reduction. An even more efficient transfer of carbon from phototrophs to heterotrophic carbon oxidizers in ancient microbial mats and stromatolites, providing higher rates of sulphate reduction, is not anticipated. Hence, the high rates of sulphate reduction measured in modern microbial mats should range among the highest rates possible in ancient sedimentary environments.

In the cyanobacterial mats of Solar Lake we measured *in situ* sulphate reduction rates of 0.3 - 3.1 μmol cm^{-3} d^{-1}, at an ambient temperature of 20°C (Figure 1), consistent with previous rate determinations on these mats[28]. To encounter a broader range in sulphate reduction rates, we incubated 2 to 3 millimeter slices from the surface 1.3 cm of the mat at temperatures from 10 to 30°C. We also explored the effect of variable sulphate concentration (65 mM, the concentration of Solar Lake water, and 20 mM) on both rates and fractionation. No difference in sulphate reduction rate was encountered when the same mat piece was incubated at both 65 mM and 20 mM sulphate (n=5). Overall, rates of sulphate reduction in our experiments ranged from 0.2 to 28 μmol cm^{-3} d^{-1}. The high rates are among the highest ever reported[25], and are also within the lower range proposed for the Archean ocean[5] (see above).

The depletion of sulphide in ^{34}S varied from 16 to 39‰ (Figure 1). These values compare with those obtained from pure cultures of sulphate-reducing bacteria[6-8,13]. At a sulphate concentration of 65 mM, the highest ^{34}S depletions were generally associated with low rates of sulphate reduction, also consistent with pure culture studies[6,8,13]. This correlation, however was not observed at 20 mM sulphate, where relatively consistent ^{34}S depletions were found through a broad range of sulphate reduction rates. For both sulphate concentrations, sulphide was depleted by 20-32‰ at

high rates of ≥ 10 μmol cm^{-3} d^{-1}. These observations indicate that sulphide is produced in the mat with a minimum ^{34}S depletion of about 20‰, independent of sulphate reduction rate, and sulphate concentration in the range of 20 to 65 mM. This value is greater than the small (0-5‰) ^{34}S depletions inferred for high rates of sulphate reduction in a sulphate-rich Archean ocean[5,12].

Further, large ^{34}S depletions of 36 to 42‰ are measured for solid phase

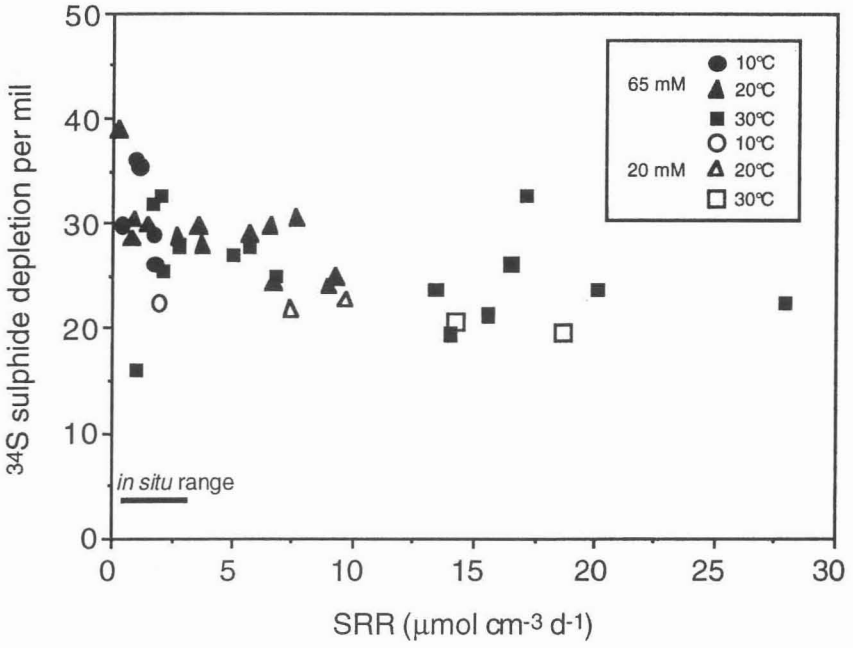

Figure 1. Depletion of sulphide in ^{34}S, compared to sulphate, versus sulphate reduction rate (SRR) for natural populations of sulphate-reducing bacteria from the microbial mats of Solar Lake, Sinai, at different temperatures and sulphate concentrations. The top 0-13 mm of the mats were sectioned into 5 depth intervals. Each mat piece was placed into a dialyses membrane and incubated in a gas-tight bag filled with N_2-purged Solar Lake water (65 mM sulphate) or diluted Solar Lake water (20 mM sulphate) and stored in dark at 10°C, 20°C or 30°C. Sulphate-reduction rates were calculated from sulphide accumulated in the bag[33]. Rates were generally linear with time, except in the surface interval where they accelerated during the incubation. The accelerated rates could have resulted from the accelerated decay of fresh cyanobacteria. After 1.5 to 8 days, the bag-water was withdrawn, and $\delta^{34}S$ values of the sulphate in the bag and the sulphide produced were measured, and calculated relative to Canyon Diablo Troilite (CDT). In all cases, less than 1% of the initial sulphate was reduced during the incubation, ensuring open-system conditions for determining ^{34}S sulphide depletion. The *in situ* rates of sulphate reduction were measured using ^{35}sulphate (ref. 24). Our bag rates at 20°C were comparable to the *in situ* rates, except in surface depth interval, where the bag rates were higher. In addition to the possible enhanced decomposition of fresh cyanobacteria in our bag incubations, *in situ* rates in the surface depth interval may have been underestimated owing to complications of rapid sulphide oxidation during the rate determination[23].

Table 1. Isotopic compositions of sulphides from Archean stromatolites and Solar Lake microbial mat.

Sample	Age (Ga)	$\delta^{34}S‰$	Location
PPRG 1408	3.0	1.5 (2)	Insuzi Group Natal S. Africa
PPRG 2119	2.75	-1.5 (8.5)	Tumbiana Formation Hamersley Basin W. Australia
PPRG 2079	2.75	0.5 (6.5)	Tumbiana Formation Hamersley Basin W. Australia
Microbial Mat	modern	-16 to -22 (-36 to -44)	Solar Lake, Sinai

Pyrite sulphur from Archean stromatolites was liberated for isotopic analysis by chromium reduction[31] of kerogen extracts. Range in isotopic values for Solar Lake mat is from both acid volatile sulphide phases (mostly FeS) and pyrites. Depletions of sulphides in ^{34}S compared to contemporaneous seawater sulphate are provided by values in brackets, with estimates for sulphate of 3.5‰ at 3.3 Ga (ref. 18), and 7‰ at 2.7 Ga (ref. 32). Isotopic composition of Solar Lake sulphate is 20‰.

sulphides in Solar Lake mats (Table 1). These sulphides are fixed in the surface few centimeters of the mat under open conditions with minimal sulphate depletion. Thus, the influence of isotopic fractionation by sulphate-reducing bacteria is preserved, with perhaps some small additional fractionation induced by the oxidative sulphur cycle[14,22]. Isotopic analysis of pyrites from Archean stromatolites from the PPRG (Precambrian Paleobiology Research Group) sample collection show values of between -1.5 to 1.5‰ . These values indicate ^{34}S depletions, compared to best-estimates for contemporaneous seawater sulphate, of between to 2 to 8.5‰. (Table 1). Such low ^{34}S depletions are far less than those measured during sulphate reduction in the Solar Lake mats (Fig. 1), or those for sulphides preserved in the mats (Table 1).

In summary, our results demonstrate that sulphide is depleted in ^{34}S by >20‰ at high rates of sulphate reduction in natural populations of sulphate-reducing bacteria exposed to sulphate levels > 20 mM. Thus, the generally small isotopic differences between sulphides from Archean and early Proterozoic sediments and stromatolites,

and contemporaneous seawater sulphate, cannot be explained by sulphide formation at high rates of sulphate reduction in a sulphate-rich ocean. Instead, as has been previously proposed[2,21], sedimentary sulphides from this time originated from either biological or mantle sources, in an ocean low in sulphate. The increase in seawater sulphate concentration is, thus, best represented by the large negative isotope shift in shale-hosted sedimentary sulphides at between 2.2 to 2.3 Ga (refs. 2, 17-19, 21). This timing correlates with the initiation of a large early Proterozoic burial pulse of organic carbon. This burial pulse released oxidants to the Earth surface[29]; first as oxygen, and subsequently as sulphate and Fe(III), as the oxygen reacted with reduced species on land and dissolved in the ocean[30]. Thus, the history of atmospheric oxygen and seawater sulphate are intimately linked[4,5,17]. This early oxygenation, however, represented only the first stage in the build-up of oxygen to present-day levels. Oxygen levels of greater than 5 to 20% present levels were probably not attained until around 0.8 Ga, near to when Metazoan life is presumed to have evolved[14].

ACKNOWLEDGMENTS

This work was supported by the Max Planck Society, der Deutschen Forschungs-Gemeinschaft, and NASA. We thank B. Thamdrup and B. B Jørgensen for helpful discussion and comments during the preparation of the manuscript. Dave Des Marais is thanked for supplying the PPRG stromatolite samples, while two anonymous reviewers are thanked for helpful and constructive criticism.

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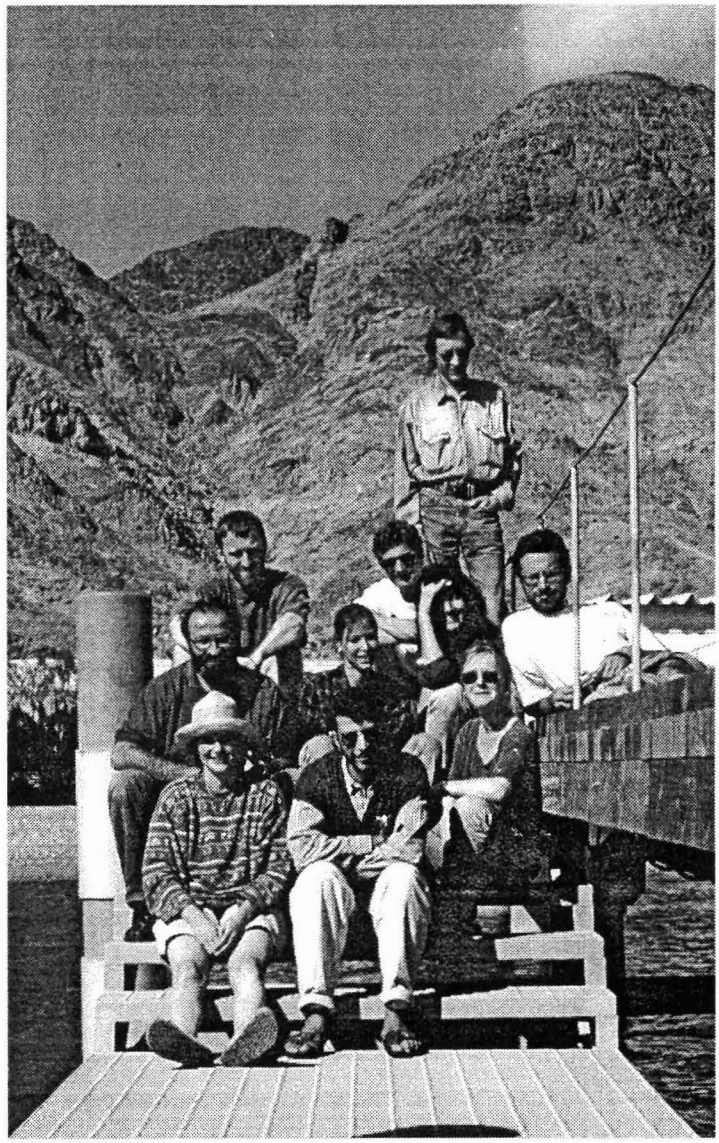

The Solar Lake Team in Eilat, Israel, November 1994

2

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

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Contents Page

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 to 37 μmol cm^{-3} d^{-1}, with the highest rates among the highest ever reported from natural sediments. The depletion of ^{34}S during dissimilatory sulfate reduction ranged from 16‰ to 42‰, with the largest ^{34}S-depletions associated with the lowest rates of sulfate reduction and the lowest ^{34}S-depletions with the highest rates. However, at high sulfate reduction rates (> 10 μmol cm^{-3} d^{-1}) the lowest fractionation was 20‰ independent of the rates. Overall, there was a similarity between the fractionation obtained by the natural population 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 $volume^{-1}$ $time^{-1}$).

Sediment sulfides (mainly FeS_2) were on average 40‰ depleted in ^{34}S 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 ^{34}S depletion of sedimentary sulfides at our study sites

INTRODUCTION

Isotope fractionation during bacterial sulfate reduction is of great importance for the interpretation of $\delta^{34}S$ values from both modern and ancient sedimentary sulfides (Cameron, 1982; Chambers, 1982; Schidlowski, 1983). Studies of isotope fractionation during dissimilatory sulfate reduction by pure cultures have shown that the kinetic isotope effect produces sulfide depleted in ^{34}S 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 ^{34}S 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 ^{34}S of up to 70 per mil 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 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 results from Solar Lake within the context of the evolution of the sulfur cycle (Habicht and Canfield, 1996, Manuscript 1).

MATERIALS AND METHODS

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 meter wide gravel bar through which seawater seeps into the lake and evaporates to a salinity of 70‰ to 180‰ (Cohen et al. 1977, Jørgensen and Cohen 1977; Revsbech et al. 1983). Mat pieces of 70x50 cm and 10 cm thickness where 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 man-made pond filled with seawater from Aqaba Bay, which had evaporated to a salinity of 90‰. The water depth in the pond was 0.5 meter.

Løgten 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 *in situ* temperature. Initial sediment handling was done within 24 hours after sampling.

Chemical and stable isotope analysis

Sediment cores were placed in an O_2-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 N_2 pressure in a pneumatic squeezer equipped with 0.45-µm filters (Reeburgh, 1967) and fixed in 1 ml of 2% $ZnCl_2$ to preserve dissolved pore water sulfide 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%; Cline, 1969). Sulfate was analyzed by non-suppressed anion chromatography using an anion exchange column and a conductivity detector (Waters), with 1 mM isophthalic acid (in 10% methanol, pH 4.7) used as eluent (S.D. 1%). For stable sulfur isotope measurements of porewater sulfide, ZnS was converted to Ag_2S as described below, and porewater sulfate was precipitated as $BaSO_4$.

Solid inorganic sulfur compounds were extracted from the sediments as acid volatile sulfur, AVS (= H_2S + FeS) and chromium reducible sulfur, CRS (= S^0 + FeS_2) by the two step chromium distillation method (Canfield et al., 1986; Fossing and Jørgensen, 1989). During the first step AVS was volatilized as H_2S after conc. HCl had been added to the sample to a final acid concentration of ca. 4N. In the second step reduced chromium, Cr^{2+}, was added and the samples were boiled to evolve H_2S from the reduction of S^0 and FeS_2 (S.D. 5%). The evolved H_2S was carried by a stream of N_2 to a trap with $AgNO_3$ which precipitated H_2S as Ag_2S. Elemental sulfur was obtained from separate portions of the ZnAc fixed sediment by Soxhlet extraction in acetone and collected as Cu_2S onto metallic Cu (Berner, 1964), which was subsequently distilled by chromium reduction (S.D. 5%). In some cases elemental sulfur was obtained from the Zn-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 Ag_2S produced during the Cr reduction and AVS distillations was weighed for concentration determination. Concentrations of FeS were calculated as the difference between AVS and porewater H_2S concentration, while the difference between CRS and S^0 concentrations yielded the sulfur in FeS_2.

Samples of Ag_2S and $BaSO_4$ were converted to SO_2 by combustion with Cu_2O 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 difference 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.

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, (^{35}S, 40 kBq/µl, Amersham) tightly sealed, and returned to the pond to maintain *in situ* temperature (20°C) and light conditions. Tracer (4 µl, 40 kBq/µl) was injected horizontally in 1-cm intervals in sediment collected from Løgten 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 minutes 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 Løgten Lagoon sediment cores were sectioned into intervals of 0.5 cm from 0 to 2 cm and, 1 cm from 2 to 10 cm. All samples were fixed in 10 ml ice-cold 20% ZnAc, homogenized and frozen. For analysis, the samples were centrifuged and a subsample from the supernatant was taken for measuring

$^{35}SO_4^{2-}$ radioactivity and SO_4^{2-} 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.

Diel cycle of S^0

In the microbial mats of Solar Lake a diel cycle of S^0 was measured. In a thermostated water-bath containing constantly aerated water from Solar Lake a 30 x 20 cm piece of mat was incubated under *in situ* light conditions at 20°C. At intervals of 3 hours, 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 the concentration was measured as described above.

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 (Ril - O- Ten; Hansen 1992, Kruse 1993) that were heat-sealed. Through a glass inlet each bag was filled with approximately 150 ml filtered, N_2-purged, Solar Lake water (65 mM SO_4^{2-}) or Aarhus Bay water (13 mM SO_4^{2-}) 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 SO_4^{2-}). 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 H_2S and SO_4^{2-} concentration measurements. When enough H_2S was produced for stable isotope analyses (> 40 µmol H_2S), the sulfidic water was sampled, filtered and fixed into 10 ml 20% ZnAc, and replaced with new N_2-purged water from the sample site. This procedure was repeated from between one to four times during 7 to 11 days depending on the rate of sulfate reduction. To limit the influence of preexisting sedimentary H_2S 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 bags was calculated from the H_2S production rate. To check that the produced H_2S 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 $^{35}SO_4^{2-}$ (40 kBq/µl). After 10 h anoxic incubation the water sample was fixed in ZnAc and analyzed for sulfate reduction rate as described above. The test showed that no sulfate reduction occurred in the water phase of the bags.

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 8 to 10 times before the fractionations accompanying elemental sulfur disproportionation were measured. To measure fractionations a series of 50 ml screw-cap bottles were

Tabel 1. Concentrations of the inorganic sulfur compounds in the microbial mats of Solar Lake.

Depth	SO_4^{2-}	H_2S	S^0	FeS	FeS_2
cm	mM			µmol cm^{-3}	
0.5	65.2	0.2	-	2.4	-
1.5	69.1	0.8	4.2	27.8	11.5
2.5	76.8	1.3	1.1	10.3	8.7
3.5	81.3	1.8	1.8	8.5	14.1
4.5	84.0	1.9	0.5	5.3	8.5
5.5	90.3	1.8	0.5	5.4	5.4
6.5	88.0	2.0	1.1	6.9	12.1
8.5	93.0	0.9	0.9	4.4	17.5

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.

RESULTS

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%

Table 2. Concentrations of the inorganic sulfur compounds in the sediments of Løgten Lagoon.

Depth	SO_4^{2-}	H_2S	FeS	CRS
cm	mM		µmol cm^{-3}	
0.25	13.2	1.7	< 1.0	20.5
0.75	12.4	2.0	< 1.0	4.4
1.5	10.4	1.6	4.3	17.5
2.5	9.3	2.2	4.8	14.8
3.5	9.3	2.3	1.7	16.8
4.5	10.6	1.2	4.8	17.1
6.0	8.3	1.4	13.2	34.2
8.0	2.2	1.1	7.5	22.4

of dry weight, respectively. In both sediments dissolved sulfide was measured within the surface 0 - 1 cm and reached maximum concentrations of up to around 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 attanining 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. Here after, the amount of S^0 decreased to reach a minimum 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 ist summertime maximum of 180‰ (Cohen et al., 1977). Rain during fall months and a lower evaporation rate at this time contributed to a reduction of the

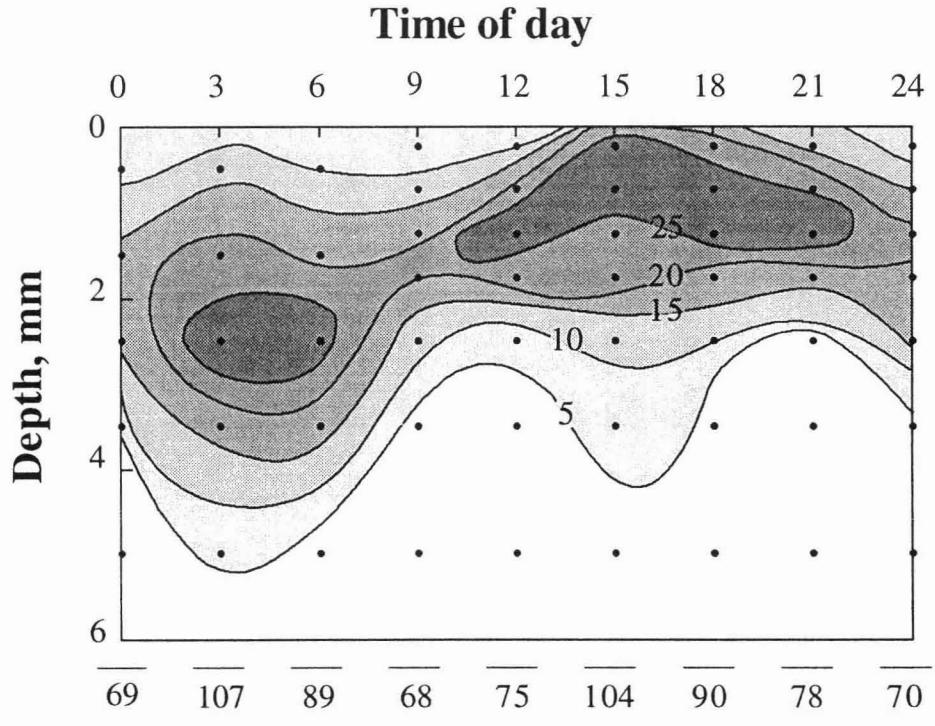


Figure 1. A diel cycle of elemental sulfur (S^0) in the surface of the microbial mats of Solar Lake. Included below the figure are the sulfur concentrations (μmol cm^{-2}) integrated over the surface 0-6 mm.

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, 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‰ at

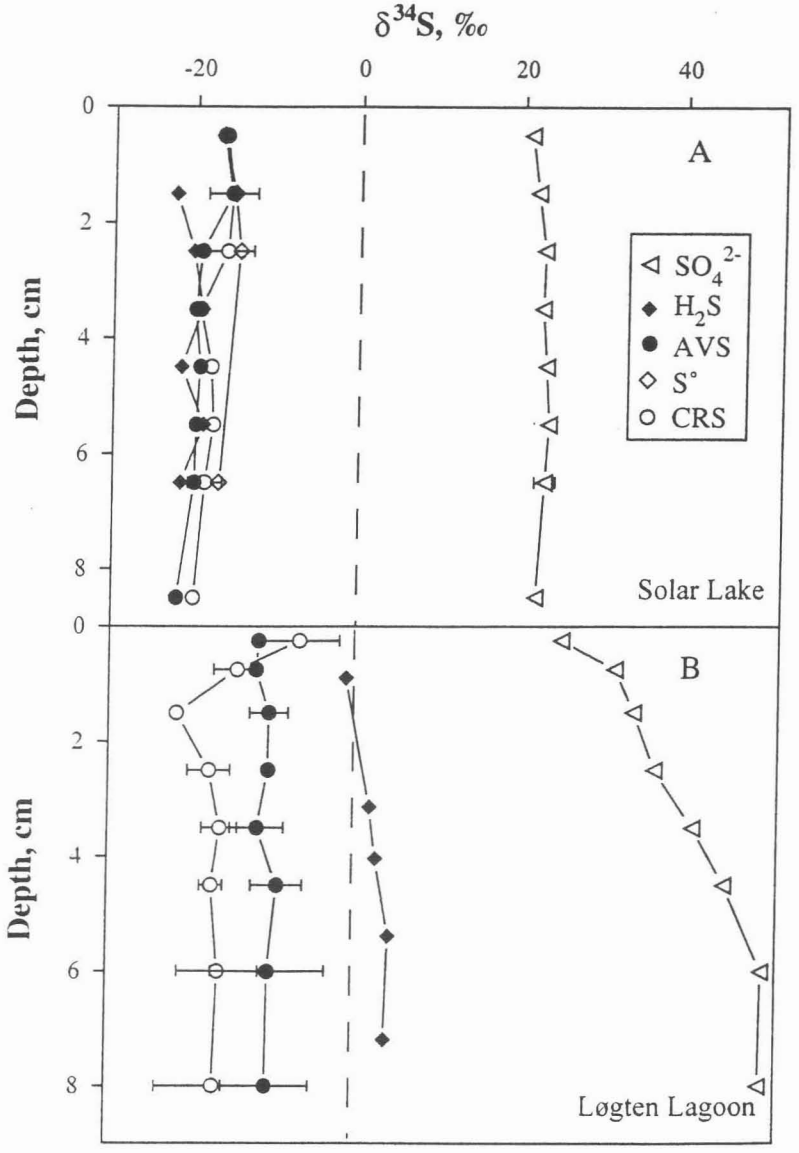


Figure 2. The stable sulfur isotopic composition ($\delta^{34}S$) of the inorganic sulfur compounds: SO_4^{2-}, H_2S, AVS (H_2S + FeS), S^0 and CRS (S^0 + FeS_2) in (A) the microbial mats of Solar Lake and (B) in the Løgten Lagoon sediments. The error bars indicate the difference between duplicate cores.

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 about -18‰ over the surface 2 cm, with no further changes with depth (Fig. 2B). These results show that the formation of, and the associated sulfur fractionation into, FeS and CRS mainly occurred in the surface 0 - 1 cm for the Solar Lake mats and from 0 - 2 cm for the sediment of Løgten Lagoon.

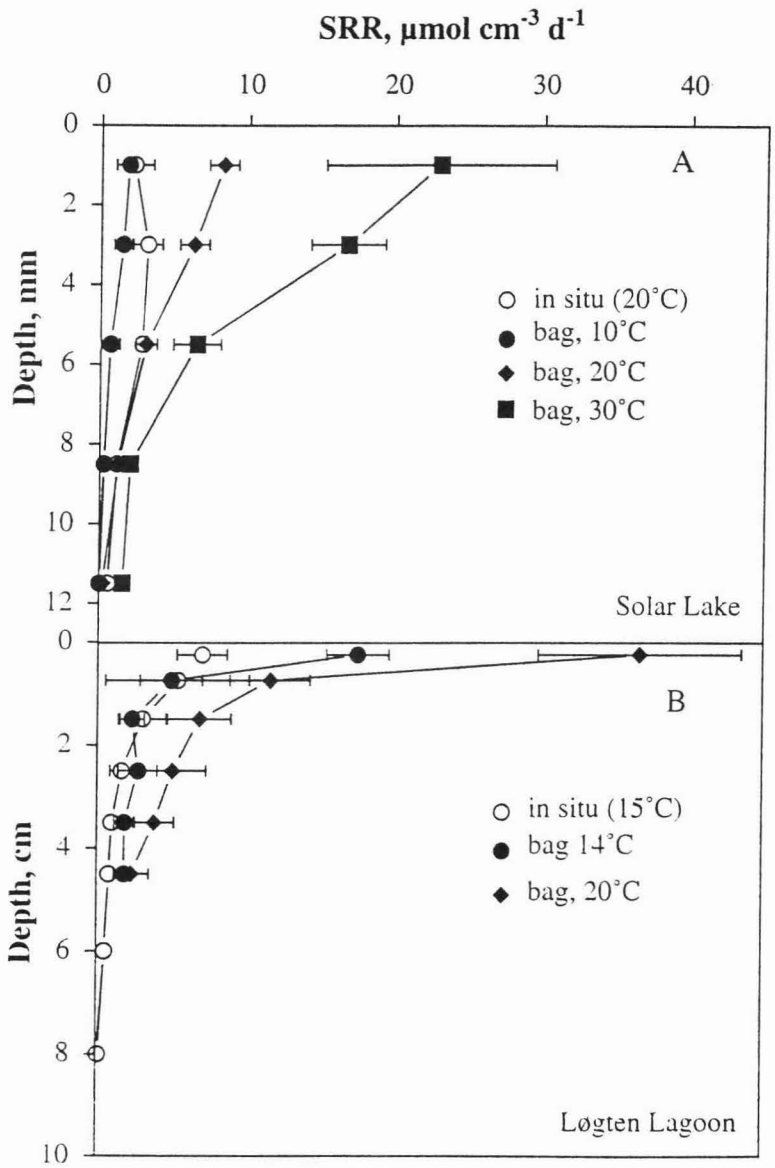


Figure 3. The rate of sulfate reduction in (A) Solar Lake and (B) Løgten Lagoon calculated from mat or sediment pieces incubated in gas-tight bags at 10°C to 30°C and from whole core incubations. See text for details. Note the different depth scales for the different sites. The error bars indicate either the standard deviation (3 - 8 samples) or the difference between duplicate measurements.

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 to 4 mm depth (Fig. 3A). In the sulfuretum of Løgten 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

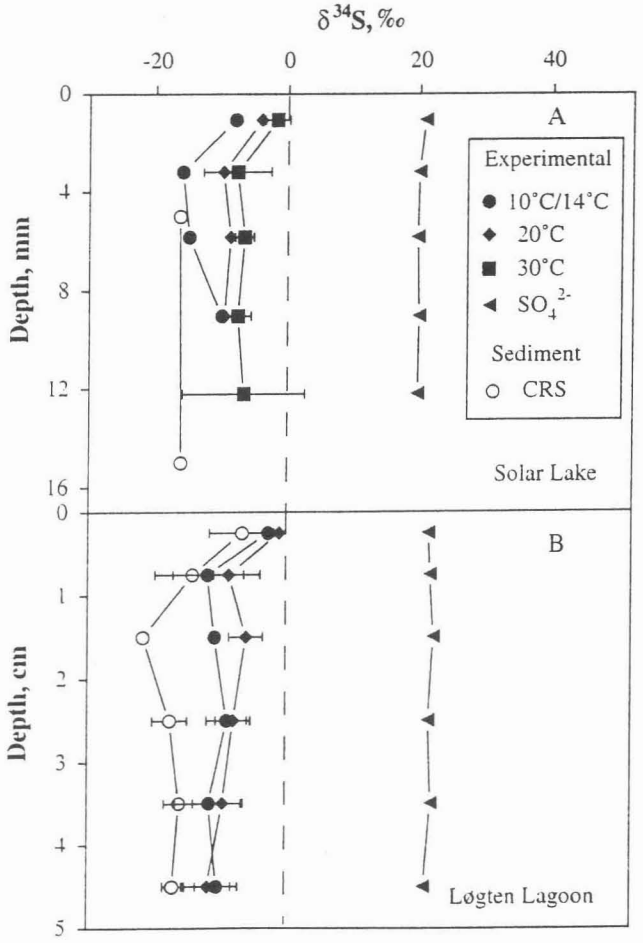


Figure 4. The fractionations during sulfate reduction by natural populations of sulfate-reducing bacteria, at different temperatures, and with sediment depth, compared to the isotopic composition of CRS. (A) Solar Lake mats incubated at 10°C, 20°C and 30°C and (B) Løgten Lagoon sediment incubated at 15°C and 20°C. Note the different depth scales for the different sites. The error bars indicate either the standard deviation (3 - 8 samples) or the difference between duplicate measurements.

bags the rates increased with time. The rate of sulfate reduction in the bags incubated at *in situ* temperatures were similar to those measured in the whole cores, except at 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 less than 1%. Sulfate concentrations of both 20 and 65 mM were used in the incubation of Solar Lake mat with no effect on rates of sulfate reduction (data not shown).

During sulfate reduction, sulfide was depleted in ^{34}S by between 16‰ to 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 or 65 mM sulfate (Fig. 4 and 5A).

In Fig. 5A we compiled all fractionation data for the two sites and show the ^{34}S depletion during sulfate reduction versus 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

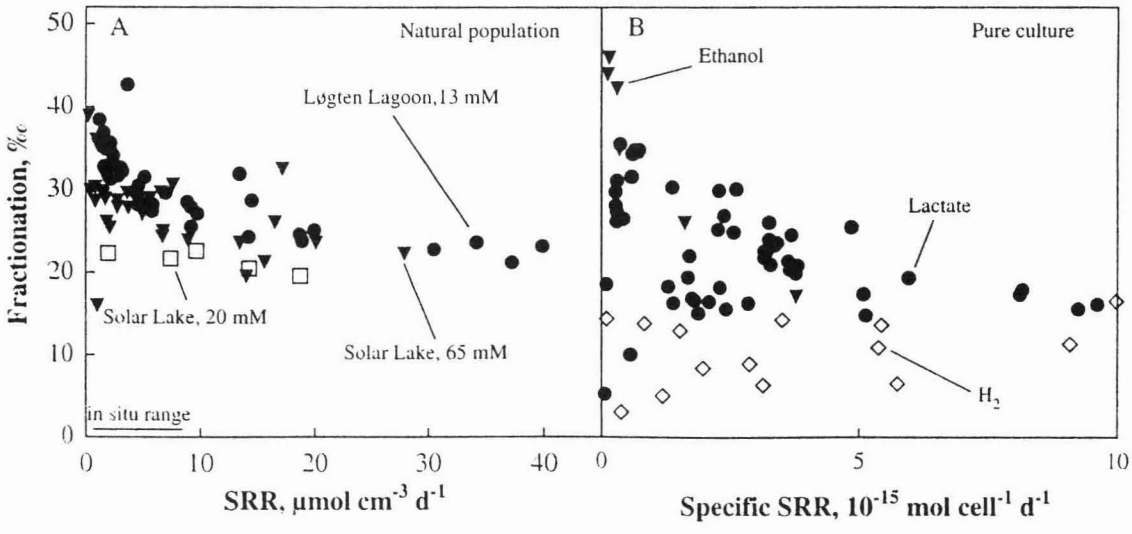


Figure 5. (A) Isotope fractionation by natural populations of sulfate-reducing bacteria measured at different rates of sulfate reduction (μmol cm^{-3}) and sulfate concentrations. (B) Isotope fractionation by pure cultures of sulfate-reducing bacteria at different specific rates of sulfate reduction (mole $cell^{-1}$ d^{-1}) and electron donors. The pure culture data are from Kaplan and Rittenberg (1964) and Chambers et al. (1975).

range of sulfate concentrations from 13 mM to 65 mM, the depletion of ^{34}S into sulfide showed little dependence on rate, with fractionations ranging from 20-25‰. These results are consistent with the observations of Habicht and Canfield (1996, Manuscript 1) on Solar Lake mat alone. Fractionation data are compared to literature results from pure culture studies in Fig. 5B.

The isotope difference between seawater sulfate and CRS, was on average 40±2‰ for Solar Lake and 37±4‰ for sediments from Løgten 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 Løgten Lagoon; Fig. 4).

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 sulfuretum at Løgten 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 Løgten Lagoon enrichments the ratio was much lower at 1.4 : 1. For both enrichment cultures the concentration of AVS decreaced and pyrit formed as previosly described for a wide variety of S^0-disproportionating cultures (Thamdrup et al., 1993; Canfield et al. in press). Also for

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

Enrichment cultures	ΔAVS - S°*) (‰)	ΔSO4^{2-} - S°*) (‰)	Production AVS/SO_4^{2-}
Solar Lake (I)	-8.9	16.7	2.1
Solar Lake (II)	-8.2	16.3	1.8
Løgten Lagoon	-7.0	14.0	1.4

*) Per mil fractionation of AVS and sulfate relative to S^0.

both enrichments sulfide was depleted in ^{34}S by 7.0‰ to 8.9‰ relative to elemental sulfur during disproportionation, while sulfate was enriched in ^{34}S by 14.0‰ to 16.7‰ (Table 3). These results are consistent with previous observations (Canfield and Thamdrup, 1994; Canfield and Thamdrup, in review).

DISCUSSION

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 sulfureta, 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 chemolithotrophic colorless sulfur bacteria. Thus, fresh organic matter produced at the sediment surface by autotrophs can be used as substrate for sulfate-reducing bacteria (Fründ and Cohen, 1992; Canfield and Des Marais, 1993). In the cyanobacterial mats of Solar Lake and in the sediment of Løgten Lagoon sulfuretum rates of sulfate reduction are 10 to 1000 times higher than maximum SRR typically measured in normal marine sediments (Jørgensen, 1982; Westrich, 1983; Skyring, 1987), although nearly comparable rates may be found in particularly organic-rich coastal sediments (Thode-Andersen and Jørgensen, 1989; Crill and Martens, 1978; Holmer and Kristensen, 1992; Thamdrup and Canfield, 1996). In both Solar Lake and Løgten Lagoon sediments rates of sulfate reduction decreased sharply with increasing sediment depth indicating that less metabolizable organic matter became available for sulfate-reducing bacteria. In the mats of Solar Lake 90% of the entire reduction of sulfate occurred within the uppermost 3 cm (Jørgensen and Cohen, 1977).

When incubated at environmental temperatues, rates of sulfate reduction in our bag-incubated sediment slices were similar to those observed *in situ*, especially at depths greater than 4 mm in the mats of Solar Lake and 1 cm in the sediments of Løgten Lagoon (Fig. 3). Higher rates of sulfate reduction for near-surface sediment incubated in the bags likely resulted from one or more of the following factors: *in situ* rates of sulfate reduction in the microbial mats of Solar Lake were measured at noon, where high rates of photosynthesis caused O_2 penetration to 2.5 mm depth (M. Kühl personal communication). An inhibition of SRR by O_2 could reduce *in situ* rates of sulfate reduction in near-surface sediment compared to rates measured in sediment incubated in bags where no O_2 was present. Rapid reoxidation of $H_2{}^{35}S$ to $^{35}SO_4^{2-}$ in the oxic zone of the sediment could also cauce an underestimate *in situ* rate of sulfate reduction, although we tried to minimize this effect with a short incubation time (30 minutes). Finally, an increase in the activity of sulfate-reducing bacteria within the bag-incubated sediment might have been spurred by organic matter liberated from the death of bacteria and algae entombed in the bags.

Isotope fractionation during bacterial sulfate reduction

The isotope 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‰ compared to 28± 3‰ and 26±3‰ at 20°C and 30°C, respectively. In the Løgten Lagoon sediments the average fractionation was 31±4‰ at 14°C and 29±4‰ at 20°C. The rate of sulfate reduction was correlated with incubation temperature (Fig. 3), and hence, a correlation was therefore also observed between rate of sulfate reduction and fractionation, particularly at low 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‰ 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 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). When H_2 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 ultilizing H_2. Thus, our stable isotope results would seem to indicate the utilization of H_2 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 culture 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 expresed 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:

$$\text{specific SRR} = \text{SRR/cell density}$$

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 rate 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 redcution are then compared to the trends from the pure culture studies (Fig. 6). We have performed this analysis separately for our Solar Lake and Løgten Lagoon results using a wide range of cell densities. For Løgten Lagoon in particular (Fig. 6B), but even for Solar Lake, our calculated trends between fractionation and specific rates of sulfate reduction converge with the results from the pure culture studies with population sizes of $< 10^{10}$ cells cm^{-3}, but $\geq 10^9$ cells cm^{-3}.

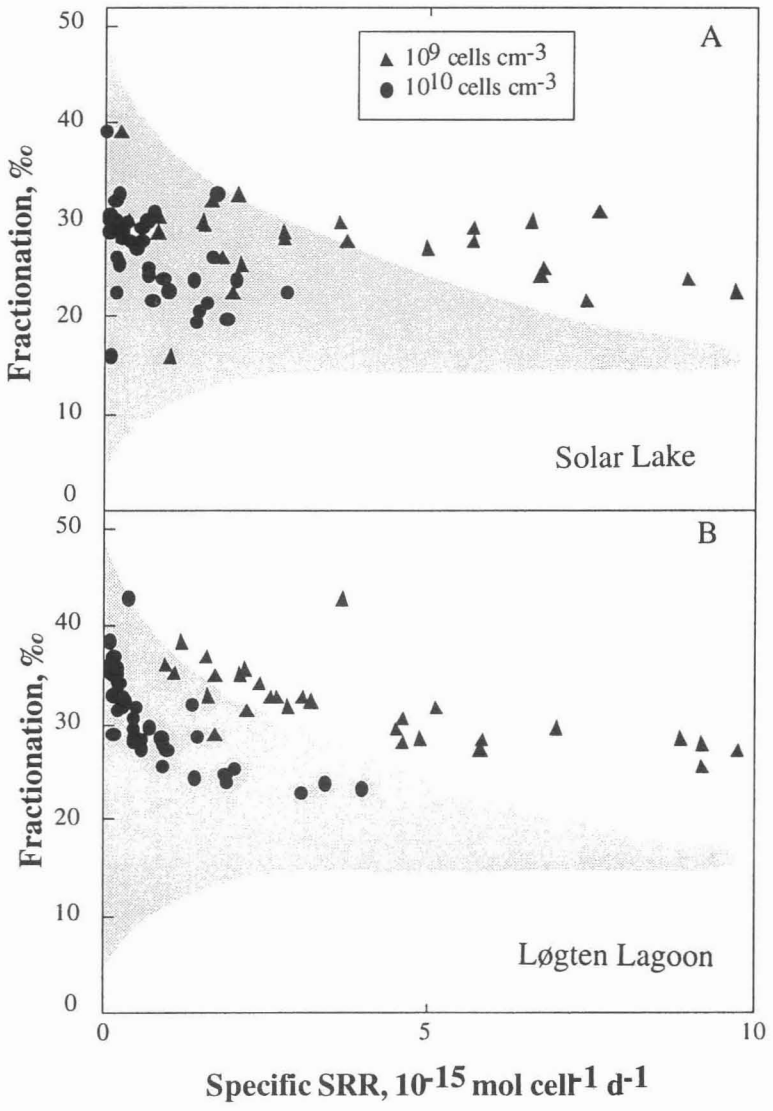


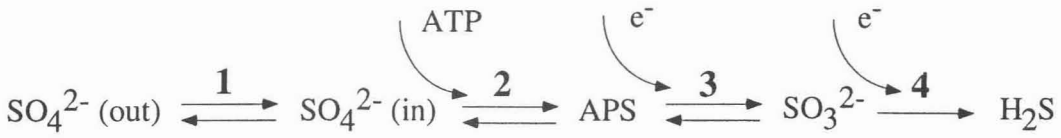
Figure 6. Isotope fractionation by natural populations of sulfate reducers in A. Solar Lake and B. Løgten Lagoon at different specific SRR (mole $cell^{-1}$ d^{-1}) predicted from assumed population densitie of 10^9 and 10^{10} cells cm^{-3}. The gray zone indicates the complete range from pure culture studies using organic electron donors. See text for details.

These population sizes are much greater than those previously measured in the mats of Solar Lake by most probable number techniques (MPN) where in the mats of Solar Lake up to 10^7 cells cm^{-3} where counted (Jørgensen and Cohen, 1977; Teske et al. Manuscript 3). Bacterial numbers estimated with the MPN technique, however, are likely 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 flourescent hybridization of bacteria collected by filtration. Furthermore, direct counts of sulfate-reducing bacteria from an organic-rich, biologically active, biofilm, yielded populations up to 2×10^9 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, 1984) and lower population sizes of sulfate-reducing bacteria. 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 4 steps, although the actual pathway

may be more complicated and might vary between bacterial species (Widdel and Hansen, 1992):



There are several steps in this scheme where fractionation is possible (Rees, 1973; Schidlowski, 1983, Thode 1991). Low or even positive fractionation values (3‰) 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‰ has been proposed for each of reactions 3 and 4 due to the splitting of S-O bounds in the reduction of APS to sulfite, and the reduction of sulfite 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 during sulfate reduction as the rate of sulfite reduction is believed to be faster than the APS reduction (Rees, 1973). A minimum fractionation of about (25‰ - 3‰ =) 22‰ is therefore expected. However, if the reduction of SO_3^{2-} to H_2S is rate-limiting, and this could be the case at low specific rates of sulfate reduction, fractionation values over 22‰ can be obtained as a sum of the individual isotope fractionations associated with Reaction 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‰) will occur. In our experiments the depletion of ^{34}S in sulfide during sulfate reduction was generally higher than about 22‰, consistent with a non-limiting 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 the Solar Lake and Løgten Lagoon nicely fits with theoretical considerations and demonstrates that fractionation is not limited by sulfate supply in 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 reemphasize that it is the specific rate of sulfate reduction and not the absolute rate that controls fractionation.

Sulfur fractionation in the sediment

In the microbial mats of Solar Lake and in the sediments of Løgten Lagoon sulfuretum the isotopic differences between seawater sulfate and CRS (mainly FeS_2) where on average 40±2‰ and 37±4‰, respectively. At *in situ* temperature we measured fractionations during sulfate reduction of 28±3‰ in the Solar Lake mats (20°C) and 31±4‰ in the sediments of Løgten Lagoon (14°C). When combined these results show that sedimentary sulfide (CRS) in Solar Lake mats were 12‰ more depleted in ^{34}S than could be accounted for by sulfate reduction alone; in Løgten Lagoon sedimnetary sulfides (CRS) were more depleted in ^{34}S by 7‰. 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 that the isotopic composition of sedimentary sulfides can record fractionations in addition to those associated with the bacterial reduction of sulfate. Additional fractionations probably results 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 both Solar Lake and Løgten Lagoon sediments in the upper 1 to 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% using the accretion rate of Jørgensen and Cohen (1977). Similar values are expected in the sediment of Løgten Lagoon sulfuretum, 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_n^{2-}, S^0, $S_2O_3^{2-}$, $S_4O_6^{2-}$ and SO_3^{2-}. Each of these compounds can then be further oxidized, reduced or disproportionated. The fractionations during chemical and bacterial oxidation of sulfide with O_2 as electron acceptor to S^0, $S_2O_3^{2-}$ or SO_4^{2-} are small (0‰ - 5‰), and also the oxidation of $S_2O_3^{2-}$ and SO_3^{2-} to sulfate is associated with small fractionations (Fry et al., 1985; Fry et al., 1986). Canfield and Thamdrup (1994) however, found rather large fractionations into both sulfide and sulfate during the bacterial disproportionation of S^0. The formation and disproportionation of other sulfur intermediates such as thiosulfate and sulfite could also provide additional fractionations leading to depletion of sulfide in ^{34}S, and these isotope systematics are currently under investigation. A repeated sulfide oxidation to S^0 and maybe other sulfur intermediates, followed by disproportionation can generate sulfide more depleted in ^{34}S than the original sulfide produced from sulfate reduction (Canfield and Thamdrup, 1994).

We observed significant isotope fractionations during disproportionation of elemental sulfur by bacterial cultures enrichted 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., in press). Furthermore, rather large concentrations of S^0 were measured in the mats of Solar Lake, varying between 2 - 30 μmol cm^{-3} in the surface 0 - 6 mm (Fig. 1). Thus, the process of elemental sulfur disproportionation likely contributes to the large ^{34}S depletions into sedimentary sulfides from Solar Lake and Løgten Lagoon.

Diel measurements showed that this 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 O_2 accumulates in the mat surface to concentrations up to 500% air saturation due to high rates photosynthesis (7 - 10 nmol O_2 cm^{-3} s^{-1}; M. Kühl personal communication). Oxygen can react with sulfide, and form S^0. Also, during anoxygenic photosynthesis by green and purple sulfur bacteria, and during chemoautotrophic oxidation of sulfide by colorless sulfur bacteria (*Beggiatoa sp.*), S^0 is produced which may accumulate as granules in the cell or be excreted to the surrounding sediment. At night we observed that S^0 was consumed which may be accounted for by either oxidation, reduction or disproportionation processes, although we cannot be certain about which processes dominate. We measured a curious accumulation of S^0 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 ^{34}S 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 ^{34}S. 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 ^{34}S-depletion could result from a relatively larger importance of direct sulfide oxidation to sulfate by anoxygenic photosynthetic bacteria or by O_2, and the possible involvement of anoxygenic phototrophs in elemental sulfur oxidation to sulfate. Also S^0-disproportionation may be inhibited to some extend by the high sulfide concentrations (Thamdrup et al. 1993) that are found in some benthic photosynthetic systems. It is unfortunate that our measurements of the diel cycling of elemental sulfur places few constraints on the pathways of its formation and turnover; quantification of these processes awaits direct determination when such techniques become available.

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 SO_4^{2-} nor did they apparently use H_2 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.

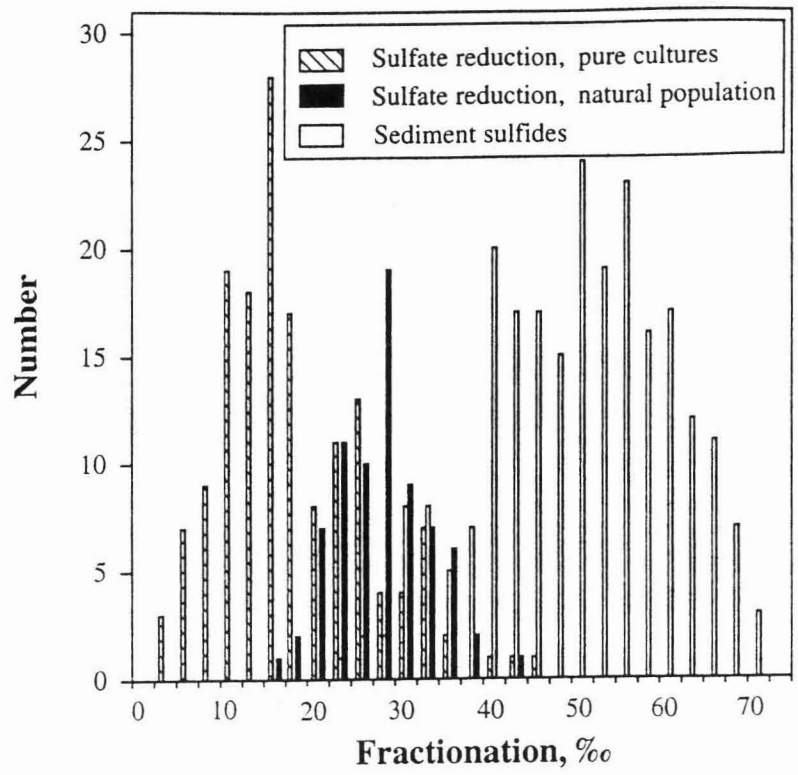


Figure 7. Comparisons of the fractionation during sulfate reduction by pure cultures and natural populations of sulfate-reducing bacteria, and the fractionation between sedimentary sulfides (mainly as FeS_2) and seawater sulfate. Sediment and pure culture data are from the literature, and natural population results from this study.

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 pervious 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 per mil, as we observed in these very active sediments, will also be encounted 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 (FeS_2) 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 disproportionating could explain the additional depletion of ^{34}S into sedimentary sulfides, and elemental sulfur-disproportionating bacteria where isolated from both the two investigated stations.

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3

Sulfate-reducing bacteria in diurnally oxic surface layers of benthic cyanobacterial mats from Solar Lake, Sinai

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Contents Page

ABSTRACT

The sulfate-reducing bacterial populations within the surface layer of the hypersaline cyanobacterial mat of Solar Lake (Sinai) were investigated with molecular and culture-dependent approaches in parallel. Cultivable sulfate-reducing populations were quantified with Most-Probable-Number counts on lactate and acetate. The upper 2 mm mat layer was oxygenated by cyanobacterial photosynthesis at daytime, but showed high sulfate-reducing activity and contained approx. 4.5 x 10^6 sulfate-reducing bacteria per ml. Incompletely and completely oxidizing sulfate reducers were found in comparable cell densities throughout the upper 13 mm mat, with slightly higher numbers of incompletely oxidizing sulfate-reducing bacteria. The filamentous sulfate reducer *Desulfonema* constituted at least 10% of the overall acetate-oxidizing sulfate-reducing population, and was found in high dilution cultures of the 0-2 and the 2-4 mm mat layers, even though filamentous bacteria are known to be underrepresented in dilution culture approaches. Sulfate reduction rates measured in parallel to the MPN sampling indicated that the MPN counts had underestimated the sulfate-reducing bacterial population by at least a factor ten. With PCR and denaturing gradient gel electrophoresis a diurnally migrating bacterial population was detected in the diurnally oxygenated mat surface layer, which was phylogenetically related to acetate-oxidizing sulfate reducing bacteria. Completely and incompletely oxidizing sulfate-reducing bacterial populations occurring within and close to the oxic surface layer sustain high photosynthetic productivity of these CO_2-limited mats by internal CO_2 recycling.

INTRODUCTION

Sulfate-reducing bacteria are almost universally distributed in marine sediments and microbial mats. Sulfate reduction is the dominant anaerobic biomineralization pathway in marine sediments, quantitatively equivalent to or exceeding aerobic respiration (Jørgensen 1982). The vertical extent of the sulfate reduction zone is inversely related to population density and activity of sulfate-reducing bacteria therein: In organic carbon-depleted marine sediments of the deep-sea abyssal plain, the zone of sulfate reduction extends over a scale of many meters, but involves only low numbers of sulfate-reducing bacteria and low activities (Ivanov et al. 1980, Canfield 1991). With increasing supply of organic nutrients, a higher primary production and accelerated sedimentation rates, population density and activity of sulfate-reducing bacteria increase. Since exogeneous energy-rich substrates are rapidly consumed before reaching deeper sediment layers, density and activity of sulfate-reducing bacteria generally increase towards the sediment surface (Jørgensen 1982), and are maintained even in the presence of oxygen (Jørgensen 1977). In marine sediments of the Kattegat (Denmark) dense sulfate-reducing populations and high sulfate reduction activity were found in oxic sediment layers close to the sediment surface (Jørgensen and Bak 1991). In sediments of an oligotrophic freshwater lake, Stechlinsee (Germany), conspicuous population and activity maxima of sulfate-reducing bacteria occurred at the oxic-anoxic interface (Sass et al. 1996, 1997).

Hypersaline cyanobacterial mats provide the most extreme example of sulfate reduction coexisting with oxic conditions. Here, photosynthetic oxygen synthesis and sulfide production from sulfate reduction overlap and create steep, diurnally shifting gradients of oxygen and sulfide. The surface mat layers are exposed to oxygen supersaturation during daylight, but simultaneously show very high cell numbers of sulfate-reducing bacteria, in the range of 10^6 - 10^8 cells cm^{-3}, and extremely high sulfate-reduction rates, in the range of 500 - 15.000 nmol SO_4^{2-} cm^{-3} d^{-1} (Jørgensen and Cohen 1977; Skyring 1987; Canfield and DesMarais 1991, 1993; Fründ and

Cohen 1992; Visscher et al. 1992; Caumette et al. 1994). Sulfate reduction rates measured under oxic conditions during daytime often exceed those observed at night under anoxia. Part of this phenomenon may be explained by elevated temperatures during the day, as sulfate reduction rates generally show an Arrhenius-like temperature dependence, with habitat- and substrate-related differences in appararent activation energies (Westrich and Berner 1988, Jørgensen 1994). Ultimately, the high sulfate reduction rates observed in hypersaline cyanobacterial mats are driven by autochthonous photosynthetic production of cyanobacteria, and do not depend on organic matter from outside sources (Cohen 1984, Cohen et al. 1994).

The benthic cyanobacterial mats of Solar Lake, a shallow, hypersaline meromiktic desert lake in the Sinai (Egypt), provide a well-documented example of this type of oxygen-tolerant, light-driven sulfate reduction (Jørgensen and Cohen 1977, Jørgensen 1983, Cohen et al. 1994). However, little is known about composition, specific populations and oxygen adaptations of sulfate-reducing bacteria in such mats. In this study, we investigated the sulfate-reducing bacteria of Solar Lake cyanobacterial mats with microbiological, molecular and geochemical methods, with focus on the oxygen-exposed mat surface layer.

MATERIALS AND METHODS

Sampling of cyanobacterial mat for Most-Probable-Number counts and sulfate reduction rates. Numbers of sulfate reducing bacteria in the Solar Lake cyanobacterial mat were determined by Most-Probable-Number counts in November 1994, using samples from a single piece (approx. 10 x 10 cm) of laminated, undisturbed mat of the shallow flat mat type (approx. 0.5 m depth) from the eastern bank of Solar Lake, Sinai (Jørgensen et al. 1983). Mat cores were sliced into the following layers: 0 - 2 mm, 2 - 4 mm, 4 - 7 mm, 7 - 10 mm, 10 - 13 mm. An oxygen penetration depth of 2.2 mm was measured with Clark-type microelectrodes when

illuminated by in situ light intensities of 1000 µEinstein m^{-2} s^{-1} (Ramsing et al. 1993). The 0 - 2 mm surface layer was oxygenated at daytime, showing maximal oxygen concentrations equivalent to 500 - 600 % air saturation which is 158 µmol O_2 at 22°C and 10% salinity at 0.5 mm depth, decreasing to approx. air saturation at 1.5 mm and 10 - 30 % air saturation at 2 mm depth (Michael Kühl, personal communication). Similar results were obtained when illuminated with 500 µEinstein m^{-2} s^{-1}. The 2 - 4 mm layer includes the chemocline during daytime, with traces of oxygen, whereas the deeper layers remain fully anoxic at all times. Mat inoculum for MPNs was harvested at noon and at midnight, to record oxygen supersaturation at daytime and anoxia at night. The same mat material was used for parallel sulfate reduction rate measurements. Rates were measured in situ with the ^{35}S-sulfate radiotracer method (Jørgensen 1994) and in bag incubations (Habicht and Canfield 1996, Manuscript 1).

Most-Probable-Number counts of sulfate reducing bacteria. Numbers of viable sulfate-reducing bacteria were estimated with most-probable-number (MPN) dilution series of the inoculum in liquid medium (American Public Health Association 1969). Media were made with artificial seawater, with the following constituents per liter of distilled water: 40 g NaCl, 5.67 g $MgCl_2$ * 6 H_2O, 6.8 g $MgSO_4$ * 7 H_2O, 1.47 g $CaCl_2$ * 2 H_2O, 0.19 g $NaHCO_3$, 0.66 g KCl, 0.09 g KBr. Compared to normal artificial seawater, which contains 26.37 g NaCl per liter, the NaCl concentration was increased to 40 g/l, to approximate the winter chlorinity and salinity of Solar Lake surface water, approx. 4 and 7 %, respectively (Cohen et al. 1977). Multipurpose medium for sulfate-reducing bacteria contained per liter artificial sea water: 1 ml non-chelated trace element mixture No. 1, 1 ml selenite-tungstate solution, 30 ml 1 M $NaHCO_3$ solution, 1 ml vitamin solution, 1 ml thiamine solution, 1 ml Vitamin B_{12} solution, and 7.5 ml Na_2S solution (Widdel and Bak 1991). The salt concentrations of the modified sea water medium used in this study are higher than those of many standard brackish or marine sulfate reducer media, and enabled the growth of rarely isolated sulfate reducers, such as *Desulfonema*, which require elevated concentrations of Ca^{2+} and Mg^{2+} (Widdel and Bak 1991).

MPN counts were performed on 20 mM lactate, 20 mM acetate, and 10 mM formate, the latter plus 2 mM acetate to account for non-autotrophic bacteria. Media were prepared anaerobically in a pressure-proof modified Erlenmeyer flask (Widdel and Bak 1991). After dispensing the medium into glass culture tubes, each tube was immediately gassed with a mixture of 90% (vol/vol) N_2 and 10% (vol/vol) CO_2 , using a gassing syringe according to the Hungate technique, and sealed with a butyl stopper. Sulfate reducers were counted by triplicate MPN dilution series from the mat layers 0 - 2 mm, 2 - 4 mm, 4 - 7 mm, 7 - 10 mm and 10 - 13 mm. Mat samples were obtained with plactic cores (2 cm diameter) and pushed out for slicing with a millimeter-graded piston. The rubber-like mat slices were homogenized manually in 9 volumes Solar Lake water for approx. 20 minutes, using a 10-ml potter homogenizer with a teflon-coated piston. Homogenisation was checked by microscopy. MPN dilution series were initially inoculated with 1 ml tenfold diluted mat homogenate, equivalent to 0.1 ml mat material, and subsequently diluted in eight 1:10 dilution steps. MPN counts of Solar Lake mat were made at noon and at midnight. Purification of sulfate-reducing bacteria from high MPN dilutions was carried out by agar dilution as described previously (Teske at al. 1996a)

Sampling of cyanobacterial mat for nucleic acid extraction and total cell counts. Solar Lake mat cores were taken in May 1994 from samples of undisturbed shallow mat (approx. 0.5 m depth) from the eastern bank of Solar Lake. Three sample sets were taken at different times, to record the changing day-night regimes of the mat: At 5°° am, during night anoxia of the surface layer, and at 12°° noon and 5°° pm, during daytime oxygenation of the surface layer. The surface centimeter of each mat core was cut in millimeter slices, using an accucate cutting device. The slices were used for nucleic acid extraction and subsequent PCR and DGGE. The upper 5 mm of the 5°° am mat and the upper cm of the 5°° pm mat were sampled in duplicates. Mat material for total cell counts with DAPI was harvested and fixed in formaldehyde as described (Ramsing et al. 1993).

Nucleic acid extraction. Nucleic acids were extracted from Solar Lake mat by hot phenol extraction. 0.2 cm^3 of each mat slice were homogenized and mixed with the same volume ice-cold AE buffer (20 mM Na-Acetate, 1 mM EDTA, pH 5.5), and kept on ice. To each sample, 500 µl phenol-chloroform-isoamylalcohol (25:24:1 vol/vol/vol; pH 5) and 5 µl 25% SDS were added. Phenolic and aequeous phases were mixed by approx. 1 minute vortexing. After 5 min of incubation at 60°C in a waterbath the samples were cooled on ice and then centrifuged for 5 min at 4000 g. The aequous phases were transferred to new vials containing 25 µl of 2 M Na-acetate, pH 5.2. Contaminating proteins and lipids were removed by subsequent twofold extraction of the aequous phase with 500 µl phenol-chloroform-isoamylalcohol. Nucleic acids were finally precipitated with 2.5 vol 96% (vol/vol) ethanol overnight at -70°C, followed by 10 min centrifugation at 4000g. The resulting white pellets were overlaid with 100 µl fresh ethanol and stored at -70°C, with a 12 h interval at -20 to 0°C during the return trip to the laboratory in Bremen. All chemicals and buffers used for the isolation of nucleic acids from Solar Lake mat, except the Phenol-Chloroform-isoamylalcohol mixture, were treated with diethyl pyrocarbonate (DEPC) to remove DNAse and RNAse activity (Sambrook et al. 1989).

PCR amplification of rDNA fragments. The 16S rRNA genes from mixed bacterial DNA or cDNA were amplified by the polymerase chain reaction. Two different primer combinations were used: GM5 (with GC-clamp) and DS907R amplified a 550 basepair fragment of the 16S rRNA gene, and 385 (with GC-clamp) and DS907R amplified a 520 base pair fragment of the 16S rRNA (Table 1). Both fragments are suitable for subsequent DGGE analysis, membrane hybridization, sequencing, and identification of the phylotype (Muyzer et al. 1995). The primer sequences, their location on the 16S rRNA gene, and the PCR conditions have been described by Muyzer et al. (1993, 1995), including a hot start and a touch-down annealing reaction to increase the specificity of the amplification and to reduce the formation of spurious by-products (Don et al. 1991). Approx. 50 ng of DNA were used as PCR template.

Table 1. Primers and probes used in this study.

PRIMER	POSITION		SEQUENCE
GM5F GC-clamp*	341 - 357	5'- GC-clamp-' CCTACGGGAGGCAGCAG -3	general eubacterial primer for 16S rRNA gene DGGE-fragment amplification (Muyzer et al. 1995)
385 GC-clamp*	385-402	5'- GC-clamp- CCTGACGCAGC(G/A)ACGCCG -3'	selective, but not specific: mostly delta-subdivision proteobacteria, incl. sulfate reducers (Amann et al. 1992, Ramsing et al. 1996)
DS907-reverse	924 - 907	5'- GTCAATTCCTTTGAGTTT -3'	general eubacterial primer for 16S rRNA gene DGGE-fragment amplification (Muyzer et al. 1995)
657	657 - 676	5'- TTCCG(C/T)TTCCCTCTCCCATA -3'	rRNA oligonucleotide probe for the genus*Desulfonema* (Fukui et al. 1997)

*GC-clamp: 5´- CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG - 3´ (Teske et al. 1996b)

DGGE analysis, blotting and hybridization analysis of DGGE gels. PCR products obtained with both primer combinations were analysed by Denaturing Gradient Gel Electrophoresis (DGGE). DGGE was performed with a BIO-RAD Protean II system, using a denaturant gradient of 30-70 %, with 100% corresponding to 7 M urea and 40% [vol/vol] formamide, as described previously (Muyzer et al. 1993, 1995). Electrophoresis was continued for eight hours at a constant voltage of 100 Volt and a temperature of 60°C. After electrophoresis the gels were stained in aequous ethidium bromide solution (0.5 mg/l), and photographed on a UV (302 nm) transillumination table with a Cybertech CS1 digital camera (Cybertech, Berlin, Germany). Small pieces of selected DGGE bands were excised from the DGGE gel, eluted, and re-amplified using the same primers, but without GC-clamp. Electroblotting of the DGGE pattern to a nylon membrane (Hybon-N+: Amersham, Amersham, UK), using a Trans-Blot SD semi Dry Transfer Cell (BIO-RAD Laboratories, Inc.), followed by UV-crosslinking of the DNA to the membrane, was performed as described (Muyzer et al. 1993, 1995).

The DGGE blot was evaluated by hybridization with rRNA-targeted probes (Table 1). The 16S rRNA region amplified by primers GM5F and DS907R, or 385GC

and DS907R, respectively, includes several target sites for general, genus- and species-specific oligonucleotide probes for sulfate-reducing bacteria (Devereux et al. 1992, Fukui et al. 1997). Probes were labeled with digoxigenin, using the digoxigenin oligonucleotide labeling kit and protocols from Boehringer Mannheim, Germany, or were obtained ready-made with 5'-Digoxigenin-label from Biometra, Göttingen. Digoxigenin-labeled probes were detected by an antibody coupled with alkaline phosphatase, which gives a chemiluminescent reaction with CSPD (Serva Tropix). Hybridization was performed as described by Muyzer et al. (1995). Probe 657 was hybridized and washed at 47°C (Fukui et al. 1997).

Phylogenetic identification. 16S rRNA sequences were aligned to those of other bacteria obtained from the Ribosomal Database Project (RDP) (Maidak et al. 1996). The SIMILARITY_RANK tool of the RDP was used to search for close evolutionary relatives. Sequence alignments were prepared with the sequence alignment editor SeqPup (Gilbert 1995). Distance matrixes were calculated with DNADIST implemented in the software package PHYLIP Version 3.5 (Felsenstein 1993, and references therein), using the Jukes-Cantor model, which assumes independent change at all sites with equal probability. Phylogenetic trees were calculated from evolutionary distances with the algorithm of Fitch and Margoliash as implemented in the program FITCH in PHYLIP 3.5. Bootstrap testing of the branching pattern was performed in 100 reruns with the bootstrap program included in PHYLIP 3.5.

RESULTS

Most Probable Number counts. Counts of sulfate reducing bacteria with lactate remain in the range of 10^6 cells/ml in all mat layers, with a possible slight increase in the upper two layers towards 10^6 - 10^7 cells/ml. In the oxic surface layer at noon we found 4.5 x 10^6 cells per ml. The numbers of lactate-utilizing sulfate-reducing

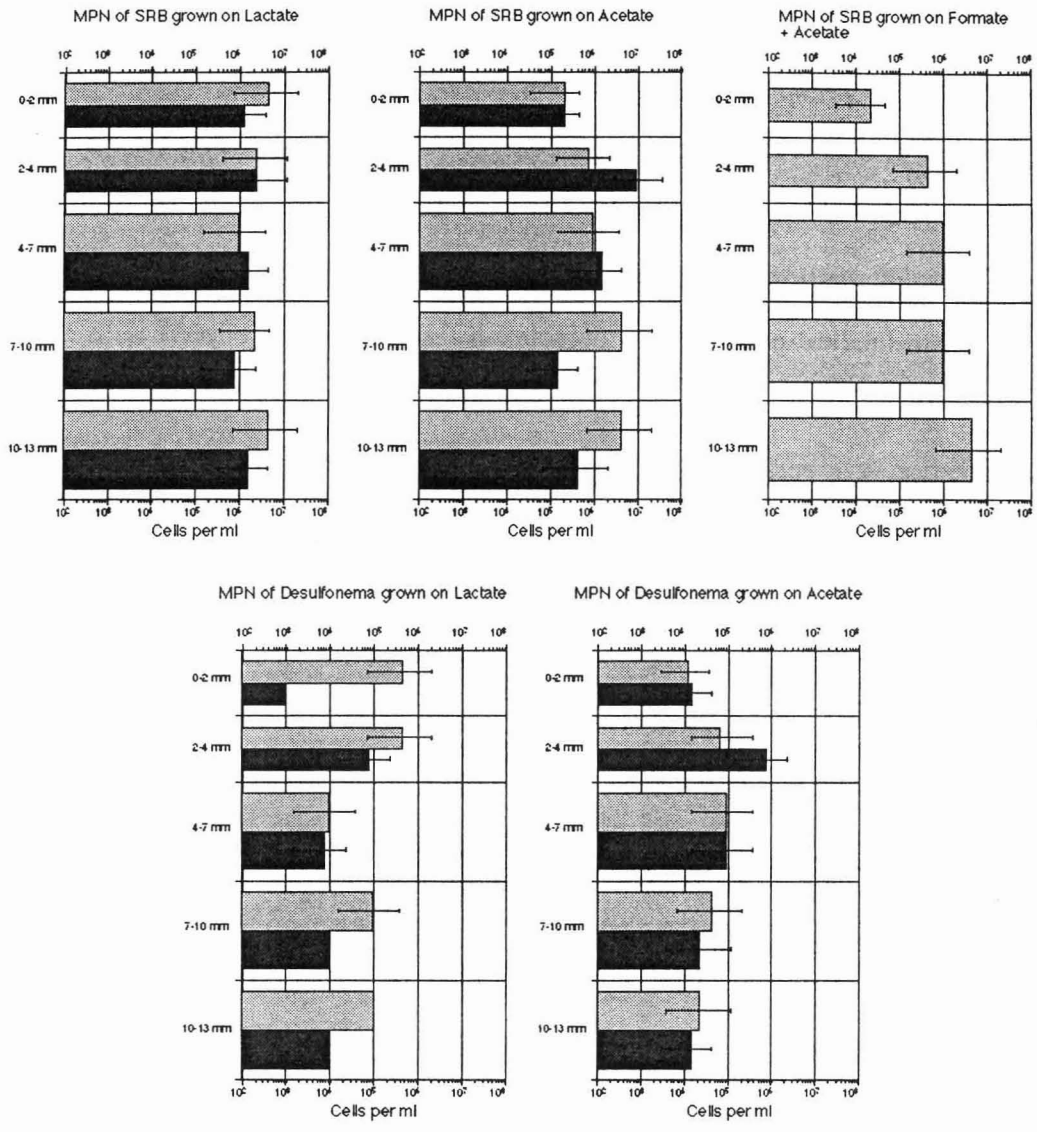

Figure 1. MPN counts of sulfate-reducing bacteria from Solar Lake cyanobacterial mat (Nov. 1994). Cell densities per ml in the mat layers 0 - 2 mm, 2 - 4 mm, 4 - 7 mm, 7 - 10 mm, 10 - 13 mm, are plotted on a logarithmical scale. Dark bars correspond to MPN counts of samples taken at midnight, bright bars correspond to MPN counts at noon. 95% confidence intervals of the MPN counts are indicated. (A) 20 mM lactate. (B) 20 mM acetate. (C) 10 mM formate plus 2 mM acetate. (D) *Desulfonema*, 20 mM lactate. (E) *Desulfonema*, 20 mM acetate.

bacteria in the diurnally oxic surface layer are thus not significantly different from those of adjacent permanently anoxic layers. The noon and midnight counts are similar, and their 95% confidence intervals overlap for all mat layers (Fig. 1A).

Acetate counts indicate approx. 10^6 cells/ml in most layers, although the estimates are altogether less homogeneous. Day and night acetate counts indicate approx. 2 x 10^5 cells/ml in the 0 - 2 mm surface layer (Fig. 1B). This is a conservative

estimate: In the day as well as in the night MPN counts, two of three dilution series reach 10^7 cells/ml, but gaps and possibly premature terminations in the third dilution lead to lower numbers. Day and night acetate counts show different patterns: The day counts indicate a gradual increase of acetate-oxidizing sulfate reducers in deeper layers, towards 10^6 - 10^7 cells/ml at 7-10 and 10-13 mm depth. The same pattern occurs in the daytime formate counts, potentially since the medium was supplemented with 2 mM acetate for non-autotrophic strains (Fig. 1C). The night acetate counts show a pronounced maximum, approx. 10^7 cells per ml, in the 2 - 4 mm layer (Fig.1B), and unusually low numbers, approx. 10^5 cells/ml, in the 7 - 10 mm layer.

Specific populations of sulfate-reducing bacteria: *Desulfovibrio*. Sulfate-reducing bacteria of the genus *Desulfovibrio* have previously been isolated from the surface layer of Solar Lake Cyanobacterial mats. Four oxygen-respiring strains have thus been obtained previously from 10^4, 10^5 and 10^6 dilutions of the upper 3 mm surface layer of a Solar Lake cyanobacterial mat (Krekeler et al. 1997b). Two of them, from 10^5 and 10^6 dilutions, were identified as the oxygen-respiring species *Desulfovibrio oxyclini*. This species was originally isolated from cyanobacterial mats of an artificial hypersaline pond which had developed from a Solar Lake mat inoculum (Krekeler et al. 1997a). From the MPN series of this study, the 16S rRNA genes of several agar-shake isolates, obtained with lactate, were PCR-amplified and partially sequenced, to search for related *Desulfovibrio* strains. A *Desulfovibrio* isolate from a 10^6 dilution was moderately related to *Desulfovibrio oxyclini*. A second isolate from another 10^6 dilution was a *Desulfovibrio*, but unrelated to the former species. Two *Desulfovibrio* isolates from 10^4 dilutions were also closely related to *Desulfovibrio oxyclini*. Therefore, *Desulfovibrio oxyclini* and closely related strains are significant - although not the only - members of the sulfate reducing community in the Solar Lake mat surface layer.

Desulfonema. After six months of storage in a dark cabinet at room temperature, filamentous bacteria had developed in many MPN dilution cultures, and covered the glass walls of the culture vials. The filaments were identified as the

filamentous sulfate reducer *Desulfonema*, a genus of nutritionally versatile, acetate-utilizing sulfate reducing bacteria previously isolated from marine habitats (Widdel et al. 1983). *Desulfonema* developed a consistent growth pattern in almost all higher dilutions of the acetate MPN, allowing an MPN estimate specifically for *Desulfonema* (Fig. 1D,E). The *Desulfonema* MPN mirrors to some extent the acetate MPN with one order of magnitude difference: Approx. 10^4 cells/ml are found at the 0-2 mm surface layer, a maximum of 10^5 cells/ml occurs at 2-4 mm and 4-7 mm, and numbers decrease gradually towards deeper layers, with 2-3 x 10^4 cells/ml at the 10-13 mm layer. The growth pattern of *Desulfonema* in the lactate MPNs is obscured by frequent blank samples without *Desulfonema*. In such cases, the bars in Fig. 1D represent dilutions with *Desulfonema* growth as they were found; i.e., a 10^4 bar without 95% confidence interval stands for a single 10^4 MPN dilution with *Desulfonema* growth. Remarkable are the high *Desulfonema* counts in the daytime lactate MPNs of the 0-2 and 2-4 mm layers, between 10^5 and 10^6 cells/ml. Since *Desulfonema* species use a wide spectrum of substrates in addition to acetate, partially spent lactate medium could have led to higher numbers. However, in many lower dilutions of the lactate MPN, *Desulfonema* has probably been overgrown and outcompeted by other, more rapidly growing sulfate reducers. The actual population density of *Desulfonema* is also likely to have been underestimated by the method employed since this sulfate reducer does not grow as single, easily dispersible cells, but in filaments of approx. 100 to 400 cells (Widdel 1980). An upper limit of the *Desulfonema* population density may be tentatively inferred from the highest positive dilutions where *Desulfonema* was observed. In the upper two mat layers, above 4 mm depth, *Desulfonema* was found twice in 10^7 dilutions (acetate, 2-4 mm, a noon and a midnight sample) and seven times in 10^6 dilutions (acetate, 0-2 mm and 2-4 mm, in both layers a noon and a midnight sample; the same for lactate, except the 0-2 mm night sample). In deeper mat layers below 4 mm, *Desulfonema* was never found in higher than 10^5 dilutions. *Desulfonema* from Solar Lake mats resemble *Desulfonema limicola* morphologically, and have similar filament diameters of 2 - 2.5 µm (Fig. 2).

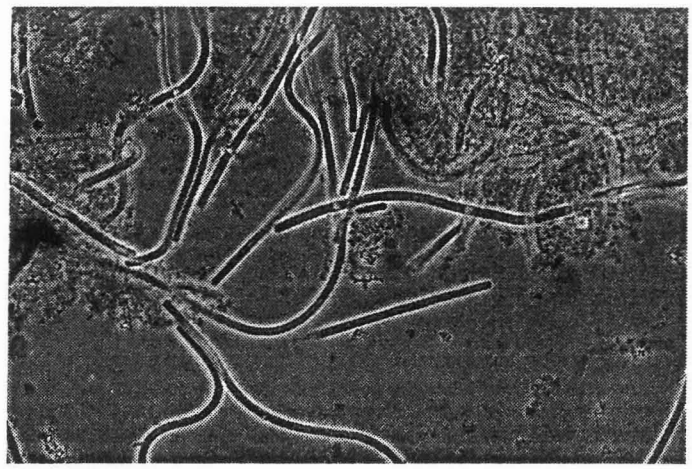

Figure 2. *Desulfonema* filaments from the 0 - 2 mm oxic surface layer of Solar Lake cyanobacterial mat, from a mat sample harvested at noon. The *Desulfonema* enrichment was growing in a 10^6 MPN dilution with lactate.

Oxygen profiles and total cell counts. Total counts of DAPI stained cells in Solar Lake mat cryosections indicated approx. 0.3 - 1.0 x 10^{11} cells/ml in all layers, independent of shifting oxygen gradients over a diurnal course (Figure 3). The oxygen concentration profile was measured with a Clark-type oxygen μ-sensor (Ramsing et al. 1993). At night, atmospheric oxygen diffuses only 0.2 millimeter into the mat, where it is rapidly consumed. At daytime, oxygenic photosynthesis of the cyanobacteria creates oxygen-supersaturated conditions, with oxygen concentrations reaching 200 - 400 μmol. Oxygen penetration reaches 1.5 mm at noon, and 2 mm at 5 pm. To monitor oxygen-related population changes in appropriate resolution, the mat was sliced in millimeter intervals, for subsequent nucleic acid extraction, PCR and DGGE. Cultivable sulfate-reducing bacteria, estimated by MPN as 10^6 - 10^7 cells/ml, represent only a small fraction of the total bacterial biomass. The molecular approach to monitor sulfate-reducing bacterial populations was therefore modified by using DGGE primers selective for sulfate-reducing bacteria (Amann et al. 1992).

Denaturing gradient gel electrophoresis and membrane hybridization. PCR-amplified 16S rDNA fragments of bacterial DNA, extracted from each millimeter layer of the upper ten millimeters of the Solar Lake cyanobacterial mat at three times during a diurnal cycle, were analyzed by Denaturing Gradient Gel Electrophoresis (DGGE). Two primer combinations were tested, primers GM5 (with

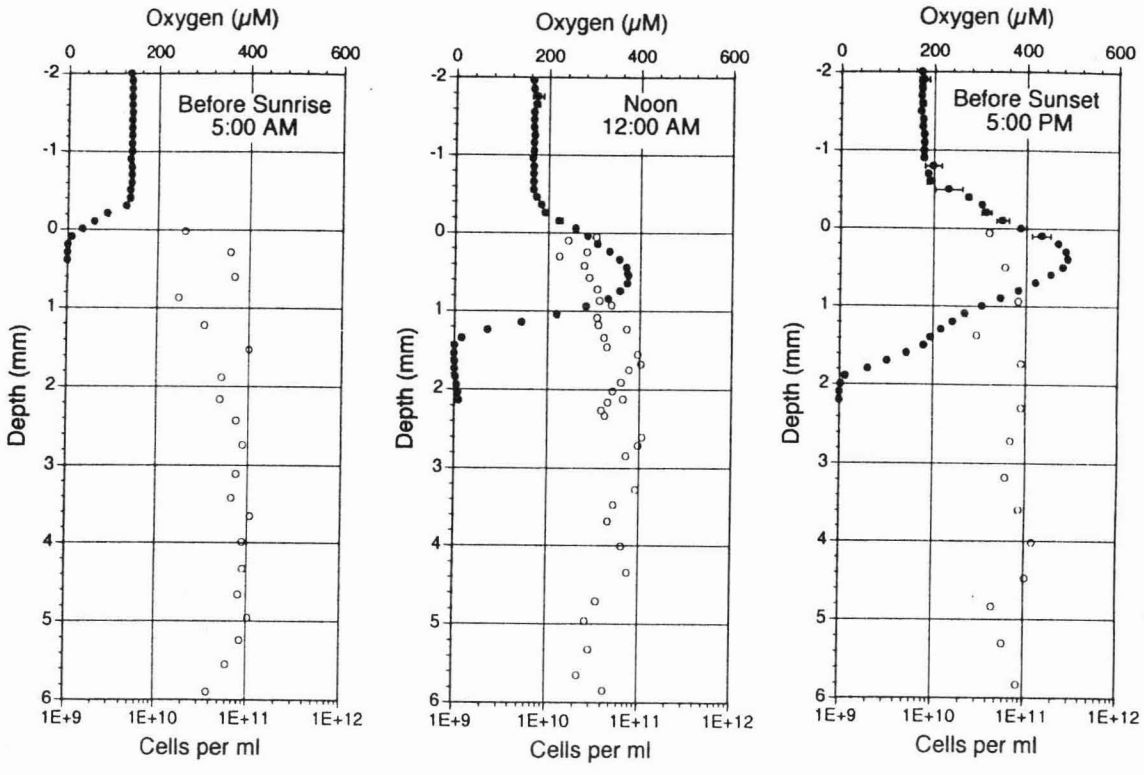

Figure 3. Total bacterial counts and shifting oxygen gradients in Solar Lake bacterial mats at different time points during a diurnal cycle (May 1994).

GC-clamp) and DS907, and 385 (with GC-clamp) and DS907 (Table 1). The former primer combination, of two general primers complementary to highly conserved 16S rRNA gene sequences, resulted in a DGGE pattern which gave consistently negative results in hybridizations with probes for sulfate-reducing bacteria (results not shown). The most conspicuous DGGE band in this pattern was excised, sequenced and identified as a *Marinobacter* sequence, a genus of facultatively anaerobic, fermentative, heterotrophic bacteria (results not shown). Since sulfate-reducing bacteria constitute only a small fraction of the total bacterial mat community, which is dominated by cyanobacteria (Krumbein et al. 1977), 16S rRNA genes of sulfate-reducing bacteria remained undetected by general PCR primers. A more specific primer combination was employed to obtain DGGE signals of sulfate-reducing bacteria, consisting of the universal bacterial primer DS907 and primer 385, which is selective, although not specific, for the delta-subdivision sulfate-reducing bacteria (Ramsing et al. 1996, Amann et al. 1992). The induced amplification bias towards delta-subdivison sulfate reducers resulted in a new set of DGGE patterns. A

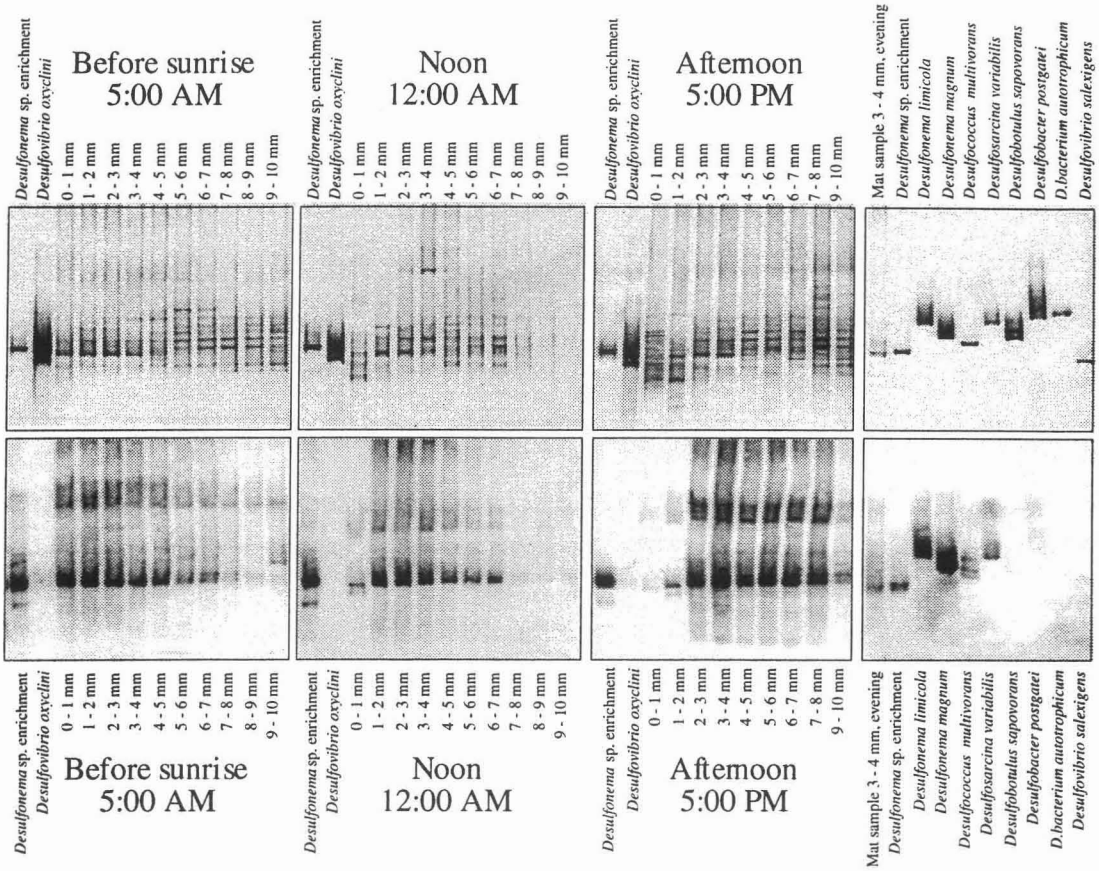

Figure 4. DGGE patterns of PCR-amplified 16S rDNA templates from Solar Lake cyanobacterial mat layers, and sulfate-reducing bacteria, obtained with primer combination 385 (GC-clamp) and 907. Gels are shown stained with ethidiumbromide (A-D) and hybridized with 16S rRNA probe 657 (E-H). (A, E) Solar Lake mat DGGE pattern at 5 am, (B,F) at noon, and (C,G) at 5 pm. (D,H) DGGE pattern of *Desulfonema* and related sulfate-reducing bacteria. The Solar Lake DGGEs include a *Desulfonema* sp. enrichment from Solar Lake mat as positive and *Desulfovibrio oxyclinae* as negative hybridization control for probe 657.

conspicuous band was found in all three sample sets (5 am, 12 noon, 5 pm), at a similar gel position as one of the hydridization controls, the DGGE band of a *Desulfonema* sp. enrichment from Solar Lake (Fig.4, A, B, C). The DGGE gels were blotted on a Hybond+ membrane and hybridized with probes, to detect particular bands derived from sulfate-reducing bacteria, and to follow them through this series of complex DGGE patterns. Detection and monitoring of particular bands, such as the conspicous main band, by hybridization is more sensitive than by ethidiumbromide staining (Fig.4. A,B,C and E,F,G). Ethidiumbromide staining will

miss bands which do not exceed background staining intensity, or are obscured by intense bands nearby.

The DGGE patterns were hybridized with 16S rRNA probe 657, which has been designed as a specific 16S rRNA probe for fluorescent in-situ hybridization detection of the genus *Desulfonema*, encompassing the species *D. magnum*, *D. limicola*, and *D. ishimotoei* (Fukui et al. 1997). To search the Solar Lake DGGE band pattern for *Desulfonema*, and also for *Desulfonema*-related sulfate reducers with one mismatch at the target site, probe 657 was used at semi-stringent conditions. This allows hybridization with *Desulfonema limicola* and *Desulfonema magnum*, but also with *Desulfococcus multivorans* and *Desulfosarcina variabilis*, which have one mismatch each in their target sequences. Two mismatches, such as in *Desulfobotulus sapovorans*, prevent hybridization (Table 2, Fig. 4D,H). In all DGGE patterns, the band already conspicuous in the ethidiumbromide-stained gels hybridized with probe 657 (Fig. 4). DGGE bands from all three diurnal sample sets were compared side by side on one gel, showing identical positions. At a later stage, sequencing confirmed their identity. The probe 657 target site of this molecular isolate included one mismatch (Table 2).

If the PCR amplification bias towards a target sequence remains constant

Table 2. 16S rRNA target regions complementary to *Desulfonema* probe 657 (Fukui et al. 1997) and to *Desulfococcus-Desulfosarcina-Desulfobotulus* probe 814 (Devereux et al. 1992).

	Probe 657	Probe 814
Probe sequence	5'-TTCCGYTTCCCTCTCCCATA-3'	5'-ACCTAGTGATCAACGTTT-3'
Target sequence	UAUGGGAGAGGGAARCGGAA	AAACGUUGAUCACUAGGU
Dn.magnum	UAUGGGAGAGGGAAGCGGAA	AAACGGUGAUCACUAGGU
Dn.limicola	UAUGGGAGAGGGAAGCGGAA	AAACGUUGAUCACCAGGU
Dn.ishimotoei	UAUGGGAGAGGGAAACGGAA	AAACGGUGAUCACUAGGU
Solar Lake Band	UAUGGGAGAGGGNAGUGGAA	AAACGGUGNUcACUAGGU
Dcs.multivorans	UAUGGGAGAGGNNAGUGGNA	AAACGUUGAUCACUAGGU
Dsa.variablilis	UAUGGGAGAGGGAAGUGGAA	AAACGUUGAUCACUAGGU
Dbo.sapovorans	UAUGGCAGAGGAAAGCGGAA	AAACGUUGAUCACUAGGU
Strain HxD3	UAUGGGAGAGGGAAGUGGAA	AAACGUUGAUCACUAGGU
MMP91	UAUGGGAGAGGGAAGUGGAA	AAACGUUGAACACUAGGU
Dbm.autotroph.	UACGGUNGAGGAAAGGGGNA	AAACGUUGUAUACUAGGU
Dbc.toluolica	UACGGGAGAGGAAAGCGGAA	AAACGAUGUACACUAGGU
Dba.vulgaris	UACGGGAGAGGAGAGAGGAA	AAACGUUGUACACUCGGU

throughout a sample series, then DGGE monitors the relative increase and decrease of this particular microbial population. At the beginning of a diurnal course at 5 am, the *Desulfonema*-probe positive DGGE band is very conspicuous in the upper 5 mm of the mat, and still visible until 8 mm depth. At noon, the band appears weaker in the surface millimeter layer, but remains fully visible between 1 and 7 mm depth. At 5 pm, the band is at best very faint in the surface layer, stronger in the second millimeter layer of the mat, fully visible between 2 and 8 mm, and still detectable at 10 mm depth. This pattern was reproducible in replica samples, taken of the upper 5 mm of the 5 am sample set and of the complete 5 pm sample set. The reduced intensity of the *Desulfonema*-probe positive DGGE band in the upper 2 millimeter layers of the mat is correlated to the gradual penetration of oxygen into the mat over a diurnal course (Fig. 3). Apparently, the corresponding bacterial population is migrating vertically, to avoid extremely high oxygen concentrations. Nevertheless, the spatial range of this bacterial population overlaps with supersaturated oxygen concentrations in the range of 200 - 400 µM at noon and in the afternoon (Fig. 3).

Phylogenetic position. The partial 16S rRNA sequence of the conspicuous molecular isolate from the Solar Lake mat surface layer was related to the sulfate-reducing bacteria *Desulfococcus*, *Desulfosarcina*, *Desulfobotulus* and *Desulfonema* (Fig. 5). *Desulfonema*, the genus of filamentous sulfate reducers, has recently been included in this phylogenetic group (Fukui et al. 1997). A multicelled magnetotactic prokaryote (DeLong et al. 1993) and the alkane-oxidizing sulfate-reducing bacterium Hdx3 (Aeckersberg et al. 1991) are other members of this group. Except for the consistent *Desulfonema* assemblage, which also includes *Desulfococcus multivorans* (Fukui et al. 1997), the branching pattern within this group differed from the phylogeny based on complete or near-complete 16S rRNA sequences: The branching order of *Desulfosarcina variabilis* and *Desulfobotulus sapovorans* appeared reversed (Devereux et al. 1989), although the instability of this branching pattern was indicated by low bootstrap values under 60. These distortions probably result from the limited sequence basis for this analysis, i.e. 16S rRNA positions 481-906 (E.coli

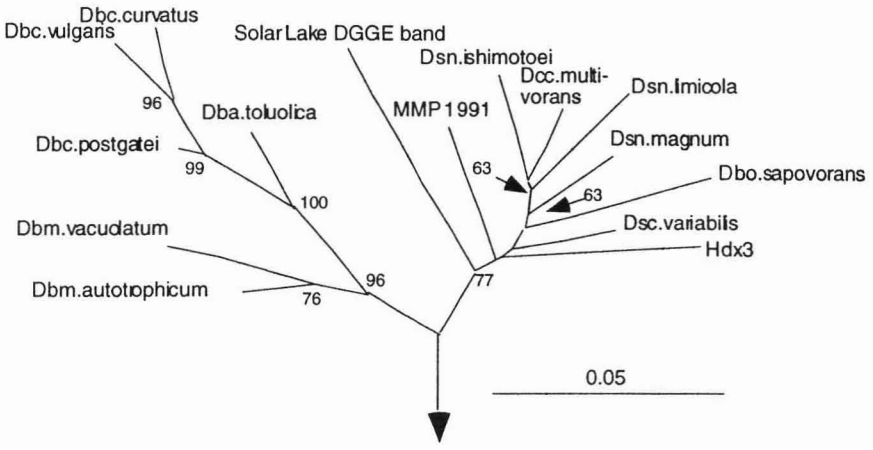

Figure 5. 16S rRNA distance tree of completely oxidizing sulfate reducing bacteria of the delta proteobacteria subdivision, and the molecular isolate from the surface layer of Solar Lake cyanobacterial mat. The tree is rooted with *Dsv. desulfuricans* as outgroup, and based on 16S rRNA sequence positions 481-906, the sequence range of the Solar Lake molecular isolate (E.coli numbering). The scale bar corresponds to 0.05 mutations per nucleotide position. Bootstrap values from distance analyses (100 replicates) refer to species distal to the associated node. Sequences used in preparing this figure were derived from the Ribosomal Database Project (RDP) (Maidak et al. 1996) and GenBank. Abbreviations: Dn., *Desulfonema*, Dcs., *Desulfococcus*, Dsa., *Desulfosarcina*, Dbo., *Desulfobotulus*, Dbm., *Desulfobacterium*, Dbc., *Desulfobacula*, Dba., *Desulfobacter*, MMP1991, iron sulfide containing multicelled magnetotactic bacterium clone MMP1991 (Delong et al. 1993), HxD3, hexadecane-oxidizing sulfate reducing bacterium (Aeckersberg et al. 1991).

positions) of the DGGE band. However, the affiliation of the Solar Lake sequence with the *Desulfonema-Desulfosarcina-Desulfococcus-Desulfobotulus* group was confirmed by 77 of 100 bootstrap test runs. *Desulfonema magnum* showed the highest individual sequence similarity to the Solar Lake sequence (0.087 Jukes-Cantor distance). The Solar Lake molecular isolate does not belong to the *Desulfobacter-Desulfobacterium* lineage, the second major phylogenetic group of completely oxidizing sulfate reducing bacteria. Oligonucleotide motifs which have been used as target sites for the group-specific *Desulfococcus-Desulfosarcina-Desulfobotulus* probe (Devereux et al. 1992) and *Desulfonema* probe (Fukui et al. 1997) occur in a similar form in the Solar Lake molecular isolate, although altered by at least one mismatch each (Table 2).

DISCUSSION

Evaluation of MPN counts. Most probable number counts indicate large populations of sulfate-reducing bacteria during daytime in the oxic surface layer of Solar Lake mat, in the range of 4.5 x 10^6 cells/ml. This is in the same range as previous counts of the upper 5 mm interval (Jørgensen and Cohen 1977). Interestingly, these numbers may underestimate the actual population density of active sulfate-reducing bacteria by at least one order of magnitude: Specific sulfate reduction rates, calculated from Solar Lake mat sulfate reduction rates (nmol SO_4^{2-} $cell^{-1}$ d^{-1}) and from cell numbers per ml, are in the order of 1 - 10 x 10^{-4} nmol SO_4^{2-} $cell^{-1}$ d^{-1}, and average 5.6 x 10^{-4}

Table 3. Numbers of sulfate-reducing bacteria in Solar Lake mat layers and corresponding sulfate reduction rates measured *in situ*, and resulting specific sulfate reduction activities per cell.

Time	Layer mm	SRBs cm^{-3} *	SO_4^{2-} reduced nmol cm^{-3} d^{-1} **	SO_4^{2-} reduced nmol $cell^{-1}$ d^{-1}
12 am	0 - 2	4.5 x 10^6	2207 ± 1258 (57%)	4.70 x 10^{-4}
12 am	2 - 4	3.0 x 10^6	3127 ± 1004 (32%)	10.4 x 10^{-4}
12 am	4 - 7	1.9 x 10^6	2780 ± 482 (17%)	14.9 x 10^{-4}
12 am	7 - 10	6.4 x 10^6	1119 ± 257 (23%)	1.75 x 10^{-4}
12 am	10 - 13	8.6 x 10^6	547 ± 164 (30%)	0.64 x 10^{-4}
12 pm	0 - 2	1.8 x 10^6	1002 ± 315 (31%)	5.6 x 10^{-4}
12 pm	2 - 4	11.6 x 10^6	1842 ± 51 (3 %)	1.6 x 10^{-4}
12 pm	4 - 7	3.0 x 10^6	1831 ± 452 (25%)	6.1 x 10^{-4}
12 pm	7 - 10	0.9 x 10^6	761 ± 76 (10%)	8.5 x 10^{-4}
12 pm	10 - 13	1.9 x 10^6	472 ± 82 (17%)	2.5 x 10^{-4}
	0 - 5	2.0 x 10^6	5400	27.0 x 10^{-4}
	(Jørgensen and Cohen 1977)			
	Specific sulfate reduction rates for pure cultures: 0.5 - 0.002 x 10^{-4} nmol SO_4^{2-} $cell^{-1}$ d^{-1} (Jørgensen 1978)			

* Counts of incompletely (lactate) and completely (acetate) sulfate-reducing bacteria combined. Overlaps between both populations are possible, since many acetate-oxidizers, i.e. *Desulfococcus*, *Desulfosarcina* and *Desulfonema* are nutritionally versatile and oxidize a variety of other compounds, including lactate (Widdel 1980).

** Measurements from three cores, with standard deviations (absolute and in %). Sulfate reduction rates of the upper two layers (0-2 mm, 2-4 mm) were higher in bag incubations than the corresponding in situ rates (Habicht and Canfield 1996, Manuscript 1).

nmol SO_4^{2-} $cell^{-1}$ d^{-1} over the upper 13 mm of the mat (Table 3). The specific rate in the oxic zone at noon is 4.7 x 10^{-4} nmol SO_4^{2-} $cell^{-1}$ d^{-1}. These calculated specific activities are at least ten times higher than the range of specific activities determined for sulfate reducing bacteria in pure culture, 0.5 - 0.002 x 10^{-4} nmol SO_4^{2-} $cell^{-1}$ d^{-1} (Jørgensen 1978). Either, sulfate-reducing bacteria in the mat are at least one order of magnitude more active than in pure culture, or the MPN counts have underestimated the actual density of sulfate reducing bacteria by this factor. Ribosomal rRNA quantifications from cyanobacterial mats of Guerrero Negro also suggest higher cell densities. These mats resemble the shallow and deep flat mat type of Solar Lake (Jørgensen et al. 1983) in environmental setting, ultrastructure, biogeochemical cycling, and sulfate-reducing activity (D'Amelio et al. 1989, Canfield and DesMarais 1991, 1993). By comparing the cellular rRNA content of actively growing *Desulfovibrio* cells to the amount of sulfate-reducing bacterial rRNA extracted from the diurnally oxic mat layer (2 mm, as in Solar Lake), total numbers of SRBs in this layer were estimated as 10^7 - 10^8 cells per gram of mat (Risatti et al. 1994).

Sulfate-reducing bacteria and their activity in the oxic surface layer. MPN counts and sulfate reduction rates both indicate active populations of sulfate reducing bacteria in the diurnally oxic surface layer (0-2 mm), with overall cell density and activity in the same order of magnitude as in deeper mat layers (Figure 1, Table 3). In this layer, the cell density of sulfate-reducing bacteria (4.5 x 10^6 cells/ml) did not differ much from the cell density averaged over of the upper 13 mm (4.9 x 10^6 cells/ml). Furthermore, sulfate reduction rates in the 0 - 2 mm surface layer corresponded to 70 % of the maximum daytime activity found in the chemocline (Table 3). Obviously, these active sulfate-reducing bacterial populations must be adapted to the conditions of the oxic chemocline layer. For example, *Desulfovibrio oxyclinae* and related *Desulfovibrio* strains from the upper Solar Lake mat layer are capable of aerobic respiration at very high rates. However, they do not grow by this metabolic mode, and do not reduce sulfate under oxic conditions in pure culture experiments (Krekeler et al. 1997a,b). Sulfate-reducing bacteria which can sustain

sulfate reduction and grow in pure culture under oxic conditions have not been isolated so far (Dannenberg et al. 1992, Marschall et al. 1993). However, a homogeneous pure culture suspension of sulfate-reducing bacteria is probably not a suitable model system for their situation in the dense matrix of a cyanobacterial mat. There, additional factors might increase viability and activity of sulfate-reducing bacteria under oxygen exposure: particle association (Fukui and Takii 1990), co-culturing with oxygen-scavenging aerobic bacteria (Gottschal and Szewzyk 1985, Teske et al. 1996) and aggregation and clump formation of motile sulfate-reducing bacteria including *Desulfonema* and *Desulfovibrio* (Widdel et al. 1983, Krekeler et al. 1997b).

Mat microheterogeneities. Sulfate reducing bacteria can survive within oxic environments in reduced microniches of at least 100 - 200 µm diameter (Jørgensen 1977). The growth patterns of many marine completely oxidizing sulfate reducers, such as aggregate formation of *Desulfobacter* and *Desulfosarcina*, and filament formation by *Desulfonema* (Widdel 1980), can contribute to the formation of microniches. The patchy distribution of sulfate-reducing bacteria in the range of approx. 100 µm was directly visualized in a photosynthetic biofilm (Ramsing et al. 1993). These results suggest similar heterogeneities could occur in mats, although support from microelectrode studies is missing so far. There is evidence for uneven distribution patterns of sulfate-reducing bacteria and activity in the surface layer of Solar Lake mat on a millimeter scale: Millimeter- and even centimeter-sized spots and streaks of increased H_2S formation have been visualized by Ag_2S formation on silverfoils inserted into the oxic mat layer (Cohen et al. 1994; Lerrer-Helman and Cohen 1996). Sulfate reduction rate measurements on three Solar Lake mat cores, sliced and processed in parallel to the MPN counts, showed the highest core-to-core variations in the surface layer, at all times of a diurnal cycle (Table 3). Ribosomal RNA hybridization analysis of hypersaline cyanobacterial mats of Guerrero Negro (Baja California, Mexico) revealed variable proportions of dominant sulfate-reducing bacteria of the surface layer, *Desulfococcus*-related populations and *Desulfovibrio*

(Risatti et al. 1994). This sample-to-sample variability occurred only in the mat surface layer, and not in deeper mat intervals.

Acetate-oxidizing sulfate-reducing bacterial populations. Completely oxiding sulfate reducers were shown to occur in high numbers in the oxic mat zone, with conservative MPN estimates in the range of 2 x 10^5 cells/ml. Acetate-oxidizing *Desulfonema* constituted at least 10% of this population, according to the *Desulfonema* growth pattern in the acetate MPN series. Occurrences of *Desulfonema* in high dilutions of the lactate series indicate higher *Desulfonema* population densities, but it remains to be seen whether these *Desulfonema* from lactate-MPNs can oxidize acetate. All Solar Lake *Desulfonema* isolates grow on isobutyrate (M. Fukui, pers. comm.). Molecular results also indicate acetate-oxidizing sulfate-reducing bacteria, although these results have to interpreted with particular caution. The conspicuous molecular isolate obtained by DGGE was phylogenetically affiliated to acetate-oxidizing sulfate-reducing bacteria of the delta-proteobacterial subdivision. However, the high metabolic diversity within this phylogenetic cluster precludes specific assumptions about the physiology of this organism, even more so since this phylogenetic group includes the alkane-oxidizing sulfate-reducing bacterium HxD3 (Aeckersberg et al. 1991) and the fatty acid-oxidizing species *Desulfobotulus sapovorans* (Devereux et al. 1989), which cannot oxidize acetate, and finally an uncultured multicelled magnetotactic bacterium of the iron sulfide type (Delong et al. 1993).

Comparable molecular results come from cyanobacterial mats of Guerrero Negro in Baja California, Mexico, which are similar to those of Solar Lake (D'Amelio et al. 1989). Bacterial rRNA, extracted directly from the mat, was blotted on membranes and hybridized with different probes for sulfate reducing bacteria (Devereux et al. 1992). In the 2 mm surface layer, rRNA which hybridized with probe 814 constituted up to 5.2 % of the total prokaryotic rRNA (Risatti et al. 1994). Probe 814 covers in its target range *Desulfococcus*, *Desulfosarcina*, *Desulfobotulus*, and, with a single mismatch, *Desulfonema limicola* and *Desulfonema ishimotoei*.

Desulfonema magnum shows two mismatches to probe 814 (Table 2). Since single mismatch discrimination in membrane blotting requires the use of competitive probes (Manz et al. 1992), the hybridization signal with probe 814 could originate partly from *Desulfonema* ribosomal RNA in addition to *Desulfococcus*, *Desulfosarcina* and *Desulfobotulus* and other related bacteria, such as the Solar Lake molecular isolate (D. Stahl, personal communication).

Acetate-stimulated sulfate reduction was observed in the cyanobacterial mat of an artificial hypersaline pond at the Heinz Steinitz Laboratory (Eilat, Israel) which had developed from Solar Lake mat inocula. The addition of acetate stimulated sulfate reduction rates within the photosynthetically active cyanobacterial layer (0-3 mm) and within the chemocline layer, which harbored anoxygenic phototrophic bacteria (3 - 5 mm), but not in the permanently reduced layer (5 - 12 mm). Lactate and ethanol, the typical substrates of *Desulfovibrio*, had no stimulating effect within the surface layer. Glycolate, a dominant photorespiration and dark fermentation product of cyanobacteria (Heyer and Krumbein 1991), had the highest stimulatory effect on sulfate reduction in the surface layer of Solar Lake mats (Fründ and Cohen 1992). A glycolate-oxidizing sulfate-reducing bacterium has been isolated from marine anoxic sediment (Friedrich and Schink 1995). This new genus and species, *Desulfofustis glycolicus*, was phylogenetically distinct from acetate-oxidizing sulfate-reducers (Friedrich et al. 1996), and would not have been detected by 16S rRNA probes 814 and 657. Therefore, cyanobacterial mats such as those of Solar Lake could harbor significant glycolate-oxidizing sulfate-reducing populations, which have not yet been detected by molecular and culture-dependent methods.

Sulfate reduction and carbon cycling in Solar Lake cyanobacterial mats. Incompletely and completely oxidizing sulfate-reducing bacterial populations are together capable of complete biomineralization of organic carbon compounds, not only in strictly anoxic deeper mat layers, but also nearby and within the photosynthetically active zone. This recycling of CO_2 probably alleviates CO_2 limitation of cyanobacterial photosynthesis. Besides other factors, isotopically

indiscriminative HCO_3^- uptake of cyanobacterial cells at reduced CO_2 concentrations could contribute to apparent reduced discrimination of cyanobacterial photosynthesis against the heavy carbon isotope ^{13}C and to unusually heavy $\delta^{13}C$ values of mat carbon (Aizenshtat et al. 1984, DesMarais et al. 1989, DesMarais and Canfield 1994).

Sulfate reduction recycles considerable quantities of CO_2 within or near the photosynthetically active mat layer, as shown by the following calculations: Photosynthetic O_2 production and CO_2 fixation rates of Solar Lake cyanobacterial mats, integrated over depth, have been estimated as 8 - 42 mmol h^{-1} m^{-2} (Krumbein et al. 1977), and more recently as 20 - 25 mmol h^{-1} m^{-2} (Revsbech et al. 1983). Depth-integrated sulfate reduction rates of approx. 2.5 mmol SO_4^{2-} h^{-1} m^{-2} correspond to 5 mmol CO_2 h^{-1} m^{-2} produced by sulfate reduction (Jørgensen and Cohen 1977). These sulfate reduction rates thus provide between 20 - 25 % of the CO_2 fixed by autochtonous photosynthetic production, according to the more constrained estimate of 20 - 25 mmol CO_2 fixed h^{-1} m^{-2} (Revsbech et al. 1983). Approx. 90 % of the total depth-integrated sulfate-reducing activity is found within the upper 3 cm of the mat, and 50% within the upper 5 mm (Jørgensen and Cohen 1977). Therefore, 10 - 12.5 % of the photosynthetic CO_2 demand can be replenished from sulfate reduction within the upper 5 mm of the mat. This tight coupling of sulfur and carbon cycles is probably a characteristic feature of hypersaline cyanobacterial mats: The most complete carbon and sulfur budgets, obtained for the mats of Guerrero Negro in Baja California, showed that sulfate reduction drives internal CO_2 recycling and provides between 15 to 50 % of the cyanobacterial photosynthetic CO_2 demand, with some seasonal variability. In the dataset obtained in November 1990, the diurnally oxic 2 mm mat surface layer accounted for approx. 40 % of the total integrated sulfate reducing activity, which provided 20% of the cyanobacterial CO_2 demand (Canfield and DesMarais 1993, 1994).

Within or nearby these photosynthetic mat surface layers, both incompletely and completely oxidizing, highly active sulfate reducing bacteria occur in high densities and show specific adaptations to oxygen exposure and to the mat matrix,

such as filamentous *Desulfonema*, oxygen-respiring *Desulfovibrio*, or the migrating molecular isolate. Since they facilitate the in-situ-turnover of organic compounds to acetate and CO_2, photosynthetic production and CO_2 recycling by sulfate reduction sustain each other in close spatial association and create highly autonomous microbial ecosystems of extremely high photosynthetic and sulfate-reducing activity. As model systems for the evolution of the sulfur cycle, they put current assumptions about sulfur and carbon cycling in archaeal microbial mats to the test (Habicht and Canfield 1996, Manuscript 1).

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4

Sulfur isotope fractionation during bacterial reduction and disproportionation of thiosulfate and sulfite

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Contents Page

ABSTRACT

In bacterial cultures we measured sulfur isotope fractionation during transformations of thiosulfate ($S_2O_3^{2-}$) and sulfite (SO_3^{2-}), pathways which might be involved in the cycling of sulfur in marine sediments and euxinic waters. We explored the fractionations during the reduction and disproportionation of $S_2O_3^{2-}$ and SO_3^{2-} by enrichments and pure bacterial cultures from marine and freshwater environments. We also measured the isotope fractionation associated with the anoxygenic phototrophic oxidation of H_2S to $S_2O_3^{2-}$ by cyanobacteria. Except for SO_3^{2-} reduction, isotope fractionations for these processes have not been previously reported. During the dissimilatory reduction of SO_3^{2-}, H_2S was depleted in ^{34}S by 6‰, and during the reduction of $S_2O_3^{2-}$ to H_2S, depletions were between 7‰ and 11‰. The largest observed isotope fractionation was associated with the bacterial disproportionation of SO_3^{2-}, which caused a ^{34}S depletion in H_2S of between 20‰ to 37‰ and a ^{34}S enrichment in sulfate of 7‰ to 12‰. During the bacterial disproportionation of $S_2O_3^{2-}$, isotope fractionations between the outer sulfane sulfur and H_2S and between the inner sulfonate sulfur and SO_4^{2-} were < 4‰. We observed isotope exchange between the two sulfur compounds of $S_2O_3^{2-}$ leading to a depletion of ^{34}S in H_2S by up to 12‰ with a comparable enrichment in SO_4^{2-} of ^{34}S. No isotope fractionation was associated with the anoxygenic phototrophic oxidation of H_2S to $S_2O_3^{2-}$. The depletion of ^{34}S into H_2S during the bacterial reduction of $S_2O_3^{2-}$ and SO_3^{2-} and during the disproportionation of SO_3^{2-}, may, in addition to sulfate reduction and the bacterial disproportionation of elemental sulfur, contribute to the generation of ^{34}S-depleted sedimentary sulfides.

INTRODUCTION

In coastal marine sediments bacterial sulfate reduction is one of the most important respiration processes accounting for ≥ 50% of the total mineralization (Jørgensen 1982; Canfield 1993). Typically 90% of the sulfide produced by sulfate reduction is reoxidized, leaving only a small amount of sulfide buried in the sediment in the form of metal sulfides, mostly pyrite (Jørgensen 1982; Berner and Westrich 1985). The pathways of sulfide oxidation to SO_4^{2-} are largely unknown. Compounds such as elemental sulfur (S^0), $S_2O_3^{2-}$, and SO_3^{2-} might, however, be important intermediate products during the reoxidation of H_2S (Pyzik and Sommer 1981; Goldhaber 1983; Fossing and Jørgensen 1990; Elsgaard and Jørgensen 1992). These compounds rapidly can be transformed either bacterially or chemically by reduction, oxidation or disproportionation (Jørgensen 1990; Widdel and Hansen 1992; Thamdrup et al. 1994; Canfield and Thamdrup 1996), and only low concentrations (< 0.5 µM - 10 µM for SO_3^{2-} and $S_2O_3^{2-}$; < 10 µmol cm^{-3} for S^0) are typically found in sediments (Troelsen and Jørgensen 1982; Thamdrup et al. 1994).

The isotopic composition of reduced sulfur compounds buried in sediments has been used to explore cycling of sulfur in both modern and ancient marine environments (Schidlowski et al. 1983; Canfield and Teske 1996; Habicht and Canfield 1996, Manuscript 1). The H_2S formed during the bacterial reduction of sulfate becomes depleted in ^{34}S, leaving the remaining sulfate enriched in ^{34}S. In studies of pure cultures and natural populations of sulfate-reducing bacteria, isotopic fractionation during sulfate reduction has been measured in the range of 5‰ to 46‰ (Kaplan and Rittenberg 1964; Kemp and Thode 1968; Chambers et al. 1975; Habicht and Canfield 1996, Manuscript 1). By contrast, the isotopic composition of marine sulfides in modern sediments and euxinic waters exhibits much greater depletions in ^{34}S, with values ranging up to 70‰ (Canfield and Thamdrup 1994; Canfield and Teske 1996). The large ^{34}S depletion of modern sedimentary sulfides is thought to result from an initial fractionation by sulfate-reducing bacteria followed by further

fractionation during the oxidative part of the sulfur cycle (Jørgensen 1990; Canfield and Thamdrup 1994). A recent study has revealed a marked isotope fractionation during the bacterial disproportionation of S^0 to H_2S and SO_4^{2-} that could explain the additional ^{34}S depletion in sedimentary sulfides (Canfield and Thamdrup 1994). Similar roles have been proposed for transformations through SO_3^{2-} and $S_2O_3^{2-}$, although the isotope systematics of these pathways have not previously been explored (Jørgensen 1990; Canfield and Thamdrup 1994).

An understanding of the isotope fractionations during $S_2O_3^{2-}$ and SO_3^{2-} transformations would allow us to better evaluate which bacterial processes might contribute to the low $\delta^{34}S$ value of sedimentary sulfides and possibly also which pathways are the most significant in the oxidative part of the sulfur cycle. We have therefore measured the sulfur isotope fractionations associated with the bacterial reduction and disproportionation of SO_3^{2-} and $S_2O_3^{2-}$. We also measured the isotope fractionation during the bacterial oxidation of H_2S to $S_2O_3^{2-}$.

MATERIALS AND METHODS

Bacterial cultures:

Pure cultures of *Desulfovibrio salexigens* (DSM 2638), *Desulfovibrio sulfodismutans* (DSM 3696), *Desulfococcus multivorans* (DSM 2059) and *Desulfocapsa thiozymogenes* (DSM 7269) were all purchased from the German Collection of Microorganisms (DSM). Strains of the cyanobacteria *Oscillatoria sp.* and *Calothrix sp.* were from the culture collection of the Department of Marine Microbiology, Bremen University, Germany, and can be obtained from there on request.

Enrichment cultures were obtained from inoculum (2% v/v) of the surface 0 - 1 cm sediments of a tidal flat at Weddewarden in the Weser estuary (Germany), the

Løgten Lagoon sulfuretum in Aarhus Bay (Denmark), and from the microbial mats of the hypersaline Solar Lake, Sinai (Egypt).

Media and cultivation techniques.

The procedures used for preparation of anoxic culture media, stock solutions and cultivation of anaerobic bacteria were as described by Widdel and Bak (1991). The marine basal medium (freshwater in brackets) had following composition, in grams per liter of distilled water: NaCl, 20 (1); $MgCl_2 \cdot 6H_2O$, 3 (0.4); $CaCl_2 \cdot 2H_2O$, 0.15 (0.1); NH_4Cl, 0.25 (0.25); KH_2PO_4, 0.2 (0.2); KCl, 0.5 (0.5). To one liter of the basal medium the following components were added from sterile stock solutions: 30 ml 1M $NaHCO_3$ and 1 ml of each solution of trace elements without chelating agent, vitamin B_{12}, thiamin, and a mixture of 7 further vitamins (Widdel and Bak 1991). The pH was adjusted to 7.2, and the medium was then dispensed aseptically into sterile 50 ml or 100 ml screw-cap glass bottles.

Desulfovibrio salexigens (marine strain) and *Desulfococcus multivorans* (freshwater strain) were cultivated with sulfate (28 mM) and lactate (20 mM). *Desulfovibrio sulfodismutans* (freshwater strain), *Desulfocapsa thiozymogenes* (freshwater strain), and all of the enrichment cultures (marine strains), were cultivated with either thiosulfate (10 mM) or sulfite (10 mM), and with acetate (2 mM) as the carbon source when appropriate. Substrates were added from sterile stock solutions to the culture bottles using glass pipettes. The cultures were incubated at 30°C in the dark without shaking. Enrichment cultures were inoculated and subsequently transferred 5 times (Solar Lake and Løgten Lagoon) or 10 times (Weddewarden) with 10% inoculum before the cultures were used for measuring the isotope fractionation during $S_2O_3^{2-}$ disproportionation.

Cyanobacteria were cultivated aerobically in modified artificial seawater medium ASN III (Rippka et al. 1979) with the following composition in grams per liter of distilled water: NaCl, 12.5; $MgCl_2 \cdot 6H_2O$, 1; $CaCl_2 \cdot 2H_2O$, 0.25; KCl, 0.25, $NaNO_3$, 0.75; Na_2CO_3, 0.02, Na_2SO_4, 4. The components: $K_2HPO_4 \cdot 3H_2O$; trace

Table 1. Overview of reactions and bacteria cultures explored in this study.

Kind of reaction	Culture	Reaction
Reduction		
SO_3^{2-}	*D'vibrio salexigens*	$3Lac + 2SO_3^{2-} + 4H^+ \rightarrow 2H_2S + 3Ace + 3CO_2 + 3H_2$
$S_2O_3^{2-}$	*D'vibrio salexigens* *D'coccus multivorans*	$2Lac + S_2O_3^{2-} + 2H^+ \rightarrow 2H_2S + 2Ace + 2CO_2 + H_2O$
Disproportionation		
SO_3^{2-}	*D'vibrio sulfodismutans* *D'capsa thiozymogenes*	$4SO_3^{2-} + 2H^+ \rightarrow H_2S + 3SO_4^{2-}$
$S_2O_3^{2-}$	*D'vibrio sulfodismutans* *D'capsa thiozymogenes* Solar Lake enrichment Løgten Lagoon enrichment Weddewarden enrichment	$S_2O_3^{2-} + H_2O \rightarrow H_2S + SO_4^{2-}$
Sulfide oxidation		
H_2S oxidation to $S_2O_3^{2-}$	*Calothrix sp.* *Oscillatoria sp.*	$2H_2S + 3H_2O \rightarrow S_2O_3^{2-} + 5H_2$ (light)

elements, iron-ammonium-citrate and vitamin B_{12} were added from sterile stock solutions to the sterile salt medium. The cultures were pre-grown autotrophically at 20°C in 1L Erlenmeyer flasks for 2 weeks at a light intensity of 44 $\mu E\ m^{-2}\ s^{-1}$.

Sulfur transformation experiments.

In Table 1 we give an overview of the experiments. For measuring isotope fractionation during the reduction and disproportionation of $S_2O_3^{2-}$ and SO_3^{2-}, a series of 50-ml screw cap bottles with basal medium were supplemented with substrates. In the reduction experiments the cultures were incubated with 20 mM lactate and 10 mM of either $S_2O_3^{2-}$ or SO_3^{2-}. To the bottles used for the disproportionation experiments 10 mM of $S_2O_3^{2-}$ or SO_3^{2-} was added, but organic substrates were omitted to exclude the dissimilatory reduction of the sulfur compounds. Anoxic stock solutions of $S_2O_3^{2-}$, SO_3^{2-} and lactate were freshly prepared before each experiment. Each bottle was inoculated with 10% inoculum with no headspace. For each experimental series, consisting of about 8 bottles, the same batch culture was used as inoculum. The cultures were incubated at 30°C in the dark.

For each sulfide oxidation experiments, cyanobacteria were incubated in between six to ten 110 ml rubber-stoppered glass bottles with artificial seawater medium. To each experimental bottle about 10 ml of dense pregrown cyanobacteria cultures were added under sterile conditions with a glass. To obtain anoxic conditions,

the culture bottles were gassed with helium for at least 15 minutes before sulfide (1.0 mM or 2.5 mM) was added. Cultures were incubated in the light at 44 $\mu E\ m^{-2}\ s^{-1}$. To obtain a high $S_2O_3^{2-}$ yield (> 100 µmol) for the isotope measurements, we added 2.5 mM of H_2S in our experiment with *Calothrix sp*. However, as the bacteria did not oxidize H_2S well at these high concentrations, we only added 1 mM of H_2S in later experiments. When all of the H_2S was oxidized to $S_2O_3^{2-}$, 1 mM of H_2S was again added to the cultures. By the time the second H_2S addition was oxidized, both *Calothrix sp.* and *Oscillatoria sp.* had begun to lose color and incubations were stopped. This was after about 4 weeks of incubation.

Time course experiments were done in all reduction and $S_2O_3^{2-}$-disproportionation experiments. In the SO_3^{2-}-disproportionation, and the H_2S-oxidation experiments, only initial and final samples were taken. After incubation, a 1 ml subsample was withdrawn from the experimental bottles and fixed into 1 ml 2% $ZnCl_2$ for concentration measurements of H_2S, $S_2O_3^{2-}$ and SO_4^{2-}. For sulfite determination, 0.1 ml of the culture sample was derivatized with DTNP (2.2'-Dithiobis(5-nitropyridine)), which fixes the SO_3^{2-} and protects it against oxidation. The DTNP-derivative was stored on Sep-Pak C_{18} cartridges (Waters) which had been rinsed according to Vairavamurthy and Mopper (1990). A further 1 ml was used to estimate bacterial population size by optical density, measured immediately after sampling at 660 nm. The remaining bottle contents were fixed with 10 ml 20% ZnAc for isotope analysis. This procedure stopped all bacterial activity and fixed H_2S as ZnS. These samples, and the samples for concentration measurements, were stored frozen until further analysis.

For isotope measurements, ZnS was filtered from the ZnAc solution, distilled with 6 N HCl, and the liberated H_2S was collected as Ag_2S. Excess Ba^{2+} was added to the supernatant solution remaining after filtration which precipitated sulfate as $BaSO_4$. We found that sulfite, but not thiosulfate, was also precipitated by Ba^{2+}, and in the sulfite disproportionation experiments, sulfite and sulfate could not be separated from each other for isotope determination. As a result we could not follow a time course,

but rather determined the initial isotopic composition of sulfite, and the isotopic composition of sulfate remaining after complete sulfite consumption by disproportionation.

The $BaSO_4$ was collected by filtration and saved for isotope determinations. The sulfonate ($-SO_3$) and sulfane ($-S^{2-}$) sulfur of $S_2O_3^{2-}$ remaining after sulfide and sulfate removeal, were separated by boiling with an excess of $AgNO_3$ (Uyama et al. 1985). This procedure precipitates the sulfane as Ag_2S and leaves the sulfonate as SO_4^{2-} in the solution, which after filtration of the Ag_2S, can be precipitated as $BaSO_4$. However, as Ag^+ also precipitates with Cl^-, which was present in high concentration in the medium, we first had to separate $S_2O_3^{2-}$ from Cl^- to minimize the AgCl precipitate. Also, Ba^{2+}, if present, needed to be removed from the $S_2O_3^{2-}$-solution to avoid a simultaneous precipitation of Ag_2S and $BaSO_4$ after boiling with the $AgNO_3$. Thiosulfate was separated from Cl^- and Ba^{2+} on a 10 cm long hand-packed anion exchange resin column (AG2-X8, 100 - 200 mesh; Biorad). Before use the column was loaded with 1M $NaNO_3$ using NO_3^- as the exchange anion. The column was loaded with the $S_2O_3^{2-}$-containing solution, washed with distilled water to remove Ba^{2+}, and subsequently eluted with 250 mM $NaNO_3$ at a flow-rate of about 1.5 ml $min.^{-1}$. Chloride was eluted first, and after 15 minutes thiosulfate was sampled until completely eluded after maximum 60 minutes. No fractionation of thiosulfate occurred during the separation procedure and no isotope effect was measured when small amounts of AgCl were present together with Ag_2S in the Ag_2S-precipitate. After separation any possible precipitation of sulfane-S with Zn^{2+} was avoided as Zn^{2+} was not retained on the anion exchange column.

Determination of stoichiometries and cell growth.

To follow changes in concentrations of sulfur species during the course of incubation, and for determination of reaction stoichiometries, a parallel series of 100 ml screw-cap bottles was inoculated at the same time as the bottles used for the stable isotope measurements. Subsamples were withdrawn frequently during the course of

incubation with sterile pipettes and fixed as described above. The volume of liquid removed was replaced by sterile glass beads and the bottles were sealed again immediately. Optical density was determined at 660 nm with 1 cm light path for monitoring cell growth (S.D. = 2%).

Concentration determinations and stable isotope analysis.

Sulfate and thiosulfate concentrations were measured by non-suppressed anion exchange chromatography with conductivity detection (Waters) using isophthalic acid (in 10% methanol, pH = 4.7) as eluent (S. D. 2%; detection limit 0.1 mM). As sulfite is an unstable sulfur compound it was oxidized to SO_4^{2-} with hydrogen peroxide. The sulfate determinations, in the experiments containing SO_3^{2-} therefore, yielded the sum of SO_4^{2-} and SO_3^{2-}. The sulfate concentration was calculated as the difference between the combined SO_4^{2-} and SO_3^{2-} concentration and the independently determined SO_3^{2-} concentration. To obtain SO_3^{2-} concentrations, the DTNP -SO_3^{2-} derivative was eluted from the Sep-Pack cartridges with methanol just before HPLC analysis. HPLC separations were performed by gradient elution on a C_{18} Spheri-Pak ODS column (3.9 cm x 15 cm) and detected by UV- absorption at 313 nm (Waters). The helium-purged eluents consisted of (a) a buffer solution containing 50 mM sodium acetate and 7.5 mM tetrabutylamonium hydrogen sulfate adjusted to pH = 3.5 and (b) acetonitrile. The gradient was as described by Vairavamurthy and Mopper (1990) at a flow rate of 1 ml per minute (S.D. 5%, detection limit 1 µM). Sulfide was quantified spectrophotometrically by the methylene blue method at 670 nm (S.D. 1%; detection limit < 1 µM; (Cline 1969).

Stable sulfur isotope values were obtained from SO_2 gas after high-temperature combustion of Ag_2S (1050°C) and $BaSO_4$ (1150°C) together with Cu_2O. The SO_2-gas was subsequently purified on a high-vacuum extraction-line and stored in Pyrex break-seal tubes until mass spectrometric analysis. The results were calculated as per mil difference relative to the Canyon Diablo Troilite (CDT; ± 0.5‰).

RESULTS

Sulfite and thiosulfate reduction.

Dv. salexigens reduced sulfite to H_2S with lactate as the substrate (Table 1 and Fig. 1A). The rate of SO_3^{2-}-reduction averaged 5.7 mM d^{-1} with concomitant growth resulting in an increase in optical density of 0.27 (data not shown). The rate of reaction in this, and in the following experiments, is calculated as the average rate from start till reaction ceased (e.g. after total consumption of the electron acceptor).

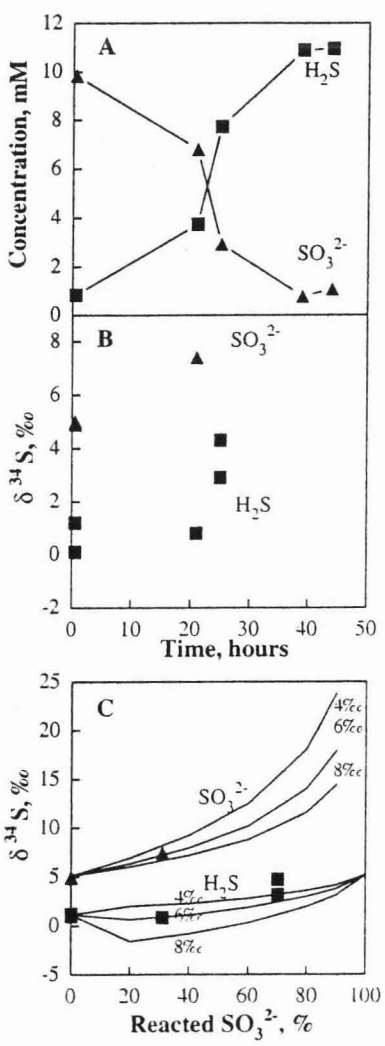

Figure 1. Reduction of SO_3^{2-} by *Dv. salexigens*. A: Concentration measurements, B: Stable sulfur isotope values and C: Model lines of $\delta^{34}S$ values obtained by different fractionation values (α) based on the Rayleigh distillation equation. Note the different scales of the X-axes.

During the course of incubation sulfite became enriched in ^{34}S from an initial $\delta^{34}S$ value of 5.0‰, to 7.4‰ after a 31% reduction of the sulfite concentration (Fig. 1B). The $\delta^{34}S$ of sulfide was 0.7‰ at the beginning of the incubation and increased to about 3.6‰ when 70% of the SO_3^{2-} had been reduced. To calculate the fractionation during sulfite reduction, model curves were generated assuming a closed system with the following Rayleigh distillation expression (Fig. 1C):

$$\delta^{34}SO_3^{2-} = (\delta^{34}SO_3^{2-}{}_{(0)} + 1000) \times f_{SO_3^{2-}}{}^{(\alpha-1)} - 1000 \quad (1)$$

$$\delta^{34}H_2S = \{[SO_3^{2-}]_0 \times (\delta^{34}SO_3^{2-}{}_{(0)} - f_{SO_3^{2-}} \times \delta^{34}SO_3^{2-}) + [H_2S]_0 \times \delta^{34}H_2S_{(0)}\} \times [H_2S]^{-1} \quad (2)$$

where the prefix δ^{34} refers to the isotopic composition of the sulfur compounds, $[SO_3^{2-}]$ and $[H_2S]$ to their concentrations, and the suffix (0) to their initial values; $f_{SO_3^{2-}}$ is the fraction of sulfite remaining, and α is the isotope fractionation factor. Equation 1 was used to generate values of $\delta^{34}SO_3^{2-}$ for different values of α, and $\delta^{34}H_2S$ was calculated by Equation 2. The best value for α was determined as the best fit between measured values and model results and was determined to be 6‰ (Fig. 1C).

Table 2. The metabolic rates and isotope fractionations during bacterial reduction of $S_2O_3^{2-}$.

Culture	$S_2O_3^{2-}$ reduction rate (mM d^{-1})	Δ sulfane - H_2S (‰)	Δ sulfonate - H_2S (‰)	Δ $S_2O_3^{2-}$ - H_2S (‰)
D'vibrio salexigens				
I	4.3	-3	-14	-8.5
II	4.9	-5	-10	-7.5
D'coccus multivorans				
I	0.6	0	-22	-11

Dv. salexigens and *Dco. multivorans* coupled the reduction of $S_2O_3^{2-}$ to H_2S to the oxidation of lactate to acetate and CO_2 (Table 2 and Fig. 2A). The reduction rate was 4.6 ± 0.4 mM d^{-1} for *Dv. salexigens* (average rate from two experiments) and 0.6 mM d^{-1} for *Dco. multivorans*. Both strains grew while reducing $S_2O_3^{2-}$ as the optical density increased by 0.30±0.03nm in both cases (data not shown). The initial $\delta^{34}S$ values were 5.4±1‰ for the inner sulfonate-S ($-SO_3$) of thiosulfate and -0.6±1‰ for the outer sulfane-S ($-S^{2-}$). During the incubation both the sulfonate and sulfane became enriched in ^{34}S (Fig. 2B). Using a Rayleigh distillation model as given above the fractionation during thiosulfate reduction was determined. Values of $\delta^{34}S$ for sulfane and sulfonate were generated independently from Equation 1 at different α-

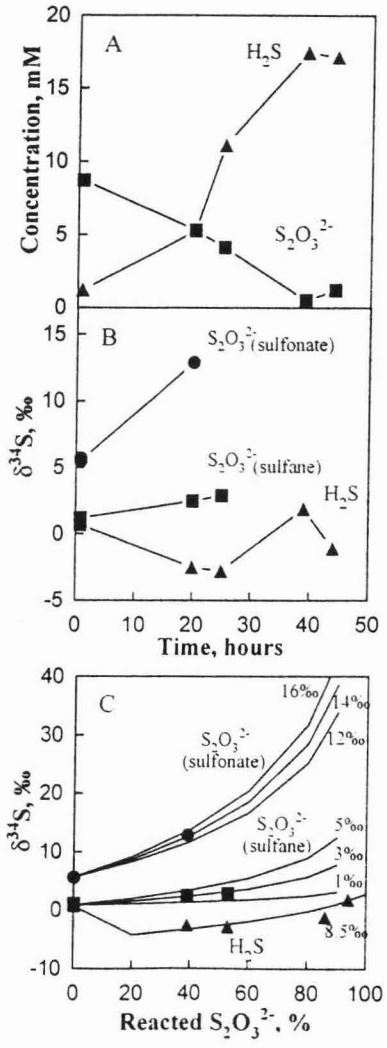

Figure 2. Reduction of $S_2O_3^{2-}$ by *Dv. salexigens*. A: Concentration measurements, B: Stable sulfur isotope values and C: Model lines of $\delta^{34}S$ values obtained by different fractionation values (α) based on the Rayleigh distillation equation. Note the different scales of the X-axes.

values. The modeled $\delta^{34}S$ values for sulfane and sulfonate were used to generate $\delta^{34}S$ values for H_2S using Equation 3:

$$\delta^{34}H_2S = \{[S_2O_3^{2-}]_0 \times (\delta^{34}S1_{(0)} + \delta^{34}S2_{(0)} - fS_2O_3^{2-} \times (\delta^{34}S1 + \delta^{34}S2) + [H_2S]_0 \times \delta^{34}H_2S_{(0)}\} \times [H_2S]^{-1} \quad (3)$$

where $\delta^{34}S1$ refers to the isotopic composition of sulfane and $\delta^{34}S2$ to the isotopic composition of sulfonate, the suffix (0) to their initial values, $[S_2O_3^{2-}]_0$ to the initial concentration of thiosulfate and $fS_2O_3^{2-}$ is the fraction of thiosulfate remaining.

The total fractionation associated with $S_2O_3^{2-}$ reduction to H_2S was 7.5‰ to 8.5‰ for *Dv. salexigens* and 11‰ for *Dco. multivorans* (Fig. 2C and Table 2). These fractionations are the average of the 10‰ to 22‰ fractionation between sulfonate and H_2S and the 0‰ to 5‰ fractionation between sulfane and H_2S. In all the reduction experiments calculated values and the measured values at the same fSO_3^{2-} or $fS_2O_3^{2-}$, respectively, correlated within an average isotope difference of ± 1‰.

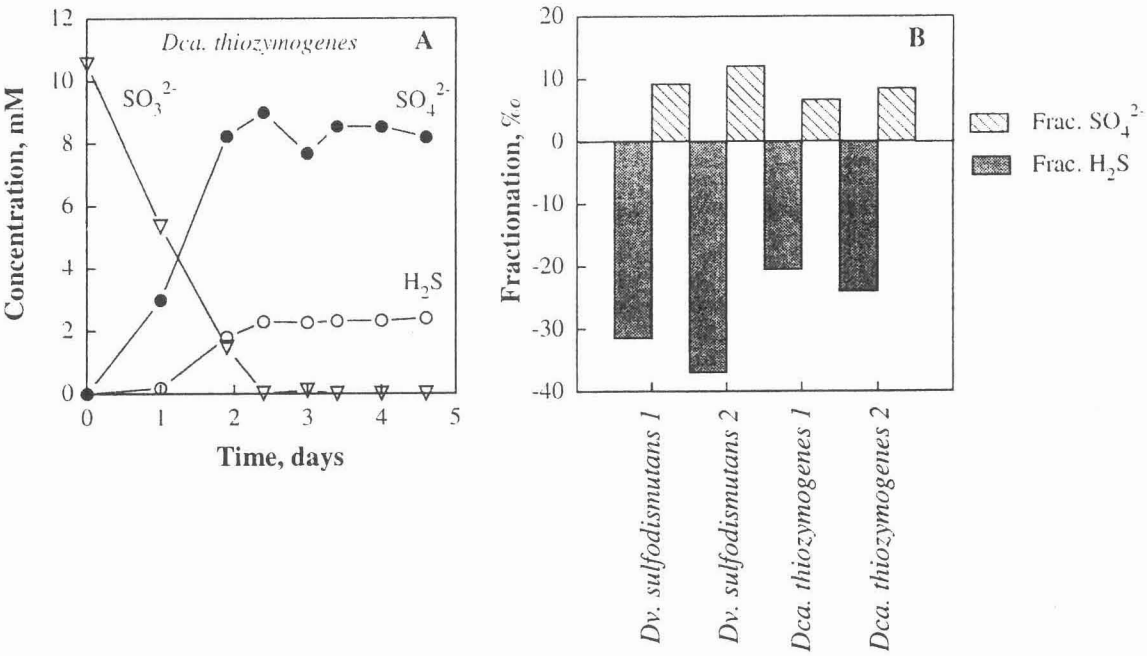

Figure 3. Disproportionation of SO_3^{2-} by *Dca. thiozymogenes* and *Dv. sulfodismutans*. A: Concentration measurements of the SO_3^{2-}-disproportionation by *Dca. thiozymogenes*. B: Isotope fractionation associated the disproportionation of SO_3^{2-} into SO_4^{2-} and H_2S relative to initial $\delta^{34}S$ values of SO_3^{2-}.

Sulfite and thiosulfate disproportionation.

Sulfite and thiosulfate were disproportionated by the freshwater strains *Dv. sulfodismutans* and *Dca. thiozymogenes* as described previously by Bak and Cypionka (1987) and Janssen et al. (1996; Fig. 3A and 4_1). In addition the marine enrichments from Løgten Lagoon, Solar Lake and Weddewarden were all able to disproportionate $S_2O_3^{2-}$ (their ability to disproportionate SO_3^{2-} was not tested). During sulfite-disproportionation, SO_4^{2-} and H_2S were produced at a ratio of about 3:1 and during thiosulfate disproportionation at a ratio of about 1:1 (Table 3, 4). The measured stoichiometries agree with the theoretical stoichiometries of the disproportionation reactions (Table 1).

The rates of SO_3^{2-} and $S_2O_3^{2-}$ disproportionation by *Dv. sulfodismutans* (Table 3, 4) were 3 - 5 times lower than previously measured by Bak and Pfennig (1987) with the same strain under similar conditions. However, in contrast to Bak and Pfennig (1987), we did not add acetate to the medium, and this might explain the low rates in our experiments. *Dv. sulfodismutans* cannot grow autotrophically and needs an organic carbon source (e.g. acetate) for biosynthesis, that is not, however, used as an electron donor. By contrast, *Dca. thiozymogenes* can disproportionate SO_3^{2-} and $S_2O_3^{2-}$ autotrophically and the rates were similar to previous measurements with the same strain (Table 3, 4; Janssen et al. 1996).

In the SO_3^{2-} disproportionation experiments the initial $\delta^{34}S$ value of SO_3^{2-}

Tabel 3. Metabolic rates, isotope fractionation and stoichiometies during bacterial disproportionation of SO_3^{2-}. Standard derivation are obtained from 3 to 4 samples incubated at the same time with the same inoculum (see also Fig. 3).

Culture	SO_3^{2-}-disp. rate (mM d^{-1})	$\Delta\ SO_3^{2-}$ - H_2S (‰)	$\Delta\ SO_4^{2-}$ - SO_3^{2-} (‰)	Prod. ratio sulfate/sulfide
D'vibrio sulfodismutans				
I	0.6	-31.5±1.3	9.2±0.6	3.4±0.3
II	0.2	-37.0±1.4	12.0±1.1	3.1±0.1
D'capsa thiozymogenes				
I	4.4	-20.5±1.3	6.9±0.3	3.0±0.2
II	1.4	-24.0±0.6	8.5±0.2	2.9±0.02

Tabel 4. Metabolic rates, $\delta^{34}S$ values and stoichiomitry during bacterial disproportionation of $S_2O_3^{2-}$. The isotope data of S_{red} (H_2S and sulfane) and S_{ox} (SO_4^{2-} and sulfonate) are the sum of the $\delta^{34}S$ values take the concentrations of the sulfur compounds in acound. The $\delta^{34}S$ differens between H_2S and sulfane and between SO_4^{2-} and sulfonate was measured at the end of the experiment.

Culture	$S_2O_3^{2-}$ disp. rate (mM d^{-1})	ΔS_{red} (end -initial) (‰)	ΔS_{ox} (end -initial) (‰)	ΔH_2S - sulfane (‰)	ΔSO_4^{2-} - sulfonate (‰)	Prod. ratio sulfate/sulfide
Desulfovibrio sulfodismutans						
I	0.4	-0.7	1.1	-2.7	-0.4	1.3
II	0.5	-1.6	0.6	-2.5	-0.7	1.2
III	0.3	-2.1	2.0	-2.6	-0.9	1.2
Desulfocapsa thiozymogenes						
I	1.7	-0.7	0.9	-	-	1.1
II	1.4	-3.2	2.5	-	-	1.1
Løgten Lagoon	0.3	-11.4	11.3	-	-	1.0
Solar Lake	0.3	-11.8	12.7	-	-	1.1
Weddewarden	0.4	-4.7	5.0	-3.9	0.6	1.0

was either 5.1±0.6‰ or -0.7±0.8‰ depending on the batch of Na_2SO_3 used. During SO_3^{2-}-disproportionation by *Dv. sulfodismutans*, sulfate was enriched in ^{34}S by 9‰ to 12‰, and H_2S depleted in ^{34}S by 32‰ to 37‰ compared to the initial isotopic composition of SO_3^{2-} (Fig. 3B and Table 3).

Dca. thiozymogenes enriched SO_4^{2-} in ^{34}S by 7‰ to 9‰ and depleted H_2S in ^{34}S by 21‰ to 24‰ during its disproportionation of SO_3^{2-}. The relative amounts of ^{34}S enrichment into SO_4^{2-}, and depletion into H_2S are in accordance with the stoichiometry of the disproportionation reaction; to maintain isotope mass balance the enrichment of ^{34}S into SO_4^{2-} should be about 1/3 of the depletion of ^{34}S into sulfide. The overall magnitude of fractionation during SO_3^{2-} disproportionation showed an inverse dependency on the disproportionation rate (Fig. 5).

In the $S_2O_3^{2-}$ disproportionation experiments the initial $\delta^{34}S$ value of sulfane S in thiosulfate was -0.3±0.6‰, and the initial $\delta^{34}S$ value of sulfonate S in thiosulfate was 4.8±0.6‰. During disproportionation of $S_2O_3^{2-}$ no (< ±1.0‰) isotope

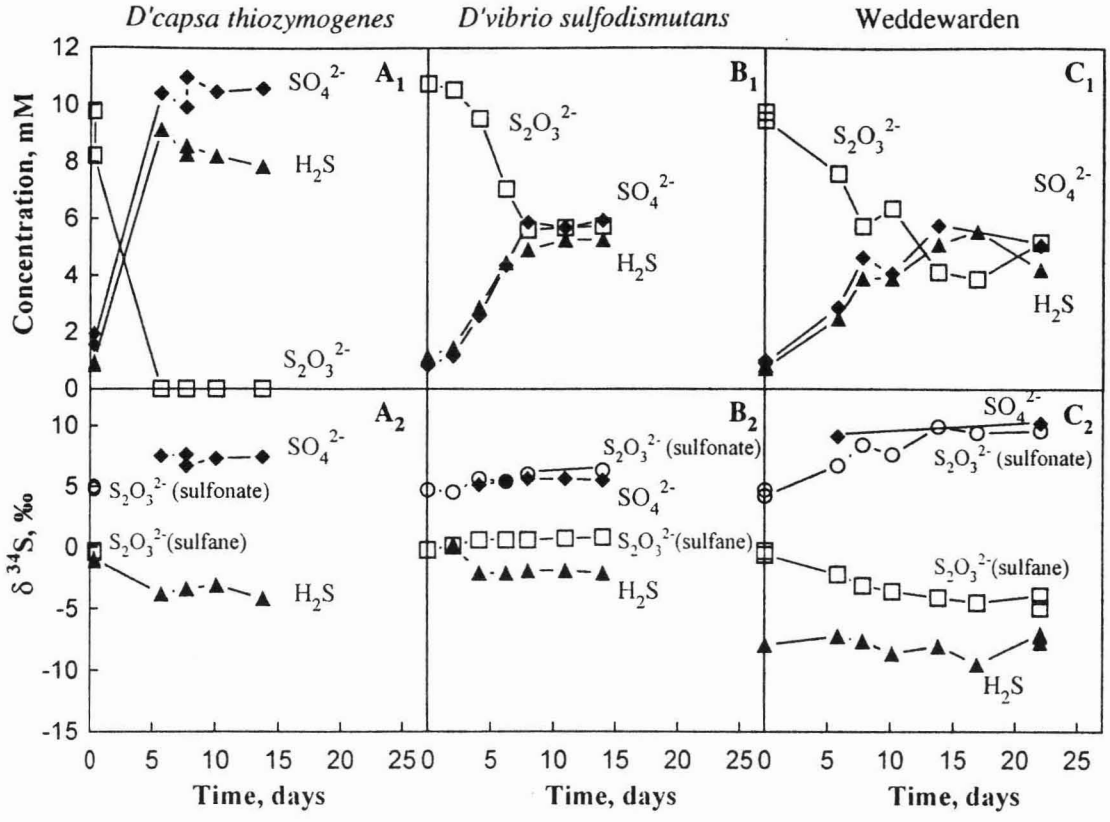

Figure 4. Disproportionation of $S_2O_3^{2-}$ by A: *Dca. thiozymogenes*, B: *Dv. sulfodismutans* and C: Weddewarden enrichment. A_1, B_1, and C_1 are concentration measurements and A_2, B_2 and C_2 are stable isotope data.

fractionation was observed between sulfonate and SO_4^{2-}, whereas H_2S was depleted in ^{34}S by -2‰ and -4‰ compared to sulfane at the end of the incubations (Table 4).

In the pure culture experiments with *Dv. sulfodismutans* and *Dca. thiozymogenes* the reduced sulfur compounds (S_{red} = H_2S + sulfane) were depleted in ^{34}S by 0.7‰ to 3.2‰, and the oxidized sulfur compounds (S_{ox} = SO_4^{2-} + sulfonate) were enriched in ^{34}S by the same amount. Similar trends were measured in the enrichment cultures, where the $\delta^{34}S$ values of the S_{red} pool and the S_{ox} pool changed by 5‰ - 12‰ during the course of incubation. In the isotope mass balance calculation for each $S_2O_3^{2-}$ disproportionation experiment we have been forced to estimate the initial isotopic compositions of either H_2S, SO_4^{2-} or both in some experiments due to low initial concentrations. These estimations have, however, minimal influence on the calculations of the isotope shifts. A good isotope balance for all experiments was achieved when considering the mass balance of the S_{red} and S_{ox} pools (± 0.5‰).

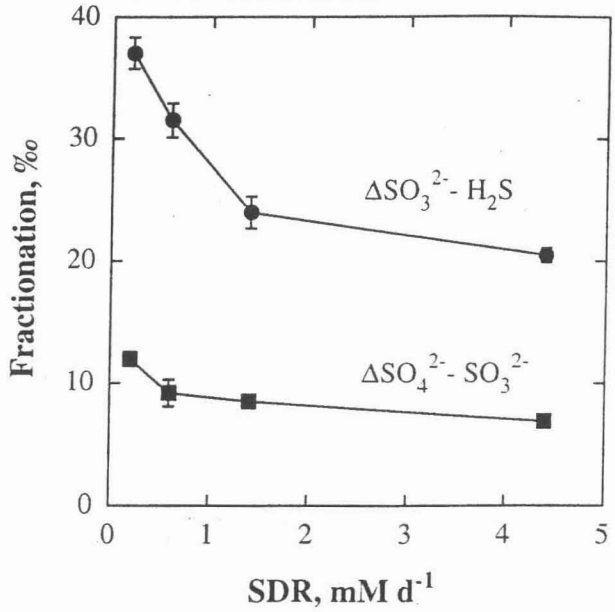

Figure 5. Isotope fractionation associated disproportionation of SO_3^{2-} at different SO_3^{2-}-disproportionation rates (SDR)

Bacterial oxidation of sulfide to thiosulfate.

During anoxygenic photosynthesis, H_2S was oxidized to $S_2O_3^{2-}$ by *Chalothrix sp.* and *Oscillatoria sp.* as previously described by Rabenstein and coworkers (1995). The recovery of added H_2S as $S_2O_3^{2-}$ varied between 70% to 100%. Due to the low H_2S tolerance of the bacteria, the yield of $S_2O_3^{2-}$ per bottle was low (about 90 µmol), and to obtain enough $S_2O_3^{2-}$ (> 100 µmol) for isotope analysis, we pooled some of the reaction bottles. The isotope values showed very little fractionation with no clear

Table 5. Isotope fractionation associated anaerobe oxidation of H_2S to $S_2O_3^{2-}$ by cyanobacteria. Between 70% to 100% of the H_2S oxidized was found as $S_2O_3^{2-}$ sulfur at the end of the experiments. Standard derivation were obtained from 3 - 5 samples.

Culture	$\delta^{34}SH_2S$, (‰)	$\delta^{34}S$sulfane, (‰)	$\delta^{34}S$sulfonate, (‰)
Chalothrix sp.			
I	14.2±1.9	16.1	na.
II	15.7±0.3	17.0±2.5	12.9±0.8
III	14.7	11.7±2.9	16.5
Oscillatoria sp.			
I	15.4±0.3	14.3±1.0	13.5±0.2
Average	15.0±1.0	14.5±2.8	13.8±1.4

na: not analyzed

trend, and taking all data together, no significant fractionation between H_2S and sulfane or between H_2S and sulfonate occurred during the bacterial oxidation of H_2S to $S_2O_3^{2-}$ (Table 5).

DISCUSSION

Influence of system closure and rates on fractionation.

During the dissimilatory reduction of SO_3^{2-} and $S_2O_3^{2-}$ to H_2S, the $\delta^{34}S$ values of all the sulfur compounds increased during the course of incubation consistent with that expected in a closed-system (Fig. 1 and 2). Thus, in a closed system, with no external supply or sink of sulfur, the $\delta^{34}S$ of H_2S will be the same as the initial $\delta^{34}S$ values of SO_3^{2-} and $S_2O_3^{2-}$, after total consumption of the electron acceptors (Fig. 1C and 2C). No net isotope fractionation can therefore be measured after all of the sulfur from either SO_3^{2-} or $S_2O_3^{2-}$ is transferred to H_2S. During the disproportionation of SO_3^{2-} by *Dv. sulfodismutans* and *Dca. thiozymogenes*, however, large isotope fractionations between H_2S and SO_3^{2-} and between SO_4^{2-} and SO_3^{2-} were measured although SO_3^{2-} was consumed to depletion (Fig. 3, Table 3). In the disproportionation reaction both H_2S and SO_4^{2-} are produced at the same time from the same precursor compound and do not follow a Rayleigh distillation model as for the reduction pathways. The depletion of ^{34}S into H_2S during the disproportionation of SO_3^{2-} is, therefore, not appreciably influenced by the extent to which the system becomes closed, as opposed to SO_3^{2-} and $S_2O_3^{2-}$ reduction. Isotope fractionation during elemental sulfur disproportionation has been shown to be also fully expressed in a closed system (Canfield and Thamdrup 1994; Canfield et al. in press).

The degree of fractionation associated with the disproportionation of SO_3^{2-} was correlated with the rate of SO_3^{2-} disproportionation; the highest fractionations between both SO_3^{2-} and H_2S and between SO_3^{2-} and SO_4^{2-} were associated with the lowest disproportionation rate (Fig. 5). However, at high rates of SO_3^{2-}

disproportionation the fractionation depended less on the rate, suggesting that a minimum fractionation might be approached. The same optical density of 0.05±0.02nm was measured at the end of all of the SO_3^{2-} disproportionation experiments implying that the bacterial population size were the same at the completion of the experiments. This means that the volume-based rate of sulfite disproportionation (mM d^{-1}), as we have measured here, correlate with the specific rates of disproportionation (mM d^{-1} $cell^{-1}$), which are equivalent to the volume based rates divided by the bacterial population density. The extent of isotope fractionation during sulfite and sulfate reduction has been shown to correlate with the specific rates (Harrison and Thode 1957; Kaplan and Rittenberg 1964; Chambers et al. 1975). The extent of isotope fractionation during sulfite disproportionation may also be influenced by the specific rate of disproportionation.

Models for fractionation during SO_3^{2-} and $S_2O_3^{2-}$ reduction and disproportionation.

To obtain a better understanding of the isotope values measured during the reduction and disproportionation of $S_2O_3^{2-}$ and SO_3^{2-}, we will examine the fractionations in terms of the biochemistry of the sulfur transformation pathways. Many sulfate-reducing bacteria have been shown to both reduce and disproportionate $S_2O_3^{2-}$ and SO_3^{2-}, and these reactions have been related to the sulfate reduction pathway (Krämer and Cypionka 1989; Cypionka 1995). Even though the details of the pathways are not completely known, we will attempt to understand the observed fractionations based on the information presented to date. The following reactions will be discussed:

Sulfate reduction:

$$SO_4^{2-} \rightleftharpoons APS \rightleftharpoons SO_3^{2-} \longrightarrow H_2S \quad (1)$$

Sulfite and thiosulfate reduction:

$$SO_3^{2-} \longrightarrow H_2S \quad (2)$$

$$S_2O_3^{2-} \rightleftharpoons H_2S + SO_3^{2-} \longrightarrow H_2S \quad (3)$$

Thiosulfate disproportionation:

$$S_2O_3^{2-} \rightleftharpoons H_2S + SO_3^{2-} \quad (4a)$$

$$SO_3^{2-} \rightleftharpoons APS \rightleftharpoons SO_4^{2-} \quad (4b)$$

$$\text{Sum: } S_2O_3^{2-} \rightleftharpoons H_2S + SO_4^{2-} \quad (4)$$

Sulfite disproportionation:

$$SO_3^{2-} \longrightarrow H_2S \quad (5a)$$

$$3SO_3^{2-} \rightleftharpoons APS \rightleftharpoons 3SO_4^{2-} \quad (5b)$$

$$\text{Sum: } 4SO_3^{2-} \rightleftharpoons H_2S + 3SO_4^{2-} \quad (5)$$

Three main biochemical pathways have been proposed to control the reduction of SO_4^{2-}, SO_3^{2-} and $S_2O_3^{2-}$, and the disproportionation of SO_3^{2-} and $S_2O_3^{2-}$:

1. The forward or reverse transformations between SO_4^{2-} and SO_3^{2-} (Reaction 1, 4b and 5b). During dissimilatory sulfate reduction, sulfate is first activated by consumption of ATP to form APS (adenosinphoshosulfate) and pyrophosphate by ATP sulfurylase. APS is further reduced to SO_3^{2-} in a reaction catalyzed by APS reductase (e. g. Hansen 1994). In the disproportionation pathways a reversal of sulfate reduction with SO_3^{2-} forming SO_4^{2-} through APS has been proposed (Krämer and Cypionka 1989).

2. The reduction of SO_3^{2-} to H_2S catalyzed by sulfite reductase. This step is included in reactions 1, 2, 3 and 5a (Bak and Cypionka 1987; Krämer and Cypionka 1989; Hansen 1994; Cypionka 1995).

3. The reduction of $S_2O_3^{2-}$ to H_2S and SO_3^{2-} catalyzed by thiosulfate reductase. This reaction occurs during the reduction and disproportionation of $S_2O_3^{2-}$ (Reaction 3 and 4a; Krämer and Cypionka 1989; Cypionka 1995).

There are several steps in these different pathways where a fractionation is possible. The overall isotope fractionation will depend on which steps in the pathway have associated fractionations, and which are rate-limiting, with the overall fractionation being the sum of the kinetic isotope effects from each step until the rate-limiting reaction (Rees 1973). In sulfate-reducing bacteria SO_4^{2-}, SO_3^{2-} and $S_2O_3^{2-}$ are reduced and disproportionated inside the cell (Cypionka 1995). Low fractionations (3‰) have been assigned the uptake of SO_4^{2-} by the bacteria. The uptake of $S_2O_3^{2-}$ and SO_3^{2-} might also be accompanied by similar low fractionations as these sulfur compounds are structurally similar to SO_4^{2-}. Furthermore, a study by Stahlmann and coworkers (1991) demonstrated that different freshwater and marine sulfate reducers were unable to distinguish between $S_2O_3^{2-}$ and SO_4^{2} during the uptake of these compounds.

In the sulfate reduction pathway, no fractionation occurs during the production of APS (Reaction 1). Instead, fractionations of 25‰ have been proposed for the reduction of APS to SO_3^{2-} and a similar fractionation of 25‰ has been proposed for the reduction of SO_3^{2-} to H_2S (Rees, 1973; Harrison and Thode, 1958). In both of these steps fractionations occur during the splitting of S—O bounds. A maximum isotope fractionation of about 25‰ might also be expected during the dissimilatory reduction of SO_3^{2-} (Reaction 2). In previos pure culture studies of sulfate-reducing bacteria the fractionations during SO_3^{2-} reduction ranged between 0‰ to maximum values of 25‰ to 33‰, and in the range of the theoretical value (Kemp and Thode 1968, Kaplan and Rittenberg 1964).

In our experiments we only measured a fractionation of 6‰ during sulfite reduction by *Dv. salexigens*; this value is lower than the 25‰ that might be expected. By analog with sulfate reduction, isotope fractionation during SO_3^{2-} reduction s hould

be reduced if the supply of SO_3^{2-} is rate determining such as might occur at high specific rates of SO_3^{2-} reduction (rate per cell) or at low SO_3^{2-} concentrations (Rees 1973). Diminished fractionation values have been measured during dissimilatory sulfate reduction at SO_4^{2-} concentrations below 1 mM (Harrison and Thode 1957). At the high concentrations of SO_3^{2-} used here (about 10 mM) we would not expect SO_3^{2-} to be limiting in concentration. It is possible that our experimental cultures maintained high specific rates of sulfite reduction. We did not, however, count cell numbers (we have instead measured optical density), and therefore we cannot compare our experimental results to the specific rates of sulfite reduction measured in previous experiments (Harrison and Thode 1957; Kaplan and Rittenberg 1964; Kemp and Thode 1968).

During the reduction of $S_2O_3^{2-}$ we observed that the largest fractionations of 10‰ to 22‰ were associated with the reduction of sulfonate to H_2S, while the reduction of sulfane to H_2S was associated with fractionations of only 0‰ to 5‰ (Table 2). The large fractionation between sulfonate and H_2S might arise during the SO_3^{2-} reduction step (Reaction 3) and a maximum fractionation of 25‰ could thus, be expected. Lower fractionations than this could arise from thiosulfate limitation within the cell, by analogy with sulfite and sulfate reduction as discussed above.

The fractionation of 0‰ to 5‰ between sulfane and H_2S can be attributed to the splitting of $S_2O_3^{2-}$ into H_2S and SO_3^{2-} (Reaction 3). During the disproportionation of $S_2O_3^{2-}$ a similar fractionation of 2‰ to 4‰ was measured between sulfane and H_2S which also could be related to the splitting of $S_2O_3^{2-}$ to H_2S and SO_3^{2-} (Table 4; Reaction 4a). The lack of a fractionation between sulfonate and sulfate in the $S_2O_3^{2-}$ disproportionation experiments implies that no fractionation is associated with the formation of SO_4^{2-} from SO_3^{2-} (Reaction 4b). This is in agreement with most results from previous studies, and results presented below, that only small fractionations are associated with the oxidation of sulfur compounds (Rees 1973; Fry et al. 1986).

In the SO_3^{2-} disproportionation experiments, large isotope differences were observed between the isotopic compositions of both SO_3^{2-} and H_2S and between

SO_3^{2-} and SO_4^{2-} (Table 3, Reaction 5a and 5b). In the discussion above we have ascribed a fractionation only to the SO_3^{2-} reduction step, whereas no fractionation is indicated during the formation of SO_4^{2-} from SO_3^{2-} in our $S_2O_3^{2-}$ disproportionation experiments. The isotope difference that we have measured between SO_3^{2-} and SO_4^{2-} might therefore be controlled by the SO_3^{2-} reduction step, and arise from the necessity of isotope balance. Thus, if the cell is viewed as a flow-through chemostate system, the production of ^{34}S-depleted sulfide from SO_3^{2-} (eqn. 5a) will leave the remaining SO_3^{2-} - subject to oxidation - enriched in ^{34}S (eqn. 5b).

In the SO_3^{2-}-disproportionation experiments using *Dca. thiozymogenes*, H_2S was depleted in ^{34}S by between 20‰ to 25‰ compared to SO_3^{2-} (Table 3). This fractionation is in the range proposed for the SO_3^{2-} reduction step (25‰). However, in the experiments with *Dv. sulfodismutans* the fractionation between SO_3^{2-} and H_2S varied between 31‰ to 37‰ which is higher than can apparently be explained by the simple breaking of S–O bonds. Thus, more information is needed on the pathways involved in the disproportionation of SO_3^{2-} for a better explanation of the biochemical aspects of the observed isotope data.

In the $S_2O_3^{2-}$ disproportionation experiments the $\delta^{34}S$ values of the sulfur compounds of H_2S, SO_4^{2-} and $S_2O_3^{2-}$ showed an unexpected development through the course of incubation. While only small fractionations of 0‰ to 4‰ were observed as sulfane S was transformed to H_2S and sulfonate S to sulfate (Table 4; Fig. 4), the reduced sulfur compounds (S_{red} = H_2S + sulfane) became depleted in ^{34}S by up to 11.8‰ with the oxidized sulfur compounds (S_{ox} = SO_4^{2-} + sulfonate) enriched in ^{34}S by the same amount (Table 4). Changes in the isotopic composition of the S_{red} and the S_{ox} pools are thus contrary to expectations. One would expect that during the disproportionation of $S_2O_3^{2-}$, as envisioned by equation 4, the isotopic compositions of the S_{red} pool and the S_{ox} pool should remain unchanged. This is because no chemical transformations between the S_{red} pool and the S_{ox} pool are expected. Thus our date indicate either an unexpected reaction path for $S_2O_3^{2-}$ disproportionation, or an isotope exchange reaction between the S_{red} pool and the S_{ox} pool during

disproportionation. A possible alternative reaction path (though unexpected) could be the reduction of sulfonate to H_2S. Such a reaction would, however, show a closed-system Rayleigh distillation path, and the $\delta^{34}S$ values of both sulfonate and H_2S should increase during consumption of $S_2O_3^{2-}$. Such a trend was not observed. Instead an isotope exchange reaction between sulfane-S and sulfonate-S of $S_2O_3^{2-}$ during $S_2O_3^{2-}$ disproportionation would explain the isotope results. The best evidence for possible isotope exchange was observed in the Weddewarden experiment where the largest ^{34}S-enrichments in the S_{ox} pool and ^{34}S-depletions in the S_{red} pool were observed (Fig 4C).

A non-bacterial isotope exchange between sulfane and sulfonate has been measured at temperatures above 100°C (Uyama et al. 1985). Thus, at equilibrium, the isotope difference between sulfane and sulfonate was measured to be 35‰ at 120°C decreasing to 29‰ at 170°C. An even greater isotope difference would be expected at equilibrium at the temperature of our experiments (30°C). We note that isotope exchange in the inorganic systems only occurred when H_2S was present in the solution. In our experiments H_2S was always present. We speculated that the low temperature intra-molecular isotope exchange between the S atoms of $S_2O_3^{2-}$ in our experiments is related to bacterial activity. We propose that the isotope exchange is catalyzed by bacterial activity because isotope exchange in inorganic reaction systems was not observed below 100°C (Uyama et al. 1985). In support of this, when the bacteria stopped disproportionating $S_2O_3^{2-}$, no further isotope exchange was measured even though $S_2O_3^{2-}$ still was present in the solution (after 7 days with *Dv. sulfodismutans* and 13 days with Weddewarden enrichment; Fig. 4). Also in marine sediments an intra-molecular exchange of ^{35}S in $S_2O_3^{2-}$ has been observed at 25°C (Bo Barker Jørgensen personal communication). In the latter sediment experiments the effect of isotope exchange was inversely dependent on the consumption rate of $S_2O_3^{2-}$, but also depended on the locality from which the sediment was sampled.

We measured the largest $\delta^{34}S$ isotope difference between sulfane and sulfonate during $S_2O_3^{2-}$ disproportionation by the enrichment cultures (Table 4). In

these experiments there was a correlation between the number of repeated transfers of the culture before the experiment began and the degree of isotope difference between S_{ox} and S_{red} pools. The enrichments from Solar Lake and Løgten Lagoon were transferred 5 times before the experiments began, and in these experiments we measured the largest isotope difference of 12‰. In the Weddewarden experiment the culture was transferred 10 times and here we measured an isotope difference between the initial and final isotope compositions of the S_{red} and S_{ox} pools of 5‰. The lowest isotope difference was found in the pure culture experiments. One explanation for these trends is that some bacteria better catalyze an isotope exchange between sulfane and sulfonate than others, and that these bacteria are gradually deselected or diluted during enrichment and isolation. We also note that the enrichment cultures are marine, whereas the pure cultures are freshwater strains, providing, possibly, further evidence that the extent of isotope exchange is related to the bacterial culture used.

Fractionations during phototrophic $S_2O_3^{2-}$ formation.

The pathway for the phototrophic oxidation of H_2S to $S_2O_3^{2-}$ by the cyanobacteria *Calothrix sp.* and *Oscillatoria sp.* is totally different from the pathways of reduction and disproportionation of $S_2O_3^{2-}$ and SO_3^{2-}. Cyanobacteria normally perform oxygenic photosynthesis using water as electron donor. However, in the presence of sulfide, oxygenic photosynthesis is inhibited and some cyanobacteria can, under such conditions, perform anaerobic photosynthesis by oxidation of sulfide to $S_2O_3^{2-}$ using Photosystem I (de Wit and van Germenden 1987; Rabenstein et al. 1995). No fractionation between H_2S and sulfane and between H_2S and sulfonate was measured during the phototrophic oxidation of H_2S to $S_2O_3^{2-}$ (Table 5). This result was surprising and showed that the sulfane and sulfonate atoms in $S_2O_3^{2-}$ formed far outside of equilibration during $S_2O_3^{2-}$ formation. A fractionation of > 35‰ would be expected under equilibrium conditions (Uyama et al. 1985). Weather or not the small isotope difference between sulfane S and sulfonate S that we observed during H_2S oxidation applies to all $S_2O_3^{2-}$ formations pathways is not clear. We were particularly

interested in $S_2O_3^{2-}$ formation, because a large isotope difference between sulfane and sulfonate S during $S_2O_3^{2-}$ formation, could induce large isotope differences between the H_2S and sulfate formed during subsequent $S_2O_3^{2-}$ disproportionation. This could be true even if the disproportionation processes itself produced only small fractionations.

Sulfur fractionation in the sulfur cycle.

Before we discuss our results in terms of the sulfur isotope data measured in natural environments, we wish to summarize the isotope fractionation measurements in this study, augmented with data from previous studies (Fig. 6). The sulfide produced during dissimilatory sulfate reduction is the primary sulfur supply in the formation of sedimentary sulfides. The fractionations measured during this reaction range between 5‰ to 46‰, with an average value of about 23‰ (Canfield and Teske 1996; Habicht and Canfield, Manuscript 2). During the reduction of $S_2O_3^{2-}$ we

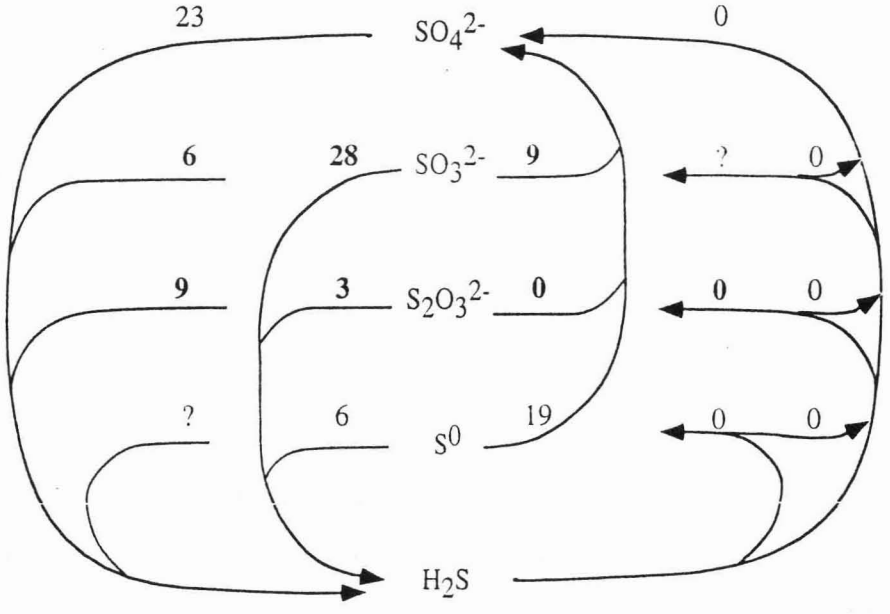

Figure 6. The sulfur cycle through S^0, $S_2O_3^{2-}$ and SO_3^{2-}. The values indicate average sulfur fractionations associated with either oxidation, reduction (outer cycles) or disproportionation (inner cycle) pathways. Bold values are from this study, the rest are literature values (see text for references).

measured an isotope fractionation of about 9‰ and during the reduction of SO_3^{2-} a fractionation of 6‰ was found. In other studies fractionations between 0‰ to 33‰ have been measured during sulfite reduction (Kemp and Thode 1968). We measured no isotope fractionation during the phototrophic oxidation of H_2S to $S_2O_3^{2-}$ by cyanobacteria. Only small (± 5‰) isotope fractionation have been found to accompany anaerobic photosynthesis by the purple sulfur bacteria *Ectothiorhodospira shaposhnikovii* and *Chromatium sp.* using H_2S, S^0, $S_2O_3^{2-}$ and SO_3^{2-} as electron donors forming either S^0 or SO_4^{2-} (Ivanov et al. 1976; Fry et al. 1985). Further, only small fractionations generally accompany the aerobic oxidation of H_2S, S^0, $S_2O_3^{2-}$ and SO_3^{2-} to either S^0 and SO_4^{2-} (Fry et al. 1986). During the disproportionation of SO_3^{2-} we measured an average isotope fractionation of 28‰ between SO_3^{2-} and H_2S, and 9‰ between SO_3^{2-} and SO_4^{2-}. An isotope fractionation has previously also been measured during the disproportionation of S^0 with average values of 6‰ between S^0 and H_2S and 19‰ between S^0 and SO_4^{2-} (Canfield and Thamdrup 1994; Canfield et al. in press). Finally we found, small isotope fractionations (< 4‰) during the disproportionation of $S_2O_3^{2-}$. However, as a result of an isotope exchange reaction between the two sulfur compounds of $S_2O_3^{2-}$, H_2S could be depleted in ^{34}S by up to 12‰ and SO_4^{2-} enriched in ^{34}S by the same amount during $S_2O_3^{2-}$ disproportionation. The only sulfur transformations in Figure 6 for which no fractionations have been determined is the reduction of S^0 and the oxidation of reduced sulfur compounds producing SO_3^{2-}. Following the reasoning presented above, large fractionations would not be expected for the oxidation pathway.

Sulfur fractionation in natural environments.

In modern marine sediments and euxinic waters, reduced sulfur compounds (mainly measured in FeS_2) are depleted in ^{34}S by up to 70‰ compared to seawater sulfate (Canfield and Teske, 1996). The activity of sulfate-reducing bacteria is an important regulator of this ^{34}S depletion. However, the fractionations measured during sulfate reduction by natural populations and pure cultures of sulfate reducers only

range up to 46‰, and cannot, therefore, explain the large ^{34}S depletions of sedimentary sulfides (Canfield and Teske 1996; Habicht and Canfield, Manuscript 2). Experimental studies have demonstrated that additional fractionations might be related to the oxidative part of the sulfur cycle (Canfield and Thamdrup 1994).

Many studies of sulfur oxidation pathways in marine sediments have focused on the transformation through $S_2O_3^{2-}$ (Jørgensen 1990; Jørgensen and Bak 1991; Elsgaard and Jørgensen 1992; Fossing and Jørgensen 1990). The *in situ* concentrations of $S_2O_3^{2-}$ normally ranges between < 0.5 µM to 10 µM (Visscher et al. 1992; Thamdrup et al. 1994) although concentrations up to 1 mM have been reported for salt marsh sediments (Luther et al. 1991). The highest concentrations of $S_2O_3^{2-}$ are mainly found in the oxic and suboxic sediment layers, where the highest amount of H_2S oxidation is expected (Troelsen and Jørgensen 1982; Bak and Pfennig 1991; Visscher et al. 1992). Sulfide can however, also be oxidized to $S_2O_3^{2-}$ in reduced sediment layers (Jørgensen and Bak 1991). Further transformations of $S_2O_3^{2-}$ can occur by either oxidation, reduction or disproportionation (Fossing and Jørgensen 1990; Jørgensen and Bak 1991). No significant ^{34}S depletion of H_2S was associated with the bacterial production of $S_2O_3^{2-}$ in our experiments, and during the disproportionation of $S_2O_3^{2-}$ a large depletion of H_2S in ^{34}S was only measured when an isotope exchange reaction between sulfane and sulfonate occurred. During $S_2O_3^{2-}$ disproportionation the most significant isotope exchange was measured in the marine cultures that were the least diluted from the original sediment. This observation might suggest that an isotope exchange during $S_2O_3^{2-}$ disproportionation could occur in the sediments and contribute to a depletion in ^{34}S of sedimentary sulfides.

We measured an isotope fractionation during reduction of $S_2O_3^{2-}$. The *in situ* concentrations of $S_2O_3^{2-}$ are generally much lower than in our experiments, where they were up to 10 mM. Diminished fractionation values could be associated with the reduction pathway at very low concentrations as observed for SO_4^{2-} at concentrations below 1 mM (Harrison and Thode 1957). Also, $S_2O_3^{2-}$ might be completely consumed, due to the low concentrations and rapid turn-over times (e.g. Jørgensen

1990; Jørgensen and Bak 1991). These circumstances might approximate a "cloced-system" for thiosulfate where net fractionations resulting from thiosulfate reduction would be low.

The significance of sulfur transformation through SO_3^{2-} in marine sediments and euxinic waters is not yet known. The *in situ* concentrations of SO_3^{2-} are, like $S_2O_3^{2-}$, normally below 10 µM (Thamdrup et al. 1994). Lower fractionation values than measured in our study could therefore also be associated with SO_3^{2-} reduction as explained above for $S_2O_3^{2-}$ reduction. Sulfur fractionation during the disproportionation of SO_3^{2-} is not influenced by a Rayleigh distillation effect and a large isotope fractionation was measured into H_2S, even after total consumption of SO_3^{2-}. Although, we worked with SO_3^{2-} concentrations far above *in situ* levels, we have no reason to believe that the isotope fractionations accompanying the disproportionation of SO_3^{2-} will be influenced by low *in situ* concentrations. The disproportionation of SO_3^{2-} could therefore be an important pathway by which additional ^{34}S depletion in sedimentary sulfides and in sulfidic waters might be explained. These results give further evidence that H_2S initially depleted in ^{34}S during sulfate reduction could be additionally depleted during the oxidative sulfur cycle (see also Canfield and Thamdrup, 1994).

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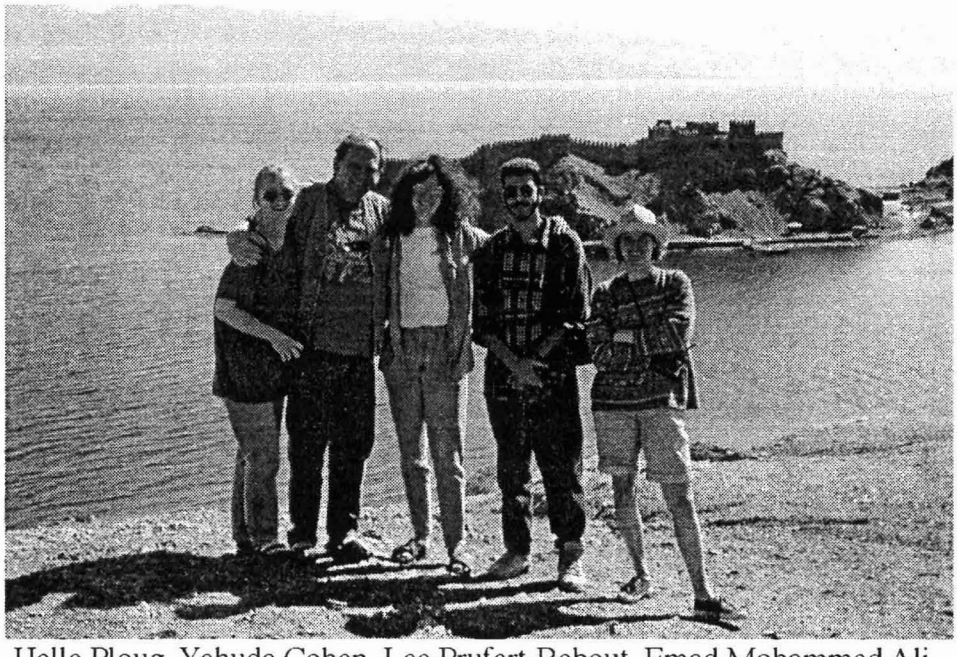

Helle Ploug, Yehuda Cohen, Lee Prufert-Bebout, Emad Mohammed Ali, and the author at the Red Sea.

SUMMARY

In this study, the sulfur fractionation during bacterial transformation of SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$ and S^0 has been quantified to provide a better understanding of the stable isotope data available from modern and ancient sediments.

For the first time, a detailed study of the sulfur fractionation during sulfate reduction by natural populations of sulfate-reducing bacteria is reported. The isotope fractionation by the natural populations was inversely related with the sulfate reduction rate (rate per volume sediment) and showed similar trends as previously measured by pure culture, where the fractionation was related to the specific rate of sulfate reduction (rate per cell). The isotope fractionation in sediments likely depends on the specific rate of sulfate reduction and not the absolute rate. While absolute rates of sulfate reduction could vary over a larger range, a large variation of the specific rate of sulfate reduction might not occur in sediments as the bacteria population size should adjust to substrate availability. Similar fractionation values as measured in this study can therefore be expected during sulfate reduction in any kind of sediment.

The sedimentary stable sulfur isotope values from the Archean and early Proterozoic are around 0‰. Interpretation of this minimal fractionation has previously been equivocal. It has been proposed that the small isotope effect could result from bacterial sulfate reduction in an Archean ocean containing both low or high concentrations of SO_4^{2-} (Cameron 1982; Ohmoto et al. 1993). High rates of sulfate reduction were proposed to give the diminished isotope values of sedimentary sulfides at high SO_4^{2-} concentrations. To distinguish between the two models, the isotope fractionation by a natural population of sulfate reducers was measured at some of the highest rates of sulfate reduction ever reported from natural systems. At SO_4^{2-} concentrations above 13 mM, isotope fractionations were least 20‰, independent of the rate. Small isotope fractionations associated with active sulfate reduction in an Archean ocean rich in sulfate are therefore not in agreement with our results. Instead, the Archean ocean may have had a low sulfate concentration. The first increase of

sulfate in the early ocean could possibly be related to the first large negative isotope shift in the sedimentary sulfides at 2.2 to 2.3 billion years (Cameron 1982). This correlates with the first large burial puls of organic matter which should release oxidants like Fe(III), SO_4^{2-} and O_2 to the ocean (Des Marais et al. 1992). The isotope fractionation between sulfide and sulfate in the Precambrian was however never higher than 35 to 40‰ (Fig. 1, p. 4). The early oxygenation of the Earth's oceans might therefore only represent the first stage in the build-up of O_2 to present-day levels (Canfield 1996). A second main event in the history of the stable sulfur isotopes is a further increase in the maximum sulfide fractionation to levels of 55‰ or more compared to seawater sulfate at around 0.8 billion years (Canfield and Teske 1996). This was associated with another carbon burial puls (Des Marais et al. 1992). This large sulfur isotope shift might be related to a promotion of the oxidative sulfur cycle as the sulfur isotope data are similar to what we see to day. During the course of this study, it has been proposed that O_2 at a concentration of at least 5% to 20% of the present level accumulated in the atmosphere at around 0.8 billion years (Canfield and Teske 1996).

In modern sediments the highest isotope fractionation during sulfate reduction by natural populations of sulfate reducers was about 40‰, which is generally not enough to explain the fractionation between $\delta^{34}S$ of the reduced sulfur compounds and seawater sulfate. Additional fractionation must therefor occur, and might be related to the oxidative part of the sulfur cycle. The sulfur fractionation during transformations of sulfur compounds which are believed to be important in the oxidative sulfur cycle was therefore measured. With the exception of SO_3^{2-} reduction, this is the first report on the fractionation connected with transformations of $S_2O_3^{2-}$ and SO_3^{2-}. An isotope fractionation of -6‰ to -22‰ occurred during reduction of $S_2O_3^{2-}$ and SO_3^{2-}, and during disproportionation of SO_3^{2-},sulfide was depleted in ^{34}S by about 28‰ and sulfate enriched in ^{34}S by 9‰. No isotope fractionation was associated with disproportionation of $S_2O_3^{2-}$ or with the bacterial oxidation of H_2S to $S_2O_3^{2-}$. Furthermore, an isotope exchange reaction between the two sulfur atoms of

$S_2O_3^{2-}$ was measured during the disproportionation of $S_2O_3^{2-}$. It was found that enrichment cultures from the microbial mats of Solar Lake and the sediments of Løgten Lagoon sulfuretum were able to disproportionate S^0. The large ^{34}S depletion in sedimentary sulfides could be explained by repeated cycling through S^0, $S_2O_3^{2-}$ and SO_3^{2-}. In sediments, the concentrations of $S_2O_3^{2-}$ and SO_3^{2-} are small and this should lead to diminished isotope effects during the reduction pathways. However, the fractionation associated with the disproportionation of SO_3^{2-} was independent on the SO_3^{2-} concentration, and this pathway might together with the disproportionation of S^0 be important in the explanation of additional isotope effects in the oxidative part of the sulfur cycle.

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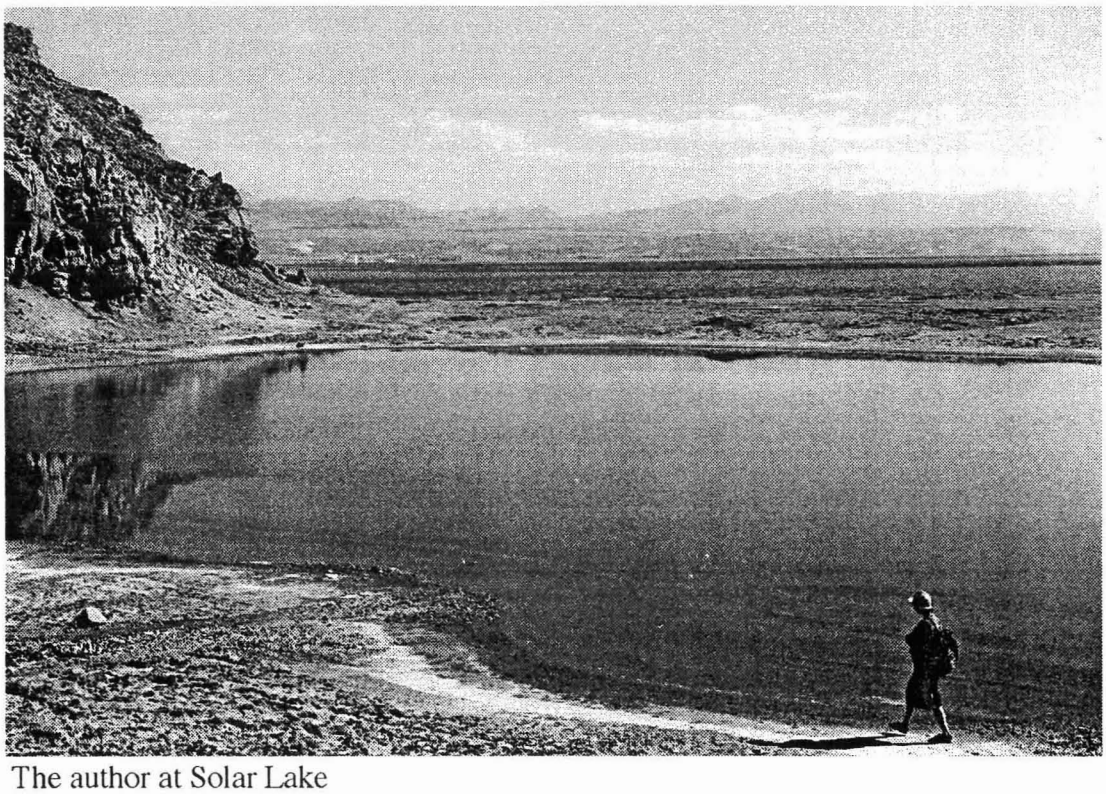

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