Hypersulfidic deep biosphere indicates extreme sulfur isotope fractionation during single-step microbial sulfate reduction

Ulrich G. Wortmann
GEOMAR Research Center for Marine Geosciences, Wiscöffstrasse I-3, D-24148 Kiel, Germany
Stefano M. Bemascioni
Geological Institute, ETH-Zürich, CH-8093 Zürich, Switzerland
Michael E. Böttcher
Max-Planck Institute for Marine Microbiology, Department of Biogeochemistry, Celsiusstrasse 1, D-28359 Bremen, Germany

ABSTRACT
Coexisting dissolved sulfide and sulfate from hypersulfidic interstitial waters of a 380-m-long sediment core show a large isotopic difference of up to 72%o caused by in situ microbial sulfate reduction. This is considerably larger than the assumed biological maximum of 46%o derived from laboratory studies with pure cultures of sulfate-reducing bacteria. Similar high fractionalations inferred from sedimentary metal sulfides have been previously explained by a multistage process, involving sulfate reoxidation and disproportionation of sulfur intermediates. Our data show that extreme isotopic differences between sulfate and the reduced sulfur species can also be generated during microbial single-step fractionation. This result indicates that the sulfate-reducing communities and/or their cellular metabolic activities in the deep biosphere may differ from those observed in near-surface sediments or the water column.

Keywords: isotope geochemistry, biogeochemistry, bacteria, continental margin, ore-forming fluids, sulfur isotopes.

INTRODUCTION
Bacterial sulfate reduction is the major pathway of organic matter oxidation in coastal-marine and continental-shelf sediments (Jorgensen, 1982b) and is a fundamental process linking the geochemical cycles of carbon, sulfur, and oxygen (Berner, 1982; Garrels and Lerman, 1984). The activity of sulfate-reducing bacteria induces a fractionation of the stable sulfur isotopes 34S and 32S (Chambers and Trudinger, 1979), resulting in a depletion of 32S in the sulfate, and enrichment of 32S in the produced sulfide. Sedimentary sulfides quite commonly record a fractionation of up to 71% relative to contemporaneous seawater sulfide (Stuebner, 1997, 1999; Canfield and Teske, 1996). This value is close to the thermodynamically predicted fractionation between H2S and SO42- of approximately 70% at 25 °C (Ohmoto and Lasaga, 1982). However, a maximum discrimination of only ~46%o was found experimentally (Kaplan and Rittenberg, 1964). Consequently, the high fractionalations inferred from sedimentary metal sulfides have been explained by a multistage process, involving sulfate reoxidation and disproportionation of sulfur intermediates (Jorgensen, 1990; Canfield and Thamnorp, 1994; Passier et al., 1999; Böttcher et al., 2000). Here, we present for the first time sulfur isotopic measurements of coexisting dissolved sulfide and sulfate from hypersulfidic interstitial waters of a 380-m-long sediment core. Our data show an apparent isotopic difference of up to 72%o caused by in situ microbial sulfate reduction. This is considerably larger than the assumed biological maximum of 46%o derived from laboratory studies with pure cultures of sulfate-reducing bacteria (Kaplan and Rittenberg, 1964).

GEOLOGIC SETTING
The continental margin of the Great Australian Bight has been the site of cool-water carbonate sedimentation since the Eocene, resulting in a succession almost 1 km thick. It is the largest area on the globe composed of such sediments (Feary and James, 1998). Although the cool-water carbonate ramp of the Great Australian Bight represents an unusual system today, it is considered a modern analogue of the Mesozoic carbonate ramps (Feary and James, 1998) that host a large share of the world’s petroleum resources. Ocean Drilling Program Leg 182 drilled several transects through this carbonate ramp (Fig. 1) and recovered 270 interstitial water samples, from depths as much as 500 m below seafloor (mbsf). Pore-water profiles measured during Leg 182 indicate that the margin of the ramp contains a complex system of different brines, with salinities up to three times that of seawater (Feary et al., 2000). These brines may have been generated during sea-level lowstands on the large adjacent shelf and emplaced into the sediments of the upper continental slope under the influence of a hydraulic head (Feary et al., 2000; Swart et al., 2000; Hine et al., 1999).

RESULTS
Leg 182 provided a unique opportunity to study the biogeochemical sulfur cycle in a deep hypersulfidic biosphere. As a result of intense in situ bacterial sulfate reduction and iron limitation, pore waters are characterized by hypersulfidic conditions with H2S concentrations of up to 9 mmol/L (compare to the 3.4 mmol/L reported by Thostersen and Mackenzie, 1971). Dissolved sulfate is present throughout the core of Site 1130 at concentrations exceeding seawater values, owing to the presence of a brine at depth. This brine reaches a chlorinity of 1320 mmol/L at 37 mbsf, remaining constant farther downcore (Fig. 2a). The ratios of the major cations and anions, as well as the 34S of sulfate, suggest that the brine was derived from evaporated seawater.
After sulfate concentrations are normalized to chlorinity (Fig. 2c), a major sulfate loss appears evident in the same interval where hydrogen sulfide is present (12 to 180 mbsf). This indicates that intense microbial sulfate reduction is occurring in situ deep in the sediment. Microbial sulfate reduction is also reflected by an increase in alkalinity to values...
up to 36 mmol/L, which represents a minimum value due to authigenic carbonate precipitation.

Although sulfate concentration increases steadily with depth, the sulfur isotopic composition of the sulfate increases with depth too, reaching a maximum value of +55% at 19.4 mbsf. This appears odd, considering that the enrichment of $^{34}$S is a function of bacterial sulfate consumption. However, the $\text{SO}_4^{2-}/\text{Cl}^{-}$ ratio (Fig. 2c) reveals that the greatest sulfate consumption occurs at 20 mbsf. As biological activity decreases with depth and sulfate is constantly replenished by the upwelling brine, the isotopic composition returns back to seawater values farther downcore. The enrichment of $^{34}$S in the sulfate is mirrored by the isotopic composition of the produced sulfide (Fig. 2d), which increases from $-27\%$ at 13 mbsf to $-19\%$ at 19.4 mbsf and then decreases significantly to values down to $-41\%$ at greater depths. The isotopic difference between the sulfate and the sulfide ranges between 65% and 72% (Fig. 2g). Similar results down to depths of 500 mbsf have been obtained from other sites of ODP Leg 182.

The extreme convex shape of the chlorinity profile shows that pore-water chemistry of Site 1130 is influenced by a strong advective component. Thus, the isotope data cannot be interpreted in terms of a closed-system Rayleigh distillation model. To quantify the process under open-system conditions, we developed a numerical transport/reaction model of Site 1130 (see Appendix 1).

The model successfully reproduces the distribution and isotopic composition of sulfate and sulfide between 20 and 300 mbsf (Fig. 2). It shows that the observed data can be explained by the assumption of a depth-invariant fractionation factor of 65%. Because the concentration gradients of $^{32}$S and $^{34}$S differ considerably, their diffusion rates differ too (Jørgensen, 1979). Consequently, the apparent in situ difference between the $^{34}$S of $\text{SO}_4^{2-}$ and the $^{34}$S of $\text{H}_2\text{S}$ varies between 60% and 75%.

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Figure 1. ODP Site 1130, located in western transect at water depth of 486.7 m, penetrated 380 m of sediment. Cored interval contains mainly Pleistocene cool-water carbonates with average carbonate content of 88 wt% (Feary et al., 2000). Figure taken from Feary et al. (2000).

Figure 2. Principal properties of interstitial waters of ODP Site 1130. Black dots indicate measured data; thick gray lines indicate results of numerical model (see Appendix 1). Differences between computed and measured $\text{H}_2\text{S}$ concentrations are most likely caused by degassing of overpressured cores (note that numerical model takes pyrite precipitation into account). Desorption of $\text{H}_2\text{S}$ from sea water under pore-water pH conditions has been shown to result in negligible sulfur isotope fractionation. Values of $\text{Cl}^{-}$ and $\text{SO}_4^{2-}$ are taken from Feary et al. (2000).
DISCUSSION

Sulfur isotope fractionation during sulfate reduction is caused by a preferential breakage of the lower bond strength of $^{32}$S-O compared to $^{34}$S-O during enzymatic catalysis. The overall isotope effect has been found experimentally to be inversely related to the cellular sulfate reduction rate (Chambers et al., 1975; Kaplan and Rittenberg, 1964) and may also be dependent on the electron donor (Kaplan and Rittenberg, 1964). However, compared to the maximum fractionation of $-46\%$ found experimentally (Kaplan and Rittenberg, 1964), sedimentary sulfides (mainly pyrite, FeS$_2$) are often significantly more depleted in $^{32}$S with respect to contemporaneous seawater sulfate (up to $-71\%$) (Strauss, 1997, 1999; Canfield and Teske, 1996). Current models consider the bacterially catalyzed disproportionation of sulfur intermediates (Bak and Cypionka, 1987) originating from the reoxidation of hydrogen sulfide as an essential step in the formation of extremely $^{32}$S enriched metal sulfides (Jørgensen, 1990; Jørgensen and Bak, 1991; Canfield and Thamdrup, 1994; Passier et al., 1999). Significant sulfur isotope fractionation has been observed in the laboratory during these microbial processes (Canfield and Thamdrup, 1994; Habicht et al., 1998; Cypionka et al., 1998), and disproportionation reactions have been demonstrated to occur in near-surface coastal marine sediments (Canfield and Thamdrup, 1996; Jørgensen, 1990). Because a large proportion of sedimentary pyrite is formed close to redox boundaries where sulfate reduction rates are highest and active sulfate reducers are most abundant (Jørgensen, 1982b; Llobet-Brossa et al., 1998; Böttcher et al., 2000), a model involving a repeated oxidation-reduction cycle can be used to explain the origin of sedimentary pyrites with low $^{32}$/S in near-surface sediments and the water column.

Coexisting pyrite and dissolved sulfate in deep buried sediments rarely reflect in situ microbial processes. However, dissolved sulfide and sulfate in interstitial waters are indicative of in situ isotope partitioning during anaerobic microbial processes. Dissolved sulfide in sediments is often present only in low concentrations, a consequence of precipitation as iron sulfide or reoxidation (Jørgensen, 1982a). However, dividing cells and geochemical evidence of sulfate reduction have been found in sediments down to about 450 mbsf (Parkes et al., 1994).

Our field results of the sulfur isotope fractionation between coexisting sulfate and hydrogen sulfide significantly exceed the magnitude of isotope effects observed so far in experiments with pure cultures of sulfate-reducing bacteria (Kaplan and Rittenberg, 1964). While a contribution from the oxidative part of the sulfur cycle can take place close to the redox boundary in near-surface sediments, where oxidants are introduced by diffusion, bioturbation or biostabilization, these processes can be excluded in deeply buried hypersulfidic sediments. It has recently been proposed that the slow release of silicate-bound Fe$^{3+}$ may be a potential oxidizing agent in deeply buried sediments (Böttcher et al., 2000). However, in the case presented here, this possibility can be excluded because (1) the investigated sediments are carbonates with a low iron content, (2) the calculated volumetric rates of sulfate reduction are orders of magnitudes higher than the dissolution-dependent release of silicate-bound iron, and (3) microbial disproportionation of elemental sulfur, the crucial process in the near-surface multistep fractionation (Canfield and Thamdrup, 1994, 1996), is energetically inhibited in the presence of high concentrations of hydrogen sulfide (Thamdrup et al., 1993).

As shown by the parallel downcore development of the hydrogen sulfide concentration and the change in the sulfate $^{34}$S, the observed hydrogen sulfide $^{34}$S values represent a pristine signature of the in situ biological processes. Because isotope exchange between the dissolved species is extremely slow under sedimentary conditions (Ohmoto and Lasaga, 1982) and because the computed volumetric sulfate-reduction rates reach values up to $3 \times 10^{-7}$ mmol-L$^{-1}$-s$^{-1}$, which is high when compared to other continental-shelf sediments (Skryng, 1987), we propose that the large fractionation may be caused by sulfate-reducing microorganisms not previously cultured for laboratory isotope discrimination studies. The occurrence and/or specific activity of these microorganisms are possibly related to the presence of the high-saline brines and may also depend on the consumption of unusual electron acceptors (Kaplan and Rittenberg, 1964).

CONCLUSIONS

The isotopic composition of the interstitial waters recovered from sediments of the Great Australian Bight suggests that extreme sulfur isotope fractionation is possible, resulting in isotope signatures similar to those inferred from $^{32}$S-depleted metal sulfides found in other marine sediments. So far, no microbial analog to natural sulfate reducers active in the investigated deep sediments has been isolated for experimental isotope studies. Because a multistep fractionation can be excluded, our results indicate that (1) not necessarily all of the isotopically light sedimentary sulfides have to be the result of a multistep fractionation, and (2) the communities of sulfate reducers in the deep biosphere may differ from those observed in near-surface sediments or the water column.

APPENDIX 1 Methods

Intertidal water samples were taken on board the JOIDES Resolution following the procedures given in Feary et al. (2000). Immediately after collection, 100 µL of a saturated zinc acetate solution was added to 5 mL of sample, to precipitate all sulfide and inhibit further activity of sulfate-reducing bacteria. Shore-based analysis of concentrations used a spectrophotometric method (Cline, 1969). For $^{34}$S measurements, the precipitated ZnS was centrifuged, washed with hot water, and dried. For $^{34}$S measurements, the samples were weighed in Sn cups together with vanadium pentoxide as a catalyst. Sulfur isotope ratios were measured on a FISONs OPTIMA mass spectrometer coupled in continuous flow with a Carlo Erba elemental analyzer. The system was calibrated using the international standards IAEA-S-1, IAEA-S-2, NBS-127, and NBS-123. Analytical reproducibility of the measurements is $\pm 0.3\%$. The data are reported in the conventional delta notation relative to V-CDT (Vienna–Canyon Diablo Troilite).

The numerical model is one-dimensional and considers advection, diffusion, volumetric sulfate consumption, and pyrite precipitation under steady-state conditions. The numeric implementation was done by solving the following equation (see, e.g., Boudreau, 1996).

$$\frac{dc}{dt} = \frac{Dc}{dx} \frac{dc}{dx} + f(x)$$

for the different ionic species, using a finite-difference approach with a resolution of 1 m. The isotopic compositions of the sulfate and sulfide reservoir were calculated as described by Jørgensen (1979). The principal input parameters of the model were (1) porosity and temperature from shipboard measurements, which were used to calculate the diffusion coefficients as a function of depth, (2) brine advection, which was obtained by fitting the conservative Cl$^-$ data against the model output, (3) bacterial activity, which was found by fitting a hypothetical biogenic activity function against the observed sulfate concentration, assuming that the pristine sulfate concentration is that of a seawater-derived brine, (4) pyrite precipitation, obtained by fitting the results of a pyrite precipitation model against the observed concentration, (5) a depth invariant fractionation factor, which was fitted against the observed sulfur isotope signature. The model shows that the sulfur isotopic composition is governed by the interplay of an upwelling brine, downward seawater transport, and bacterial sulfate consumption. The conservative chlorinity profile indicates that the upper 20 mbsf cannot be explained by a diffusion–advection-reaction process alone. This points to nonlocal transport processes (i.e., seawater irrigation). Therefore, the concentration profiles of Cl$^-$ were fixed to the measured concentrations at 0 and 18 mbsf (i.e., used as boundary conditions). It should be noted that these peculiar hydrologic conditions are unrelated to the observed isotopic fractionation, because similar results are obtained from other Leg 182 sites, which show no advection.
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