The Production of ^{34}S-Depleted Sulfide During Bacterial Disproportionation of Elemental Sulfur

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Bacteria that disproportionate elemental sulfur fractionate sulfur isotopes such that sulfate is enriched in sulfur-34 by 12.6 to 15.3 per mil and sulfide is depleted in sulfur-34 by 7.3 to 8.6 per mil. Through a repeated cycle of sulfide oxidation to S^0 and subsequent disproportionation, these bacteria can deplete sedimentary sulfides in sulfur-34. A prediction, borne out by observation, is that more extensive sulfide oxidation will lead to sulfides that are more depleted in sulfur-34. Thus, the oxidative part of the sulfur cycle creates circumstances by which sulfides become more depleted in sulfur-34 than would be possible with sulfate-reducing bacteria alone.

Pure cultures of sulfate-reducing bacteria produce sulfide depleted in ^{34}S (enriched in ^{32}S) by 4 to 46 per mil compared with the starting sulfate (*1*). By contrast, sulfides in sediments and euxinic waters are commonly depleted in ^{34}S by 45 to 70 per mil relative to seawater sulfate (*2*), far beyond the apparent capabilities of sulfate-reducing bacteria. This dilemma is well demonstrated in the Black Sea where enrichment cultures of sulfate-reducing bacteria produce sulfide depleted in ^{34}S by 26 to 29 per mil, yet water-column sulfide is depleted by 62 per mil (*3*). In this report, we show that cycles of fractionation accompanying the bacterial disproportionation of S^0, followed by sulfide oxidation, can generate the large ^{34}S depletions of many marine sulfides.

Sulfur-disproportionating bacteria (SDB) have been found to grow autotrophically, requiring only S^0, basic inorganic nutrients, and a sulfide scavenger (*4*). These bacteria become inactive at dissolved sulfide concentrations above 1 mM. They disproportionate S^0 completely to sulfate and sulfide; no sulfur intermediates have been detected. The overall stoichiometry of disproportionation is

$$4S^0 + 4H_2O \rightarrow 3H_2S + SO_4^{2-} + 2H^+ \quad (1)$$

However, when iron oxides provide a sulfide scavenger, the net stoichiometry is altered as some of the sulfide is reoxidized back to S^0 (*4*)

$$H_2S + 4H^+ + 2Fe(OH)_3 \rightarrow 2Fe^{2+} + S^0 + 6H_2O \quad (2)$$

and the sum of Eqs. 1 and 2 becomes

$$3S^0 + 2Fe(OH)_3 \rightarrow 2FeS + 2H_2O + SO_4^{2-} + 2H^+ \quad (3)$$

Typically 90% of the sulfide produced by sulfate reduction in coastal sediments is reoxidized (*5*). The significance of oxidation intermediates is not well known, but S^0 is an important sulfide oxidation product (*6*) and is routinely found in surficial marine sediments where reoxidation occurs (*7*). SDB are abundant and have been enriched from coastal sediments ranging from the North Sea to the Mediterranean coast, and south to Chile (*4*, *8*).

Our initial experiments exploring isotopic fractionation by SDB ($ÅB_1$ and $ÅB_2$) were conducted on enrichment cultures from Århus Bay, Denmark [the same ones used in (*4*)]. Cultures were inoculated with S^0 and ferric oxides (ferrihydrite) and were incubated anaerobically for several days. In control experiments with S^0 and ferric oxides but no bacteria, no sulfide or SO_4^{2-} was formed (*4*). In the presence of bacteria, both SO_4^{2-} and sulfide formed, indicative of disproportionation. In duplicate incubations, sulfide became depleted in ^{34}S, relative to S^0, by 7.3 to 8.6 per mil, while SO_4^{2-} became enriched in ^{34}S by 12.6 to 15.3 per mil (Table 1). Such fractionations are consistent with the idea that thiosulfate or polythionates are intermediates in the disproportionation process (*9*), although the pathways of disproportionation are not known.

We next explored whether fractionation as seen in the laboratory occurs under more "natural" conditions. It is difficult to measure the isotopic consequences of S^0 disproportionation in nature because S^0 is rapidly cycled and is normally found in fairly low concentrations (1 to 20 μmol cm^{-3}) (*7*), and its continuous formation requires an input of oxidant. We thus incubated sediment with added S^0 to boost the rate of disproportionation. We assumed that the fate of our added S^0 was the same as that of naturally generated S^0 and that any associated fractionations would mimic those naturally occurring. We collected ferric oxide–rich (95 μmol of ferric iron per cubic centimeter by dithionite extraction) sediment from near the surface at Wedderwarden, a tidal mud flat located at the mouth of the Weser Estuary in northern Germany. Sediment was amended, in duplicate, with S^0 to a concentration of 190 μmol cm^{-3} [S^0_1 and S^0_2 experiments (Table 1)], incubated anaerobically at 24°C, and sampled regularly. Two separate unamended splits of sediment acted as controls. We also enriched SDB from the site by inoculating a mixture of S^0 and ferric oxides (ferrihydrite) in anoxic saltwater medium (*4*) with 2% sediment by volume [Fe/S experiment (Table 1)]; these enrichments were also sampled regularly. All experiments were monitored for the concentrations and isotopic compositions of SO_4^{2-}, acid-volatile sulfide (AVS), and S^0. We measured rates of sulfate reduction, using $^{35}SO_4^{2-}$ in the S^0_1, S^0_2, and control experiments.

Table 1. Production of SO_4^{2-} and AVS (acid volatile sulfide) and associated fractionations during S^0 disproportionation (*18*).

Experiment	Δ_{AVS-S^0}* (per mil)	$\Delta_{SO_4^{2-}-S^0}$* (per mil)	Production AVS/SO_4^{2-}
$ÅB_1$	−8.6 ± 0.5	15.3 ± 0.5	†
$ÅB_2$	−7.3 ± 0.5	12.6 ± 0.5	†
S^0_1	−7.9 ± 1.0	12.6 ± 1.0	2.0
S^0_2	−7.4 ± 1.0	12.6 ± 1.0	1.6
Fe/S	−7.7 ± 0.5	12.8 ± 0.5	1.5

*Per mil fractionations of AVS and SO_4^{2-} relative to S^0. †An accurate quantification of SO_4^{2-} and AVS production was not made in these experiments.

After a small lag period during which, presumably, SDB colonized the S^0 grains, S^0 disproportionation in the S^0_1 and S^0_2 experiments accelerated as evidenced by the concomitant production of AVS and SO_4^{2-} (Fig. 1A). The ratio of AVS to SO_4^{2-} production varied between 1.6 and 2 (Table 1) (these values are corrected for the small amount of SO_4^{2-} reduced and the AVS produced by sulfate reduction). In the Fe/S experiment, the rate of AVS production was 1.5 times that of SO_4^{2-}. Together, these ratios are similar to, although a bit lower than, what is anticipated for S^0 disproportionation in a ferric oxide–rich environment (Eq. 3). The missing sulfide could be attributed to reducing equivalents retained as growing bacterial biomass with a growth yield of 0.15 mol of carbon fixed for each mole of S^0 disproportionated.

Isotopic fractionation clearly accompanied disproportionation (Fig. 1B and Table 1). To determine fractionation factors, we first determined the isotopic influences of sulfate reduction. Next we generated a series of curves with the isotopic compositions of SO_4^{2-} and AVS added from disproportionation as the only variables (Fig. 1C). When model results are compared to the measured values (Fig. 1C and S^0_2 results), SO_4^{2-} was added with a δ^{34}S

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of 30.5 ± 1 per mil and AVS with a $\delta^{34}S$ of 10.2 ± 1 per mil. These values, together with the initial $\delta^{34}S$ for S^0 of 17.9 ± 0.2 per mil, yield an enrichment of ^{34}S into SO_4^{2-} of 12.6 ± 1 per mil and a depletion of ^{34}S into AVS of 7.4 to 7.9 ± 1 per mil.

Overall, SDB in both natural sediments and enrichment cultures fractionate S^0 during disproportionation, and we conclude that this ability is likely a basic consequence of their metabolism. We propose that isotopic fractionations associated with S^0 disproportionation may account for the large isotopic depletions of ^{34}S commonly found in sedimentary and water-column sulfides. Through a repeated cycle of sulfide oxidation to S^0 and subsequent disproportionation, sulfides may be generated that are much more depleted in ^{34}S than those produced in the initial reduction of sulfate to sulfide (Fig. 2). For example, the sulfides in the Black Sea water column that are depleted by 62 per mil (relative to Black Sea sulfate) could be generated by such a mechanism. At the chemocline, sulfide oxidation is extensive (*10*) and S^0 formation is observed (*11*). Also, S^0 is an important component of the reduced sulfur pool settling through the water column (*12*). Disproportionation of this S^0 as it settles would yield sulfide isotopically lighter (depleted in ^{34}S) than the original S^0. Reoxidation of this sulfide to S^0 and subsequent disproportionation will generate even lighter sulfide still.

More extensive sulfide oxidation should produce sulfides more depleted in ^{34}S. Some evidence for this effect is found in sedimentary sulfides from coastal locations in the United States (Table 2), where a clear correlation (−0.323 ± 0.063 per mil per percent of S oxidized; R^2 = 0.866, standard linear regression) is found between $\Delta_{\text{sulfide-SO}_4^{2-}}$ (Table 2) and the extent of sulfide oxidation. For two sites in Table 2 (FOAM and NWC), the sulfides are, in fact, more depleted in ^{34}S than the maximum fractionations that would be achieved by sulfate-reducing bacteria (*1*). Finally, the correlation between sulfate reduction rate and $\Delta_{\text{sulfide-SO}_4^{2-}}$ is rather poor (0.019 ± 0.018 per mil mM^{-1} $year^{-1}$; R^2 = 0.208, standard linear regression), although sulfate reduction rate has often been considered a key parameter in controlling ^{34}S depletion (*1*, *13*).

After an initial fractionation by sulfate reduction, fractionations accompanying S^0 disproportionation can generate the large ^{34}S depletions often observed in sediment and water-column sulfides. Hence, because sediments have differing degrees of sulfide oxidation, much of the spread in sedimentary sulfur isotopic values may be attributable to this mechanism. Also, as proposed by Jørgensen (*14*), other elements of the oxidative sulfur cycle, such as thiosulfate disproportionation, may produce isotopic fractionations contributing to the depletion of sedimentary sulfides in ^{34}S.

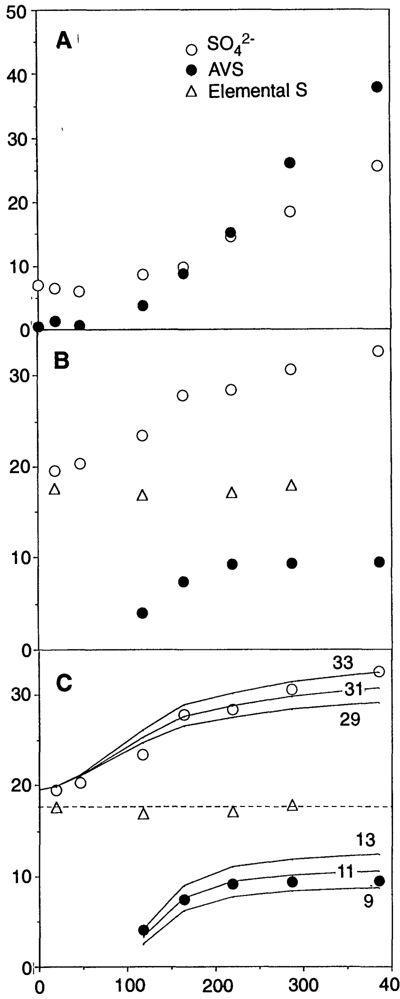

Fig. 1. (**A**) The production of AVS and sulfate as a function of time in the S^0_1 experiment. In addition to exhibiting S^0 disproportionation, these results are also influenced by sulfate reduction, which increased AVS and decreased SO_4^{2-} at a rate of 8.5 ± 0.1 nmol cm^{-3} $hour^{-1}$ (see text for details). Similar results were obtained for the S^0_2 experiment. (**B**) Isotopic compositions of SO_4^{2-}, AVS, and S^0 in the S^0_1 experiment. The S^0_2 experiment gave similar results. (**C**) Model calculation (*15*) predicting the isotopic composition ($\delta^{34}S$ per mil) of SO_4^{2-} and AVS added from disproportionation. The numbers on the plots refer to $\delta^{34}S_{\text{disp}}$ (*15*). Dotted line indicates the initial isotopic composition of the added elemental sulfur.

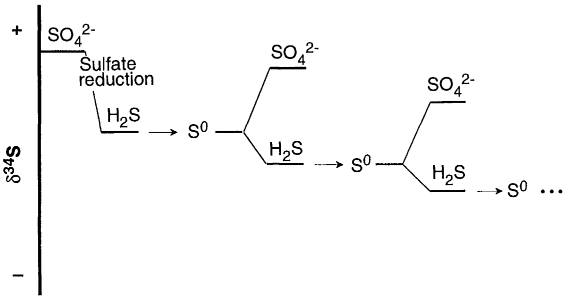

Fig. 2. A generalized scheme showing how repeated sulfide oxidation to S^0, followed by disproportionation, can generate sulfides more depleted in ^{34}S than the original sulfide produced from sulfate reduction. The scheme is for a system open to sulfate, which is characteristic of most sediments near the sediment surface. In such systems, pore water sulfate is often readily exchanged by benthic organisms with overlying water (*16*). If organisms are for some reason excluded, diffusion ensures that somewhat open conditions will be found over several centimeters near the surface (at least), where sulfate reduction is fastest, and most sulfide is normally fixed (*16*, *17*). In the generalized scheme we also assume that there is no significant fractionation during the oxidation of sulfide to S^0.

Table 2. Isotopic composition of sulfide, sulfide retention, and maximum sulfate reduction rate (SRR) for coastal marine sediments. Isotopic values for NWC (Northwest Control, Long Island Sound) are from (*16*), FOAM (Friends of Anoxic Mud, Long Island Sound) from (*19*), Sachem and Black Hole (Long Island Sound) from (*20*), SBB (Santa Barbara Basin) from (*21*), and CLB (Cape Lookout Bight, North Carolina) from (*13*). The SRR for Sachem, Black Hole, NWC, and FOAM are from (*16*), for SBB from (*22*), and for CLB from (*23*). Sulfur oxidized (the percentage of sulfide produced by sulfate reduction but lost and not buried in the sediment) for Sachem, Black Hole, NWC, and FOAM are from (*24*), for SBB from (*22*), and for CLB from (*13*).

Site	$\Delta_{\text{sulfide-SO}_4^{2-}}$* (per mil)	S oxidized (%)	Maximum SRR (mM $year^{-1}$)
Sachem	−32 (2–3)†	33 (0–9)	180
CLB	−34 (0–3)	27 (0–20)	400
Black Hole	−41 (3–4)	72 (0–7)	600
SBB	−43 (0–9)	61 (0–9)	9
FOAM	−49 (4–5)	88 (0–12)	100
NWC	−58 (120–130)	94 (0–150)	60

*Per mil isotopic composition of sedimentary sulfides relative to seawater sulfate with $\delta^{34}S$ = +20 per mil. Isotopic analysis from depths where minimal sulfate depletion has occurred and the "closed system" addition of heavy sulfide is not important. †Depth interval in centimeters is given in parentheses.

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$$\delta^{34}S = \frac{[(^{34}S/^{32}S)_{sam} - (^{34}S/^{32}S)_{CDT}]}{(^{34}S/^{32}S)_{CDT}} \times 10^3$$
where sam is the sample. The symbol δ is used only to present isotopic values relative to CDT. In many cases isotopic values are presented or discussed relative to some specified secondary reference point such as seawater sulfate or the S^0 used in the experiments.
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$$\delta^{34}S_t[X]_t = \delta^{34}S_{t-1}[X]_{t-1} + \delta^{34}S_{SR}\Delta[X]_{SR} + \delta^{34}S_{disp}\Delta[X]_{disp}$$
where $[X]_t$ is the measured concentration of either AVS or $SO_4{}^{2-}$ at time *t*, $\delta^{34}S_t$ is the model predicted isotopic composition of *X*, and $\delta^{34}S_{t-1}[X]_{t-1}$ is the product $\delta^{34}S_t[X]_t$ from the previous time point; $\delta^{34}S_{t-1}$ is the isotopic composition of *X* either removed or added as a result of sulfate reduction (SR). In the control experiments sulfate reduction produced sulfide depleted in ^{34}S by 20 per mil relative to sulfate, and this value has been used. The quantity $\Delta[X]_{SR}$ is the change in the concentration of *X* between *t* and *t* −1; $\Delta[X]_{disp}$ is the change in *X* due to disproportionation, and $\delta^{34}S_{disp}$ is the isotopic composition of *X* added as a result of the fractionations accompanying disproportionation. This is the variable in the equation, and the lines are generated for different values of this parameter.
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Isotopic Composition of Old Ground Water from Lake Agassiz: Implications for Late Pleistocene Climate

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A uniform oxygen isotope value of −25 per mil was obtained from old ground water at depths of 20 to 30 meters in a thick deposit of clay in the southern part of the glacial Lake Agassiz basin. The lake occupied parts of North Dakota and southern Manitoba at the end of the last glacial maximum and received water from the ice margin and the interior plains region of Canada. Ground water from thick late Pleistocene–age clay deposits elsewhere, a till in southern Saskatchewan, and a glaciolacustrine deposit in northern Ontario show the same value at similar depths. These sites are at about 50°N latitude, span a distance of 2000 kilometers, and like the Lake Agassiz sites, have a ground-water velocity of less than a few millimeters per year. The value of −25 per mil is characteristic of meltwater impounded in the southern basin of Lake Agassiz. This value corresponds to an estimated air temperature of −16°C, compared with the modern temperature of 0°C for this area.

At some locations in the glaciated regions of North America and elsewhere, the stable-isotope content of old ground water provides a terrestrial record of pre-Holocene climate. Old ground water is usually associated with large confined aquifers with long residence times and long flow paths. However, complex mixing may dampen climate signals. Old ground water in thick unfractured aquitards also has long residence times but short travel paths and limited mixing. These aquitards may preserve isotopic signatures characteristic of the water that was incorporated at the time of deposition. On the basis of field measurements of hydraulic gradients and hydraulic conductivities and calculations of average linear ground-water velocities, we anticipate that original pore water is still present in thick aquitards deposited during the last glaciation (*1*). The isotopic signature of pore water from such aquitards could potentially yield climate information from the late Pleistocene.

In this report, we describe ground-water conditions at four field sites located in the aquitard deposited in the southern basin of glacial Lake Agassiz (Fig. 1). At the end of the last glacial maximum, Lake Agassiz occupied parts of North Dakota and southern Manitoba and was the receiving body for meltwater coming directly from the ice margin and from meltwater channels originating in western Canada (*2*). The sediments comprising the aquitard were deposited from about 11,700 to 9500 years ago, following a southward readvance of the ice sheet. The sediments range in thickness from about 10 to 80 m.

At the Montcalm site, about 50 km south of Winnipeg, 40 m of glaciolacustrine clay overlie 30 m of till. Dolostone underlies the glacial sediments. At the Emerson site, less than 1 km north of the Canada–United States boundary, 30 m of glaciolacustrine clay and 30 m of till overlie the same dolostone. At the Drayton site, 50 km south of the international boundary and 21 km west of the Red River, 76 m of Lake Agassiz deposits overlie 18 m of till and shale. At the Manvel site, 100 km south of the border, 28 m of clay overlie a thin till (1 to 2 m thick) underlain by sandstone.

At the Montcalm, Emerson, and Manvel sites, sediment cores were obtained. Monitoring wells were installed in vertical clusters through the clay (*3*), except at Emerson, where one till and one bedrock well were

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