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$^{34}S/^{32}S$ Fractionation in Sulfur Cycles Catalyzed by Anaerobic Bacteria

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Stable isotopic distributions in the sulfur cycle were studied with pure and mixed cultures of the anaerobic bacteria, *Chlorobium vibrioforme* and *Desulfovibrio vulgaris*. *D. vulgaris* and *C. vibrioforme* can catalyze three reactions constituting a complete anaerobic sulfur cycle: reduction of sulfate to sulfide (*D. vulgaris*), oxidation of sulfide to elemental sulfur (*C. vibrioforme*), and oxidation of sulfur to sulfate (*C. vibrioforme*). In all experiments, the first and last reactions favored concentration of the light ^{32}S isotope in products (isotopic fractionation factor $\varepsilon = -7.2$ and $-1.7‰$, respectively), whereas oxidation of sulfide favored concentration of the heavy ^{34}S isotope in products ($\varepsilon = +1.7‰$). Experimental results and model calculations suggest that elemental sulfur enriched in ^{34}S versus sulfide may be a biogeochemical marker for the presence of sulfide-oxidizing bacteria in modern and ancient environments.

Consortia of sulfate-reducing bacteria and photosynthetic bacteria can catalyze a complete anaerobic sulfur cycle. Sulfate-reducing bacteria provide sulfide and CO_2 used by photosynthetic bacteria for carbon fixation. Photosynthetic bacteria oxidize sulfide to elemental sulfur and to sulfate; the sulfate is used by sulfate-reducing bacteria for anaerobic respiration of carbon substrates. Because sulfide and sulfate are rapidly cycled between consortium partners, only small amounts of sulfur (<1 mM) are required to sustain this sulfur cycle (14). The major overall requirements for growth of these anaerobic consortia are light for photosynthetic bacteria and a continued carbon supply for sulfate-reducing bacteria.

Although consortia are ubiquitous features of microbial communities, they have been neglected in investigations of stable sulfur isotopic abundances. Isotopic differences of up to 74 to 85‰ should occur at equilibrium between sulfate and sulfide isotopic values (18), and it has been suggested that rapid bacterial cycling of sulfur might catalyze such equilibria (17). Although large differences (50 to 70‰) have been reported between isotopic compositions of sulfide and sulfate in some natural environments, studies with pure cultures of sulfate-reducing bacteria and photosynthetic bacteria have not shown effects of this magnitude; isotopic fractionations accompanying sulfate reduction, sulfide oxidation, and elemental sulfur oxidation are usually much smaller in laboratory studies (0 to −30‰, +1 to +2.5‰, and about 0‰, respectively) (2, 5). We therefore undertook a study of pure and mixed cultures to test whether isotopic fractionations increase when a complete sulfur cycle is active. A second objective was to determine whether sulfur isotopic abundances provide a biogeochemical marker for the presence of sulfide-oxidizing bacteria. Large isotopic differences of 30 to 70‰ between sulfate and sulfide isotopic compositions have been used as evidence for the presence of sulfate-reducing bacteria (15). We investigated whether the isotopic differences between sulfide and elemental sulfur might be similarly used to infer the presence of sulfide-oxidizing bacteria.

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MATERIALS AND METHODS

Organisms and cultures. Cultures of the sulfate-reducing bacteria *Desulfovibrio baculatus* Norway, *D. baculatus* ethylica, *Desulfovibrio multispirans*, and *Desulfovibrio vulgaris* were obtained from T. Lissolo, Department of Biochemistry, University of Georgia, Athens. These bacteria were grown routinely in medium of the following composition (per liter): NH_4Cl, 0.5 g; K_2HPO_4, 2.5 g; Na_2SO_4, 4.0 g; $FeSO_4 \cdot 7H_2O$, 10 mg; sodium citrate, 0.3 g; yeast extract, 1.0 g; sodium DL-lactate syrup (60%), 15 ml. The medium was boiled, 0.5 g of cysteine hydrochloride was added, and the pH was adjusted to 7.6. After the medium was autoclaved and cooled, sterile $CaCl_2 \cdot 2H_2O$ (0.1 g) and $MgSO_4$ (1.23 g) were added. For experiments in which samples were taken from isotopic determinations, cysteine was omitted and the iron level was reduced (2.5 mg of $FeSO_4 \cdot 7H_2O$ per liter).

Cultures of the green photosynthetic bacterium *Chlorobium vibrioforme* subsp. *thiosulfatophilum* were obtained from M. Madigan, Department of Microbiology, Southern Illinois University, Carbondale. Medium for routine culture was assembled from three solutions as follows. (i) The following components were added to 300 ml of deionized water: disodium EDTA, 0.01 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.05 g; NaCl, 0.4 g; KH_2PO_4, 1 g; $Na_2S_2O_3 \cdot 5H_2O$, 1 g; ammonium acetate, 0.5 g; NH_4Cl, 0.4 g; vitamin B_{12}, 20 μg. Trace element solution (1 ml) and chelated iron solution (2 ml) described previously (6) were also added. (ii) Three milliliters of 10 M NaOH was added to 600 ml of deionized water. (iii) One-half gram of $Na_2S \cdot 9H_2O$ was added to 100 ml of deionized water. The solutions were autoclaved and cooled; after the addition of 1 g of sterile $NaHCO_3$, solution ii was bubbled for 15 min with sterile CO_2, and all three solutions were combined for the final medium (pH 6.8 to 7.0). For pure-culture experiments involving isotopic determinations, thiosulfate was omitted for several transfers and cultures were maintained by the addition of sulfide only.

Viable mixed cultures were produced by combining cultures of *C. vibrioforme* grown without thiosulfate but with sulfide and cultures of *D. vulgaris*. A simplified medium consisting of three components was developed for these mixed cultures. (i) The following constituents were added to 350 ml of deionized water: sodium DL-lactate syrup (60%), 0.2 ml; 10 N NaOH, 2.5 ml. (ii) The following constituents were added to 650 ml of deionized water: disodium EDTA, 0.01 g; $MgSO_4 \cdot 7H_2O$, 1.3 g; $CaCl_2 \cdot 2H_2O$, 0.05 g; NaCl, 0.4 g; vitamin B_{12}, 20 μg; sodium citrate, 0.6 g; KH_2PO_4, 2.0 g; ammonium acetate, 1.0 g; NH_4Cl, 0.8 g; chelated iron solution, 2 ml; trace element solution, 1 ml. (iii) The remaining component was 1.0 g of sodium bicarbonate. The three components were autoclaved separately, cooled, and combined. The final pH was 7.0, and the medium was stored in completely filled glass bottles.

For experimental work involving isotopic determinations, actively growing cultures were pelleted by centrifugation and resuspended in fresh medium to give an initial absorbance of 50 to 200 Klett-Summerson photometer units (filter no. 66). For studies of sulfate reduction, several vessels were inoculated and then their entire contents were harvested to give a time course series. Sulfide was precipitated by the addition of zinc acetate and separated from sulfate by centrifugation. Sulfate present in supernatant fluids was measured gravimetrically as $BaSO_4$ after the addition of $BaCl_2$. Sulfide-containing pellets were suspended in deionized water to remove occluded sulfate, centrifuged again, and then oxidized with bromine water (3 ml of bromine per 100 ml of water). Resultant sulfate was determined gravimetrically as $BaSO_4$. The fractional extent of reaction in each vessel was determined as the weight ratio [sulfate sulfur/(sulfate + sulfide sulfur)].

In mixed-culture experiments and pure-culture work with *C. vibrioforme*, serial samples were extruded from 1-liter vessels (Roux bottles) whose headspace was maintained under a positive pressure of oxygen-free argon. Incubation temperatures were 20 to 25°C. Concentration measurements and separations for isotopic determinations were made as follows. For each extruded sample, sulfide concentrations were determined (3) and cells and elemental sulfur were then pelleted by centrifugation. Zinc acetate was added to the supernatant, and the resulting sulfide precipitate was separated by centrifugation; thiosulfate formed during bacterial metabolism and sulfate remaining in the supernatant were analyzed spectrophotometrically (13, 16). Further treatment of sulfide and sulfate samples were done by procedures described above for work with *D. vulgaris*. Elemental sulfur was converted to SCN^- by cyanolysis (16) and separated from cells by filtration, concentrations were measured spectrophotometrically, and the remaining SCN^- was oxidized to sulfate with bromine water.

For isotopic determinations, all samples were thus converted to sulfate. Sulfates were precipitated with $BaCl_2$ from hot acid solution, and the resulting $BaSO_4$ was decomposed under vacuum to yield SO_2 (8). Isotopic values of SO_2 were determined with a Nuclide 6-60 isotope ratio mass spectrometer as follows:

$$\delta^{34}S = [(R_{sample}/R_{standard}) - 1] \times 1{,}000$$

where $R = {}^{34}S/{}^{32}S$. Reagent sulfate and sulfide used in media were employed as isotopic standards.

Per mille isotopic fractionation factors, ε values (11), were calculated from experimental data. For irreversible reactions at low yield, these fractionation factors express the expected difference in δ units (per mille) between a product and the substrate from which it is formed. Negative ε values signify a normal isotope effect in which products are depleted in ^{34}S relative to substrates; since mass balance is preserved, residual reactants become correspondingly enriched in ^{34}S during such reactions. The opposite effect, in which products are enriched in ^{34}S and substrates are depleted in ^{34}S, is observed in some reactions. These reactions occur with inverse (rather than normal) isotope effects, and ε values are positive to signify this difference.

Both concentrations and isotopic data are used to calculate ε values, since isotopic compositions change in a predictable manner with substrate concentration. The fraction f of substrate remaining during a reaction was calculated from concentration measurements, and isotopic $\delta^{34}S$ values were determined with a mass spectrometer. Linear regression yielded ε as the slope of best-fit lines for isotopic and transformed concentration data (11). Reactant and product data were combined for the determination of ε in each experiment since slopes of regression lines for reactants and products did not differ significantly. The indicated error limits for ε are 95% confidence intervals.

RESULTS AND DISCUSSION

Experiments with pure cultures. Before sulfur isotope effects in mixed cultures of *D. vulgaris* and *C. vibrioforme* were measured, studies with pure cultures were initiated. Sulfate reduction by *D. vulgaris* occurred with a normal isotopic fractionation of $\varepsilon = -6.5 \pm 0.5$‰ at temperatures of 15 to 25°C, and product sulfides were depleted in ^{34}S relative to the sulfate substrate (Fig. 1). This −6.5‰ fractionation is small relative to −20 to −60‰ effects commonly observed for sulfate reduction in natural systems but within the range reported for several laboratory studies (2, 12). Further experiments with other species of sulfate-reducing bacteria, *D. baculatus* Norway, *D. baculatus* ethylica, and *D. multi-*

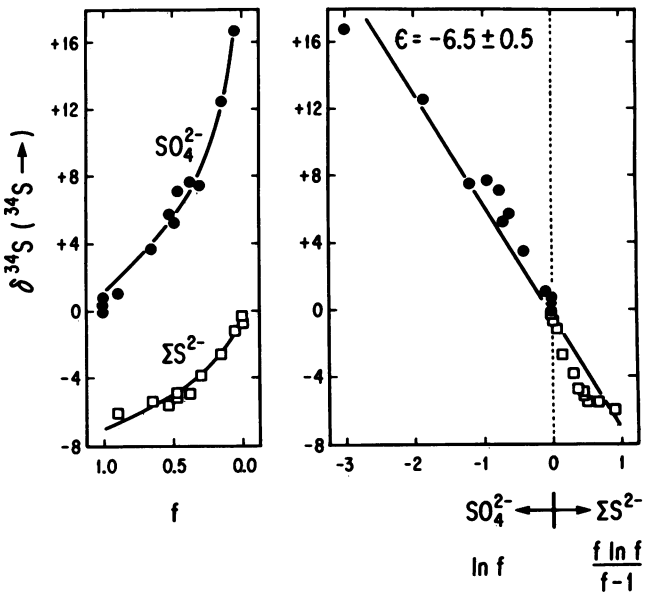

FIG. 1. Isotopic changes in sulfate (●) and sulfide (□) during sulfate reduction by *D. vulgaris*. (Left) Isotopic values as a function of f, the fraction of remaining sulfate substrate; data were combined from experiments at 15 and 25°C. (Right) Calculation of isotopic fractionation factor: ε = the slope of the best-fit line for sulfate and sulfide data combined.

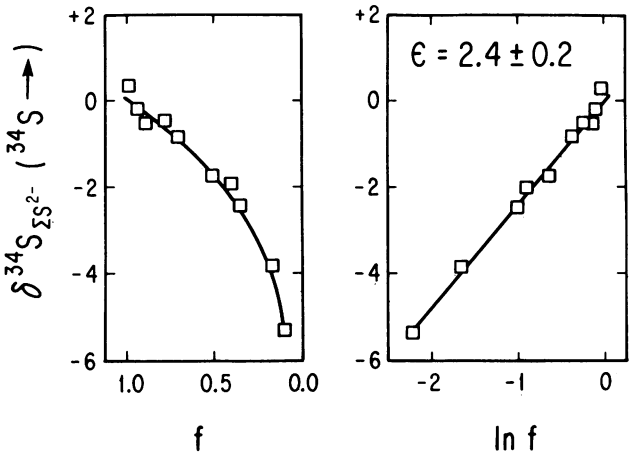

FIG. 2. Isotopic changes during sulfide oxidation by the photosynthetic bacterium *C. vibrioforme*. (Left) Isotopic values as a function of f, the fraction of remaining sulfide substrate. (Right) Calculation of isotopic fractionation factor: ε = slope of best-fit line for sulfide data.

spirans, showed similar isotopic fractionations (ε for combined data = −5.9 ± 0.9‰; N = 11); therefore, small isotopic fractionations during sulfate reduction were common to several species maintained in our laboratory. On the other hand, oxidation of sulfide to elemental sulfur by *C. vibrioforme* was accompanied by an inverse isotope effect of ε = +2.4 ± 0.2‰, favoring concentration of ^{34}S in the product and ^{34}S depletion in the residual sulfide substrate (Fig. 2). Inverse isotope effects averaging +1.8 to +2.4‰ have been previously reported for the oxidation of sulfide to elemental sulfur by photosynthetic bacteria (6). This inverse isotope effect associated with sulfide oxidation may reflect bacterial use of dissolved H_2S that is enriched in ^{34}S by an equilibrium isotope effect (7).

Experiments with mixed cultures. We report three kinds of mixed-culture investigations: initial experiments, experiments with sequential reduction and oxidation of sulfur species, and experiments with simultaneous oxidation and reduction of sulfur species. The main reactions involved in these experiments were sulfate reduction, sulfide oxidation, and elemental sulfur oxidation. Other possible reactions such as reduction of elemental sulfur to sulfide by *D. vulgaris* (1) were seldom observed and were of little quantitative importance.

(i) Initial experiments. Two experiments with mixed cultures of *D. vulgaris* and *C. vibrioforme* showed that rates of sulfate reduction and sulfide oxidation were difficult to balance. Either sulfate or sulfide accumulated to the exclusion of the other in growing consortia maintained in the light. Sulfate accumulated when sulfate reduction was the slowest reaction in the sulfur cycle, whereas sulfide accumulated when sulfide oxidation was the slowest reaction. Sulfide and sulfate $\delta^{34}S$ were constant under these conditions of continued growth with only one detectable sulfur species.

To more closely examine isotope effects associated with sulfur cycling in mixed cultures, cultures that accumulated sulfate in the light were used. Doubling times for these cultures were 8.5 to 15 h. Measurable sulfate reduction occurred when the lights were turned off, as sulfide accumulated and sulfate disappeared. Subsequent illumination of sulfide-rich cultures resulted in rapid, transient oxidation of sulfide to elemental sulfur, followed by a slower oxidation of elemental sulfur to sulfate. The use of dark-light cycles thus resulted in a phased functioning of three reactions that constitute a sulfur cycle, i.e., sulfate reduction, sulfide oxidation, and sulfur oxidation. Initial experiments with cultures maintained in the dark showed that isotopic fractionations (ε values) associated with sulfate reduction were −7.4 to −9.3‰ (Table 1, mixed culture experiments 1 and 2), values similar to the −6.5‰ value obtained in studies with pure cultures (Fig. 1 and Table 1).

(ii) Sequential reduction and oxidation of sulfur. Sequential functioning of sulfate reduction, sulfide oxidation, and elemental sulfur oxidation in a mixed culture experiment was achieved by a combination of dark-light periods and lactate limitation. In an initial dark period, low lactate levels limited sulfate reduction so that sulfate was not completely consumed (Fig. 3, 0 to 42 h). Growth was minimal in this initial period, and optical density measurements did not increase significantly (Fig. 3). Illumination of the culture at 42 h resulted in rapid formation of elemental sulfur from sulfide; sulfur globules accumulated extracellularly in the cultures, accounting for the observed rapid increase in optical density (Fig. 3). Elemental sulfur accumulation was followed by oxidation of sulfur to sulfate and declines in optical density as sulfur globules disappeared from the culture. The net increases in optical density from about 190 (preillumination) to 300 (postillumination, after sulfur oxidation) represented real growth of *C. vibrioforme*, the photosynthetic component of the mixed culture. Subsequent dark incubation showed that sulfate reduction was still inactive (i.e., no sulfide accumulation or sulfate disappearance at 52.5 to 65.5 h [Fig. 3]). The addition of lactate at 67 h and incubation in the dark stimulated sulfate reduction, again indicating that lactate had been previously limiting sulfate reduction.

The manipulations of dark-light cycles and lactate levels thus resulted in a sequential functioning of sulfate reduction, sulfide oxidation, and elemental sulfur oxidation. Isotopic determinations showed that both normal and inverse isotope effects occurred during this reaction sequence. Sulfate reduction occurred with a normal isotope effect, as reactant sulfate became enriched in ^{34}S and product sulfide was

TABLE 1. Isotopic fractionation factors (ε) for sulfur cycle reactions

Expt	ε ± 95% confidence limits (N), ‰		
	$SO_4^{2-} \rightarrow \Sigma S^{2-}$	$\Sigma S^{2-} \rightarrow S^0$	$S^0 \rightarrow SO_4^{2-}$
Pure cultures			
D. vulgaris	−6.5 ± 0.5 (23)		
C. vibrioforme		+2.4 ± 0.2 (10)	
Mixed cultures of *D. vulgaris* and *C. vibrioforme*[a]			
1	−7.4 ± 1.9 (11)		
2	−9.3 ± 1.0 (17)		
3	−5.8 ± 0.6 (11)[b] −8.3 ± 1.0 (8)[b]	+2.4 ± 0.8 (8)	−2.4 ± 1.1 (5)
4	−7.5 ± 0.9 (15)	+2.0 ± 0.6 (15)	−1.0 ± 0.3 (13)
5	−7.0 ± 0.9 (17)	~0	
$\bar{x}$	−7.4	+1.7	−1.7

[a] Results of experiments 3 to 5 are shown in Fig. 3 to 5, respectively.
[b] Isotope effects were measured in two separate phases of the experiment.

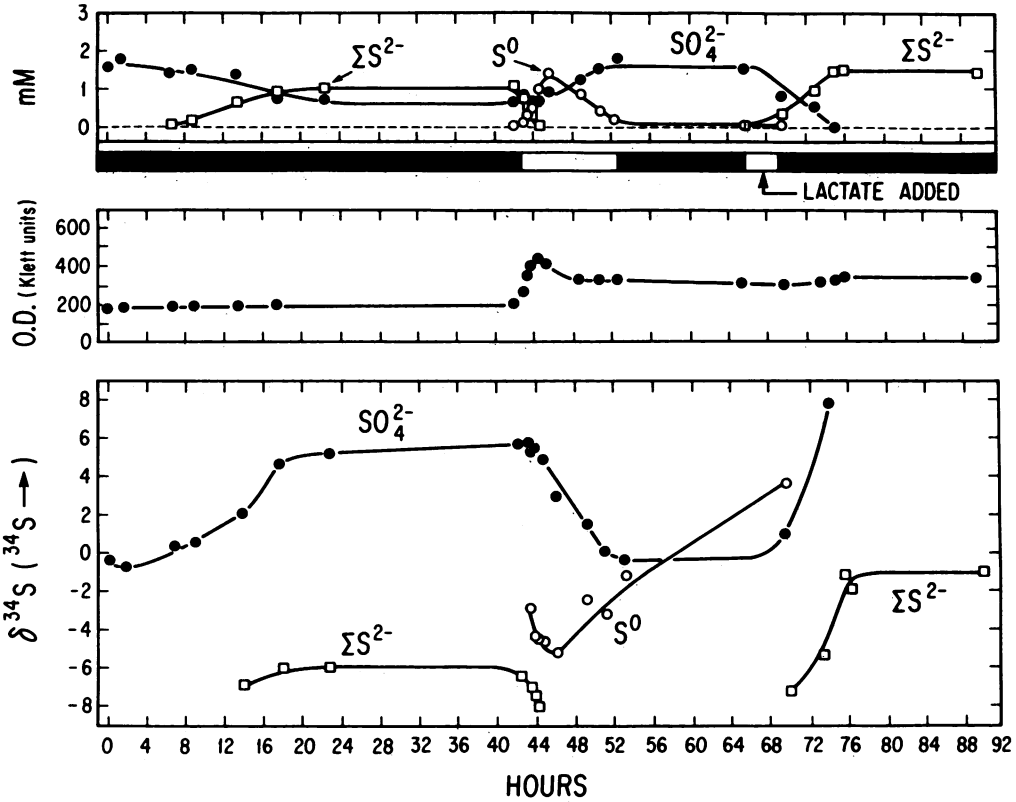

FIG. 3. Changes in concentrations, optical density, and isotopic values for experiment 3 (Table 1) with mixed cultures of *D vulgaris* and *C. vibrioforme*. In this experiment, sulfate reduction, sulfide oxidation, sulfur oxidation, and sulfate reduction again occurred independently and in sequence.

initially depleted in ^{34}S (Fig. 3). However, as observed in the experiment with a pure culture of *C. vibrioforme*, sulfide oxidation occurred with an inverse effect: upon illumination at 42 h, reactant sulfide became depleted in ^{34}S and product sulfur was enriched in ^{34}S (Fig. 3). Subsequent oxidation of elemental sulfur to sulfate and the subsequent reduction of sulfate to sulfide occurred with normal isotope effects, since reactant sulfur became enriched in ^{34}S, followed by ^{34}S enrichment in sulfate and ^{34}S depletion in the sulfide product (Fig. 3).

The magnitudes of the isotopic fractionations for the experiment shown in Fig. 3 were $\varepsilon = -5.8$ and $-8.3‰$ for sulfate reduction, $-2.4‰$ for sulfur oxidation, and $+2.4‰$ for sulfide oxidation (Table 1, experiment 3). The values for sulfate reduction and sulfide oxidation are similar to values found in experiments with pure cultures (Table 1); the $-2.4‰$ value for the oxidation of elemental sulfur is significantly less than 0‰, the value found in a previous study of sulfur oxidation by a photosynthetic bacterium (6).

(iii) Synchronous reduction and oxidation of sulfur. Two final experiments with mixed cultures were conducted to assay isotopic fractionations in situations in which synchronous oxidation and reduction of sulfur species occur. In the first experiment, sulfate reduction occurred during an initial dark phase, whereas sulfide and elemental sulfur oxidation occurred during a subsequent light phase (Fig. 4). High initial lactate levels were used to insure continued sulfate reduction during the light period; increases in optical density throughout this phase confirmed active sulfate reduction since continued growth was dependent on functioning of both reductive and oxidative reactions in the sulfur cycle of

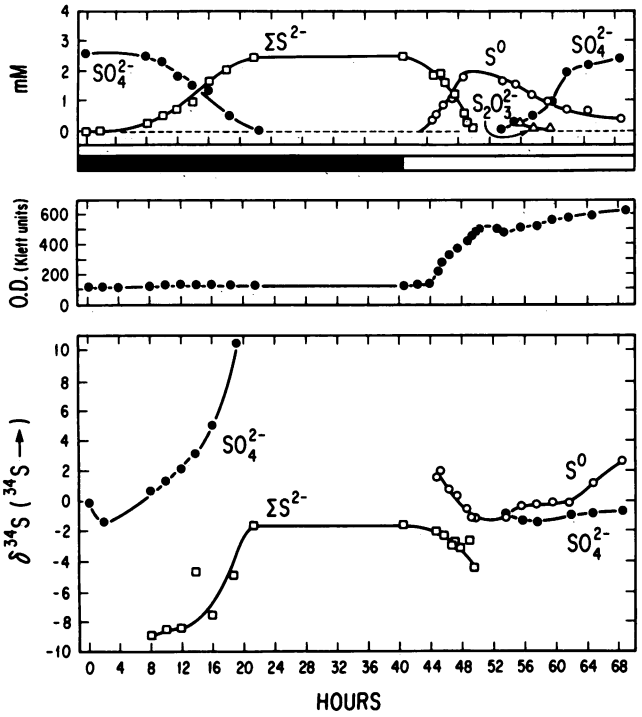

FIG. 4. Changes in concentrations, optical density, and isotopic values for experiment 4 (Table 1) with mixed cultures of *D. vulgaris* and *C. vibrioforme*. In the light (second half of the experiment), sulfate reduction, sulfide oxidation, and sulfur oxidation occurred simultaneously.

the mixed culture. Although all three sulfur cycle reactions were thus simultaneously operative in the light, isotopic compositions of sulfide, sulfur, and sulfate changed in a manner similar to that observed when oxidation and reduction of sulfur species were sequential rather than simultaneous (compare Fig. 3 and 4). Calculated isotopic fractionations for sulfate reduction, sulfide oxidation, and elemental sulfur oxidation were not significantly different for the sequential and simultaneous operations of the sulfur cycle (Table 1, experiments 3 and 4, respectively).

Transient, low levels of sulfide were observed in a last mixed-culture experiment after a brief dark phase (Fig. 5). Elemental sulfur accumulated slowly, and its isotopic composition was lower than that of sulfide (Fig. 5). Examination of the isotopic compositions of sulfate, sulfide, and elemental sulfur showed that the values were characteristic of residual reactant, instantaneous product, and accumulating product for sulfate reduction (11). A combined plot of values based on this assumption yielded an isotope effect of $\varepsilon = -7.0‰$ (Fig. 6). The intermediate values for sulfide in this experiment more strongly reflect the "upstream" normal isotope effect involved in sulfide formation than the "downstream" inverse isotope effect involved in sulfide consumption.

Models. The experimental work indicated that isotope effects associated with sulfate reduction and sulfide oxidation are not significantly different in mixed and pure cultures. However, steady-state coexistence of sulfate, elemental sulfur, and sulfide could not be achieved under experimental conditions. In lieu of experimental measurement, a simple model can be formulated to show expected isotopic distributions in a steady-state sulfur cycle. The model consists of three pools (sulfate, sulfide, and elemental sulfur), with isotopic fractionations assigned to flows connecting the pools (Fig. 7). Because inputs and outputs to each pool must have the same isotopic composition at steady state, the following approximations hold true:

$$\delta^{34}S_{sulfate} + \varepsilon_1 = \delta^{34}S_{sulfide} + \varepsilon_2 = \delta^{34}S_{sulfur} + \varepsilon_3$$

(The exact equations are: $R_{sulfate}(\alpha_1) = R_{sulfide}(\alpha_2) = R_{sulfur}(\alpha_3)$, where $\alpha = 1 + (\varepsilon/10^3)$ and $R = {}^{34}S/{}^{32}S$ [11].) Setting $\delta^{34}S_{sulfate}$ equal to 0 as an arbitrary reference and giving ε_1, ε_2, and ε_3 the average ε values of -7.2, $+1.7$, and $-1.7‰$ found in this study for sulfate reduction, sulfide oxidation, and sulfur oxidation, respectively (Table 1 and experiments with *D. baculatus* and *D. multispirans*), the expected steady-state sulfur isotopic compositions are as follows: $\delta^{34}S_{sulfate} = 0‰$; $\delta^{34}S_{sulfide} = -8.9‰$; and $\delta^{34}S_{sulfur} = -5.5‰$. (The corresponding exact solutions using R and α values are 0, -8.88, and $-5.51‰$ for sulfate, sulfide, and sulfur, respectively.) These calculations show that at steady state, sulfide oxidation slightly increases the sulfate/sulfide isotopic difference expected from sulfate reduction alone, i.e., from -7.2 (ε_1) to $-8.9‰$ (ε_1 and ε_2). Also, the isotopic composition of elemental sulfur ($-5.5‰$) is more closely related to that of sulfide ($-8.9‰$) than that of sulfate (0‰). This becomes more apparent when the isotope effect accompanying sulfate reduction is large, as it usually is in field situations (e.g., if $\varepsilon_1 = -50‰$ instead of $-7.2‰$ for sulfate reduction, expected

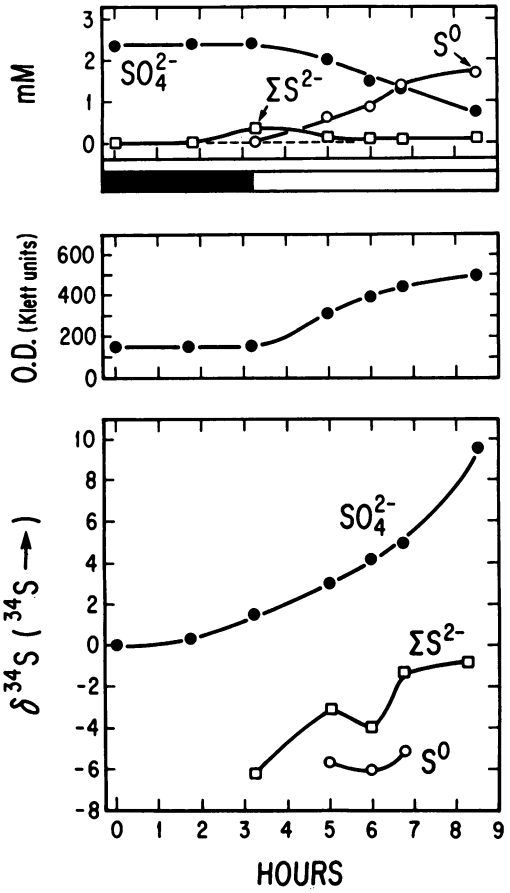

FIG. 5. Changes in concentrations, optical density, and isotopic values for experiment 5 (Table 1) with mixed cultures of *D. vulgaris* and *C. vibrioforme*. Sulfate reduction, sulfide oxidation, and sulfur oxidation occurred simultaneously in the light.

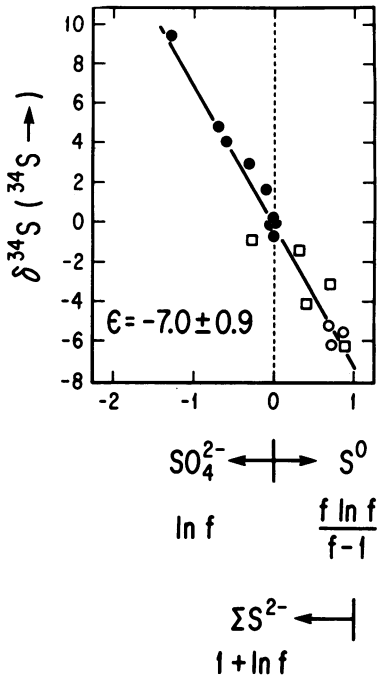

FIG. 6. Regression analysis for experiment 5 (Table 1) showing a $-7‰$ isotopic fractionation characteristic of sulfate reduction. In this analysis, sulfide (□) was considered to be an instantaneous product (11), elemental sulfur (○) represented the cumulative product, and sulfate (●) was the residual reactant.

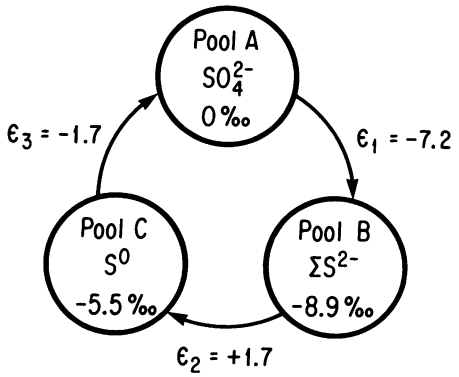

FIG. 7. Three-pool model of steady-state sulfur cycle. With an arbitrary reference of $\delta^{34}S_{sulfate}$ = 0‰, the expected isotopic compositions of sulfide and sulfur are −8.9 and −5.5‰, respectively, using average fractionation factors (ε values) measured in this study.

$\delta^{34}S$ values for sulfate, sulfide, and sulfur are 0, −51.7, and −48.3‰, respectively).

Comparison with field studies. Recent field studies have examined sulfur isotopic changes in microbial sulfureta of the Baltic Sea (9, 10). Both sulfate reduction and photosynthetic sulfide oxidation occurred in clear acrylic boxes placed over sediments at 10 m. Apparent isotopic fractionations for sulfate reduction (−20‰) and sulfide oxidation (−8‰) were significantly greater in the field study than in our laboratory study (−7.2 and +1.7‰, respectively); fractionations during the oxidation of elemental sulfur were similar (ε = −1.7‰ [laboratory] versus 0‰ [field]). The −20‰ value for sulfate reduction in the Baltic Sea is typical of many field studies (2), and smaller laboratory fractionations such as the −7.2‰ value we observed have been attributed to the relatively rapid rate of sulfate reduction in laboratory as opposed to field situations (2, 12). Discrepancies between estimates of the fractionation during sulfide oxidation are harder to explain. Our results and those of previous pure-culture studies with photosynthetic bacteria indicate a small inverse isotope effect of ε ≃ +2‰ for the oxidation of sulfide to sulfur rather than a normal isotope effect of ε ≃ −8‰. The small inverse effect has been explained in terms of an equilibrium exchange that enriches H_2S in ^{34}S at the expense of HS^- (7), with sulfide-oxidizing bacteria forming sulfur from the ^{34}S-enriched H_2S (6). We expect that this mechanism also operates in field situations, and in a study of isotopic changes in a meromictic lake, slight ^{34}S enrichments of 2 to 5.5‰ in sulfur versus sulfides have been measured near a midwater bacterial plate that contains sulfate-reducing and photosynthetic sulfide-oxidizing bacteria (4). These direct field measurements can be contrasted with the Baltic Sea results for which isotopic compositions of elemental sulfur were inferred from model calculations rather than directly measured (9). It is also possible that the presence of sediments in the Baltic Sea sulfureta complicated interpretation of the results since sediments can, at various times, act as sources, sinks, or simply exchange reservoirs for various sulfur species.

In conclusion, experimental work shows that isotope effects accompanying sulfide and elemental sulfur oxidation are generally much smaller than those accompanying sulfate reduction. Model studies show that the small inverse isotope effect in sulfide oxidation results in a small enhancement of the sulfur isotopic difference that can be measured between sulfate and sulfide; observed isotopic differences between sulfate and sulfide are primarily controlled by sulfate reduction. On the other hand, the isotopic difference between sulfide and elemental sulfur is controlled by sulfide-oxidizing bacteria. Experimental results and model calculations show that elemental sulfur formed from sulfide by photosynthetic bacteria is slightly enriched in ^{34}S. Because this enrichment most probably arises during an equilibrium exchange reaction involving HS^- and H_2S, we expect that other sulfide-oxidizing bacteria using H_2S will also form ^{34}S-enriched sulfur. Further study should show whether the occurrence of elemental sulfur that is enriched in ^{34}S versus sulfide is a generally useful marker for the presence of sulfide-oxidizing bacteria.

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