ARE SULFUR ISOTOPE RATIOS SUFFICIENT TO DETERMINE THE ANTIQUITY OF SULFATE REDUCTION

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Abstract. Sulfur isotope fractionation values have been measured in sedimentary sulfides of varying ages, The 'Antiquity and evolutionary status of bacterial sulfate reduction . . .' has been inferred from these measurements by Schidlowski (1979). However, under experimental conditions, the isotope values vary widely due to inadequately controlled variables. Thus the direct extrapolation of sulfur isotope fractionation values measured in the laboratory to those measured in sedimentary rocks is unwarranted. New sulfur transforming microbes have been described and recent measurements indicate that inorganic processes affect sulfur isotope fractionation values. This information is summarized here; at present sulfur isotope fractionation values are insufficient to determine the antiquity of sulfate reduction.

The major trends in metabolic evolution during the early Earth's history may rarely be directly determined from microfossils and stromatolites. The indirect fossil record, available as carbon-rich sediments and fractionated carbon and sulfur isotopes may indicate past microbial activity and thus may be useful for inference of metabolic evolution. From the stable sulfur isotope fractionation values ($\delta^{34}S$)* of the Precambrian fossil record, Schidlowski (1979) inferred that respiratory sulfate reduction arose 2.8–3.1 billion years ago. As discussed here, Schidlowski's conclusions can be questioned on several grounds:

(1) the interactions between microbial members of the sulfur transforming community and how these interactions affect fractionation values are not understood (Cohen *et al.*, 1977; Jorgensen and Cohen, 1977; Jorgensen *et al.*, 1979; Cohen, 1979),

(2) the mechanism(s) of sulfur fractionation is (are) poorly understood (Chambers and Trudinger, 1979; Postgate, 1979),

(3) alternative explanations often exist for the same set of sulfur fractionation values (Trudinger *et al.*, 1972; Rye and Ohmoto, 1974), and

(4) coordinated laboratory and field experiments to corroborate laboratory $\delta^{34}S$ values have not been undertaken.

These points will be expanded after a brief background discussion.

Sulfur exists as a mixture of stable isotopes, ^{32}S and ^{34}S predominating. Primordial terrestrial sulfur apparently has a composition close to, if not the same as that found in the stoichiometric ferrous sulfide mineral troilite, from meteorites. This is conventionally

$$* \delta^{34}S‰ = \frac{^{34}S/^{32}S \text{ sample} - {}^{34}S/^{32}S \text{ standard}}{^{34}S/^{32}S \text{ standard}} - 1 \times 1000$$

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expressed in its $\delta^{34}S$ form as 0‰. Fractionation of sulfur isotopes occurs during chemical as well as biological transformations of sulfur, yielding isotopic compositions which deviate from the primordial 0‰. Preferential enrichment of the lighter isotope by *Desulfovibrio desulfuricans* during respiratory reduction of sulfate to sulfide was initially demonstrated by Thode and his co-workers (1951). This result suggested the use of stable isotopes as a tool to distinguish minerals in which sulfides were biogenically produced (presumably those of low $\delta^{34}S$ values), with sulfide of abiogenic origin (presumably those of high $\delta^{34}S$ values).

The ways in which sulfur isotope ratios are altered in nature must be understood before they can be used as a base from which evolutionary inferences can be made. How sulfur is transformed in nature is primary to applying isotopes as effective tools. Sulfur compounds mainly move through the biological-sediment interface by microbial metabolic conversions. During the early part of this century, the work of Baas Becking (1925) Beijerinck, and Winogradsky (Goldhaber and Kaplan, 1974; Trudinger, 1976a, b; Postgate, 1979) provided the framework for understanding the marine sulfur cycle by associating certain microorganisms with specific biochemical reactions (Figure 1). These works emphasized the marine environment, though it is likely that when sulfur is available similar reactions occur in fresh water. The areas in which communities of sulfur metabolizing organisms are functioning were referred to as 'sulfureta' by Baas Becking (1925). Our concept of how sulfur is transformed in the 'sulfuretum' is not yet complete. New microorganisms were recently isolated under strictly anaerobic conditions by variation

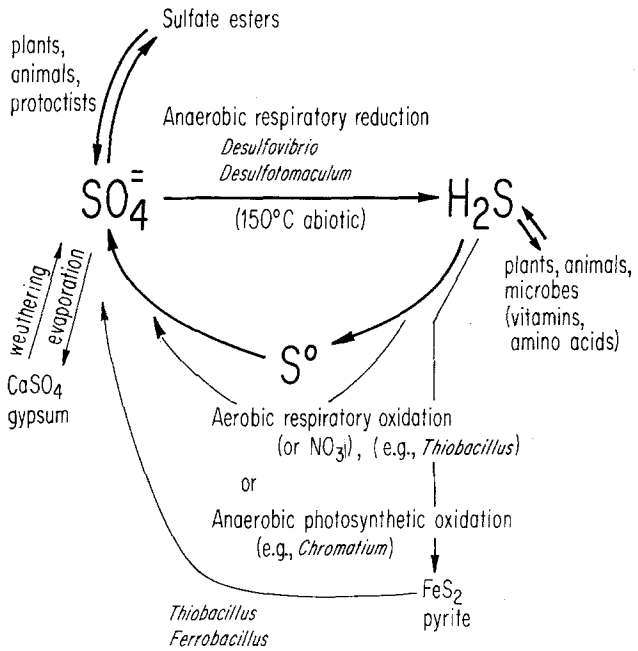

Fig. 1. Marine sulfur cycle. (After Trudinger, 1976a, b; Fenchel and Blackburn, 1979; Postgate, 1979.)

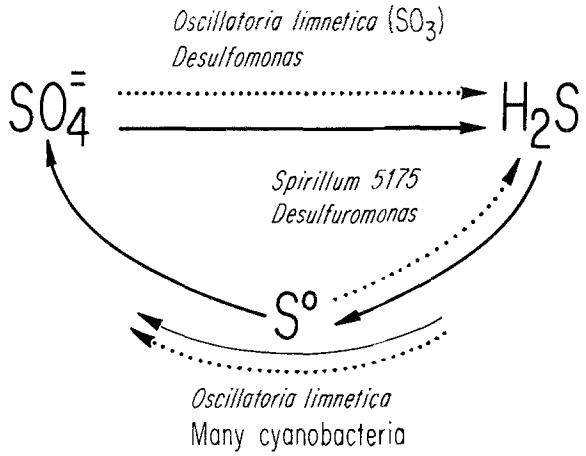

Fig. 2. Marine sulfur cycle, follow closed arrows. New organisms of cycle noted, follow open arrows. See Figure 3 for references

of the electron donors and acceptors. These genera augment the classical marine sulfur scheme (Figure 2). Table I summarizes the data relevant to a marine sediment study of sulfate reducers and draws attention to the metabolic diversity found among these microbial populations. 'Sulfureta', in which the evaporation rates are high and where light, organic compounds, sulfate and some oxygen are available, serve as models for metalliferous sulfide formation (Roberts, 1975; Renfo, 1974), emphasizing the continuity through time of 'sulfureta'. Communities of microbial species are responsible for sulfur transformation in these environments and therefore the eventual mineralization.

Several sulfur containing gases, i.e. dimethyl sulfide, carbonyl disulfide, carbon disulfide – though they have not yet been associated with specific microbial genera – are measured over marine sediments and/or marshlands. Among these, the methylated sulfide, dimethyl sulfide, has been postulated as the dominant vehicle returning sulfur from the sea to the land through the atmosphere (Lovelock *et al.*, 1972). It had previously been assumed that the major sulfur volatile playing this role was H_2S (Kellogg *et al.*, 1973; Babich and Stotsky, 1978; Zinder *et al.*, 1978). Dimethyl sulfoxide, an atmospheric oxidation product of dimethyl sulfide was discovered to be an electron acceptor for a mud-inhabiting, anaerobic spiral microbe (Zinder and Brock, 1978).

The current scheme for the sulfur cycle (Figure 1) is merely a compilation of information based on studies of pure cultures of bacteria rather than what has actually been observed to occur in sediment-atmosphere interactions. Only recently have there been attempts to study the biochemistry and geochemistry of microbial communities in nature (Van Gemerden, 1967; Cohen *et al.*, 1977; Jorgensen and Cohen, 1977; Jorgensen *et al.*, 1979). The sulfur cycling, its controls, and the effect on sedimentary sulfur of diagenetic processes requires interdisciplinary approaches of this type.

Most organisms reduce sulfur for incorporation into proteins, a process called assimilatory reduction though few release reduced sulfur into their environment (Postgate, 1979). The most significant group of sulfide producing organisms in marine sedimentary

TABLE I
Sulfate reduction in a marine sediment
(After Ferry, 1978)

Organism	Usual donor	Acceptor	Product	Reference
Desulfovibrio gigas	lactate, ethanol	SO_4^{-2}, SO_3^{-2}	acetate, H_2S	Buchanan and Gibbons
D. desulfuricans	pyruvate	" "	CO_2, H_2S	(1974)
	H_2, fumarate	" "	H_2S	
Desulfotomaculum nigrificans	glucose	SO_4^{-2}, SO_3^{-2}	acetate, H_2S	Buchanan and Gibbons
	lactate, ethanol	" "	CO_2	(1974)
	pyruvate	" "	H_2S	
D. acetoxidans	acetate, ethanol	SO_4^{-2}, SO_3^{-2}	CO_2, H_2S	Widdel and Pfennig
	butanol	" "		(1977)
Desulfomonas pigra	lactate	SO_4^{-2}, SO_3^{-2}	acetate, H_2S	Ferry (1978)
	pyruvate	" "	CO_2, H_2S	
Desulfuromonas acetoxidans	acetate, ethanol	S^0, fumarate	CO_2, H_2S	Pfennig and Biebl (1976)
Sulfate producers	lactate	S^0	CO_2, H_2S	Biebl and Pfennig (1977)
Spirillum 5175	H_2, HCO_2H, formate	S^0, SO_3	CO_2, H_2S	Wolf and Pfennig
		S_2O_3, fumarate		(1977)
Unidentified	acetate, lactate	SO_3, S_2O_3	H_2S	Tuttle and Jannasch
marine bacterium	pyruvate	" "	"	(1973)
Various strains of marine bacteria	methane, lactate	SO_4	CO_2, H_2S	Panganiban *et al.* (1979)
Strain DL-1	lactate, succinate	dimethyl sulfoxide	acetate, DMS	Zinder and Brock
	H_2 if acetate present	SO_3, S_2O_3, S^0		(1978)
		methionine,		
		sulfoxide, nitrate		
Desulfovibrio vulgaris	acetate + CO_2	SO_4 + H_2	H_2S	Badziong and Thauer (1978)
(Marburg & Madison strains)	lactate	SO_4	H_2S	
Methanogenic bacteria	CO_2, acetate	H_2	CH_4, H_2O	Cappenberg (1974)
	methanol, formate			
Oscillatoria limnetica		SO_4	H_2S	Cohen (1979)

environments are the respiratory reducers. These anaerobic microorganisms such as *Desulfovibrio, Desulfotomaculum,* and *Desulfuromonas,* generate large quantities of reduced sulfur as H_2S on a regular basis and thus have a significant impact on sulfide mineralization at temperatures below 150°C (Trudinger, 1976a, b: Postgate, 1979).

Although nearly thirty years have elapsed since the pioneering work of Thode, satisfactory explanations of sulfur isotope ratios observed in live organisms and sediments are not available. Models have been proposed (i.e. Rees, 1973). These are based on the current biochemical understanding of sulfate reduction pathways which is often controversial or lacking (Goldhaber and Kaplan, 1974; Chambers and Trudinger, 1979; LeGall *et al.*, 1979; Postgate, 1979). The demonstration of isotopic fractionation during the reverse reaction, sulfide oxidation, in *Desulfovibrio desulfuricans* has made a clear cut explanation more difficult (Trudinger and Chambers, 1973). An example of the current confusion comes from the work of McCready *et al.* (1975, 1976). The sulfide produced from sulfite reduction by *Clostridium pasteurianum*, an anaerobic, sporeforming, rod-shaped bacteria, which resides in marine muds with *Desulfovibrio*, showed a large range of $\delta^{34}S$ values +23‰ to −15‰. The small amount of sulfide measured from sulfate reduction yields $\delta^{34}S$ values +1.6‰ to +2.2‰. The production of both positive and negative values is unusual and was attributed to either fractionation occurring along the reverse pathway ('inverse fractionation effects') or to the internal recycling of sulfur along the reduction pathway. Thus, only inadequate explanations for the behavior of *Clostridium pasteurianum* are available. A review of sulfur isotope fractionation and metabolic mechanisms has recently been published (Chambers and Trudinger, 1979).

To interpret $\delta^{34}S$ values measured in sedimentary sulfides, the effect of organisms on isotope values must be ascertained. Two types of measurements have been made: (1) Those experiments with microbes in pure cultures showing kinetic discriminatory processes (Figure 3), and (2) rock and sediment measurements which determined the patterns in the environment. No simple relation between the two is observed.

All members of the same genus of sulfate reducing bacteria do not always fractionate sulfur to the same extent (Chambers and Trudinger, 1979). The genus of organism and even more importantly the experimental conditions (e.g. nature of electron donor, temperature, and rate of reduction) alter the final sulfur isotope value. By varying these conditions, $\delta^{34}S$ values between +3‰ to −46‰ could be measured. Fractionation values from five species of *Desulfovibrio* (*D. africanus, D. desulfuricans, D. gigas, D. salexigens,* and *D. vulgaris*) and *Desulfotomaculum nigrificans*, which were maintained under identical conditions and maximum reduction rates, were compared (Chambers *et al.*, 1976). A $\delta^{34}S$ value of approximately −15‰ was measured for all under the same conditions as long as reduction proceeded rapidly. The alteration of culture conditions caused dramatic differences in the final fractionation value in other cases. For example, when *Desulfovibrio desulfuricans* reduced sulfate with either hydrogen or an organic donor, a different isotopic pattern resulted in each case (Chambers and Trudinger, 1979). These results strongly suggest that the actual isotope pattern for *Desulfovibrio* depends more heavily on the environmental conditions during reduction than on the genetic makeup of the microorganisms.

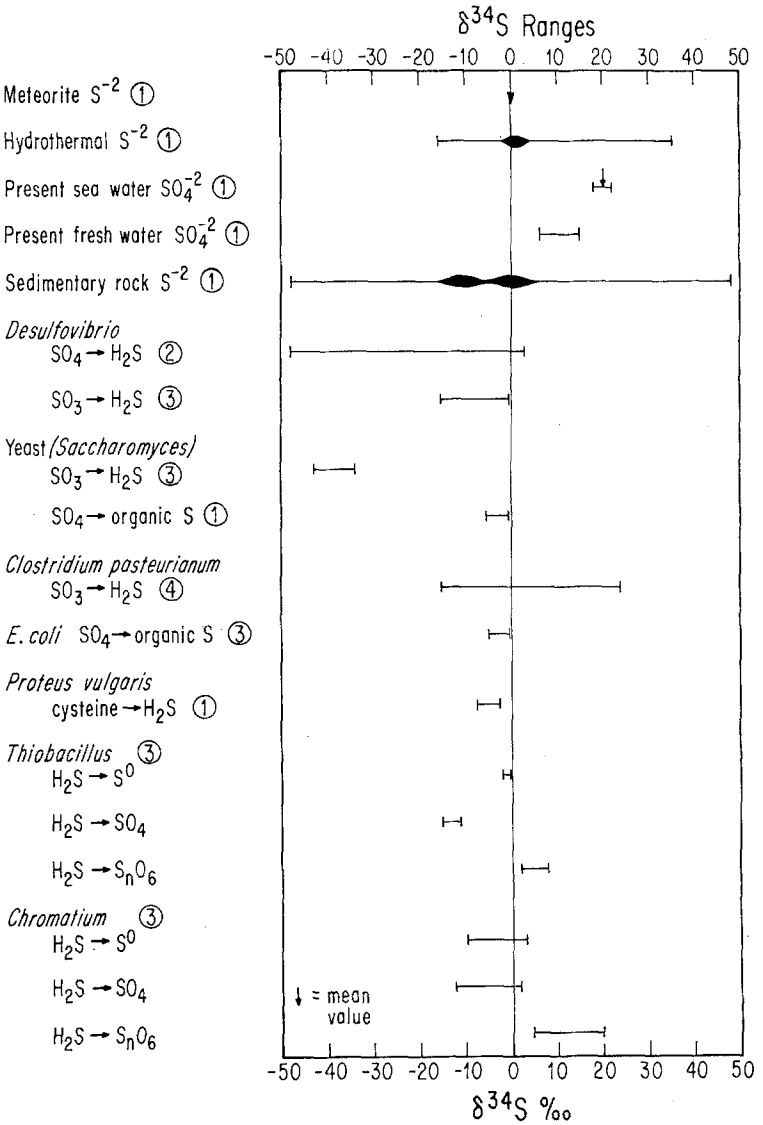

Fig. 3. Comparison of sulfur isotope fractionation ($\delta^{34}S$) from geological samples and pure cultures of organisms. References as follows: ① Goldhaber and Kaplan (1974). ② Rees (1973). ③ Kaplan and Rittenberg (1964). ④ McCready *et al.* (1975, 1976).

In nature, similar patterns of fractionation are often recognized. Microbially derived sulfides from a modern marine basin of Santa Barbara, California, were found to vary in $\delta^{34}S$ values between $-18‰$ to $-54‰$ (Kaplan and Goldhaber, 1974). *Desulfovibrio desulfuricans* to which they attributed the H_2S produced, was isolated from these muds. However, it was not clearly shown that *Desulfovibrio desulfuricans* is responsible for all the sulfide produced. As mentioned previously, all isolated sulfate reducing organisms

have not yet been studied in pure culture to determine if they fractionate sulfur to the same extent as *Desulfovibrio*. Reduced compounds other than H_2S may also be fractionated and reflected in the light sulfides measured in the basin study. How the interactions within a community of microbes affect the fractionation values, and the importance of the environmental fluctuations on the isotope values are not known.

Metalliferous sedimentary sulfide deposits in the geological record vary in $\delta^{34}S$ values from +50‰ to −50‰ (Trudinger, 1976b). Since the pH, eH, and temperature conditions at which *Desulfovibrio* thrive are compatable with those of metalliferous sulfide formation, it is likely that microorganisms are pivotal in sedimentary sulfide formation below 150°C. Biological fractionation may therefore be responsible for the light sulfur seen in sediments. But the isotope data have been over interpreted since inorganic processes can produce isotopic patterns similar to biogenically produced ones.

Inorganic processes can affect sulfur isotope fractionation values. Harrison and Thode (1957) reduced sulfate with hypophosphorous acid and measured H_2S of $\delta^{34}S$ value −22‰. Chromous ions in the presence of halide catalysts show fractionation values from −12‰ to −22‰ (Chambers and Trudinger, 1979). However, these particular conditions would be unlikely in nature. Changes in the ore fluids in the chemical environment can account for broad ranges in hydrothermal deposits of sulfide as well as changes in the $\delta^{34}S$ values (Rye and Ohmoto, 1974). For example, a slight change in pH can shift the $\delta^{34}S$ value +5‰ of pyrite. Thus, environmental conditions influence both chemically and biologically derived $\delta^{34}S$ values. Stratiform sulfide deposits which appear to have had similar magmatic origins may differ widely in sulfur isotope fractionation values, e.g. the Mogul of Ireland (^{34}S value +50‰ to −40‰) and the Pine Point of the Northwest Territory (^{34}S value +10‰ to −25‰). In contrast, very different ore formations may have similar values and ranges. The Darwin Deposit of California is a replacement ore with values +5‰ to −8‰, whereas the Kuroko Ore of Japan was reduced and precipitated from sea water, its δ^{34} values +10‰ to −8‰ (Rye and Ohmoto, 1974).

The dramatic spread in the isotope values of pyrites between the time of deposition of the rocks of the Isua Formation of Greenland (3.76×10^9 years $\delta^{34}S$ values +2‰ to −2‰) to those of the Michipicotan and Woman River Banded Iron Formations (2.75×10^9 years; $\delta^{34}S$ values +10‰ to −10‰) is representative of some change in sulfur transforming activity (Schidlowski, 1979). However, this broadening in the range might be due to an evolutionary biological, geological or atmospheric base, or, most likely a complex interaction of all of these.

The information currently available to interpret sulfur isotope values is not sufficient to determine when biological sulfate reduction first appeared. All the fractionation values available for respiratory sulfate reduction are for *Desulfovibrio* production of H_2S. No data exists for newly described genera or for other reduced compounds of sulfur. No model exists to predict isotope effects under specific environmental conditions and no adequate model can exist until the biochemical and geological processes are better understood. The isotope fractionation data from different laboratory measurements of microbial activity have not been compiled in a manner condusive for meaningful com-

parisons. Furthermore, although they are exceptions to the general trends, microbial sulfate reduction can produce positive $\delta^{34}S$ values and inorganic processes negative $\delta^{34}S$ values. The variation of fractionation values measured in cultured microbes emphasizes the importance of knowing those variables to which the final sulfur isotopic fractionation values are sensitive, and the transformations between organisms and their environment. The above discussion reiterates the need to understand the sulfur cycle, the organisms involved, their fractionation values and their interactions before meaningful paleontological inferences can be made from sulfur isotope values.

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