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A revised isotope fractionation model for dissimilatory sulfate reduction in sulfate reducing bacteria

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Abstract—Sulfur isotope fractionation during dissimilatory sulfate reduction has been conceptually described by the widely accepted Rees model as related to the stepwise reduction of sulfate to sulfide within the cells of bacteria. The magnitude of isotope fractionation is determined by the interplay between different reduction steps in a chain of reactions. Here we present a revision of Rees' model for bacterial sulfate reduction that includes revised fractionation factors for the sulfite-sulfide step and incorporates new forward and reverse steps in the reduction of sulfite to sulfide, as well as exchange of sulfide between the cell and ambient water. With this model we show that in contrast to the Rees model, isotope fractionations well in excess of −46‰ are possible. Therefore, some of the large sulfur isotope fractionations observed in nature can be explained without the need of alternate pathways involving the oxidative sulfur cycle. We use this model to predict that large fractionations should occur under hypersulfidic conditions and where electron acceptor concentrations are limiting.

1. INTRODUCTION

Sulfur isotope fractionation by sulfate-reducing bacteria is the result of a sequence of reactions leading to the reduction of sulfate to sulfide. A conceptual model of sulfate reduction was introduced by Rees (1973) and recently adapted by Farquhar et al. (2003) to incorporate the less abundant isotope ^{33}S. The Rees model is commonly accepted, but field observations, their interpretation, and advances in the understanding of the metabolism of sulfate-reducing bacteria demand an update of some of the underlying assumptions. The revisions we discuss in this paper lead to the conclusion that the maximum possible sulfur isotope fractionation induced by bacterial sulfate reduction can be in the order of −70‰. This is well in excess of the value −46‰ that previously was considered to be the theoretical maximum, and sheds new light on the issue of sulfur isotope effects by sulfate-reducing bacteria.

In natural environments sulfides are commonly depleted in ^{34}S by −45‰ to −70‰ relative to seawater sulfate (Ohmoto et al., 1990). On the other hand, laboratory culture experiments with *Desulfovibrio desulfuricans* and other strains of sulfate-reducing bacteria yielded a maximum sulfur isotope difference between produced sulfides and residual sulfate of around −46‰ (Kaplan and Rittenberg, 1964; Thode, 1991; Bolliger et al., 2001), and the theoretical model of Rees (1973) predicts a maximum sulfur isotope fractionation effect of about −46‰. An elegant explanation for the apparent discrepancy between the maximum isotope fractionation value observed in laboratory cultures and in natural environments was presented by Canfield and Thamdrup (1994). These authors postulated that sulfides produced by sulfate reduction are oxidized to elemental sulfur, which in turn is disproportionated to sulfide and sulfate. This process can be repeated several times. A consequence of this cycling is a multistep sulfur isotope fractionation leading to the strongly depleted sulfur isotope composition of sulfides. The importance of this process in marine sediments was subsequently demonstrated by Habicht and Canfield (2001). Thiosulfate disproportionation has also been proposed as a possible mechanism to increase the fractionation between sulfate and sulfide because the two S atoms in the thiosulfate have different sulfur isotope compositions (Jørgensen, 1990).

However, there are some environments where this model cannot necessarily be applied. In the hypersulfidic interstitial waters from the Great Australian Bight sediments drilled during ODP Leg 182, sulfur isotope offsets between pore-water sulfates and sulfide in the range of 60‰ to 72‰ have been observed (Wortmann et al., 2001). Using a diffusion-advection model, Wortmann et al. (2001) concluded that the offsets at ODP Site 1130 could be explained by a depth invariant fractionation of −65‰. From hypersulfidic pore-waters in the Cariaco Basin, Werne et al. (2003) report an isotope offset between pore-water sulfate and pore-water sulfide of −55‰ to −65‰. The large fractionation observed in these two hypersulfidic environments is interesting, because under high sulfide concentrations elemental sulfur disproportionation as additional sulfur isotope fractionation process becomes thermodynamically unfavorable, and is thus inhibited (Canfield and Thamdrup, 1994; Rabus et al., 2000). The energy yield of the sulfur disproportionation reaction is strongly influenced by sulfate and sulfide concentrations and pH, whereby low sulfate and sulfide concentrations and high pH are necessary for the reaction to yield energy. These conditions are neither met in the hypersulfidic interstitial waters from the Great Australian Bight sediments nor in the hypersulfidic pore-waters in the Cariaco Basin. The energy yield of thiosulfate disproportionation also is strongly dependent on sulfide concentrations and becomes inhibited at higher sulfide concentrations (Frederiksen and

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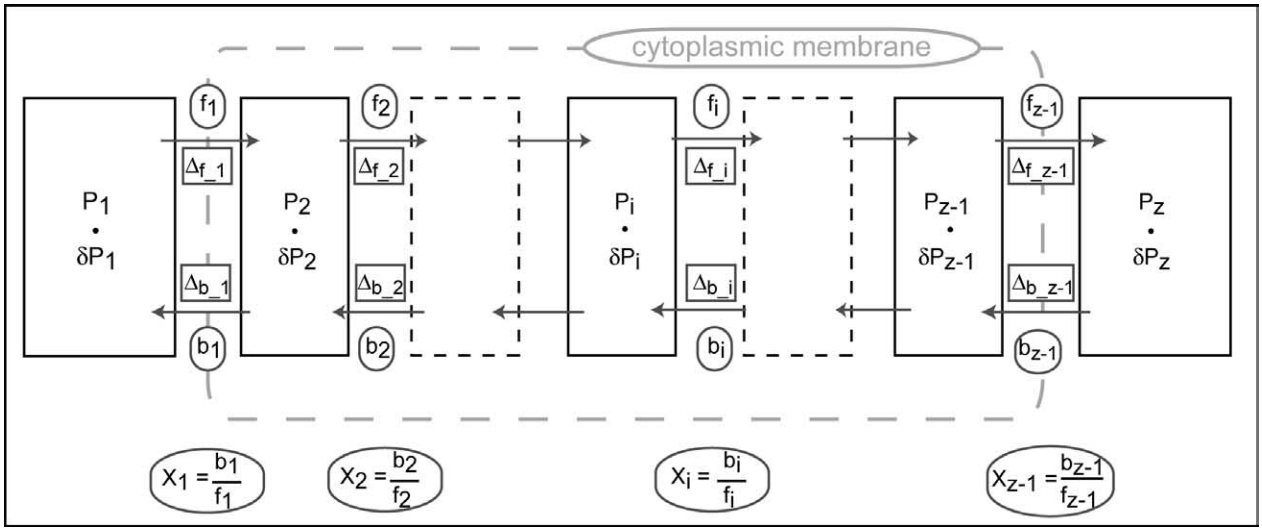

Fig. 1. Pathway of an enzyme-catalyzed chemical reaction. A chemical compound (P_i) with an isotopic composition δP_1 is reacted to a product (P_2) with an isotopic composition δP_2 by enzyme-catalyzed steps within a bacterium. Forward (f_i) and backward fluxes (b_i) connect compounds (P_i) of intermediate compounds (X_i equals the b_i:f_i ratios). Each forward and backward reaction is associated with kinetic isotope fractionation effects (Δ_{f_i}, Δ_{b_i}).

Finster, 2004). In addition, the concentration of reactive oxides in the Cariaco Basin (Werne et al., 2003) and in the Great Australian Bight sediments (Wortmann et al., 2001) are very low, making the partial reoxidation of sulfide to elemental sulfur through sulfide-metal reactions unlikely. Based on these considerations, we postulate that sulfur isotope fractionation by sulfate reducers alone is likely to be the cause for these large fractionations.

Rudnicki et al. (2001) observed an extreme enrichment in sulfate $\delta^{34}S$ in pore-waters from deep ocean sediments at elevated temperatures and modeled the evolution of the sulfur isotope composition of sulfate using a diffusion-advection model. They calculated sulfur isotope fractionation factors for bacterial sulfate reduction of $-77‰ \pm 7‰$. Because disproportionation can only produce strongly ^{34}S-depleted sulfide, but not extremely enriched sulfate, Rudnicki et al. (2001) attributed the observed isotope effect solely to bacterial sulfate reduction. In a recent study, Claypool (2004) used a diffusion-advection model to reanalyze published data on the sulfur isotope fractionation in pore-waters from DSDP and ODP drilling programs. He concluded that the average sulfur isotope fractionation by bacterial sulfate reduction in marine sediments is $-75‰$ and that the observed variability is due to the different degree of diffusive replenishment of nonfractionated seawater sulfate at the different sites.

There are other modern environments where large fractionations between sulfate and sulfide are observed, such as the Black Sea water column (e.g. Neretin et al., 2003). However, because these environments are close to anoxic-oxic interfaces, the presence of an active oxidative sulfur cycle cannot be excluded. Because the oxidative sulfur cycling induces additional isotope fractionations (Canfield 2001; Sørensen and Canfield, 2004), these environments cannot unequivocally be used as a supporting argument for the occurrence of high isotope fractionations by sulfate reducers alone. Summarizing, the above discussion indicates that the theoretical maximum isotope fractionation value for microbial sulfate reduction of $\sim -46‰$ may be too low, and that at least for some environments, disproportionation of elemental sulfur and the reactions of the oxidative sulfur cycle may not be used to explain the high fractionations observed.

In the first part of this paper, we introduce and review some concepts of isotope fractionation processes in a reaction chain, and the relation between kinetic and equilibrium isotope fractionation effects. We then discuss the commonly accepted reaction scheme for the stepwise bacterial reduction of sulfate (in the following called "Rees-model" [Rees, 1973]). In a third part, we introduce our modifications and demonstrate that a maximum sulfur isotope fractionation of $\sim -70‰$ is possible and suggest that it is likely that such an extreme fractionation occurs in hypersulfidic environments when electron acceptors are limiting.

1.1. Isotope Fractionation in a Reaction Chain

Sulfur isotope fractionations caused by dissimilatory sulfate-reducing bacteria have been interpreted to be related to a sequence of enzyme-catalyzed kinetic isotope fractionation steps (Rees, 1973; Habicht and Canfield, 1997). A general reaction description of such a chain of reactions is depicted in Figure 1.

In this framework, isotope effects are caused by a stepwise reaction of a compound P_1 to a compound P_z. Each intermediate step includes forward (f_i) and backward (b_i) fluxes that can be related by a ratio ($X_i = b_i/f_i$) and kinetic isotope fractionation factors (Δ_{f_i}, Δ_{b_i}) that are associated with each transfer flux.

At a constant reaction rate, the sizes of the internal pools (P_2 to P_{z-1}) are constant, and the reaction rate equals the difference between the forward and backward fluxes. Under this condition the total isotope fractionation effect caused by an enzyme-catalyzed reaction (Δ_{total}) can be calculated (for a derivation see Appendix):

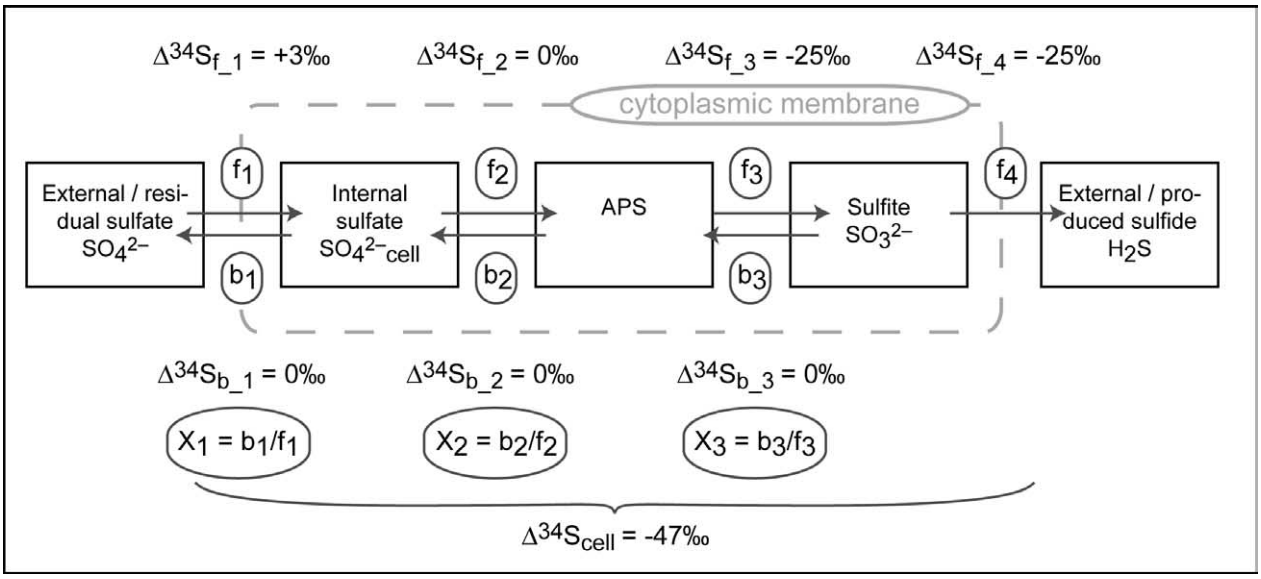

Fig. 2. Pathway of dissimilatory sulfate reduction (modified after Rees, 1973; Fritz et al., 1989): Sulfate is transformed to sulfide by enzyme-catalyzed steps within the sulfate-reducing organism. Forward (f_i) and backward fluxes (b_i) connect pools of intermediate sulfur compounds (X_i equals the b_i:f_i ratios). Sulfur isotope fractionation is caused by uptake of sulfate into the cell (step 1) and splitting of S-O bonds (steps 3 and 4). It is assumed that backward fluxes do not cause sulfur isotope fractionation. The theoretical maximal sulfur isotope effect by the cell ($\Delta^{34}S_{cell}$) equals the sum of the isotope fractionation steps.

$$\Delta_{total} = \Delta_{f\_1} + \sum_{u=1\ldots(z-2)} \left(\prod_{v=1\ldots u} X_v \right) \cdot \Delta_{f\_u+1} - \sum_{u=1\ldots(z-1)} \left(\prod_{v=1\ldots u} X_v \right) \cdot \Delta_{b\_u}$$

where Δ_{total} = total isotope effect; z = total number of pools; z − 1 = total number of steps; u, v = counter for reaction steps; Δ_{f_u} = kinetic isotope fractionation in a forward step "u"; Δ_{b_u} = kinetic isotope fractionation in a backward step "u"; X_v = ratio between backward and forward flux in step "v"; $\Sigma_{u=1\ldots(z-2)}$ = expression for the sum of u elements; $\Pi_{v=1\ldots u}$ = expression for the product of v elements.

A corresponding equation calculated with fractionation factors expressed as α instead of the Δ-notation used here, has been derived by Comstock (2001). An additional discussion of the calculation of isotope fractionations in a reaction network can be found in Hayes (2001).

1.2. Kinetic Isotope Effects and Their Relation to Equilibrium Isotope Effects

In reversible reactions, the equilibrium isotope effect can be understood as the result of a bi-directional kinetic isotope fractionation at chemical equilibrium. If the kinetic isotope effect in one direction and the equilibrium isotope effect are known, the kinetic isotope effect in the opposite direction can be determined (O'Leary, 1977). This relation, for example, has been used for the determination of the kinetic carbon isotope fractionation of in the CO_2-HCO_3^- system (Zeebe and Wolf-Gladrow, 2001). The kinetic sulfur isotope effects can be calculated in a similar way. In a reaction chain, thermodynamic equilibrium is reached when the backward and forward fluxes become equal (X_i = 1). In this case, the equilibrium isotope effect equals the sum of all kinetic isotope effects. This relation holds for the isotope effects of an entire reaction chain (i.e., the reduction of sulfate to sulfide) as well as for individual reaction steps (i.e., the reduction of sulfate to sulfite and the reduction of sulfite to sulfide). The equilibrium isotope fractionation factors can be determined based on theoretical physicochemical concepts and experimental data (e.g., Urey 1947; Ohmoto and Lasaga, 1982). Therefore, if the equilibrium isotope fractionation between two compounds is known and an estimate for the kinetic isotope fractionation in one direction has been determined, the kinetic isotope fractionation in the opposite direction can be calculated. We will apply this concept to estimate kinetic isotope effects in dissimilatory sulfate reduction.

2. THE REES MODEL

Rees (1973) developed his reaction and isotope fractionation scheme for the pathway of dissimilatory sulfate reduction (Fig. 2) based on work by Peck (1959, 1961, 1962) and Kemp and Thode (1968). In the following, we review Rees' considerations and assumptions and identify points where, based on new evidence, a revision may be necessary.

The reaction pathway for sulfate reduction consists of four principal enzyme-catalyzed steps:

Step 1. Sulfate is transferred into the cell.
Step 2. Cell-internal sulfate is activated with adenosine triphosphate (ATP) to adenosine5′ phosphosulfate (APS).
Step 3. The APS is reduced to sulfite.
Step 4. Sulfite is reduced to sulfide by the enzyme sulfite reductase.

As already pointed out by Rees (1973), the reduction of

sulfite to sulfide could be either a single- step or a multistep reaction. A single-step (6 electron) reduction of sulfite to sulfide without production of intermediates was supported by studies with ^{35}S labeled sulfite by Chambers and Trudinger (1975). Other studies (Lee at al., 1971; Kobayashi et al., 1972; Kobayashi et al., 1974; Vainshtein et al., 1980; Fitz and Cypionka, 1990; Sass et al., 1992), however, have shown that intermediates such as trithionate and thiosulfate were also produced. Rees (1973) considered all flows as reversible with the exception of the reduction of sulfite to sulfide. He assumed that under normal conditions this final reaction step was probably fast, and consequently the backward reaction would be inexistent. However, the production of intermediates in the reduction of sulfite to sulfide indicates that reverse reaction cannot a priori be considered inexistent. Therefore, in our revision of the Rees model, we suggest that a backward flow in this step should be introduced.

For the assignment of kinetic sulfur isotope fractionation factors at the different steps, Rees considered work of Harrison and Thode (1958), Kaplan and Rittenberg (1964), and Kemp and Thode (1968). He postulated that, with the exception of the breakage of sulfur-oxygen bonds and the uptake of sulfate into the cell, the isotope effects of the forward and backward steps are small. This is due to the fact that they are associated either with reactions where the oxidation state of sulfur is not altered or with sulfur oxidation, which is considered to produce only small isotope fractionation. Therefore, in the Rees-model all isotope fractionation factors except $\Delta^{34}S_{f\_1}$, $\Delta^{34}S_{f\_3}$, $\Delta^{34}S_{f\_4}$ were set equal to zero. The assumption that the oxidation of sulfur compounds does cause only small isotope fractionations has been supported by studies of Fry et al. (1984), Fry et al. (1985), and Habicht et al. (1998), who observed small sulfur isotope effects in experiments where sulfur compounds were oxidized by bacterial cultures. However, as for bacterial reduction of sulfate, bacterial oxidation of sulfur compounds is likely to be a stepwise process: thus the measured fractionations are dependent not only on the isotope fractionation of the oxidation reaction but also on the cell internal-reaction pathways. It can be expected that maximum kinetic isotope effects are not fully expressed in oxidation experiments with bacterial cultures; thus, fractionations observed from abiotic oxidation of sulfide are better estimates. Abiotic oxidation of sulfide by oxygen has been shown to produce an average sulfur isotope fractionation of $-5.2‰ \pm 1.4‰$ (Fry et al., 1988). Therefore, sulfur isotope fractionation related to oxidative backward fluxes needs to be considered in the revision of the Rees model.

From the Rees-model assumption that all backward fluxes cause small isotope effects, a simplified equation for the total sulfur isotope effect produced by a bacterial cell can be derived:

$$\Delta^{34}S_{cell} = \Delta^{34}S_{f\_1} + X_1 \cdot X_2 \cdot \Delta^{34}S_{f\_3} + X_1 \cdot X_2 \cdot X_3 \cdot \Delta^{34}S_{f\_4}$$

The subscript "cell" in $\Delta^{34}S_{cell}$ indicates that the described sulfur isotope effect is created by a single sulfate-reducing bacterium. Hereafter, the expression "single sulfate-reducing bacterium" is abbreviated as "a cell".

For this model two end members can be identified. In the first case, which could correspond to a sulfate limited system, no backward fluxes occur and consequently the values for X_1, X_2, and X_3 all become zero. The total sulfur isotope effect by a cell becomes then

$$\Delta^{34}S_{cell} = \Delta^{34}S_{f\_1}.$$

Rees (1973) hypothesized that at very low sulfate concentrations, the forward reactions should proceed as fast as sulfur is supplied, and that therefore, no backward flows are established. In laboratory experiments, Harrison and Thode (1958) found an overall reverse isotope effect of $+3‰$ at very low sulfate concentrations (10 μM). Consequently, Rees (1973) assigned the value of $+3‰$ to the uptake of sulfate into the cell of the bacterium ($\Delta^{34}S_{f\_1} = +3‰$). For different freshwater and marine natural populations of sulfate reducers Habicht et al. (2002) report small sulfur isotope fractionation at low sulfate concentrations (<50 μM) in a range of $-5.9‰$ to $+4.5‰$. These findings indicate that two competing isotope fractionation processes may contribute to the isotope effects (positive and negative) observed at very low sulfate concentrations. These isotope effects are likely to be related to the uptake of sulfate from ambient water into the cell via secondary transport systems in symport with cations, a reversible process (Cypionka, 1995).

The opposite extreme case in the Rees model is when the backward fluxes equal the forward fluxes and the values for X_1, X_2, and X_3 are close to unity, and the total sulfur isotope effect by a cell equals the total of the sulfur isotope fractionation steps:

$$\Delta^{34}S_{cell} = \Delta^{34}S_{f\_1} + \Delta^{34}S_{f\_3} + \Delta^{34}S_{f\_4}$$

This relation was used by Rees (1973) to estimate the values for $\Delta^{34}S_{f\_3}$ ($-25‰$) and $\Delta^{34}S_{f\_4}$ ($-25‰$). The estimates were mainly based on two observations from bacterial sulfate-reduction experiments and on an assumption concerning the reaction kinetics. Rees observed that the largest observed isotope fractionation effect in culture experiments was less than $-50‰$ and that isotope fractionation factors larger than $-25‰$ were reported from only a minority of experiments. With the assumption that the transformation of sulfite to sulfide (step 4) is rapid, Rees (1973) concluded that in most cases the sulfite pool was depleted immediately after the supply of sulfite from the reduction of APS. Therefore, no backward flux to APS could occur ($b_3 = 0$, $X_3 = 0$). He concluded that the fractionation effect caused by step 4 would contribute to the measured total fractionation effect by a cell in only a few exceptions, most likely the few cases in which fractionation factors observed in cultures were larger than $-25‰$. Rees, therefore, assigned a fractionation factor of $-25‰$ to step 3 ($\Delta^{34}S_{f\_3}$) to match the "normal" conditions. Taking into account the $+3‰$ isotope fractionation that Rees assigned to the uptake of sulfate into the cell of the bacterium ($\Delta^{34}S_{f\_1} = +3‰$), an assignment of $-28‰$ to the fractionation by the reduction of sulfate to sulfite ($\Delta^{34}S_{f\_3} = -28‰$) would have been more appropriate. The value of $-25‰$ for $\Delta^{34}S_{f\_3}$ is supported by the calculations of Farquhar et al. (2003), who calculated the theoretical equilibrium sulfur isotope fractionation between sulfate and sulfite to be around $-24‰$ at 25°C and by laboratory experiments indicating that sulfite reoxidation takes place when $\Delta^{34}S_{cell}$ exceed a value of $-25‰$ (Brunner et al., 2005).

In culture experiments with *Desulfovibrio desulfuricans,* a

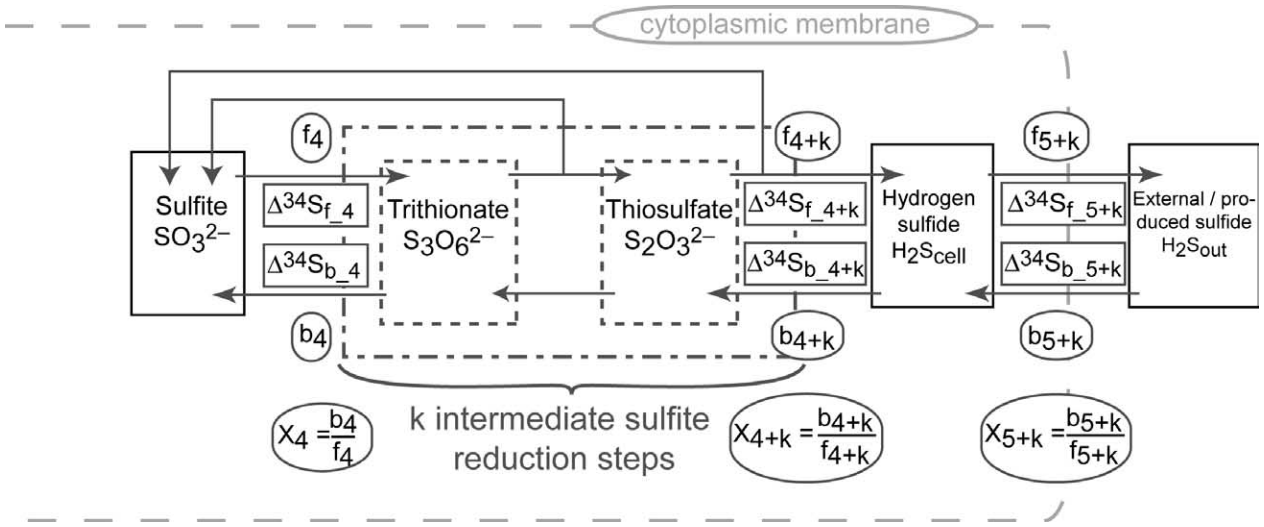

Fig. 3. Multistep reaction scheme for the reduction of sulfite to sulfide: Sulfite is transformed to sulfide by several (k) reversible enzyme-catalyzed steps. In a final step, sulfides are excreted through the cytoplasmic membrane. As an example for a multistep reaction, we depict the trithionate pathway. For a detailed description see text and Cypionka (1995).

maximum sulfur isotope difference between produced sulfides and residual sulfate of around −46‰ (Kaplan and Rittenberg, 1964; Thode, 1991, and references therein) was observed. Rees (1973) concluded that a fractionation factor of −25‰ for step 4 ($\Delta^{34}S_{f\_4}$) would be an appropriate estimate to explain the maximal experimental values. Summarizing, using the assumptions of Rees, the sulfur isotope fractionation factor by a cell can be written as follows:

$$\Delta^{34}S_{cell} = +3‰ - X_1 \cdot X_2 \cdot 25‰ - X_1 \cdot X_2 \cdot X_3 \cdot 25‰$$

However, as was already pointed out by Rees (1973), the assumption for the sulfur isotope effect related to the reduction of sulfite represents a minimum estimate only. In culture experiments the maximum sulfur isotope effect for the reduction of sulfite to sulfide was in the range of −25‰ to −33‰ (Kaplan and Rittenberg, 1964; Kemp and Thode, 1968). This indicates that the commonly used value of −25‰ for the isotope effect related to sulfite reduction is probably at the lower limit of the possibilities.

3. REVISION OF THE REES MODEL

Based on the above considerations, we propose the following modifications to the Rees model: an exchange flux of sulfide between the cell and ambient water is introduced, the fractionation factors for the sulfite-sulfide step are changed, and the reaction chain at the reduction of sulfite to sulfide is modified with the introduction of multiple steps and the inclusion of reverse flows.

3.1. Reverse Multistep in Sulfite-Sulfide Reaction

Although there is still debate on the exact mechanisms and enzymes involved in the reduction of sulfite to sulfide, it is clear that the final step of sulfite reduction to sulfide can involve a series of intermediates, in particular thiosulfate, trithionate and bisulfite (Akagi, 1995; Cypionka, 1995). Culturing experiments of Chambers and Trudinger (1975), where no intermediates were observed, were carried out under excess electron donor supply, whereas in the experiments showing the production of intermediates, bacterial growth was limited by electron donor availability (Cypionka, 1995). The production and excretion of these intermediates allows for the reverse reaction to take place. For example, the trithionate pathway involves three enzymes, a sulfite reductase forming trithionate from three sulfite molecules, a trithionate reductase forming thiosulfate and sulfite, and a thiosulfate reductase forming sulfide and sulfite (for chemical equations and discussion see Cypionka [1995]). The production of sulfite in these reactions indicates that a backward flux is an integral part of this pathway. Therefore, these additional reverse fluxes are incorporated in the model, and we propose to add, as an alternative pathway, a multistep reaction scheme for the sulfite reduction (Fig. 3) to the Rees model. The depicted multistep reaction pathway for the reduction of sulfite to sulfide involves a number (k) of intermediate sulfur compounds.

Further, we add a final step in the reaction scheme (Figs. 3 and 4), the excretion of produced sulfide (either diffusive as H_2S gas or as HS^- transported by electroneutral symport with H^+) through the cytoplasmic membrane to ambient water (Cypionka, 1995). This step is reversible, because under neutral conditions, approximately half of the sulfide is present as the very diffusible H_2S. High sulfide concentrations are known to inhibit the growth of sulfate-reducing bacteria, although the inhibiting mechanism is not fully understood (e.g., Reis et al., 1991).

3.2. Reversible Sulfite-Sulfide Reaction

We agree with Rees (1973) that under most conditions, for example, when sulfide concentrations are low, a backward flux is unlikely to occur. However, there is no evidence that, in principle, the reduction of sulfite to sulfide is irreversible.

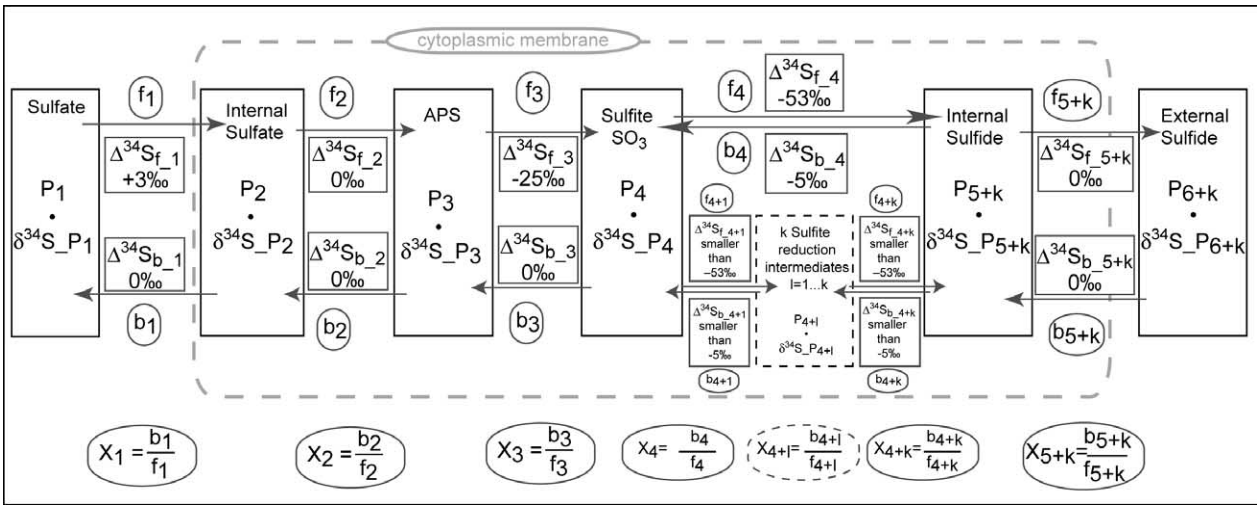

Fig. 4. Revised pathway of dissimilatory sulfate reduction (modified after Rees [1973] and Fritz et al. [1989]): Sulfate is transformed to sulfide by enzyme-catalyzed steps within the sulfate-reducing organism. Forward (f_i) and backward fluxes (b_i) connect pools of intermediate sulfur compounds (X_i equals the b_i:f_i ratios). Sulfite is either transformed to sulfide in a single step mechanism or in a multistep process. In the latter, the variable "k" represents the number of intermediates in the reduction step of sulfite to sulfide. For derivation of the values assigned to sulfur isotope fractionation steps, see text.

Membrane-associated dissimilatory sulfite reductase has been shown to catalyze the oxidation of sulfide (Einsele et al., 2001). In addition, the observation of the formation of intermediates such as thiosulfate and trithionate described by Fitz and Cypionka (1990) indicates that a backward flux cannot be excluded and supports the stepwise reduction pathway with reverse reactions proposed by Kobayashi et al. (1974).

3.3. Reassessment of Kinetic Fractionation Factors

As pointed out above, the small sulfur isotope fractionations determined from experiments where sulfur compounds were oxidized by bacterial cultures (Fry et al., 1984; Fry et al., 1985; Habicht et al., 1998) cannot be used as a direct estimate for the sulfur isotope fractionation related to oxidation steps. This is because the bacterial oxidation of sulfur compounds itself is likely to be a stepwise process. However, isotope fractionation factors determined from abiological oxidation of sulfur compounds represent good estimates if the oxidation process occurred unidirectionally. The abiotic oxidation of sulfide by oxygen produces an average sulfur isotope fractionation of $-5.2‰ \pm 1.4‰$ (Fry et al., 1988), while the abiotic oxidation of sulfite to sulfate produces a negligible sulfur isotope fractionation of $-0.6‰$ to $-0.3‰$ (Fry et al., 1985) with an average value of $-0.4‰$ (Fry et al., 1986). We, therefore, suggest to assign a sulfur isotope fractionation of $-5‰$ to the oxidation of sulfide to sulfite and to keep the value for the oxidation of sulfite to sulfate at 0‰. Because secondary isotope effects are small, we further assume that sulfur isotope effects in backward fluxes that do not involve oxidation reactions are around 0‰.

However, as pointed out above, it is likely that both the uptake of sulfate into the cell and the release of sulfate from the cell do create isotope effects. Currently, there are no data that would permit us to assign specific values to the isotope effects related to the uptake of sulfate into the bacterium or the release of sulfate from the cell. Therefore, we suggest keeping the values for the isotope effects that have been attributed by Rees (1973) to the uptake of sulfate into the cell ($\Delta^{34}S_{f\_1} = +3‰$) and to the release of sulfate from the cell ($\Delta^{34}S_{b\_1} = +0‰$) until this problem can be assessed based on additional data. A higher value of $+4.5‰$ has been observed by Habicht et al. (2002) at low sulfate concentrations ($<5 \times 10^{-5}$ M), indicating that $+3‰$ may not be a maximum value.

Our revision of the isotope effect for the reduction of sulfite to sulfide is based on the following observations: in culture experiments of sulfate reducing bacteria growing on sulfite (Kaplan and Rittenberg, 1964; Kemp and Thode, 1968), a sulfur isotope effect of $-25‰$ to $-33‰$ was observed. This indicates that the factor of $-25‰$ used by Rees (1973) is at the lower limit of the possibilities. The assumption of Rees (1973) for the maximum isotope effect by sulfate reducing bacteria ($-46‰$) was based on maximum values derived from culture experiments where the cells were exposed to extreme conditions (resting suspensions of *Desulfovibrio desulfuricans,* ethanol as hydrogen donor [Kaplan and Rittenberg, 1964], and nutrient-free experiments of Ford [1957]). Rees (1973) assumed that under these conditions, the backward fluxes were almost equal to the forward fluxes (X_i close to unity). More recent experiments have shown that in a few cases, similar high isotope fractionations of $-42‰$ (Detmers et al., 2001) and $-47‰$ (Bolliger et al., 2001) can occur at optimized growth conditions when, for instance, sulfate and an electron donor exist in excess and temperature is in the optimum growth range. In these experiments, the cell- specific sulfate-reduction rate was in the range of the majority of other sulfate-reducing bacterial cultures, where much smaller isotope effects were observed. Since a significant cell-specific sulfate-reduction rate was observed, it cannot be concluded that the backward fluxes equaled the forward fluxes, as this would mean that no net sulfate reduction is taking place. Therefore, it is unlikely that in

these experiments, the ratios between backward and forward fluxes were close to unity. Consequently, in order to fully express an isotope effect around −46‰ with X_i ratios smaller than unity, the theoretical maximum isotope fractionation between sulfite and sulfide has to be larger than the postulated −25‰.

The kinetic isotope fractionation in the forward reaction of sulfite to sulfide equals the difference between the corresponding reverse reaction and the equilibrium sulfur isotope fractionation between sulfite and sulfide. The equilibrium sulfur isotope fractionation between sulfite and sulfide was calculated to be around −48‰ at 25°C (Farquhar et al., 2003). As discussed above, the sulfur isotope fractionation caused by the oxidation of sulfide to sulfite is around −5‰. Consequently, the kinetic isotope fractionation of the reduction of sulfite to sulfate equals the difference between the equilibrium isotope effect and the isotope effect related to the oxidation of sulfide to sulfite ($\Delta^{34}S_{f\_4} = -53‰$). This suggestion for a reassessment of the kinetic sulfur isotope fractionation of the reduction of sulfite to sulfide doubles the estimate by Rees (1973). However, we emphasize that Rees was aware that his assumption for the isotope effect related to the reduction of sulfite to sulfide was only a minimum value. For the case, where this reduction is a stepwise process, the total of the involved isotope fractionation steps sums up to −53‰. Figure 4 depicts a summary of the proposed revisions of the Rees model.

According to the fluxes and values depicted in Figure 4, the sulfur isotope fractionation caused by a sulfate reducing bacterium can be recalculated for the modified Rees model. This results in:

$$\Delta^{34}S_{cell} = 3‰ + X_1 \cdot X_2 \cdot -25‰ + X_1 \cdot X_2 \cdot X_3 \cdot X' - 53‰ - X_1 \cdot X_2 \cdot X_3 \cdot X'' \cdot -5‰$$

In the case of the formation of two intermediate sulfur compounds (as in Fig. 3) during the reduction of sulfite to sulfide, X′ and X″ equal a combination of the ratios between backward and forward fluxes:

$$X' \cdot -53‰ = \Delta^{34}S_{f\_4} + X_4 \cdot \Delta^{34}S_{f\_5} + X_4 \cdot X_5 \cdot \Delta^{34}S_{f\_6}$$

with:

$$-53‰ = \Delta^{34}S_{f\_4} + \Delta^{34}S_{f\_5} + \Delta^{34}S_{f\_6}$$

$$X'' \cdot -5‰ = X_4 \cdot \Delta^{34}S_{b\_4} + X_4 \cdot X_5 \cdot \Delta^{34}S_{b\_5} + X_4 \cdot X_5 \cdot X_6 \cdot \Delta^{34}S_{b\_6}$$

with:

$$-5‰ = \Delta^{34}S_{b\_4} + \Delta^{34}S_{b\_5} + \Delta^{34}S_{b\_6}$$

In the case of a single-step reduction of sulfite to sulfide, X′ equals 1 and X″ equals the ratio between the backward and forward flux during sulfite reduction:

$$X' = 1$$

$$X'' = X_4$$

As with the Rees model, we can model two extreme cases. When sulfate reduction is unidirectional (no backward fluxes), for instance, because sulfate is present in very low concentrations, the total sulfur isotope effect by a sulfate-reducing bacterium becomes:

$$\Delta^{34}S_{cell} = +3‰$$

In the opposite extreme, backward fluxes equal the forward fluxes. In this case the ratios between backward and forward fluxes equal unity and the total sulfur isotope effect by a sulfate-reducing bacterium becomes:

$$\Delta^{34}S_{cell} = 3‰ - 25‰ - 53‰ - 5‰ = -70‰$$

In such an extreme scenario, the sulfur isotope fractionation by sulfate reduction can reach −70‰ (in the range of calculated sulfate-sulfide equilibrium sulfur fractionation factor at temperatures between 0°C and 25°C; Tudge and Thode, 1950; Ohmoto and Lasaga, 1982; Farquhar et al., 2003). This finding is not at all new—Kaplan and Rittenberg (1964) concluded, "Assuming the reversibility of the entire sequence then an active exchange between SO_4^{2-} and H_2S could occur, and a fractionation factor of 1.074 might be approached." The sulfate-sulfide equilibrium isotope effect calculated for a temperature of 5°C is ~ −80‰ (Farquhar et al., 2003). Therefore, theoretically, isotope effects by sulfate-reducing bacteria well in excess of −70‰ could occur in a temperature range that corresponds to marine environments. Such an extreme sulfur isotope fractionation by sulfate-reducing bacteria has never been observed in laboratory culture studies. However, from natural environments, there is evidence that such extreme sulfur isotope fractionations might occur.

4. DISCUSSION AND INTERPRETATION

A major implication of our revisions of the Rees model is that the theoretical maximum sulfur isotope fractionation by sulfate-reducing bacteria is ~ −70‰, instead of the previously reported −46‰. This finding is relevant, because in natural environments, sulfides are often depleted in ^{34}S by −45‰ to −70‰ relative to seawater sulfate (Ohmoto et al., 1990). Previously, the −45‰ to −70‰ depletion of sulfides relative to sulfate had to be explained by additional sulfur isotope fractionation processes besides that of sulfate reduction. Canfield and Thamdrup (1994) proposed such a process, where sulfides produced by sulfate reduction are oxidized to elemental sulfur, which in turn is disproportionated to sulfide and sulfate. This process can be repeated several times. A consequence of this cycling is a multistep sulfur isotope fractionation leading to the strongly depleted sulfur isotope composition of sulfides (Habicht and Canfield, 2001). The now revised (theoretical) maximum sulfur isotope fractionation of −70‰ for sulfate reduction that is based on a larger estimate for the fractionation related to the reduction of sulfite to sulfide suggests that sulfate reduction in natural environments may create large sulfur isotope fractionation without additional disproportionation of elemental sulfur. Two lines of evidence support this hypothesis. The first is based on observations from natural environments indicating that extreme sulfur isotope fractionations by sulfate-reducing bacteria can occur. Based on a numerical model integrating diffusion, advection, and sulfate reduction, Wortmann et al. (2001) determined sulfur isotope fractionation factors of −65‰ in hypersulfidic interstitial waters from the Great Australian Bight sediments. In the Cariaco Basin, Werne et al. (2003) observed an offset between pore water sulfate and pore water sulfide of −55‰ to

−65‰. As discussed in the introduction, under hypersulfidic conditions disproportionation, as additional sulfur isotope fractionation process, is inhibited; hence, sulfur isotope fractionation by sulfate reducers is likely to be the cause for these large fractionations. From deep ocean sediments at elevated temperatures, Rudnicki et al. (2001) report sulfur isotope fractionation factors of −77‰ ± 7‰, which were determined using a diffusion-advection model. They attribute the observed isotope effect solely to bacterial sulfate reduction and speculate that the large fractionation is related to extremely low sulfate reduction rates. The second argument favoring sulfur fractionation by sulfate-reducing bacteria up to 70‰ is that alternative fractionation pathways (i.e., disproportionation of elemental sulfur) create large isotope fractionations by repeating reactions with smaller isotope fractionations. Thus, one would expect that, depending on the number of repetitions, a much broader range of fractionations between sulfides and sulfate should be observed (i.e., isotope fractionations larger than −70‰) than what is reported from natural environments. Sulfides are commonly depleted in ^{34}S by −45‰ to −70‰ relative to seawater sulfate (Ohmoto et al., 1990). The existence of an upper fractionation limit of ~ −70‰ in natural environments, which is close to the thermodynamic equilibrium fractionation between sulfate and sulfide, therefore indicates that a single step fractionation process (without repetitions) with a maximal fractionation of ~ −70‰ may be important.

We are aware that the reports of large sulfur isotope fractionations by sulfate-reducing organisms cannot be considered as definite proof for sulfur isotope fractionation by sulfate-reducing organisms in excess of −47‰ because the reported isotope fractionations have not been determined directly (e.g., from in situ measurements), and the involved organisms have not been identified. However, other explanations involving anaerobic sulfide oxidation and bacterial disproportionation of elemental sulfur under hypersulfidic conditions by bacteria with a currently unknown metabolic pathway (Werne et al., 2003) are rather speculative.

The main limitation of our model is that to date fractionations larger than 47‰ have neither been observed in laboratory experiments with sulfate-reducing bacteria (Kaplan and Rittenberg, 1964; Thode, 1991; Bolliger et al., 2001) nor in studies of natural populations (e.g., Habicht and Canfield, 2001). In the following, we discuss some possible explanations for the lower fractionations observed in the laboratory.

4.1. Conditions for Extreme Sulfur Isotope Fractionation

Our model predicts that high isotope fractionations are expressed when the ratios between the cell internal backward and forward fluxes (X-values) are close to unity. This is best achieved by a low reaction rate of the last fractionation-relevant step relative to the other processes, for instance, a low reaction rate of the one step-reduction of sulfite to sulfide. In this case, the backward fluxes preceding the last fractionation-relevant step become important and the corresponding X-values can approach unity. Such a situation is either caused by a reduction of the reaction rate in the last fractionation-relevant step or an acceleration of the preceding steps.

The observation of extremely large isotope fractionations in hypersulfidic environments indicates that a high sulfide concentration might be a trigger for large fractionations. A high total sulfide concentration (H_2S and HS^-) is known to inhibit sulfate reduction (Okabe et al., 1995), and molecular hydrogen sulfide (H_2S) has been found to be the major toxic form of sulfide, reducing the activity of sulfate-reducing bacteria with a decrease in substrate consumption (Reis et al., 1991; Reis et al., 1992). If the inhibition of sulfate reduction by molecular hydrogen sulfide occurs at the last fractionation-relevant step (i.e., the reduction of sulfite to sulfide), high sulfur isotope fractionations might be observed. However, to our knowledge, there is no report of increasing sulfur isotope fractionations with increasing sulfide concentrations in laboratory experiments. Therefore, it is unlikely that hypersulfidic conditions alone result in high sulfur isotope fractionation.

The large isotope fractionation of −77‰ ± 7‰ from deep ocean sediments at elevated temperatures was related to extremely low bulk sulfate reduction rates (Rudnicki et al., 2001). Thus, low sulfate reduction rates might be another condition for extreme sulfur isotope fractionations to be expressed. However, Detmers et al. (2001) observed that cell-specific sulfate reduction rates and isotope fractionation factors did not correlate. Their laboratory investigations at optimized growth conditions rather suggest that energy supply could play an important role for isotope fractionation. Sulfate reducers that oxidized the carbon source completely to carbon dioxide (energetically less favorable) showed greater fractionations than sulfate reducers that released acetate as the final product of carbon oxidation (energetically more favorable). Interestingly, the formation of intermediates in the reduction of sulfite to sulfide has been observed in cultures where the electron donor was limiting (Cypionka, 1995 and references therein), whereas the experiments of Chambers and Trudinger (1975) that did not show formation of intermediates had an excess of electron donor. A strong effect of substrate limitation on sulfur isotope fractionation has been recently observed by Hoek et al. (2004) in experiments with the thermophile sulfate reducer *Thermodesulfatator indicus* grown with hydrogen as electron donor and CO_2 as primary carbon source. In their experiments the sulfur isotope fractionation increased from values between −1.5‰ and −10‰ in cultures with high H_2 concentrations to values between −24‰ and −37‰ in cultures grown under H_2 limitation. In addition to the energy yield, the physiology of a specific bacterial strain plays a major role for the magnitude of the sulfur isotope fractionation (Kleikemper et al., 2004). Extreme sulfur isotope fractionations, therefore, could be strongly dependent on the consortia of bacteria.

4.2. How Can Sulfur Isotope Fractionations Larger Than −46‰ be Observed in Laboratory Experiments with Sulfate Reducing Bacteria?

In the above discussion, a unique precondition for extreme sulfur isotope fractionation could not be identified. We speculate that a combination of low energy supply with hypersulfidic conditions could trigger larger sulfur isotope fractionation effects by sulfate-reducing bacteria than the reported maximum value ~ −46‰. To achieve the anticipated results, we propose to investigate different cultures of sulfate-reducing bacteria under combined hypersulfidic and substrate-limited conditions at nonlimited supply of sulfate.

5. CONCLUSIONS

The commonly accepted model for the isotope effects by dissimilatory sulfate reduction (Rees, 1973) cannot fully explain the sulfur isotope effects related to the reduction of sulfite to sulfide. The main consequence of our revised model is that the sulfur isotope fractionation by dissimilatory sulfate reduction can reach values above −70‰ (instead of the previously reported value of ~ −46‰). This would account for observations from natural environments, where fractionation factors up to −77‰ have been observed (Rudnicki et al., 2001; Wortmann et al., 2001; Werne et al., 2003). We obviously do not disregard the involvement of processes related to the oxidative cycle of sulfur in near-surface environments, but our model presents a possible alternative way to obtain large isotope fractionations observed in the geologic record. Further experimental culturing work under well-constrained growth conditions is necessary to test our model.

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APPENDIX 1. Calculation of the Total Sulfur Isotope Fractionation by a Sulfate-Reducing Bacterium

For a similar calculation (using α instead of Δ) see Rees (1973) and Comstock (2001).

In a laboratory steady-state setup, where the $\delta^{34}S$ of sulfate, sulfate, and nutrient concentration and physical and chemical parameters (e.g., temperature and pH) are kept constant, the sulfur isotopic composition of the produced sulfides is also constant. The total sulfur isotope fractionation by the sulfate reduction equals the difference between $\delta^{34}S_{sulfide}$ and $\delta^{34}S_{sulfate}$. This value is the result of the combination of different forward and

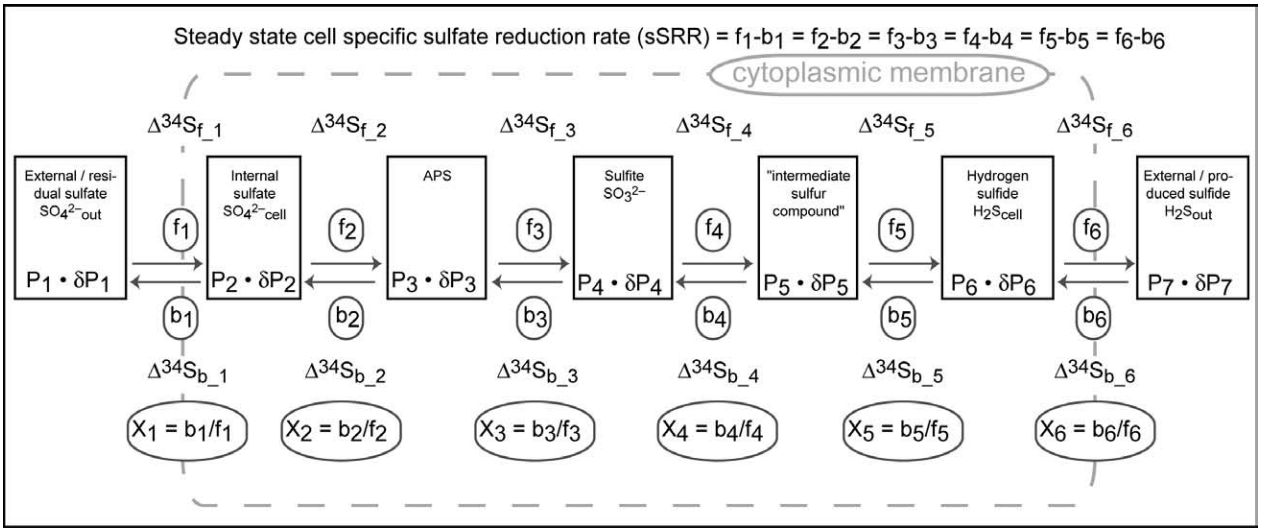

Fig. 5. Pathway of dissimilatory sulfate reduction (modified after Rees [1973] and Fritz et al. [1989]): Sulfate is transformed to sulfide by enzyme-catalyzed steps within the sulfate-reducing organism (dashed line = cytoplasmic membrane). Forward- (f_i) and backward fluxes (b_i) connect pools (P_i) of intermediate sulfur compounds (X_i equals the b_i:f_i ratios). The cell specific sulfate reduction rate (sSRR) equals the difference between forward and backward fluxes. For simplicity, the sulfur pools and their isotope composition are abbreviated, e.g., external sulfate = P_1 and isotope composition of external sulfate = δP_1.

backward fluxes in the cascade of enzymatic transformations of sulfur compounds within the cell and can be calculated by steady-state mass balances for each sulfur pool. Here, we develop the equation for a seven step sulfate reduction scheme (Fig. 5); however, the calculation is valid for any multistep reaction chain. The derived equation thus can be used for any number of reaction steps.

The following equations are derived from the steady-state assumption above:

Step 7 = last step (external sulfide):

Change of amount of S-compound in pool 7:

$$\frac{d}{dt}P_7 = +f_6 - b_6 = sSRR$$

Change in amount and isotope composition of S compound in pool 7:

$$\frac{d}{dt}\left(P_7 \cdot \delta P_7\right) = \frac{d}{dt}P_7 \cdot \delta P_7 + \frac{d}{dt}\delta P_7 \cdot P_7 = +f_6 \cdot \left(\delta P_6 + \Delta_{f\_6}\right) - b_6 \cdot \left(\delta P_7 + \Delta_{b\_6}\right)$$

Assuming a steady state, there is no change in the amount of S-compound in pool 7:

$$\frac{d}{dt}\delta P_7 = 0$$

The equations for pool 7 are rearranged and solved for the isotope composition of the S-compound in pool 6:

$$\frac{d}{dt}P_7 \cdot \delta P_7 = sSRR \cdot \delta P_7 = \left(+f_6 - b_6\right) \cdot \delta P_7 = +f_6 \cdot \left(\delta P_6 + \Delta_{f\_6}\right) - b_6 \cdot \left(\delta P_7 + \Delta_{b\_6}\right) \Rightarrow sSRR \cdot \delta P_7 = +f_6 \cdot \left(\delta P_6 + \Delta_{f\_6}\right) - b_6 \cdot \left(\delta P_7 + \Delta_{b\_6}\right)$$

$$f_6 \cdot \left(\delta P_6 + \Delta_{f\_6}\right) = sSRR \cdot \delta P_7 + b_6 \cdot \left(\delta P_7 + \Delta_{b\_6}\right)$$

$$\left(\delta P_6 + \Delta_{f\_6}\right) = \frac{b_6}{f_6} \cdot \left(\delta P_7 + \Delta_{b\_6}\right) + \frac{sSRR}{f_6} \cdot \delta P_7$$

The calculations made for pool 7 are repeated for pool 6 to pool 2.

Step 6 (internal sulfide):

$$\frac{d}{dt}\left(P_6 \cdot \delta P_6\right) = 0 = +f_5 \cdot \left(\delta P_5 + \Delta_{f\_5}\right) - b_5 \cdot \left(\delta P_6 + \Delta_{b\_5}\right) - \left(+f_6 \cdot \left(\delta P_6 + \Delta_{f\_6}\right) - b_6 \cdot \left(\delta P_7 + \Delta_{b\_6}\right)\right)$$

$$0 = +f_5 \cdot \left(\delta P_5 + \Delta_{f\_5}\right) - b_5 \cdot \left(\delta P_6 + \Delta_{b\_5}\right) - sSRR \cdot \delta P_7 \Rightarrow sSRR \cdot \delta P_7 = +f_5 \cdot \left(\delta P_5 + \Delta_{f\_5}\right) - b_5 \cdot \left(\delta P_6 + \Delta_{b\_5}\right)$$

$$\left(\delta P_5 + \Delta_{f\_5}\right) = \frac{b_5}{f_5} \cdot \left(\delta P_6 + \Delta_{b\_5}\right) + \frac{sSRR}{f_5} \cdot \delta P_7$$

Step 5 (“intermediate sulfur compound”):

$$\frac{d}{dt}\left(P_5 \cdot \delta P_5\right) = 0 = +f_4 \cdot \left(\delta P_4 + \Delta_{f\_4}\right) - b_4 \cdot \left(\delta P_5 + \Delta_{b\_4}\right) - \left(+f_5 \cdot \left(\delta P_5 + \Delta_{f\_5}\right) - b_5 \cdot \left(\delta P_6 + \Delta_{b\_5}\right)\right) \Rightarrow sSRR \cdot \delta P_7 = +f_4 \cdot \left(\delta P_4 + \Delta_{f\_4}\right) - b_4 \cdot \left(\delta P_5 + \Delta_{b\_4}\right)$$

$$\left(\delta P_4 + \Delta_{f\_4}\right) = \frac{b_4}{f_4} \cdot \left(\delta P_5 + \Delta_{b\_4}\right) + \frac{sSRR}{f_4} \cdot \delta P_7$$

Step 4 (sulfite):

$$\frac{d}{dt}\left(P_4 \cdot \delta P_4\right) = 0 = +f_3 \cdot \left(\delta P_3 + \Delta_{f\_3}\right) - b_3 \cdot \left(\delta P_4 + \Delta_{b\_3}\right) - \left(+f_4 \cdot \left(\delta P_4 + \Delta_{f\_4}\right) - b_4 \cdot \left(\delta P_5 + \Delta_{b\_4}\right)\right) \Rightarrow sSRR \cdot \delta P_7 = +f_3 \cdot \left(\delta P_3 + \Delta_{f\_3}\right) - b_3 \cdot \left(\delta P_4 + \Delta_{b\_3}\right)$$

$$\left(\delta P_3 + \Delta_{f\_3}\right) = \frac{b_3}{f_3} \cdot \left(\delta P_4 + \Delta_{b\_3}\right) + \frac{sSRR}{f_3} \cdot \delta P_7$$

Step 3 (APS_sulfate):

$$\frac{d}{dt}\left(P_3 \cdot \delta P_3\right) = 0 = +f_2 \cdot \left(\delta P_2 + \Delta_{f\_2}\right) - b_2 \cdot \left(\delta P_3 + \Delta_{b\_2}\right) - \left(+f_3 \cdot \left(\delta P_3 + \Delta_{f\_3}\right) - b_3 \cdot \left(\delta P_4 + \Delta_{b\_3}\right)\right) \Rightarrow sSRR \cdot \delta P_7 = +f_2 \cdot \left(\delta P_2 + \Delta_{f\_2}\right) - b_2 \cdot \left(\delta P_3 + \Delta_{b\_2}\right)$$

$$\left(\delta P_2 + \Delta_{f\_2}\right) = \frac{b_2}{f_2} \cdot \left(\delta P_3 + \Delta_{b\_2}\right) + \frac{sSRR}{f_2} \cdot \delta P_7$$

Step 2 (internal sulfate):

$$\frac{d}{dt}\left(P_2 \cdot \delta P_2\right) = 0 = +f_1 \cdot \left(\delta P_1 + \Delta_{f\_1}\right) - b_1 \cdot \left(\delta P_2 + \Delta_{b\_1}\right) - \left(+f_2 \cdot \left(\delta P_2 + \Delta_{f\_2}\right) - b_2 \cdot \left(\delta P_3 + \Delta_{b\_2}\right)\right) \Rightarrow sSRR \cdot \delta P_7 = +f_1 \cdot \left(\delta P_1 + \Delta_{f\_1}\right) - b_1 \cdot \left(\delta P_2 + \Delta_{b\_1}\right)$$

$$\left(\delta P_1 + \Delta_{f\_1}\right) = \frac{b_1}{f_1} \cdot \left(\delta P_2 + \Delta_{b\_1}\right) + \frac{sSRR}{f_1} \cdot \delta P_7$$

The above results for the pools 7 to 2 can be summarized as follows:

$$\left(\delta P_6 + \Delta_{f\_6}\right) = \frac{b_6}{f_6} \cdot \left(\delta P_7 + \Delta_{b\_6}\right) + \frac{sSRR}{f_6} \cdot \delta P_7$$
$$\left(\delta P_5 + \Delta_{f\_5}\right) = \frac{b_5}{f_5} \cdot \left(\delta P_6 + \Delta_{b\_5}\right) + \frac{sSRR}{f_5} \cdot \delta P_7$$

$$\left(\delta P_4 + \Delta_{f\_4}\right) = \frac{b_4}{f_4} \cdot \left(\delta P_5 + \Delta_{b\_4}\right) + \frac{sSRR}{f_4} \cdot \delta P_7$$
$$\left(\delta P_3 + \Delta_{f\_3}\right) = \frac{b_3}{f_3} \cdot \left(\delta P_4 + \Delta_{b\_3}\right) + \frac{sSRR}{f_3} \cdot \delta P_7$$
$$\left(\delta P_2 + \Delta_{f\_2}\right) = \frac{b_2}{f_2} \cdot \left(\delta P_3 + \Delta_{b\_2}\right) + \frac{sSRR}{f_2} \cdot \delta P_7$$
$$\left(\delta P_1 + \Delta_{f\_1}\right) = \frac{b_1}{f_1} \cdot \left(\delta P_2 + \Delta_{b\_1}\right) + \frac{sSRR}{f_1} \cdot \delta P_7$$

Model considerations: The cell specific sulfate reduction rate equals the difference between the forward- and backward flux in each step:

$$sSRR = f_1 - b_1 = f_2 - b_2 = f_3 - b_3 = f_4 - b_4 = f_5 - b_5 = f_6 - b_6$$

Using the ratio of backward- to forward flux, the forward fluxes (f_i) and the cell specific sulfate reduction rate (sSRR) can be substituted by backward- to forward ratios (X_i):

$$X_i = \frac{b_i}{f_i}$$
$$\frac{sSRR}{f_i} = \frac{f_i - b_i}{f_i} = 1 - \frac{b_i}{f_i} = 1 - X_i$$

Substitution of f_i and sSRR by X_i for the equations for pool 7 to 1:

$$\left(\delta P_6 + \Delta_{f\_6}\right) = X_6 \cdot \left(\delta P_7 + \Delta_{b\_6}\right) + \left(1 - X_6\right) \cdot \delta P_7 = X_6 \cdot \Delta_{b\_6} + \delta P_7$$
$$\left(\delta P_6 + \Delta_{f\_6}\right) = X_6 \cdot \Delta_{b\_6} + \delta P_7 \Rightarrow \delta P_6 = -\Delta_{f\_6} + X_6 \cdot \Delta_{b\_6} + \delta P_7$$
$$\left(\delta P_5 + \Delta_{f\_5}\right) = X_5 \cdot \left(\delta P_6 + \Delta_{b\_5}\right) + \left(1 - X_5\right) \cdot \delta P_7$$
$$\left(\delta P_4 + \Delta_{f\_4}\right) = X_4 \cdot \left(\delta P_5 + \Delta_{b\_4}\right) + \left(1 - X_4\right) \cdot \delta P_7$$
$$\left(\delta P_3 + \Delta_{f\_3}\right) = X_3 \cdot \left(\delta P_4 + \Delta_{b\_3}\right) + \left(1 - X_3\right) \cdot \delta P_7$$
$$\left(\delta P_2 + \Delta_{f\_2}\right) = X_2 \cdot \left(\delta P_3 + \Delta_{b\_2}\right) + \left(1 - X_2\right) \cdot \delta P_7$$
$$\left(\delta P_1 + \Delta_{f\_1}\right) = X_1 \cdot \left(\delta P_2 + \Delta_{b\_1}\right) + \left(1 - X_1\right) \cdot \delta P_7$$

Calculation for the isotope composition of S-compound in pool 6:

$$\delta P_6 = -\Delta_{f\_6} + X_6 \cdot \Delta_{b\_6} + \delta P_7$$

Calculation for the isotope composition of S-compound in pool 5, using result for calculation of S-compound in pool 6:

$$\delta P_5 = -\Delta_{f\_5} + X_5 \cdot \left(\delta P_6 + \Delta_{b\_5}\right) + \left(1 - X_5\right) \cdot \delta P_7$$
$$\delta P_5 = -\Delta_{f\_5} + X_5 \cdot \left(-\Delta_{f\_6} + X_6 \cdot \Delta_{b\_6} + \delta P_7 + \Delta_{b\_5}\right) + \left(1 - X_5\right) \cdot \delta P_7$$
$$\delta P_5 = -\Delta_{f\_5} + X_5 \cdot \left(\Delta_{b\_5} - \Delta_{f\_6} + X_6 \cdot \Delta_{b\_6}\right) + \delta P_7$$

Calculation for the isotope composition of S-compound in pool 4, using result for calculation of S-compound in pool 5:

$$\delta P_4 = -\Delta_{f\_4} + X_4 \cdot \left(\delta P_5 + \Delta_{b\_4}\right) + \left(1 - X_4\right) \cdot \delta P_7$$
$$\delta P_4 = -\Delta_{f\_4} + X_4 \cdot \left(-\Delta_{f\_5} + X_5 \cdot \left(\Delta_{b\_5} - \Delta_{f\_6} + X_6 \cdot \Delta_{b\_6}\right) + \delta P_7 + \Delta_{b\_4}\right) + \left(1 - X_4\right) \cdot \delta P_7$$
$$\delta P_4 = -\Delta_{f\_4} + X_4 \cdot \left(\Delta_{b\_4} - \Delta_{f\_5} + X_5 \cdot \left(\Delta_{b\_5} - \Delta_{f\_6} + X_6 \cdot \Delta_{b\_6}\right)\right) + \delta P_7$$

Calculation for the isotope composition of S-compound in pool 3, using result for calculation of S-compound in pool 4:

$$\delta P_3 = -\Delta_{f\_3} + X_3 \cdot \left(\delta P_4 + \Delta_{b\_3}\right) + \left(1 - X_3\right) \cdot \delta P_7$$
$$\delta P_3 = -\Delta_{f\_3} + X_3 \cdot \left(-\Delta_{f\_4} + X_4 \cdot \left(\Delta_{b\_4} - \Delta_{f\_5} + X_5 \cdot \left(\Delta_{b\_5} - \Delta_{f\_6} + X_6 \cdot \Delta_{b\_6}\right)\right) + \delta P_7 + \Delta_{b\_3}\right) + \left(1 - X_3\right) \cdot \delta P_7$$
$$\delta P_3 = -\Delta_{f\_3} + X_3 \cdot \left(\Delta_{b\_3} - \Delta_{f\_4} + X_4 \cdot \left(\Delta_{b\_4} - \Delta_{f\_5} + X_5 \cdot \left(\Delta_{b\_5} - \Delta_{f\_6} + X_6 \cdot \Delta_{b\_6}\right)\right)\right) + \delta P_7$$

Calculation for the isotope composition of S-compound in pool 2, using result for calculation of S-compound in pool 3:

$$\delta P_2 = -\Delta_{f\_2} + X_2 \cdot \left(\delta P_3 + \Delta_{b\_2}\right) + \left(1 - X_2\right) \cdot \delta P_7$$
$$\delta P_2 = -\Delta_{f\_2} + X_2 \cdot \left(\begin{array}{l} \Delta_{b\_2} - \Delta_{f\_3} \\ +X_3 \cdot \left(\begin{array}{l} \Delta_{b\_3} - \Delta_{f\_4} \\ +X_4 \cdot \left(\begin{array}{l} \Delta_{b\_4} - \Delta_{f\_5} \\ +X_5 \cdot \left(\Delta_{b\_5} - \Delta_{f\_6} + X_6 \cdot \Delta_{b\_6}\right) \end{array}\right) \end{array}\right) \end{array}\right) + \delta P_7$$
$$\delta P_1 = -\Delta_{f\_1} + X_1 \cdot \left(\delta P_2 + \Delta_{b\_1}\right) + \left(1 - X_1\right) \cdot \delta P_7$$
$$\delta P_1 = -\Delta_{f\_1} + X_1 \cdot \left(\begin{array}{l} \Delta_{b\_1} - \Delta_{f\_2} \\ +X_2 \cdot \left(\begin{array}{l} \Delta_{b\_2} - \Delta_{f\_3} \\ +X_3 \cdot \left(\begin{array}{l} \Delta_{b\_3} - \Delta_{f\_4} \\ +X_4 \cdot \left(\begin{array}{l} \Delta_{b\_4} - \Delta_{f\_5} \\ +X_5 \cdot \left(\Delta_{b\_5} - \Delta_{f\_6} + X_6 \cdot \Delta_{b\_6}\right) \end{array}\right) \end{array}\right) \end{array}\right) \end{array}\right) + \delta P_7$$

The sulfur isotope difference between isotope composition of hydrogen sulfide produced and sulfate consumed equals the difference in the isotope composition of the S-compounds between pool 7 and pool 1:

$$-\Delta_{cell} = \delta P_1 - \delta P_7 = -\Delta_{f\_1} + X_1 \cdot \left(\begin{array}{l} \Delta_{b\_1} - \Delta_{f\_2} \\ +X_2 \cdot \left(\begin{array}{l} \Delta_{b\_2} - \Delta_{f\_3} \\ +X_3 \cdot \left(\begin{array}{l} \Delta_{b\_3} - \Delta_{f\_4} \\ +X_4 \cdot \left(\begin{array}{l} \Delta_{b\_4} - \Delta_{f\_5} \\ +X_5 \cdot \left(\Delta_{b\_5} - \Delta_{f\_6} + X_6 \cdot \Delta_{b\_6}\right) \end{array}\right) \end{array}\right) \end{array}\right) \end{array}\right)$$

The overall isotope effect caused by a single bacterium is equal to the isotope difference above:

$$\Delta_{cell} = \Delta_{f\_1} + X_1 \cdot \left(\begin{array}{l} \Delta_{f\_2} - \Delta_{b\_1} \\ +X_2 \cdot \left(\begin{array}{l} \Delta_{f\_3} - \Delta_{b\_2} \\ +X_3 \cdot \left(\begin{array}{l} \Delta_{f\_4} - \Delta_{b\_3} \\ +X_4 \cdot \left(\begin{array}{l} \Delta_{f\_5} - \Delta_{b\_4} \\ +X_5 \cdot \left(\Delta_{f\_6} - \Delta_{b\_5} + X_6 \cdot -\Delta_{b\_6}\right) \end{array}\right) \end{array}\right) \end{array}\right) \end{array}\right)$$

The above equation can be written in an abbreviated algebraic form, allowing for a formulation of an equation that is valid for any number of pools (z) and of reduction steps (z-1):

$$\Delta_{total} = \Delta_{f\_1} + \sum_{u=1\ldots(z-2)} \left(\prod_{v=1\ldots u} X_v\right) \cdot \left(\Delta_{f\_u+1} - \Delta_{b\_u}\right) - \prod_{v=1\ldots u} X_v \cdot \Delta_{b\_(z-1)}$$
$$\Delta_{total} = \Delta_{f\_1} + \sum_{u=1\ldots(z-2)} \left(\prod_{v=1\ldots u} X_v\right) \cdot \Delta_{f\_u+1} - \sum_{u=1\ldots(z-1)} \left(\prod_{v=1\ldots u} X_v\right) \cdot \Delta_{b\_u}$$