THE EFFECT OF BIOSYNTHETIC NETWORKS ON MASS-DEPENDENT SULFUR ISOTOPE FRACTIONATIONS. J. Farquhar[1], D.T. Johnston[1], B.A. Wing[1], K.S. Habicht[2], D.E. Canfield[2], S. Airieau[3], M.H.Thiemens[3], [1]Department of Geology and Earth Systems Science Interdisciplinary Center, University of Maryland, College Park Maryland 20742, [2]Danish Center of Earth System Science and Insitute of Biology, University of Southern Denmark, Campusvej 55, DK-5230, Odense M, Denmark [3]Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California 92093.

Introduction: Biosynthetic reaction networks can influence mass-dependent isotopic fractionations of multiple sulfur isotopes and an understanding of these relationships is necessary in order to use sulfur isotope data as supporting evidence of specific sulfur metabolisms.

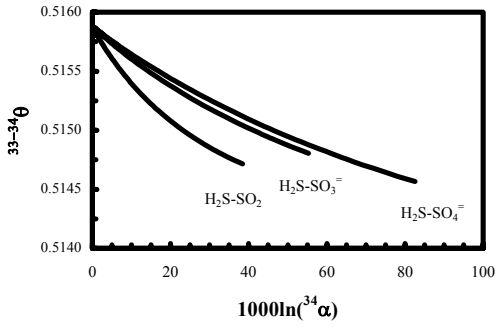
In the case of sulfur, mass-dependent isotopic fractionations are reflected in the terrestrial environment by the fractionation array of $\delta^{33}S \sim 0.515\ \delta^{34}S$. This array takes this form because the fractionation of ^{34}S from ^{32}S depends on an ~2 atomic mass unit mass difference and the fractionation of ^{33}S from ^{32}S depends on an ~1 atomic mass unit difference between the isotopes. It is well known that different mass-dependent fractionation processes can produce mass-dependent fractionations that deviate by a few percent from $\delta^{33}S \sim 0.515\ \delta^{34}S$ [1-4], and recent work has shown that it is possible to differentiate between different mass-dependent fractionation processes with high precision isotopic measurements.

Notation: We follow current convention (cf. Angert et al. [1-5]) and describe mass-dependent fractionation by:

(1) $^{33}\lambda = \ln(1+\delta^{33}S/1000)/\ln(1+\delta^{34}S/1000)$,

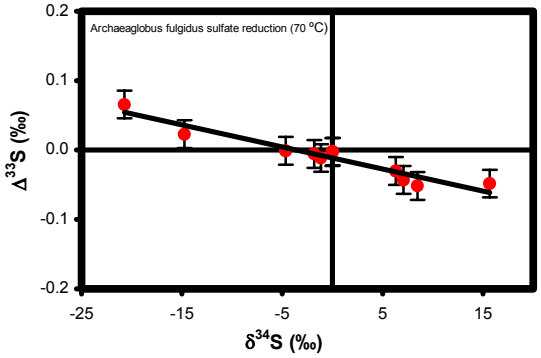
(2) $^{33}\theta = \ln(^{33}\alpha)/\ln(^{34}\alpha)$,

where λ refers to fractionation relationships between measured quantities (e.g., fractionation arrays), and θ refers to relationships due to the fractionation process itself (e.g., fractionation factors).



Equilibrium isotope fractionation effects such as those described by Urey [6] obey relationships that are determined principally by changes in vibrational energies associated with isotopic substitution. In Figure 1, we have plotted the values of $^{33}\theta$ versus $\ln^{34}\alpha$ that we have calculated for several equilibrium isotopic exchange reactions. These values have been calculated using the theory presented by [6, 7] and vibrational spectra presented by [7-10]. The values of θ for equilibrium sulfur isotope exchange fall in a narrow range that is similar to the high temperature limit calculated using methods presented in prior studies [1, 2, 4].

Kinetic fractionations are defined as being produced by unidirectional processes. Unidirectional processes include some chemical reactions and physical processes such as diffusion. Mass fractionation relationships for kinetic processes have been described [2, 4, 5] and generally obey fractionation relationships that have values of $^{33}\theta$ closer to 0.500. The values for $^{33}\theta$ can calculated for diffusion of hydrogen sulfide and sulfate using [2, 4, 5] to range from 0.503 to 0.511.



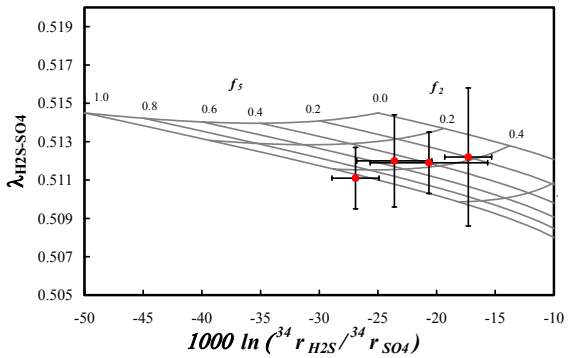
Values of λ measured for fractionations of sulfur isotopes by dissimilatory sulfate reduction (0.5117±0.0012 (2σ)[11] raise the possibility that some biological processes may fractionate isotopes with λ that are intermediate between the θ of equilibrium and kinetic processes. Research on oxygen isotope fractionation during respiration and photosynthesis by Luz and colleagues [12-14] indicates that similar differences occur for λ in the triple isotope composition of oxygen.

The λ for sulfur fractionation can be traced to the pathways that describe the flow of sulfur through the biosynthetic pathway associated with dissimilatory sulfate reduction [11]. One of the basic building blocks of these sulfur networks describes the relationship between reactants and products with back reaction:

$$A \underset{3}{\overset{1}{\rightleftarrows}} B \xrightarrow{2} C$$

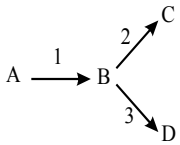
, where A, B, and C are sulfur-bearing species. In this network, C is the final products of the network; A is the starting species, and B is an intermediate species. The arrows labeled 1,2, and 3 diagram the paths by which sulfur can be transferred between the phases. For this network we are

interested in determining the fractionation relationship between C and A. The difference between the λ for dissimilatory sulfate reduction and the θ for equilibrium exchange can be demonstrated to be rooted in the dissimilatory sulfate reduction network. This arises because the network acts like a distillation process. In the figure 3, we have plotted the experimental sulfate reduction data on a grid that is constructed for the model of sulfate reduction of [15].
This figure is constructed for a network with three fractionation factors and two branch points suggested by [15]. We also assumed that all fractionations followed mass fractionation relationships with θ = 0.5145. The branching ratios at these two branch points (f_2 and f_5) are described by the relationship between rate limiting steps involving $SO_3^=$ reduction relative to transport of $SO_4^=$ into and out of the cell (f_2) or relative to $SO_4^=$ reduction to $SO_3^=$ by way of APS.



This signature of these types of biosynthetic networks arises from the structure of the networks themselves and should be considered as necessary condition for their identification during searches for their operation early in Earth history or in extraterrestrial samples. As measurement capabilities improve, these criteria may also be useful for understanding the conditions under which biosynthetic processes occur.

Note however, that not all biosynthetic networks are expected to have this characteristic. An example of one that does not do this is a component of metabolisms that disproportionate an intermediate to form two products (e.g., sulfite to sulfate and hydrogen sulfide). This network has a component:



in which the fractionation of interest is between C and D. In this case the fractionations between C and D yield a λ that is a weighted average of the θ for the constituent steps. It may not be possible to use λ to distinguish this type of biosynthetic process from the θ for equilibrium exchange. The relationships between the reactant A and either of the products (C and D) however is dependent on the branching ratio at branch B and obeys a similar relationship to that observed for the network described above for reactants and products with back reaction.

References: [1]J. R. Hulston and H. G. Thode (1965), *Journal of Geophysical Research, 70*, 3475-3484.
[2]Y. Matsuhisa, J. R. Goldsmith and R. N. Clayton (1978), *Geochimica et Cosmochimica Acta, 42*, 173-182.
[3]M. F. Miller (2002), *Geochimica Et Cosmochimica Acta, 66*, 1881-1889.
[4]E. D. Young, A. Galy and H. Nagahara (2002), *Geochimica Et Cosmochimica Acta, 66*, 1095-1104.
[5]W. G. Mook (2000), Environmental Isotopes in the hydrological cycle principles and applications, V I: Introduction-Theory, Methods, Review,
[6]H. C. Urey (1947), *Journal of the Chemical Society,* 562-581.
[7]G. Herzberg (1962), Infrared and raman spectra of polyatomic molecules,632 pp.
[8]H. C. Urey and C. A. Bradley (1931), *Physical Reviews, 38*, 1969-1978.
[9]K. Nakamoto (1997), Infrared and Raman Spectra of inorganic and coordination compounds part A: Theory and Applications in inorganic chemistry,387 pp.
[10]L. Peter and B. Meyer (1985), *Inorganic Chemistry, 24*, 3071-3073.
[11]J. Farquhar, D. T. Johnston, B. A. Wing, K. S. Habicht, D. E. Canfield, S. A. Airieau and M. H. Thiemens (2003), *Geobiology, 1*, in review.
[12]A. Angert, S. Rachmilevitch, E. Barkan and B. Luz (2003), *Global Biogeochemical Cycles, 17*, in press.
[13]B. Luz and E. Barkan (2000), *Science, 288*, 2028-2031.
[14]B. Luz, E. Barkan, M. L. Bender, M. H. Thiemens and K. A. Boering (1999), *Nature, in press*,
[15]C. E. Rees (1973), *Geochimica Et Cosmochimica Acta, 37*, 1141-1162.