the maximum insolation anomaly), and this age is used here. The third point (90.7 m) refers to the decline of Mediterranean elements and is assigned an age of 122.6 ka (event 5.51 of the SPECMAP time scale) on the basis of joint pollen and isotopic data from marine cores (*23*). Finally, the uppermost point (83.2 m), after which NAP percentages begin to exceed 50%, represents the transition from the last interglacial to the ensuing stadial and is assigned an age for the MIS 5e/5d boundary of ~116 ka (*22*).

26. We thank R. C. Preece, T. C. Atkinson, and M. R. Chapman for comments and S. Boreham and H. Sloane for technical advice and assistance. We are grateful to the director-general and the staff at the Department of Energy Resources of the Institute of Geology and Mineral Exploration in Athens (particularly Y. Broussoulis) for their cooperation and for making the core available. M.R.F. acknowledges a NERC Studentship and a Fellowship from St. John's College, Cambridge; P.C.T. acknowledges a NERC Advanced Fellowship and a Fellowship from Robinson College, Cambridge.

24 May 1999; accepted 28 July 1999

Iron Isotope Biosignatures

Brian L. Beard,[1]* Clark M. Johnson,[1] Lea Cox,[2] Henry Sun,[2] Kenneth H. Nealson,[2] Carmen Aguilar[3]

The $^{56}Fe/^{54}Fe$ of Fe-bearing phases precipitated in sedimentary environments varies by 2.5 per mil ($\delta^{56}Fe$ values of +0.9 to −1.6 per mil). In contrast, the $^{56}Fe/^{54}Fe$ of Fe-bearing phases in igneous rocks from Earth and the moon does not vary measurably ($\delta^{56}Fe = 0.0 \pm 0.3$ per mil). Experiments with dissimilatory Fe-reducing bacteria of the genus *Shewanella algae* grown on a ferrihydrite substrate indicate that the $\delta^{56}Fe$ of ferrous Fe in solution is isotopically lighter than the ferrihydrite substrate by 1.3 per mil. Therefore, the range in $\delta^{56}Fe$ values of sedimentary rocks may reflect biogenic fractionation, and the isotopic composition of Fe may be used to trace the distribution of microorganisms in modern and ancient Earth.

Fractionation of light stable isotopes such as C, O, N, and S is controlled by inorganic processes related to temperature changes and phase transitions, and by biological processes (*1*). This dual control can make it difficult to interpret the origin of isotopic differences in rocks. For example, excursions in $\delta^{13}C$ values in deep-sea sediments can be interpreted as a function of changes in the productivity of the oceans or the partial pressure of CO_2 of the atmosphere (*2*). In contrast, intermediate-mass elements such as Fe may not be fractionated substantially by inorganic processes because the relative mass difference between Fe isotopes is less than that of C, O, N, or S isotopes. However, biological processes may produce measurable Fe-isotopic fractionation because the metabolic processing of Fe involves a number of steps, such as transport across membranes and uptake by enzymes (*3*), that may fractionate isotopes.

Few studies have documented biological fractionation of transition metal elements (*4*) because of the difficulty of measuring precisely the isotopic ratios of transition metals (*5*). Thermal ionization mass spectrometry (TIMS) can produce high-precision isotope ratio measurements of these metals and, in the case of Fe, is not subject to large interferences by Ar-containing species (for example, $^{40}Ar^{16}O$ and $^{40}Ar^{14}N$), as is inductively coupled plasma mass spectrometry. However, TIMS produces large mass-dependent isotope fractionations during the course of a measurement, which must be corrected before the natural isotopic composition of a sample can be determined. Previous attempts to correct instrumental, mass-dependent isotopic fractionation of Fe used an empirical approach that produced data with a 1σ precision of only 2 to 3 per mil for $^{56}Fe/^{54}Fe$ (*6*), an uncertainty that exceeds the range in nature. Here we used a mixed double spike to correct for instrumental mass bias (*7–9*). With the use of this technique, it is possible to make Fe isotope ratio measurements that are precise to ±0.2 to 0.3 per mil (1σ) for $^{56}Fe/^{54}Fe$. We report Fe-isotopic ratios in conventional per mil notation:

$$\delta^{56}Fe = [(^{56}Fe/^{54}Fe)_{measured}/(^{56}Fe/^{54}Fe)_{E\text{-}M} - 1] \times 1000$$

where $(^{56}Fe/^{54}Fe)_{E\text{-}M}$ is the average $^{56}Fe/^{54}Fe$ measured for 15 terrestrial igneous rocks, ranging in composition from peridotite to rhyolite, and five high-Ti lunar basalts. The average $^{56}Fe/^{54}Fe$ measured for the Earth-moon system is 15.7028 (*7*). Terrestrial and lunar rocks comprise an isotopically homogenous igneous iron reservoir that is thought to represent the bulk isotopic composition of Earth and the moon.

Two sets of experiments were performed to determine the magnitude of Fe-isotopic fractionation that might be produced by microorganisms. Experiment 1, run in duplicate at the University of Wisconsin–Milwaukee (U.W.-Milwaukee), used *S. algae* (strains BCM 8 and BrY) grown on a ferrihydrite substrate in an LM growth medium (*10*). After inoculation of the ferrihydrite + growth medium solution with the cells, the bacteria were allowed to reduce Fe for 8 hours. One run was contained in a 10,000 molecular weight dialysis bag (ferrihydrite + cells + growth medium) suspended in a 100-ml flask of LM growth medium, which allowed the ferrihydrite and cells to be removed from the growth medium. The other run was done in a 100-ml flask, followed by separation of the ferrihydrite and cells from the growth medium + hydrolyzed Fe(II), by filtration. Ferrous iron in the growth medium solution was precipitated by adding ultrapure ammonia, to increase the pH to 9 to 10 immediately after the solution was exposed to the atmosphere, and then allowed to sit for 3 to 5 days. Abiological control experiments were run in parallel; addition of ammonia did not precipitate any iron, confirming that no substantial amount of ferrous Fe was generated. The ammonia precipitation procedure effectively removes the Fe(II) from the growth medium as a ferric oxyhydroxide, which was centrifuged and washed three times in doubly distilled H_2O. The precipitate was dissolved in 6 M HCl, after which followed chemical processing and isotopic analysis with the methods of (*7, 8*).

Experiment 2, performed at the Jet Propulsion Laboratory (JPL), used *S. algae* BrY grown on ferrihydrite in an LB growth medium (*11*). Three runs were made, harvesting Fe 13, 15, and 23 days after inoculation, which produced Fe(II) contents of 5.5, 11.1, and 35.6 parts per million (ppm) Fe, respectively (*12*). Each solution was sterilized with a 0.2-μm filter. Reacted ferrihydrite from runs 2 and 3 was saved for Fe isotope analysis. Splits (50 ml) of the Fe(II) solutions were evaporated to dryness and the organic material combusted in quartz crucibles in a muffle furnace at 700°C for 8 hours. The remaining solids were dissolved in 6 M HCl and processed for Fe isotope analysis as in (*7, 8*). A parallel set of 50-ml aliquots from each run was processed, for comparison with the samples that were combusted, where Fe was harvested as an oxyhydroxide using 10 ml of 30% H_2O_2 in ammonia to bring the pH to 9 to 10. The precipitated Fe oxyhydroxide was treated in the same manner as in the U.W.-Milwaukee experiments. The JPL experiments also included an abiological control, which was harvested by the H_2O_2 + ammonia precipitation technique, as well as by the evaporation and combustion technique.

The high levels of Fe(II) that are produced

[1]Department of Geology and Geophysics, University of Wisconsin-Madison, 1215 West Dayton Street, Madison, WI 53706, USA. [2]Jet Propulsion Laboratory, MS 183-301, 4800 Oak Grove Road, Pasadena, CA 91109–8009, USA. [3]Institute for Great Lakes Study, University of Wisconsin-Milwaukee, 600 East Greenfield Avenue, Milwaukee, WI 53204, USA.

*To whom correspondence should be addressed. E-mail: beardb@geology.wisc.edu

in the experiments that contain bacteria, relative to those in the abiological controls, are interpreted to reflect biologically produced Fe(II) because the $^{54}Fe/^{56}Fe$ of the Fe(II) is significantly less than the $^{56}Fe/^{54}Fe$ ratio of the starting ferrihydrite substrate (Fig. 1 and Table 1). These data show that the Fe-reducing bacteria *S. algae* preferentially reduce ^{54}Fe relative to ^{56}Fe, as reflected in a 1.3 per mil negative shift in $\delta^{56}Fe$ values. The U.W.-Milwaukee experiments (contained in dialysis bags) do not display as large an iron isotope fractionation as the experiments that were run in flasks—the differences in the isotopic compositions are smaller than the analytical error of the measurements—but the results are consistent (Table 1). Although Fe(II) concentrations were not measured in the U.W.-Milwaukee experiments, clumping of the ferrihydrite in the dialysis bag experiments effectively reduced the amount of substrate surface area available to the bacterium. The armoring of the ferrihydrite by clumping probably drove the reaction further to completion, toward the original isotopic composition of the starting ferrihydrite, which would have produced higher $\delta^{56}Fe(II)$ values in the experiments that were contained in dialysis bags, compared with the flask experiments.

The JPL experiments were scaled so that the isotopic composition of the abiological control could be analyzed. This control has an anomalously low $\delta^{56}Fe$ compared with the Fe(II) from the bacterial experiment. Moreover, there are significant differences between the Fe-isotopic compositions determined by the precipitation technique and the evaporation and combustion technique, for harvest 1 as well as for the abiological control (Fig. 1 and Table 1). The low $\delta^{56}Fe$ of the abiological control can be interpreted in two ways. One explanation is that partial dissolution of ferrihydrite occurs in the complex organic medium, and this produces significant Fe isotope fractionation. If the low $\delta^{56}Fe$ of the abiological control is a result of isotopic fractionation caused by dissolution of ferrihydrite in the growth medium, the fractionation factor (Fe(II)-ferrihydrite) would be about −2.6 per mil for $^{56}Fe/^{54}Fe$, as calculated from the Fe contents of the abiological control (0.34% of the total ferrihydrite). An alternative explanation is that the low $\delta^{56}Fe$ value of the abiological control represents a mixture between Fe of normal isotopic composition (no isotopic fractionation during partial dissolution of ferrihydrite) and the Fe blank of the growth medium with an anomalously low $\delta^{56}Fe$. The JPL growth medium has an Fe blank of 0.32 ppm, the bulk of which comes from the tryptone and yeast extract. We have been unable to measure precisely the Fe isotopic composition of the growth medium because of the low Fe contents and the processing complexities associated with the organic matrix. If the low $\delta^{56}Fe$ of the abiological control reflects a mixture of growth medium blank and normal Fe (partially dissolved ferrihydrite), then mass balance calculations show that the growth medium blank must have a $\delta^{56}Fe$ of −8 per mil (Fig. 1A). Such a low $\delta^{56}Fe$ could be produced through multicyclic processing of Fe by yeast during preparation of the commercial yeast extract.

The Fe-isotopic compositions of the Fe(II) produced by Fe-reducing bacteria grown on ferrihydrite are consistent only with biologically produced Fe isotope fractionation, despite the fact that the abiological control produced low $\delta^{56}Fe$ values. The Fe(II) contents of the biological experiments (5.5 to 35.6 ppm Fe) are too large to have been affected by the Fe blank of the growth medium or the abiological control, even if the abiological control reflects only isotopic fractionation

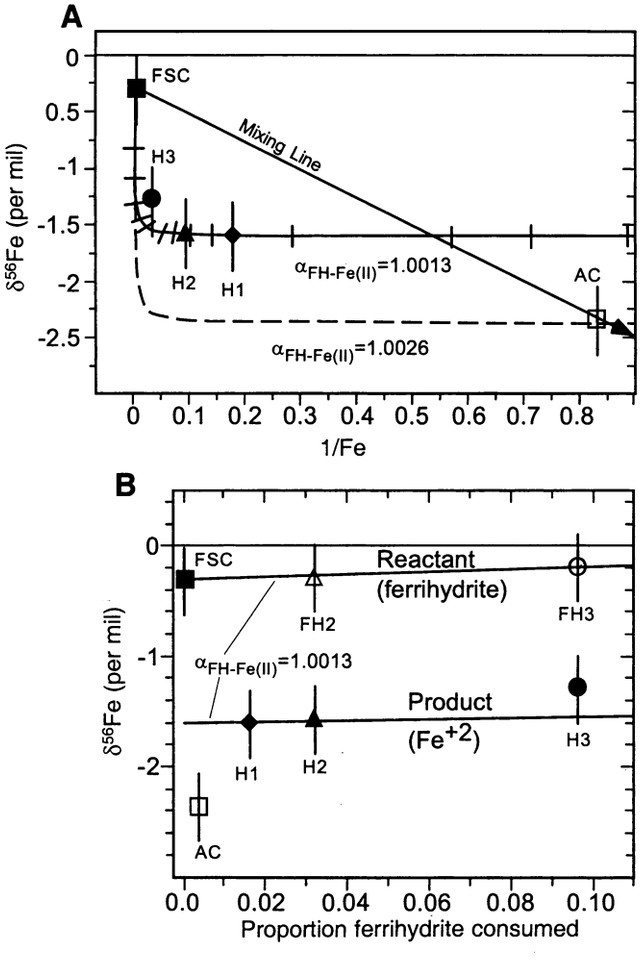

Fig. 1. (**A**) $\delta^{56}Fe$ versus the inverse of iron concentration (in parts per million) of the Fe produced from Fe-reducing bacteria, starting ferrihydrite, and abiological control of the experiments conducted at JPL. The Fe-isotopic composition of the abiological control can be explained by mixing a low $\delta^{56}Fe$ value that is inferred from the growth media and the high $\delta^{56}Fe$ value of the ferrihydrite (see text for discussion). The low $\delta^{56}Fe$ of the iron reduced by bacteria is best explained by mass-dependent fractionation caused by bacteria preferentially reducing ^{54}Fe relative to ^{56}Fe. The curves are closed system Rayleigh distillation curves with $\alpha_{ferrihydrite\text{-}Fe(II)}$ values of 1.0013 (solid curve) and 1.0026 (dashed curve). Tick marks for the curve with an α value of 1.0013 represent the proportion of ferrihydrite consumed and from right to left are 0.003, 0.004, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.1, 0.2, 0.4, 0.6 and 0.8. FH, ferrihydrite; FSC, ferrihydrite starting composition; AC, abiological control; H1, Fe(II) from harvest 1; H2, Fe(II) from harvest 2; H3, Fe(II) from harvest 3. (**B**) $\delta^{56}Fe$ versus the proportion of ferrihydrite consumed by Fe-reducing bacteria. The Fe isotope composition of the ferrous Fe produced by bacteria and the reacted ferrihydrite may be consistent with a closed system Rayleigh distillation process that has a fractionation factor of 1.3 per mil for the $^{56}Fe/^{54}Fe$. FH2, ferrihydrite harvest 2; FH3, ferrihydrite harvest 3.

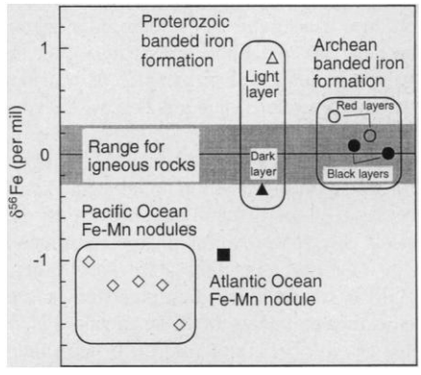

Fig. 2. $\delta^{56}Fe$ of iron deposited in sedimentary environments. The shaded band is the baseline for the isotopic composition of inorganic Fe, as determined from analysis of a variety of igneous rocks from Earth and the moon (*7*). The measured range of Fe isotope compositions in natural samples is 2.5 per mil, which is an order of magnitude larger than the analytical uncertainty. Variations in the measured Fe isotope composition of these natural samples are best explained by biological processes (see text for discussion).

produced during the dissolution of ferrihydrite (Fig. 1). For example, if there was no biological fractionation of Fe isotopes and the bacteria served only as a catalyst for the ferrihydrite dissolution that was accompanied by Fe isotope fractionation, then the products harvested from the three experiments should lie along a Rayleigh distillation curve that defines a fractionation factor (Fe(II)-ferrihydrite) of −2.6 per mil (Fig. 1). Alternatively, if the Fe(II) in the experiments reflects a mixture of biologically produced Fe that was not isotopically fractionated and a "blank" of low δ^{56}Fe (abiological control), then the isotopic compositions of the biological experiments should plot along a mixing line defined by the δ^{56}Fe of the stock ferrihydrite and the abiological control (Fig. 1). Neither of these explanations is consistent with the relation observed between δ^{56}Fe and 1/Fe, and it seems most likely that Fe(II) produced in the bacteria-containing experiments undergoes biologically produced Fe-isotopic fractionation.

The differences between the Fe-isotopic compositions of the Fe(II) harvested by the precipitation technique, and of that harvested by the combustion technique for the abiological control and harvest 1 (which contained the lowest Fe contents) probably reflect differing proportions of biologically produced Fe and Fe blank. The evaporation and combustion method includes Fe(II) that has been produced by bacteria and any Fe blank in the growth media (which may have a low δ^{56}Fe, as indicated by the abiological control). In contrast, the Fe precipitation method most likely contains only Fe that has been processed by bacteria if the blank Fe of the growth media is complexed by an organic ligand.

The Fe-isotopic fractionation produced by *S. algae* BrY grown on a ferrihydrite substrate may follow a Rayleigh distillation law (Fig. 1B), where the fractionation factor (ferrihydrite-Fe(II)) is 1.3 per mil for ^{56}Fe/^{54}Fe. If confirmed by longer experimental runs, extreme isotopic fractionations may be produced in the remaining ferrihydrite substrate. For example, when 80% of the substrate is consumed, the remaining fraction would have a δ^{56}Fe of +1.8 per mil and the ferrous iron produced would have a δ^{56}Fe of −0.8 per mil. Low ^{57}Fe/^{56}Fe values have been reported for Fe that has been processed by Fe-reducing bacteria (*13*).

We have measured anomalous Fe-isotopic compositions in iron-bearing minerals from sedimentary environments, including Fe-Mn nodules from the Pacific and Atlantic Oceans and individual layers from banded iron formations (Fig. 2 and Table 1). Modern sediments (Fe-Mn nodules) and ancient sedimentary rocks (Precambrian banded iron formations) show 2 to 3 per mil Fe-isotopic variations that can be explained by bacterially produced fractionation, given the constancy of Fe-isotopic compositions measured in igneous rocks from Earth and the moon (*7*).

Two banded Fe formations have been analyzed, a Proterozoic sample from the Empire Mine in Michigan and an Archean sample from the Sudan Mine in Minnesota. δ^{56}Fe values of the dark-colored Fe-rich layers from the Archean and Proterozoic samples are close to zero. In contrast, the light-colored, Fe-poor layers have positive δ^{56}Fe values: The δ^{56}Fe values of the red layers of the Archean sample overlap that of the bulk earth, whereas the green layer from the Proterozoic sample has a positive δ^{56}Fe value of +0.9 per mil.

Our experimental results predict that the light-colored, Fe-poor layers of banded iron formations should have positive δ^{56}Fe values if Fe-reducing bacteria were involved in Fe mobilization. Because mobile (reduced) Fe should have negative δ^{56}Fe values (Fig. 1), we infer that loss of reduced Fe during the development of banded iron formations should produce Fe-poor residues with high δ^{56}Fe values. It has been proposed that Fe(III) in sediments may be reduced by bacteria, dissolved, and transported from its place of deposition during the genesis of banded iron formations (*14*), which would leave behind a substrate with a positive δ^{56}Fe. In contrast, if dark, Fe-poor layers result from inorganic Fe precipitation, then no isotopic anomalies would be expected. It is unclear if the lack of large Fe-isotopic anomalies in the Archean formation indicates a lack of biologic activity or if the Fe-isotopic composition of this sample was homogenized by supergene enrichment, as suggested by the specular hematite veins that cross cut layers in the analyzed sample. High ^{57}Fe/^{56}Fe in aquifer sediment leachates and low ^{57}Fe/^{56}Fe for groundwater from the same site (*4*) are thought to reflect mass balance in a biologically mediated system.

Table 1. Fe isotope compositions of sedimentary Fe and bacteria-mediated Fe products. Pacific Ocean nodules collected at the following: sample 1, 16°N, 125°W; sample 2, 57°S, 95°W; sample 3, 10°N, 136°W; sample 4, 35°N, 160°W. Atlantic nodule sample 5 was collected from the Blake Plateau. Analytical methods are reported in (*7, 8*). Isotopic ratios are the averages of "*n*" duplicate isotopic analyses. Errors in the δ^{56}Fe values reflect internal statistics ($n = 1$) or external error based on duplicates ($n \geq 2$).

Sample	^{54}Fe/^{56}Fe	^{57}Fe/^{56}Fe	^{58}Fe/^{56}Fe	δ^{56}Fe	1SD	*n*
Pacific Ocean Fe-Mn nodule						
Sample 1	0.063748	0.023076	0.0030584	−1.01	0.21	2
Sample 2	0.063762	0.023073	0.0030577	−1.24	0.13	3
Sample 3 (outer layer)	0.063760	0.023074	0.0030578	−1.21	0.25	4
Sample 3 (inner layer)	0.063763	0.023073	0.0030577	−1.25	0.13	2
Sample 4	0.063786	0.023069	0.0030566	−1.62	0.22	1
Atlantic Ocean Fe-Mn nodule						
Sample 5	0.063744	0.023077	0.0030585	−0.96	0.04	2
Banded Fe formation Proterozoic						
Dark layer	0.063705	0.023083	0.0030604	−0.34	0.31	1
Light layer	0.063625	0.023098	0.0030646	0.91	0.21	4
Banded Fe formation Archean						
Red layer	0.063661	0.023091	0.0030624	0.35	0.19	2
Black layer	0.063679	0.023088	0.0030616	0.07	0.01	2
Red layer	0.063673	0.023089	0.0030619	0.16	0.34	3
Black layer	0.063683	0.023087	0.0030614	0.00	0.26	2
U.W.-Milwaukee experiments						
Stock ferrihydrite	0.063693	0.023086	0.0030609	−0.16	0.19	7
Shewanella alga (dialysis bag)	0.063738	0.023078	0.0030589	−0.87	0.07	4
Shewanella alga (filtered)	0.063771	0.023072	0.0030573	−1.38	0.30	3
BCM 8 (dialysis bag)	0.063746	0.023076	0.0030585	−0.99	0.24	3
BCM 8 (filtered)	0.063768	0.023072	0.0030574	−1.34	0.26	3
JPL experiments						
Harvest 1 (precipitation)	0.063786	0.023069	0.0030566	−1.62	0.26	6
Harvest 1 (combustion)	0.063815	0.023064	0.0030559	−2.07	0.23	4
Harvest 2 (precipitation)	0.063779	0.023070	0.0030570	−1.50	0.20	6
Harvest 2 (combustion)	0.063784	0.023070	0.0030567	−1.58	0.10	1
Harvest 3 (precipitation)	0.063768	0.023072	0.0030575	−1.33	0.02	3
Harvest 3 (combustion)	0.063766	0.023073	0.0030576	−1.30	0.20	4
Stock ferrihydrite	0.063703	0.023084	0.0030605	−0.31	0.05	2
Ferrihydrite harvest 2	0.063702	0.023084	0.0030605	−0.30	0.29	3
Ferrihydrite harvest 3	0.063696	0.023085	0.0030608	−0.20	0.11	4
Control (precipitation)	0.063834	0.023061	0.0030544	−2.37	0.20	4
Control (combustion)	0.063853	0.023057	0.0030535	−2.66	0.32	5
Fe from igneous rocks						
Average Earth-moon	0.063683	0.023087	0.0030614	0.00	0.27	40

References and Notes

1. I. Friedman and J. R. O'Neil, *U.S. Geol. Surv. Prof. Pap. 440KK* (1977); M. Schidlowski, J. M. Hayes, I. R. Kaplan, in *Earth's Earliest Biosphere: Its Origins and Evolution*, J. W. Schopf, Ed. (Princeton Univ. Press, Princeton, NJ, 1983), pp. 149–186.

2. K. Caldeira, M. R. Rampino, T. Volk, J. C. Zachos, in *Extinction Events in Earth History*, E. G. Kaufman and O. H. Walliser, Eds. (Springer-Verlag, Berlin, 1990), pp. 333–345.
3. E. Baker and E. H. Morgan, in *Iron Metabolism in Health and Disease*, J. H. Brock, J. W. Halliday, M. J. Pippard, L. W. Powell, Eds. (Saunders, Philadelphia, PA, 1994), pp. 63–96.
4. T. D. Bullen and P. M. McMahon, *Min. Mag.* **62A**, 255 (1998).
5. C. N. Marechal, P. Telouk, F. Albarede, *Chem. Geol.* **156**, 251 (1999).
6. P. R. Dixon *et al.*, in *Water Rock Interaction*, vol. 2, *Moderate and High Temperature Environments*, Y. K. Kharaka and A. S. Maest, Eds. (Balkema. Rotterdam, Netherlands, 1992), pp. 915–918.
7. B. L. Beard and C. M. Johnson, *Geochim. Cosmochim. Acta*, in press.
8. C. M. Johnson and B. L. Beard, *Int. J. Mass Spectrom. Ion Proc.* **63**, 1653 (1999).
9. A mixed double spike composed of 7.8% ^{58}Fe and 92.2% ^{54}Fe was added to an aliquot of each analyzed sample to normalize the measured iron isotope ratios for instrumental mass bias.
10. U.W.-Milwaukee experiments were performed in a Coy Laboratory anaerobic hood; ferrihydrite was produced according to the method of C.-F. Lin and M. Benjamin [*Environ. Sci. Technol.* **24**, 126 (1990)]; LM growth medium is 0.1 g of peptone, 0.2 g of yeast extract, and 1 g of NaCl per liter of H_2O [C. R. Myers and J. M. Myers, *J. Appl. Bacteriol.* **76**, 253 (1993); B. Little *et al.*, in *CORROSION/97* (Paper No. 215, NACE International, Houston, TX, 1997). Each experiment was run with 2×10^7 cells/ml.
11. LB growth medium is 10 g of tryptone, 5 g of yeast extract and 1 g of NaCl per liter of H_2O. Ferrihydrite was made according to the method of U. Schwertmann and R. M. Cornell [*Iron Oxides in the Laboratory: Preparation and Characterization* (V.C.H., New York, 1991)]. Each experiment was run in 1-liter polycarbonate bottles filled with the LB growth media, 0.5 g of ferrihydrite, and 5×10^6 cells/ml.
12. Fe(II) contents were determined with the ferrozine technique [L. C. Stookey, *Anal. Chem.* **42**, 779 (1970)]. Ferrozine (1 g/liter) in Hepes buffer (50 mM) at pH 7 was used. A 0.1-ml sample, filtered through a 0.2-μm syringe filter, was mixed with 5 ml of the ferrozine solution. After about 5 min, the absorbance was measured at 562 nm on a spectrophotometer, which had been calibrated with Fe(II) solutions.
13. T. D. Bullen and P. M. McMahon, *Eos* **78**, S173 (1997).
14. K. H. Nealson and C. R. Myers, *Am. J. Sci.* **290A**, 35 (1990).
15. This work was supported by NASA grant NAG5-6342, NSF grant OPP-9713968, and the NASA Astrobiology Institute. We thank P. Brown, and C. Bowser for donating samples for Fe isotope analysis.

16 April 1999; accepted 19 July 1999

Oxygen and Iron Isotope Studies of Magnetite Produced by Magnetotactic Bacteria

Kevin W. Mandernack,[1]* Dennis A. Bazylinski,[2] Wayne C. Shanks III,[3] Thomas D. Bullen[4]

A series of carefully controlled laboratory studies was carried out to investigate oxygen and iron isotope fractionation during the intracellular production of magnetite (Fe_3O_4) by two different species of magnetotactic bacteria at temperatures between 4° and 35°C under microaerobic and anaerobic conditions. No detectable fractionation of iron isotopes in the bacterial magnetites was observed. However, oxygen isotope measurements indicated a temperature-dependent fractionation for Fe_3O_4 and water that is consistent with that observed for Fe_3O_4 produced extracellularly by thermophilic Fe^{3+}-reducing bacteria. These results contrast with established fractionation curves estimated from either high-temperature experiments or theoretical calculations. With the fractionation curve established in this report, oxygen-18 isotope values of bacterial Fe_3O_4 may be useful in paleoenvironmental studies for determining the oxygen-18 isotope values of formation waters and for inferring paleotemperatures.

The biomineralization of Fe_3O_4 is known to occur by either biologically induced mineralization or biologically controlled mineralization (*1*). In the former, Fe_3O_4 is formed extracellularly, as a result of dissimilatory reduction of Fe^{3+} (*2*). In the latter, the Fe_3O_4 particles are produced intracellularly by magnetotactic bacteria and some higher organisms and are well-ordered crystals that exhibit narrow size distributions and species- or strain-specific morphologies (*1*). The Fe_3O_4 particles in the magnetotactic bacteria are within the single-domain size range (35 to 120 nm in length) and are usually arranged in a chain motif, providing the cell with a permanent dipole moment. This arrangement causes the cell to align along Earth's inclined geomagnetic field lines, functioning as an efficient means of locating and maintaining an optimal position in vertical chemical gradients (*3*).

Fe_3O_4-producing magnetotactic bacteria produce a number of crystal morphologies, including cubo-octahedra, elongated hexa- and octahedra, and bullet- or arrowhead-shaped forms (*1*). The presence of single-domain Fe_3O_4 crystals with these apparently unique morphologies, termed magnetofossils (*4*), as determined by electron microscopy of magnetic material separated from sediments and soils, has been used to identify the presence of magnetotactic bacteria (*5*). These apparently unique crystal forms of Fe_3O_4 have been used to distinguish a bacterial (intracellular) origin from an inorganic origin (*1, 4–9*), although not without controversy. A recent study showed that a statistical analysis of the sizes and shapes of fine-grained Fe_3O_4 crystals might prove to be a robust criterion for distinguishing between biogenic and nonbiogenic Fe_3O_4 (*10*). The presence of single-domain Fe_3O_4 with some of these morphologies has been used as evidence for the biomineralization of Fe_3O_4 by magnetotactic bacteria occurring as early as 2 billion years ago on Earth (*8*) and as partial evidence for ancient life on Mars (*11*). Stable O and Fe isotopic ratios might provide additional criteria for distinguishing between inorganically and biologically produced Fe_3O_4.

Fe_3O_4-producing magnetotactic bacteria are ubiquitous in marine and freshwater environments, where they generally inhabit the microaerobic oxic-anoxic interface (*1*). Because of their preference for environments having little or no O_2, magnetotactic bacteria may have been more widespread during Earth's ancient past, when an oxidizing aerobic atmosphere was developing (*12*). The O isotope composition [reported as $\delta^{18}O$ values (*13*)] of bacterially produced metal oxides may reflect chemical or biological origins, temperature of formation, $\delta^{18}O$ values of the formation waters, and whether molecular O_2 is incorporated during biomineralization (*14, 15*). Bacterial Mn^{4+} manganates precipitated extracellularly incorporate as much as 50% of the O from O_2, thus serving as potentially important paleo-oxygen indicators. The bacterial manganates show different $\delta^{18}O$ values from chemically produced Mn^{4+} manganates, presumably because of differences in reaction pathways or mechanisms (or both) that result in varying proportions of O derived from O_2 and H_2O being incorporated into the mineral (*15*). Because O isotopes of a variety of materials have been used to assess paleoenvironmental conditions (*16–19*), the $\delta^{18}O$ values of single-domain Fe_3O_4 particles preserved in Earth's sedimentary rock record might provide important paleoenvironmental information.

[1]Department of Chemistry and Geochemistry, Colorado School of Mines, Golden, CO 80401, USA. [2]Department of Microbiology, Iowa State University, Ames, IA 50011, USA. [3]U.S. Geological Survey, 973 Denver Federal Center, Denver, CO 80225, USA. [4]Water Resources Division, U.S. Geological Survey, MS 420, 345 Middlefield Road, Menlo Park, CA 94025, USA.

*To whom correspondence should be addressed. E-mail: kmandern@mines.edu