Mass-dependent and -independent signature of Fe isotopes in magnetotactic bacteria

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Magnetotactic bacteria perform biomineralization of intracellular magnetite (Fe3O4) nanoparticles. Although they may be among the earliest microorganisms capable of biomineralization on Earth, identifying their activity in ancient sedimentary rocks remains challenging because of the lack of a reliable biosignature. We determined Fe isotope fractionations by the magnetotactic bacterium *Magnetospirillum magnetotacticum* AMB-1. The AMB-1 strain produced magnetite strongly depleted in heavy Fe isotopes, by 1.5 to 2.5 per mil relative to the initial growth medium. Moreover, we observed mass-independent isotope fractionations in 57Fe during magnetite biomineralization but not in even Fe isotopes (56Fe, 54Fe, and 52Fe), highlighting a magnetic isotope effect. This Fe isotope anomaly provides a potential biosignature for the identification of magnetite produced by magnetotactic bacteria in the geological record.

Magnetotactic bacteria synthesize magnetite [Fe(II)Fe(III)2O4] nanoparticles under a genetically controlled pathway (1). They are morphologically, physiologically, and phylogenetically diverse (2, 3). Nanoparticles of biogenic magnetite are produced in these cells in organelles called magnetosomes, consisting of a bilayered lipid membrane surrounding the magnetite crystal (4). Magnetosomes are assembled in chains inside the cell and provide it with a permanent magnetic dipole (4, 5). Magnetotactic bacteria are markers of oxic/anoxic transition zones in sediments and aquatic systems (1). In addition, these bacteria have been proposed to represent some of the most ancient microorganisms capable of biomineralization (6, 7), but the identification of their activity in the fossil record remains poorly resolved (8). Specifically, it is challenging to distinguish intracellular magnetite nanoparticles from abiotic or extracellular biogenic magnetites produced by Fe-metabolizing bacteria [i.e., Fe(III)-reducing and Fe(II)-oxidizing bacteria] (5, 6, 8, 9). Moreover, magnetite nanoparticles can experience variable transformations during diagenetic and/or metamorphic processes and may thus have lost some of their original physical characteristics (10). Iron isotopes have been proposed as a tool to infer the presence of Fe(III)-reducing and Fe(II)-oxidizing bacteria in the Precambrian geological record (11–13). However, a previous study reported no fractionation of Fe isotope compositions obtained from cultures of magnetotactic bacteria, *Magnetospirillum magnetotacticum* MS-1 and *Magnetotrichia blakemorei* MV-1 (15), and only oxygen isotope measurements showed a temperature-dependent fractionation between magnetite and water.

We measured the Fe isotope fractionation produced by another magnetotactic bacterium, *M. magneticum* strain AMB-1, which was cultured in batches with either Fe(III)-quinate or Fe(II)-ascorbate to investigate the effect of various Fe sources on the Fe isotopic signatures in magnetite (see the supplementary materials). A small fraction of the growth media was sampled before and after AMB-1 cultures for subsequent Fe isotope analyses. Magnetites were magnetically separated from bacterial lysates (i.e., plasmic membranes, periplasm, and cytoplasm) according to an optimized procedure, which ensured that the mineral was analyzed with no organic residue (9). Both organic and biomineralized fractions were analyzed for Fe isotope compositions.

Magnetite produced by AMB-1 was strongly depleted in the heavy Fe isotopes relative to the initial growth medium. Two replicates for each culture condition provided consistent results, with δ56Fe values of -1.00 ± 0.08‰ and -1.80 ± 0.13‰ for Fe(II)-ascorbate and Fe(III)-quinate experiments, respectively (Fig. 1 and table S1). Overall, the net Fe isotope fractionation between Fe sources and magnetite ranged between 1.5 and 2.5‰. Iron is usually assumed to reside either in magnetite or in the residual growth medium (16). Yet we found that bacterial lysates can represent up to 70% of cellular Fe (supplementary materials). In the two culture conditions of our experiments, bacterial lysate δ56Fe values were enriched in the heavy isotopes by 0.3 to 0.8‰ relative to initial Fe sources. Although several studies reported heavy Fe(II) adsorbed on solid surfaces or ligated into organic complexes (17, 18), Fe in the lysates is more likely to be present as Fe(III). This is consistent with the low δ56Fe values of magnetite, which suggest partial reduction of mass-dependent and mass-independent isotope compositions from cultures of magnetotactic bacteria. δ56Fe values (relative to international standard IRMM-014) of Fe sources (initial growth media, circles), growth media after AMB-1 cultures (squares), magnetite samples (triangles), and bacterial lysates (diamonds) in experiments using either Fe(III)-quinate (solid black symbols) or Fe(II)-ascorbate (open symbols) as Fe sources. The gray bar represents the range of Fe isotope compositions for initial growth media and can be regarded as a reference. Magnetite samples are enriched in light Fe isotopes relative to Fe sources, by ~1.5 to 2.5‰. Growth media are also enriched in light isotopes, with fractionation between 0.5 and 1.5‰. In contrast, bacterial lysates are enriched in heavy isotopes relative to Fe sources, by 0.5 to 0.75‰.

![Fig. 1. Iron isotope compositions obtained from cultures of magnetotactic bacteria.](http://science.sciencemag.org/)
Fe(III), whose residuals will be included in the lysates (17, 19).

Based on our experimental results and previous models for magnetite precipitation in magnetotactic bacteria (1, 16, 20), we can propose a geochemical view of Fe cycling in AMB-1 cultures (Fig. 2). Iron initially present as Fe(II) or Fe(III) in the growth medium was incorporated into the cell and stored as Fe(III), possibly in the form of ferritine (16). Fe(III) was then partially reduced for trafficking to magnetosomes, where Fe(II) was precipitated as magnetite in processes involving cytochromes (20, 22) or similar proteins. This geochemical model of magnetite precipitation in AMB-1 is in line with Fe isotope fractionations determined in Fe(III)-reducing bacteria and in abiotic magnetite precipitation (17, 19, 22). Bacterial Fe(III) reduction releases Fe(II) depleted in the heavy isotopes by ~3‰ (17). Then precipitation enriches magnetite in the heavy isotopes relative to Fe(II) by 0.8 to 1.5‰ (depending on kinetic or equilibrium control) in both abiotic and biotic systems (19, 22). Overall, the net isotope fractionation between initial Fe(III) and magnetite would be 2.2 to 1.5‰. This range of values is consistent with our experimental results obtained for bacterial lysates and magnetite (Fig. 1).

We also observed that magnetite and bacterial lysates show distinct but homogeneous isotopic compositions whatever the initial source of Fe (Fe(II) or Fe(III)). This contrasts with the prior observations of the absence of fractionation (15) and may result from differences between the strains and their culture conditions (supplementary materials). The presence of a large intracellular pool of Fe distinct from magnetite in our study may be important for explaining such differences. This extra Fe-bearing reservoir may have originated from the larger Fe concentrations in the initial growth media (150 μM versus 30 to 70 μM; supplementary materials).

Finally, we observed a statistically significant deviation from mass-dependent fractionation, expressed here as Δ57Fe (23) (Fig. 3 and table S1). In Fe(III)-quinate experiments, both growth media after AMB-1 cultures and magnetite showed unambiguous deviations from mass-dependent fractionations, with Δ57Fe values ranging between 0 and +0.23 ± 0.08‰ (2 SD). Each isolate mass balance lies within Δ57Fe ~ 0.00 ± 0.04‰ for every experiment, confirming the isotope anomalies (supplementary materials). Moreover, a negative linear correlation between Δ57Fe and Δ56Fe, with magnetite showing the largest deviation from mass-dependent fractionation line, reveals a mass-independent contribution referred to here as mass-independent fractionation (MIF) (Fig. 3). Such a linear correlation may represent a mixing between intracellular Fe with Δ57Fe values different from zero and the pool of Fe in the growth medium.

We explored several possible mechanisms that could have produced this MIF signature. We first considered whether an apparent mass-independent isotope signal could be due to the mass conservation among mass-dependent isotope fractionations, similar to S isotope fractionation by sulfate-reducing bacteria (24). Indeed, mixing between two reservoirs with Δ57Fe = 0 and contrasted isotopic compositions would produce an apparent MIF. Mixing between two Fe reservoirs in AMB-1 with extreme δ56Fe values of -3 and +3‰, respectively, should result in variations of Δ57Fe smaller than 0.003‰ from the mass-dependent line, which remains within error of our measurements (figs. S4 and S5). Such variations are therefore too small to account for Δ57Fe values up to +0.23 ± 0.08‰ (Fig. 3).

Magnetic isotope effects (MIEs) can discriminate isotopes according to their nuclear spins and magnetic moments rather than by their masses and are affected by external magnetic fields (25). They are usually observed in reactions involving free radicals and paramagnetic species, and primarily affect odd as compared to even isotopes (25). Fe(II) (in high spin configuration) and Fe(III) ions are both paramagnetic. As a consequence, the isotope δ57Fe would behave differently from δ54Fe, δ56Fe, and δ58Fe. To test this hypothesis, we attempted to determine potential isotope anomalies on δ58Fe, expressed as Δ58Fe (23) (fig. S10). No significant anomaly on Δ58Fe is predicted with a MIE. Due to the very low abundance of δ58Fe (0.282% of the total Fe), only a few samples were measured with an acceptable precision better than ±1.5‰ (2 SD) on Δ58Fe. These samples show Δ58Fe ~ 0‰ within analytical uncertainties (fig. S10), suggesting that only δ57Fe is associated with a mass-independent signature and supporting a MIE as the process enriching magnetite in δ7Fe relative to δ54Fe, δ56Fe, and δ58Fe.

In contrast with the Fe(III)-quinate experiments, no detectable MIF was observed in Fe(II)-ascorbate experiments (Fig. 3 and table S1). The likely

Fig. 2. Geochemical view of Fe cycling in AMB-1 showing the main Fe reservoirs and fluxes. Iron cycling in cultures with (A) Fe(III)-quinate and (B) Fe(II)-ascorbate. Fe(II) in the lysate fraction may be associated to ferritines either in the cytoplasm or periplasm for Fe storage in the cell (16). δ56Felys, δ56FeM, and δ56Femag. mean δ56Fe values of the lysate, growth medium after AMB-1 culture, and magnetite samples, respectively. Δ57Fe: deviation from mass-dependent Fe isotope fractionation (23) in the growth medium, lysate, and magnetite samples. Starting Fe sources had Δ57Fe = 0‰ within analytical uncertainties.
Fig. 3. MIF of odd Fe isotope in cultures. Plot of $\delta^{57}\text{Fe}$ (23) versus $\delta^{56}\text{Fe}$ in growth media before (circles) and after (squares) AMB-1 cultures, bacterial lysates (diamonds), and magnetite samples (triangles). The gray horizontal lines correspond to $\delta^{57}\text{Fe} = 0\%_\text{o}$. (A) Experiments using Fe(III)-quinate as a Fe source (solid symbols). $\Delta^{57}\text{Fe}$ values of magnetite samples and growth media after AMB-1 cultures are significantly different from zero, even considering analytical uncertainties (2 SD). Data define a linear correlation between $\Delta^{57}\text{Fe}$ and $\delta^{56}\text{Fe}$ (correlation coefficient of 0.92). (B) Experiments using Fe(II)-ascorbate as a Fe source (open symbols). No MIF is observed.

**REFERENCES AND NOTES**

14. Iron isotope data are reported using conventional delta notation relative to the international standard IRMM-014 and defined as $\delta^{56}\text{Fe}_{\text{sample}} = (\text{Fe}_{\text{sample}} / \text{Fe}_{\text{standard}} - 1) \times 1000$, where xx is 56, 57, or 58.
23. Capital delta notation $\Delta^{57}\text{Fe}$ illustrates the deviation of Fe isotope fractionation from single-stage low-temperature equilibrium exchange reactions, as defined for S isotopes (24). In other words, $\Delta^{57}\text{Fe}$ quantifies the deviation from mass-dependent fractionation and is expressed as $\Delta^{57}\text{Fe} = \delta^{57}\text{Fe} - 1000 \times [1 + (\delta^{56}\text{Fe} / \delta^{55}\text{Fe}) - 1]$, where xx is 57 or 58 and $\delta^{56}\text{Fe}$ corresponds to the variation of $^{56}\text{Fe} / ^{54}\text{Fe}$ and $^{57}\text{Fe} / ^{54}\text{Fe}$ mass ratios (i.e., 1.475) or $^{56}\text{Fe} / ^{54}\text{Fe}$ mass ratios (i.e., 1.932).
demography

Inequality in mortality decreased among the young while increasing for older adults, 1990–2010

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Many recent studies point to increasing inequality in mortality in the United States over the past 20 years. These studies often use mortality rates in middle and old age. We used poverty level rankings of groups of U.S. counties as a basis for analyzing inequality in mortality for all age groups in 1990, 2000, and 2010. Consistent with previous studies, we found increasing inequality in mortality at older ages. For children and young adults below age 20, however, we found strong mortality improvements that were most pronounced in poorer counties, implying a strong decrease in mortality inequality. These younger cohorts will form the future adult U.S. population, so this research suggests that inequality in old-age mortality is likely to decline.

Poorer people tend to have shorter lives and are more likely to die than richer people at all ages. Understanding the evolution of these inequalities in mortality is a central concern of economists, policy-makers, and the public. Not surprisingly, a great deal of highly publicized research has investigated changes in inequality in life expectancy and mortality in the United States over the past 20 years. A preponderance of the existing evidence points to alarming increases in inequality in mortality over this time period (1–16). Some studies investigating mortality trends across educational groups and geographic areas argue not only that inequality in life expectancy is widening, but that overall life expectancy is actually falling among the most disadvantaged groups (11–13).

However, much of the recent literature focuses on adults, and in particular on life expectancy at age 40 or 50, exploiting rich data sets that link individuals’ career earnings to deaths at older ages (1–8). By construction, these analyses omit children, teens, and young adults. A second strand of research analyzes demographic subgroups defined by education, location, and/or race (9–16).

These studies typically focus on overall life expectancy at birth. Life expectancy at birth is a summary measure that collapses all of the age-specific mortality rates observed in a given year (and in a certain demographic subgroup) into a single number. It provides information about how long a cohort of newborns can expect to live, under the assumption that the age-specific mortality rates observed in that given year remain constant into the future. This assumption is unlikely to hold in the United States, given that mortality rates at all ages have been continuously changing (mostly improving) over the past century (17).

Changes in infant and childhood mortality have been shown to be important predictors of a cohort’s health and mortality at later ages, and such data may therefore be more informative about the development of future death rates for the current young. Moreover, mortality at young ages is considered a sensitive indicator of social conditions because it responds relatively quickly to changes affecting the entire population, whereas old-age mortality is partly determined by conditions in the past. The infant mortality rate has been shown to be an important indicator of health for whole populations and one that is highly correlated with more complex measures such as disability-adjusted life expectancy (18).

Therefore, to study how inequality in mortality changes over time, it is important to understand age-specific mortality trends and in particular those at younger ages. Life expectancy at birth masks potential differences in age-specific trends, and the measure is also dominated by changes in old-age mortality because that is when most deaths occur. A recent study by Case and Deaton (19) highlights the relevance of examining age-specific mortality rates: They document increases in middle-age mortality for non-Hispanic whites, a striking development that would not be detectable in overall life expectancy at birth.

We followed an empirical approach, based on placing counties into groups, that allows us to analyze trends in age-specific mortality while taking into account population shifts across groups. We ranked all counties in 1990, 2000, and 2010 by their poverty level and then divided them into 20 groups, each representing roughly 5% of the overall U.S. population (fig. S1 and table S1). This enables us to compare, for example, the 5% of the population living in the poorest counties in 1990 with the 5% of the population living in the richest counties in 1990, and analyze how the mortality differences between these groups change over time. We refer to the county groups with the highest fractions of their populations in poverty as the poorest counties, and those with the lowest fractions of their populations in poverty as the richest counties.

Our approach reassigns county groups in 1990, 2000, and 2010 to adjust for changes in county ranking and population size. That is, we compare the poorest counties representing 5% of the population in 1990 with the poorest counties representing 5% of the population in 2010, even if they are not exactly the same counties. The advantages of this procedure and a comparison with other approaches are discussed below. Our county grouping approach is similar to that of Singh and Siahpush (9), who investigated life expectancy trends ranking U.S. counties by a deprivation index (comprising a set of county characteristics) up to 2001. Our approach differs from theirs in that they did not analyze age-specific mortality, analyzed data only up to 2001, and did not reorder county groups over time.

Mortality rates were constructed at the levels of county group, gender, and age by dividing death counts from the U.S. Vital Statistics by population counts from the decennial Census. We focused on 3-year mortality rates for Census years 1990, 2000, and 2010, based on a total of 21,175,011 deaths. Life expectancy was calculated by constructing a life table based on 19 age groups (see the supplement for additional details regarding the construction of mortality rates and life expectancy). Socioeconomic county characteristics, including poverty rate, median and per capita income, and percentage of high school...
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Science 352 (6286), 705-708. [doi: 10.1126/science.aad7632]

Editor's Summary

An isotope record of magnetic bacteria

Microorganisms have shaped Earth's oceans and atmosphere over billions of years. Ancient microbes left very little direct morphological evidence of their existence in the rock record, thereby requiring geochemical clues for evidence of their activity. Amor et al. show that magnetotactic bacteria impart a distinct isotopic signature to their internal iron nanoparticles. Cultures of a modern magnetic bacterium fractionated $^{57}$Fe isotopes independent of their mass, in contrast to fractionation patterns often observed for other isotopes. Because this signature is not produced abiotically or by other iron-metabolizing bacteria, it could serve as a reliable biomarker of this ancient magnetic microbial lifestyle.

Science, this issue p. 705

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Supplementary Materials for

Mass-dependent and -independent signature of Fe isotopes in magnetotactic bacteria

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Published 6 May 2016, Science 352, 705 (2016)
DOI: 10.1126/science.aad7632

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Materials and Methods

*Magnetospirillum magneticum* AMB-1 cultures

*Magnetospirillum magneticum* AMB-1 (ATCC700264) was cultured in batches. The composition of AMB-1 growth medium is given by the ATCC under AMB-1 reference. Either Fe(III)-quinate or Fe(II)-ascorbate were added to growth media at 150 μM to constrain the effect of various Fe sources on Fe isotope fractionation. Fe(II)-ascorbate solutions were first prepared in a glove box under anoxic conditions, and transferred to the growth media just before AMB-1 inoculation. For each culture condition (i.e., Fe(III)-quinate and Fe(II)-ascorbate), AMB-1 was cultured during 6 days (no stirring) at pH 6.75 in 2-L glass bottles (fig. S1). Twelve bottles were prepared for each condition. Immediately after inoculation, bottles were stored at 30°C in a light-blocking incubator to prevent photochemical reactions in AMB-1 cultures. At the end of experiments, the pH of growth media was about 7.5. Small fractions of the growth media (10 mL) were sampled before and after AMB-1 cultures (two bottles for each condition) and immediately filtered (0.22 μm, relative to 2-3 μm bacterial length) for subsequent Fe isotope analyses. All experiments were carried out in duplicate (twelve 2-L bottles for one replicate). For each replicate, the twelve bottles reproduced exactly the same conditions. We ensured that the mass-independent fractionations observed in this study did not result from bottle pooling and consequent mixing effects (fig. S9): considering a mixing between two Fe reservoirs with δ^{56}Fe of -3 and +3 ‰, respectively, Δ^{57}Fe deviation from zero would not be larger than 0.003‰. Thus, this mechanism cannot explain the high Δ^{57}Fe values (up to +0.23±0.08‰) observed in magnetite.

*Magnetite extraction and purification*
Cultured bacteria from the twelve bottles were pooled into a single volume (200 mL) using an automatic tangential filtration system (KrosFlo Research IIi tangential filtration system). Magnetite extraction and purification protocols are described in details elsewhere (9) and only summarized herein. Briefly, chains of magnetosomes were extracted from cultured bacteria with a high-pressure homogenizer (EmulsiFlex-C5, Avestin), and recovered with a magnet. Magnetosome membranes surrounding magnetite were removed by severe digestion in strong detergent (Triton X-100 and SDS) and phenol solutions. Finally, magnetite was leached with EDTA and Milli-Q water to get rid of adsorbed Fe-bearing components.

Small magnetite nanoparticles (i.e., <30 nm in size) are superparamagnetic at room temperature and may not have been fully recovered by magnetic extraction. Thus, it is important to estimate the isotopic effect of uncomplete magnetite extraction from bacterial lysates. If 10% of magnetite with $\delta^{56}$Fe of -1‰ were left in the lysate sample, then it would decrease $\delta^{56}$Fe value of the lysate by only 0.2‰, which is negligible compared to the large variations observed in our experiments.

We have ensured that purification did not alter nor dissolve magnetite by monitoring the size of the nanoparticles (figs. S2 and S3). High-resolution TEM observations of the nanoparticles showed that crystal surfaces and edges were undistinguishable in samples before and after treatments, revealing well-defined crystal edges. Typical {111}, {110} and {100} faces were observed in untreated and treated magnetite samples (9). Typical TEM images of (i) AMB-1 bacteria harvested after culture experiments, (ii) extracted chains of magnetosomes and (iii) digested/leached magnetite nanoparticles are shown
in figure S2. We also checked that no remnants of organic matter were associated to the magnetite fraction (fig. S2), according to a procedure developed in (9).

Although size distribution and TEM observations indicate that magnetites were apparently not dissolved during our extraction and purification process, we evaluated potential Fe isotope effect related to magnetite dissolution by EDTA leaching. Only few isotope fractionations associated with Fe oxide dissolution by organic compounds have been reported in the literature, but to our knowledge none of them focused on magnetite. Brantley and co-workers showed that Fe released from goethite (Fe(III)O(OH)) dissolving in the presence of the siderophore desferrioxamine mesylate is isotopically unchanged (34). On the other hand, goethite dissolution by oxalic acid has been shown to produce Fe isotope fractionation, with a dissolved fraction enriched by +0.5‰ in the heavy isotopes when >1% of goethite was dissolved (35). Assuming a fractionation of 0.5‰ associated with magnetite dissolution by EDTA, a release of 10% of Fe contained in magnetite would shift δsuperscript-56Fe values from -1‰ to -1.05‰, which is within our analytical uncertainty. We thus consider that, if any, magnetite dissolution during purification procedure had a negligible effect on Fe isotope composition.

Iron isotope analyses

Growth media before and after AMB-1 cultures, bacterial lysates, and magnetite samples were digested in pure nitric acid at 100°C overnight in a clean room at Institut de Physique du Globe de Paris. Before isotope analyses, iron was separated from matrix elements by anion-exchange chromatography in HCl medium (36). The procedural yield was always >94%. The Fe blank level of the present protocol has been evaluated by systematic analyses of one blank in each sample series, prepared as described above but
without any sample powder or solution. The blank was always below 10 ng Fe, thus representing less than 2% of the bulk Fe.

Iron isotope compositions were measured using a Neptune ThermoFinnigan MC-ICP-MS (Multiple Collector Inductively Coupled Plasma Mass Spectrometer). Iron isotopes were measured simultaneously at masses 54, 56, 57 and 58, while the contributions of Cr and Ni on masses 54 and 58 were monitored and corrected for by using ion intensities measured at masses 53 and 60, respectively. Masses 53, 54, 56, 57, 58 and 60 were collected in the Faraday cups Low 3, Low 2, C, High 1, High 2 and High 4, respectively. Iron isotopes were fully resolved from argide interferences using the high-resolution mode of the Neptune (37). Sample solutions were nebulized and introduced to the mass spectrometer using the ESI Apex-HF desolvating apparatus. The samples were analyzed in 0.3 M HNO₃ at a concentration of ~0.5 ppm Fe. Nebulizer uptake rate was between 40 to 60 µL/min. Signal intensity on the mass 56 was between 15 and 20 volts in the high resolution mode. Fe isotope measurements were performed only when a flat Fe shoulder-peak plateau with a width > 200 ppm was achieved. The analytical routine included baseline correction measured for 120 s before each acquisition. Each sample measurement consisted of 1 block of 15 cycles with 7.2 seconds duration. The take up time before analysis and wash time after analysis were fixed at 70 and 120 seconds respectively.

Instrumental mass discrimination was corrected using the conventional sample-standard bracketing (SSB) approach (38). The SSB method was shown to give results as accurate and precise as the Cu-doping method for correction of instrumental mass bias during Fe isotope measurement (39). The $^{56}$Fe/$^{54}$Fe, $^{57}$Fe/$^{54}$Fe and $^{58}$Fe/$^{54}$Fe ratios were expressed in the usual δ notation in per mil (‰) as:
\[
\delta^{56}Fe_{\text{sample}} = \left[ \frac{(^{56}Fe/^{54}Fe)_{\text{sample}}}{(^{56}Fe/^{54}Fe)_{\text{standard}}} - 1 \right] \times 1000 \quad \text{(Eq. S1)}
\]

\[
\delta^{57}Fe_{\text{sample}} = \left[ \frac{(^{57}Fe/^{54}Fe)_{\text{sample}}}{(^{57}Fe/^{54}Fe)_{\text{standard}}} - 1 \right] \times 1000 \quad \text{(Eq. S2)}
\]

\[
\delta^{58}Fe_{\text{sample}} = \left[ \frac{(^{58}Fe/^{54}Fe)_{\text{sample}}}{(^{58}Fe/^{54}Fe)_{\text{standard}}} - 1 \right] \times 1000 \quad \text{(Eq. S3)}
\]

where the standard is IRMM-014, a pure synthetic Fe metal from the Institute for Reference Materials and Measurements. We assessed the accuracy and analytical precision through the analysis of multiple preparations of the international rock geostandard IF-G (40). IF-G corresponds to a 3.8 Ga old BIF (Banded Iron Formation) from Isua (Greenland) with high Fe content (39.10 wt%). Figures S4 and S5 provide long-term \(\delta^{56/54}Fe\), \(\delta^{57/54}Fe\), and \(\Delta^{57}Fe\) measurements of IRMM-014 and IF-G. In the present work, mean \(\delta^{56}Fe\) and \(\delta^{57}Fe\) values were 0.00±0.06‰ and 0.00±0.10‰ for IRMM-014, and +0.65±0.05‰ and +0.96±0.08‰ for IF-G (2 SD, i.e. Standard Deviation), in good agreement with available data for these geostandards (40). Importantly, both standards show \(\Delta^{57}Fe\) values of 0.00±0.07‰ (2 SD), indicating that deviation from mass-dependent fractionation can be assessed with a precision better than ±0.07‰ (external reproducibility). These results demonstrate the accuracy of our measurements.
Supplementary Text

Isotopic compositions of growth media after AMB-1 cultures

Initial growth media had similar Fe isotope compositions for Fe(III)-quinate ($\delta^{56}\text{Fe} = +0.39\pm0.02\%$) and Fe(II)-ascorbate ($\delta^{56}\text{Fe} = +0.43\pm0.03\%$) experiments (Fig. 1). At the end of the experiments, Fe concentration in the growth media showed variable degrees of depletion. The proportion of initial Fe remaining in the growth medium ranged from 2 to 10% for Fe(III)-quinate experiments, and from 18 to 74% for Fe(II)-ascorbate experiments (fig. S6; table S1). Iron in the residual growth media was depleted in the heavy Fe isotopes ($\delta^{56}\text{Fe}$ between -1.17 and +0.19‰) relative to initial conditions (Fig. 1). AMB-1 cell density was similar in all cultures (fig. S1), suggesting that the total surface for Fe adsorption on bacterial membranes was identical in all experiments. Accordingly, the variability of Fe depletion in growth media and associated Fe isotope composition cannot be explained by passive adsorption on cell surfaces only, but is rather linked to bacterial Fe uptake into the cell. AMB-1 preferentially uptakes heavy Fe isotopes since growth media are enriched in the light Fe isotopes over the course of the bacterial cultures. If Fe uptaken into the cell and precipitated as magnetite did not equilibrate anymore with the growth medium, then the effect of Fe depletion in the growth medium can be modeled as a Rayleigh distillation (fig. S6). In a first approximation, we attempted to apply a Rayleigh distillation model to our system, which describes an exponential depletion in heavy Fe isotopes in growth medium and is calculated as:

$$\delta^{56}\text{Fe}_{\text{growth media}} = \left(\frac{\delta^{56}\text{Fe}_{\text{sources}}}{1000} + 1\right) \times f^{(\alpha_{\text{growth media-bacteria}}^{-1})} - 1 \times 1000 \quad (\text{Eq.S4})$$
where $\delta^{56}\text{Fe}_{\text{growth media}}$ and $\delta^{56}\text{Fe}_{\text{Fe sources}}$ are the isotopic compositions of growth media and Fe sources provided to the media, respectively, $f$ is the fraction of initial iron remaining in the growth media and $\alpha_{\text{growth media-bacteria}}$ is the Fe isotope fractionation factor between growth media and bacteria. The two solid curves dark grey and light grey in fig. S6 represent two Rayleigh models with $\delta^{56}\text{Fe}$ of the initial source at $\sim 0.4\%\text{o}$ and $\alpha_{\text{growth media-bacteria}}$ of 0.9997 and 0.9993, respectively. For Fe uptake lower than 45% of initial Fe, experimental data corresponding to Fe(II)-ascorbate cultures are well explained by a Rayleigh distillation process and a fractionation factor ($\alpha_{\text{growth media-bacteria}}$) of 0.9993 (fig. S6). For higher Fe uptake (>45% of initial Fe) in both Fe(II)-ascorbate and Fe(III)-quinate experiments, most of the measurements are not compatible with a Rayleigh distillation. The data scattering suggests that a fraction of intracellular Fe is able to diffuse back to the growth medium. This conclusion is in line with the loss of MIF signature in Fe(II)-ascorbate experiments and the positive $\Delta^{57}\text{Fe}$ values observed in growth media after AMB-1 cultures in Fe(III)-quinate conditions (see main text and Fig. 3).

Assessing mixing effects on mass-dependent isotope fractionations

Following the classical theory for mass-dependent fractionations (MDF), a plot of $\ln\alpha_{57/54}$ versus $\ln\alpha_{56/54}$ is linear, with different slopes for equilibrium or kinetic reactions (41). In contrast, a plot of $\delta^{57}\text{Fe}$ versus $\delta^{56}\text{Fe}$ corresponds to a curve but is usually assumed to be linear over a short interval. As a consequence, a mixing between two Fe reservoirs with very distinct $\delta^{56}\text{Fe}$ and $\delta^{57}\text{Fe}$ values should produce deviation from the curve describing MDF. When low fractionations occur (typically few %\text{o}), such a deviation from MDF is not measurable. However, Farquhar and collaborators (24) showed that large sulfur isotope fractionations by sulfate-reducing bacteria led to
detectable apparent mass-independent fractionations (24). To test a potential impact of mixing effects on $\Delta^{57}$Fe deviations from zero, we applied the model of Farquhar and collaborators (24) to Fe isotope system. Results are presented in fig. S9. In considering a mixing between two extreme Fe reservoirs with $\delta^{56}$Fe values of $-3$ and $+3\%$, respectively, the maximum variation on $\Delta^{57}$Fe would be $\sim 0.003\%$. Such a low deviation cannot explain our data, since $\Delta^{57}$Fe values are up to $+0.23 \pm 0.08\%$ (2 SD).

Iron reservoirs in AMB-1

The relative concentration of Fe in bacterial lysates with respect to magnetite in AMB-1 was assessed in the two culture conditions from two different methods: (1) direct measurement of Fe concentration in lysate and magnetite fractions (Table S1) and (2) isotope mass balance based on the following equations:

$$\delta^{56}Fe_{cell} = \delta^{56}Fe_{lysate} \times f_1 + \delta^{56}Fe_{magnetite} \times (1-f_1)$$  \hspace{1cm} (Eq. S5)

where $\delta^{56}Fe_{cell}$, $\delta^{56}Fe_{lysate}$ and $\delta^{56}Fe_{magnetite}$ are the isotopic compositions of whole cell, bacterial lysates and magnetite respectively, and $f_1$ is the fraction of cellular Fe contained in the bacterial lysate. For each culture condition, $\delta^{56}Fe_{lysate}$ and $\delta^{56}Fe_{magnetite}$ are given in Table S1. The value of $\delta^{56}Fe_{cell}$ can be obtained from:

$$\delta^{56}Fe_{initial} = \delta^{56}Fe_{cell} \times f_2 + \delta^{56}Fe_{final} \times (1-f_2)$$ \hspace{1cm} (Eq. S6)

where $\delta^{56}Fe_{initial}$ and $\delta^{56}Fe_{final}$ are the isotopic compositions of growth media before and after AMB-1 culture respectively, and $f_2$ is the fraction of Fe in the initial growth medium uptaken by AMB-1 and incorporated into the cell. Finally, $\delta^{56}Fe_{final}$ can be expressed as:
\[ \delta^{56}Fe_{final} = \Delta^{56}Fe_{medium-cell} + \delta^{56}Fe_{cell} \]  
(Eq. S7)

where \( \Delta^{56}Fe_{medium-cell} \) is the fractionation between growth medium and AMB-1 cells.

From Eq. S6 and S7, \( \delta^{56}Fe_{cell} \) in Eq. 1 can be expressed as:

\[ \delta^{56}Fe_{cell} = \delta^{56}Fe_{initial} - \Delta^{56}Fe_{medium-cell} \times (1 - f_2) \]  
(Eq. S8)

The fraction \( f_2 \) can be calculated from Fe concentrations in table S1, and \( \Delta^{56}Fe_{medium-cell} \) is given in fig. S6. As Fe(II) is capable of diffusing from the cell to the growth medium (see main text), we chose to consider the Rayleigh model calculated for the Fe(II)-ascorbate condition as the most representative model for Fe uptake by AMB-1. In this case, the fraction of Fe remaining in growth medium was high. Thus, the isotopic contamination of Fe in growth media by Fe(II) diffusing from the cell should have been limited, as it is suggested by the correlation between results of the Fe(II) ascorbate experiment and the Rayleigh model. Considering a fractionation between growth media and bacteria of -0.7‰ (fig. S6), the fraction \( f_1 \) in Eq. S5 was calculated from Eq. S8 and gave similar values of ~0.7 for Fe(III)-quinate and Fe(II)-ascorbate cultures.

**Mass balance of \( \delta^{56}Fe \)**

We ensured that isotope mass balance lies within \( \delta^{56}Fe \sim 0\%_o \) following the mass balance equation:

\[ \delta^{56}Fe_{sources} = \delta^{56}Fe_{growth media} \times f_3 + \delta^{56}Fe_{lysate} \times f_4 + \delta^{56}Fe_{magnetite} \times f_5 \]  
(Eq. S9)
where $\delta^{56}\text{Fe}_{\text{sources}}$, $\delta^{56}\text{Fe}_{\text{growth media}}$, $\delta^{56}\text{Fe}_{\text{lysate}}$ and $\delta^{56}\text{Fe}_{\text{magnetite}}$ represent the $\delta^{56}\text{Fe}$ values for Fe sources, Fe remaining in growth media after AMB-1 cultures, the bacterial lysate and magnetite respectively, and $f_3$, $f_4$ and $f_5$ represents the relative concentration of Fe in growth media, lysate and magnetite respectively. In each culture condition, $\delta^{56}\text{Fe}$ values are given in table S1 for all samples. The fractions of Fe in growth media, lysate and magnetite samples were calculated from Fe concentrations (in mg of Fe per L of AMB-1 culture) in table S1. For each replicate, $\delta^{56}\text{Fe}_{\text{sources}}$ values were 0‰ within analytical uncertainty.

Mass balance of $\Delta^{57}\text{Fe}$

We ensured that isotope mass balance lies within $\Delta^{57}\text{Fe} \sim 0‰$ following the mass balance equation:

$$\Delta^{57}\text{Fe}_{\text{initial}} = \Delta^{57}\text{Fe}_{\text{final}} \approx \Delta^{57}\text{Fe}_{\text{lysate}} \times f_6 + \Delta^{57}\text{Fe}_{\text{magnetite}} \times (1 - f_6) \quad (\text{Eq. S10})$$

where $\Delta^{57}\text{Fe}_{\text{initial}}$, $\Delta^{57}\text{Fe}_{\text{final}}$, $\Delta^{57}\text{Fe}_{\text{lysate}}$ and $\Delta^{57}\text{Fe}_{\text{magnetite}}$ represent the $\Delta^{57}\text{Fe}$ values for Fe source, all Fe components after AMB-1 growth, the bacterial lysate and magnetite respectively, and $f_6$ represents the relative concentration of Fe in the bacterial lysate with respect to the concentration of Fe in magnetite. The initial values of $\Delta^{57}\text{Fe}$ are 0‰ within analytical uncertainties. The fraction of Fe remaining in growth media after AMB-1 cultures was very low in Fe(III)-quinate experiments, ranging between 2 and 10% of the initial Fe fraction. Thus, we considered that almost all Fe was contained either in the bacterial lysate or magnetite. The fraction $f_6$ has been estimated to be $\sim 0.7$ in Fe(III)-
quinate experiments (see above). $\Delta^{57}$Fe values are given in table S1. For the two replicates, we calculated $\Delta^{57}$Fe = 0.00±0.04‰.

**Comparison with previous results**

The differences between Fe isotope fractionations reported previously in (15) and in the present work may derive from different bacterial strains, and their specific biomineralization pathways and/or various experimental conditions such as initial Fe concentrations in growth media and temperature. Mandernack and collaborators tested two MTB strains, *Magnetospirillum magnetotacticum* MS-1 and *Magnetovibrio blakmorei* MV-1, while we used *Magnetospirillum magneticum* AMB-1. Different MTB strains could synthesize magnetite nanoparticles through different mechanisms, leading to contrasting Fe isotope fractionations. AMB-1 forms magnetosomes under microaerophilic conditions (42), whereas MS-1 is an obligate microaerophile requiring molecular O$_2$ to synthesize magnetite (15) closely related to AMB-1, and MV-1 produces magnetite nanoparticles under anaerobic conditions (15). The three strains are denitrifying microorganisms. Different culture conditions used in (15) for MS-1 and MV-1 produced negligible Fe isotope fractionation (<0.3‰).

Even if AMB-1 may show very specific magnetite biomineralization pathways, the similar results previously obtained for MS-1 and MV-1 suggest that variability in Fe isotope fractionations more likely originates from different culture conditions rather than from differences between strains. Various temperatures of growth experiments tested by Mandernack and collaborators (15) did not evidence any variation in the Fe isotope fractionation. The main difference between the work of Mandernack and collaborators and the present study is the initial Fe concentration: MS-1 and MV-1 strains were cultivated with Fe at 30-70 µM, whereas AMB-1 was cultivated with Fe at
150 µM in the present study. High Fe concentrations in the initial growth media may lead to Fe accumulation apart from magnetites in AMB-1 cells, yielding significant Fe concentrations in lysates, as observed here (Table 1). An absence of extra-magnetite Fe pool in MS-1 and MV-1 would imply a complete precipitation of intracellular Fe. In this case, magnetite would be the only Fe reservoir in bacteria, and the fractionation between growth medium and magnetite would be small (Fig. 1; fig. S6). Mandernack and collaborators proposed maximum difference in $\delta^{56}$Fe between Fe sources and magnetite of 0.3‰ (15). Some growth media after AMB-1 culture in this study are compatible with an apparent fractionation factor between growth media and bacteria ($\alpha_{\text{growth media-bacteria}}$) of 0.9997 (fig. S6), meaning that in some conditions the difference in $\delta^{56}$Fe between Fe sources and bulk AMB-1 may indeed be of ~0.3‰ as proposed by Mandernack and collaborators.

**Natural magnetite samples**

We calculated $\Delta^{57}$Fe (23) values of natural magnetite samples from $\delta^{56}$Fe and $\delta^{57}$Fe data available in the literature (13, 32, 33). It was not mentioned in these studies whether $\delta^{56}$Fe and $\delta^{57}$Fe showed covariance. Therefore, we estimated the maximum errors on $\Delta^{57}$Fe as the sum of the errors on $\delta^{56}$Fe and $\delta^{57}$Fe:

\[
2SD \Delta^{57}Fe = 2SD \delta^{57}Fe + 1000 \times ((1 + \frac{2SD \delta^{56}Fe}{1000})^\beta - 1)
\]  

(Eq. S11)
Fig. S1. AMB-1 growth curves for Fe(III)-quinate and Fe(II)-ascorbate experiments. The evolution of optical density at 565 nm ($OD_{565\text{ nm}}$) with time of culture is similar in the two culture conditions. Each point corresponds to the mean value of four replicates.
Fig. S2. TEM observations of magnetite samples during the purification process.

TEM images of AMB-1 whole cells (A, B), extracted chains of magnetosomes (C, D) and purified magnetite nanoparticles (E, F) in Fe(III)-quinate (A, C, E) and Fe(II)-ascorbate (B, D, F) experiments.
Fig. S3. Size distributions of magnetite nanoparticles. Size distributions of magnetites extracted from AMB-1 (A, C) and purified magnetite nanoparticles (B, D) in Fe(III)-quinine (A, B) and Fe(II)-ascorbate (C, D) experiments. The consistent mean and median sizes of nanoparticles show that magnetites were not dissolved or altered by the purification process.
Fig. S4. Long-term analyses of IRMM-014 geostandard. Long-term δ⁵⁶/⁵⁴Fe, δ⁵⁷/⁵⁴Fe, δ⁵⁷/⁵⁴Fe and Δ⁵⁷Fe measurements of IRMM-014. All values are near 0 ‰, as expected for a standard-standard bracketing. The uncertainties represent 2 SD external reproducibility for several sessions over two years.
Fig. S5. Long-term analyses of IF-G geostandard. Long-term $\delta^{56/54}$Fe, $\delta^{57/54}$Fe, $\delta^{55/54}$Fe and $\Delta^{57}$Fe measurements of IF-G. All values are in good agreement with available data for this geostandard (40).
Fig. S6. Evolution of Fe isotope compositions in the growth media. $\delta^{56}$Fe values (relative to IRMM-014) of filtered AMB-1 growth media are represented as a function of the fraction of Fe remaining in the growth media after six days of culture. Filled black circles: Fe(III)-quinate iron source; open circles: Fe(II)-ascorbate iron sources. Data are compatible with a fractionation factor between growth media and bacteria ranging between 0.9997 and 0.9993. This corresponds to fractionation between growth media and bacteria ranging between -0.3 and -0.7 ‰, respectively.
Fig. S7. Mass-independent fractionation (MIF) of Fe isotope in MTB cultures using Fe(III)-quinate as Fe source. Plot of $\delta^{57}\text{Fe}$ versus $\delta^{56}\text{Fe}$ that suggests MIF on odd isotope ($^{57}\text{Fe}$). Experimental data shows a linear correlation ($R^2=0.9986$) with a slope (1.38) significantly different from the one predicted for mass-dependent fractionation (MDF) (1.48) (41).
Fig. S8. Mass-dependent fractionation (MDF) of Fe isotopes in MTB cultures using Fe(II)-ascorbate as Fe source. Plot of $\delta^{57}\text{Fe}$ versus $\delta^{56}\text{Fe}$ that suggests the absence of MIF of Fe isotopes. Experimental data shows a linear correlation ($R^2=0.9996$) with a slope (1.46) almost identical to the one predicted for MDF at equilibrium (1.48) (41).
Fig. S9. Deviation from mass-dependent fractionation induced by mixing effects. Variation of $\Delta^{57}\text{Fe}$ produced by mixing of two endmembers related by mass-dependent fractionations. The evolution of $\Delta^{57}\text{Fe}$ was calculated by applying the model proposed by Farquhar and collaborators (24). Considering a mixing between two Fe reservoirs with $\delta^{56}\text{Fe}$ of -3 and +3 ‰, respectively, $\Delta^{57}\text{Fe}$ deviation from zero would not be larger than 0.003‰. Thus, this mechanism cannot explain the magnitude of $\Delta^{57}\text{Fe}$ shown in Figure 3.
Fig. S10. Mass-dependent fractionation of even Fe isotopes in MTB cultures. Plot of $\Delta^{58}\text{Fe}$ versus $\delta^{56}\text{Fe}$ that suggests the absence of mass-independent fractionation on even isotopes ($^{54}\text{Fe}$, $^{56}\text{Fe}$, $^{58}\text{Fe}$). Symbols represent growth media after AMB-1 cultures (squares) and magnetite samples (triangles) in experiments using either Fe(III)-quinate (black filled symbols) or Fe(II)-ascorbate (open symbols) as Fe sources. Error bars are 2 SD. The thick horizontal grey line corresponds to $\Delta^{58}\text{Fe} = 0\text{‰}$. 
### Table S1
Iron concentrations (in mg of Fe per L of AMB -1 culture) and δ\(^{56}\)Fe, δ\(^{57}\)Fe, δ\(^{58}\)Fe, Δ\(^{57}\)Fe and Δ\(^{58}\)Fe values for filtered growth media before and after AMB-1 cultures, bacterial lysates and magnetite samples using Fe(III)-quinate and Fe(II)-ascorbate Fe sources, respectively.

<table>
<thead>
<tr>
<th>Sample</th>
<th>[Fe] (mg/L of culture)</th>
<th>δ(^{56})Fe ± 2 SD (‰)</th>
<th>δ(^{57})Fe ± 2 SD (‰)</th>
<th>δ(^{58})Fe ± 2 SD (‰)</th>
<th>Δ(^{57})Fe ± 2 SD (‰)</th>
<th>Δ(^{58})Fe ± 2 SD (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fe(III)-Quinate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Medium before growth #1</td>
<td>3.10 ± 0.39</td>
<td>+0.58 ± 0.08</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medium before growth #2</td>
<td>3.39 ± 0.37</td>
<td>+0.56 ± 0.06</td>
<td>-</td>
<td>-0.01 ± 0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medium before growth #3</td>
<td>3.15 ± 0.41</td>
<td>+0.56 ± 0.06</td>
<td>-</td>
<td>-0.04 ± 0.06</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medium before growth #4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medium after growth #1</td>
<td>0.06 ± 0.19</td>
<td>+0.29 ± 0.06</td>
<td>-</td>
<td>0.00 ± 0.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medium after growth #2</td>
<td>0.19 ± 0.51</td>
<td>-0.61 ± 0.24</td>
<td>+0.16 ± 0.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medium after growth #3</td>
<td>0.33 ± 0.37</td>
<td>-0.45 ± 0.06</td>
<td>+0.10 ± 0.06</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medium after growth #4</td>
<td>0.28 ± 1.17</td>
<td>-1.60 ± 0.22</td>
<td>+0.15 ± 0.06</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacterial lysate #1</td>
<td>2.09 ± 0.93</td>
<td>+1.34 ± 0.06</td>
<td>-</td>
<td>-0.04 ± 0.08</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacterial lysate #2</td>
<td>2.39 ± 0.79</td>
<td>+1.14 ± 0.12</td>
<td>-</td>
<td>-0.03 ± 0.10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Magnetite #1</td>
<td>0.90 ± 1.76</td>
<td>-2.37 ± 0.14</td>
<td>+0.25 ± 0.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Magnetite #2</td>
<td>0.60 ± 1.66</td>
<td>-2.25 ± 0.12</td>
<td>+0.22 ± 0.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Fe(II)-Ascorbate</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Medium before growth #1</td>
<td>2.69 ± 0.42</td>
<td>+0.61 ± 0.22</td>
<td>-</td>
<td>-0.01 ± 0.01</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Medium before growth #2</td>
<td>2.06 ± 0.47</td>
<td>+0.68 ± 0.06</td>
<td>-</td>
<td>-0.03 ± 0.01</td>
<td>-</td>
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<tr>
<td>Medium before growth #3</td>
<td>2.37 ± 0.42</td>
<td>+0.58 ± 0.10</td>
<td>-</td>
<td>-0.04 ± 0.06</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medium before growth #4</td>
<td>2.41 ± 0.39</td>
<td>+0.56 ± 0.12</td>
<td>-</td>
<td>-0.02 ± 0.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medium after growth #1</td>
<td>1.25 ± 0.12</td>
<td>-0.20 ± 0.06</td>
<td>-</td>
<td>-0.02 ± 0.08</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medium after growth #2</td>
<td>1.51 ± 0.23</td>
<td>+0.34 ± 0.10</td>
<td>-</td>
<td>0.00 ± 0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medium after growth #3</td>
<td>0.42 ± 0.23</td>
<td>-0.33 ± 0.20</td>
<td>+0.01 ± 0.06</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medium after growth #4</td>
<td>1.51 ± 0.11</td>
<td>+0.17 ± 0.04</td>
<td>-</td>
<td>0.00 ± 0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacterial lysate #1</td>
<td>0.97 ± 1.25</td>
<td>+1.84 ± 0.06</td>
<td>-</td>
<td>-0.02 ± 0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacterial lysate #2</td>
<td>0.91 ± 1.24</td>
<td>+1.78 ± 0.10</td>
<td>-</td>
<td>-0.06 ± 0.06</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Magnetite #1</td>
<td>0.24 ± 0.93</td>
<td>-1.33 ± 0.12</td>
<td>+0.06 ± 0.05</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Magnetite #2</td>
<td>0.24 ± 0.94</td>
<td>-1.38 ± 0.16</td>
<td>-1.72 ± 0.11</td>
<td>+0.02 ± 0.03</td>
<td>0.13 ± 1.0</td>
<td>-</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
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</tbody>
</table>
REFERENCES AND NOTES


14. Iron isotope data are reported using conventional delta notation relative to the international standard IRMM-014 and defined as

\[ \delta^{xx}\text{Fe}_{\text{sample}} = \left( \frac{^{xx}\text{Fe}_{\text{sample}}}{^{xx}\text{Fe}_{\text{IRMM-014}}} - 1 \right) \times 1000 \]

where \( xx \) is 56, 57, or 58.


23. Capital delta notation $\Delta^{xx}Fe$ illustrates the deviation of Fe isotope fractionation from single-step low-temperature equilibrium exchange reactions, as defined for S isotopes (24). In other words, $\Delta^{xx}Fe$ quantifies the deviation from mass-dependent fractionation and is expressed as $\Delta^{xx}Fe = \delta^{xx}Fe \times 1000 \times \left[1 - \beta \left(\frac{Fe^{57}}{Fe^{56}}\right)^{xx} \right] - 1$ where $xx$ is 57 or 58, and $\beta$ corresponds to the variation of $^{57}Fe/^{56}Fe$ and $^{58}Fe/^{56}Fe$ mass ratios (i.e., 1.475) or $^{56}Fe/^{54}Fe$ mass ratios (i.e., 1.932).


26. D. Schüler, E. Baeuerlein, Iron-limited growth and kinetics of iron uptake in 


39. R. Schoenberg, F. von Blanckenburg, An assessment of the accuracy of stable Fe isotope ratio measurements on samples with organic and inorganic matrices by high-

