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Discussions

Interactive comment on "Interpretation of kinetic isotope fractionation between aqueous Fe(II) and ferrihydrite under a high degree of microbial reduction" *by* Lei Jiang et al.

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We sincerely thank Reviewer 1 to provide feedback on our manuscript.

1. As the authors realize, ferrihydrite readily transforms into secondary minerals in the presence of Fe(II) depending on, among other factors, Fe(II) concentration. Hence, it can be assumed that different types of secondary iron minerals have been formed in the experiments depending on the rates and extent of Fe(III) reduction. Changes in mineralogy, obviously, effect isotope fractionation and without quantitative information of the Fe isotope signature of the various Fe species it is very difficult to interpret fractionation factor.

Author response: Thank you for your suggestion, it would have been benificial to determine the mineralogy of Fe minerals and Fe isotope fractionation as a function of time. We surely haven't done more research about this area except for SEM/TEM study. Previous studies suggested that biogenic magnetite retained morphologic and size features of ferrihydrite, wheareas siderite (another readily formed secondary mineral during ferrihydrite bioreduction) was generally observed as rhombohedral crystallites (Zachara et al., 2002). Determined by SEM, the reduction end-product in our experiment surely produced a little of magnetite. This is consistent with the research of Wu et al., (2013), whose materials and methods we refered to. When magnetite is the only secondary mineral in the HFO reduction experiment, Fe isotope fractionation is mainly associtated with HFO reduction rate (Johnson et al., 2005). In a long-term laboratory experiment at low Fe(III) reduction rate, the Fe(II)aq-magnetite fractionation have been achieved at constant of -1.3‰ which is interpreted to be the equilibrium fractionation factor at 22âĎČ. However, at a high ferrihydrite reduction rate experiment, Fe isotope fractionation between Fe(II)aq and ferrihydrite substrate is essentially associated with rapid sorption of Fe(II) to HFO. Moreover, magnetite is usually produced at the second of half of the experiment. As our experiments performed at a more higher reduction rate than Jonhnson'sïijŇand the reaction is short, so the influence of magnetite on Fe isotope fraction was limited. We will state it more clearly in the revised version.

2. I also have several other concerns about the interpretation of the data: According to the methodology about 1 g Fh were added to 50 mL medium. This should yield a Fe concentration of about 120 mM. This implies that only around 50 % of total Fe was recovered, which questions the isotope values for Fe(III) when the digestion was not quantitative. The trend that Fe recovery decreases with progressing reaction might reflect Fe mineral transformation (e.g. magnetite formation).

Author response: We are sorry for making a clerical error. The amount of ferrihydrite added to the 50 mL medium was 0.1 g, which should yield a final Fe concentration of about 20 $\sim$ 22 mM according to the controversial ferrihydrite chemical formula of

5Fe2O3.9H2O or Fe5HO8.4H2O. The concentrations of Fe0.1 M HCl and Fe0.5 M HCl in Table 1 represent the extracts (not the reactor), and the volumes of 0.1 M HCl and 0.5 M HCl extracts used for extraction are 15 ml and 20ml, respectively. However, the concentration of Feaq in Table 1 represents the reactor of which the volume is 50 ml. So, this leads to the misunderstanding that the recovery is only around 50%. We will correct it and state clearly in the revised version. 3. The ratio Fe(II)(0.1M HCl)/(Fetot(0.1M HCl) +Fetot(0.5M HCl)) exceeds 0.25, which is larger than a realistic concentration of surface sites (about 0.2 per Fe for HFO). This implies that not all extracted Fe(II) is adsorbed Fe but includes structurally bound Fe(II).

Author response: The ratio doesn't exceed realistic concentration of surface sites, the detail reason see response 2. We will state it clearly in the manuscript.

4. The authors do not mention anything about pH. Does the pH change throughout the reaction (no buffer is present in the medium) and how would pH effect fractionation. Considering these uncertainties, I am sceptic that the data set could be used to rigorously discussing fractionation mechanisms or deriving reliable fractionation factors.

Author response: Our apologies for no mention about pH and the effect of its variation on the rate and extent of Fe isotope exchange in the manuscript. In fact, the pH of each aqueous fraction was determined by HQ 40d in our experiments. The initial value of S. piezotolerans WP3 and S. oneidensis MR-1 reduction experiments were 6.3 and 6.6, respectively. With the proceeding of reaction, the pH increased to 6.4$\sim$6.8 in S. piezotolerans WP3 reactor, as well as 6.8$\sim$7.3 in S. oneidensis MR-1 reactor. The effect of pH on Fe isotope fractionation is essentially attributed to that Fe(II) sorption onto ferric minerals (Reddy et al., 2015). We will add the pH section and disscuss its influence on Fe isotope fractionation in the revised version.

5. I have also a couple of minor comments: Why did the authors vary the pressure? The experimental design is not justified. Varying the reduction rates or manipulating the Fe(II)/Fe(tot) ratios could have been easier achieved by adapting the bacteria/Fh

ratio. Using different organisms and pressures creates unnecessary ambiguity.

Author response: We agree with your comments. We chosen pressure and bacterial strains as a way to modulate reduction rates and extents. The results haven show that the effect of pressure on the extent of bioreduction and Fe isotope fractionation is not obvious. However, the bacterial strains have significantly impact on the rate and extent of bioreduction, and Fe isotope fractionation. In order to clarify the fact that Fe isotope exchange will be inhibited under high degree of bioreduction and the comments you give, we will remove the pressure part in the revised version.

6. Fh is produced by neutralizing a Fe(III)NO3 solution with KOH. The authors do not mention any purification step before freeze drying, implying that the solid should contain considerably amounts of nitrate. I presume the organisms can both use nitrate as electron acceptor or not? What would be the implications of the presence of nitrate.

Author response: Our apologies for no mention about the purification steps. Before freeze drying, ultrapure water was added to the suspension and centrifuged to isolate the nitrate fraction, repeating this operation 10 times. We will add this part in the revised manuscript.

7. Minor text related comments: The first two sentences in the abstract do not help to grasp the content of the study but obscure the subject. My first impression was that the authors argue that isotopic fractionation is the cause for the cessation of iron reduction.

Author response: We agree with your comments, and we will remove the two sentences in the reviesed version.

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