

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 thank the Anonymous Referee #2 for his comprehensive comments, we will respond to each below.

General comments:

1. The main goal of the study and the research questions are not clearly stated. The use of pressure is not discussed. It is unclear what the pressure experiments bring to the study. In itself, the role of pressure on Fe isotope fractionation is a valid question (with an adequate experimental design), but it is only mentioned in the abstract and is

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apparently used in the study as a way to modulate Fe(III) reduction rate.

Authors response: In order to obtain the different extents of bioreduction and its relationship with Fe isotope fractionation, we chosen pressure and bacterial strains as a way to modulate it. The results 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 will be impacted under higher degree of bioreduction. In order to clarify the fact that Fe isotope exchange will be inhibited under high degree of bioreduction, we will remove the pressure section in the revised paper.

2. It would have been beneficial to determine the mineralogy of Fe minerals as a function of time. Fe(II) catalyzes mineral transformation at the surface of Fe(II) oxides and mineral transformation might potentially influence Fe fractionation.

Authors response: Please see the response 1 to Anonymous Referee #1.

Specific comments:

3. First sentence of abstract is unclear about what the topic of the study is.

Author response: We agree with your comments, it surely obscures the subject. We will write it clearly in the revised manuscript.

4. I.55-56 reference missing for the procedure (Schwertmann & Cornell)

Author response: Thank you for your suggestion. It will be done.

5. I.57-60 should be moved to “Iron isotope measurements section”.

Author response: We agree with your comment. We will move it to “Fe isotope measurements section”.

6. I.71 It is unclear what the DIR experiment media are

Author response: We will clarify it in the manuscript. 50 ml 2216E and LB media were

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added into sterile plastic syringes, respectively, followed by addition of 0.1 g ferrihydrite and bacteria. The syringes were then sealed with PE material stoppers and placed in the high pressure steel vessel for the DIR experiments.

7. I.72 what is the concentration of ferrihydrite? What is the starting cell concentration?

Author response: We add 0.1g ferrihydrite to the 50 ml medium. It should yield a concentration of about 20 ~ 22 mM according to the controversial ferrihydrite chemical formula of $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$ or $\text{Fe}_5\text{HO}_8 \cdot 4\text{H}_2\text{O}$. The starting cell concentration was about $7.22 \times 10^{-6} \text{ L}^{-1}$. We will state it clearly in the manuscript.

8. Figure 1: why is the total concentration of Fe(II) decreasing at incubations of WP3 at ambient pressure?

Author response: We have no idea about this. It may be due to the formation of secondary minerals or experimental operation error.

9. I.112-113 What does the ratio of Fe(II) sorbed to Fe(III) aqueous indicate? What is its significance?

Author response: Thank you for your suggestion. This is a problematic statement, we will remove these sentences in the revised version.

10. I. 131-135 The effects of pressure on Fe(III) reduction have been previously investigated for *S. piezotolerans* WP3 (Wu et al. 2013 Geobiology) and for *Shewanella profunda* (Picard et al. 2015 Frontiers). I.137-139 not all appropriate references are used.

Author response: We will clarify it.

Technical comments:

11. I.31 “forming a wide range of soluble Fe(II)”: replace by “producing soluble Fe(II)”
I.35 replace “, in showing” by “have shown”
I.38 Replace “Seems to no effect on ” by
“does not seem to impact”
I.46 Replace “less” by “low”
I.61 Plural of medium is “Media”

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Figure 1: typo in piezotolerans in the figure panels a and c. Also found throughout the manuscript. I.102 and after: mM instead of mM L-1

Author response: We thank you for pointing out the errors in the manuscript. We will amend these errors in the manuscript.

12. I.36-37 Be specific: are you talking about the yield of the reaction (how much Fe(III) is reduced overall during the experiment) or the rate at a specific time.

Author response: Yes, we want to show the degree of hematite and goethite bioreduction is lower than 4% in a lasting 280 days experiment. But, we made a clerical error. The “rate” in I.37 should be replaced by “extent”.

13. I.109-110 It is a well-established fact that low crystalline Fe(III) minerals are reduced more and faster than crystalline Fe(III) oxides. Use appropriate references

Author response: Yes, this is also the reason that we choose ferrihydrite as terminal ferric substance to perform DIR experiments. We will cite the appropriate references in the revised manuscript.

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