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Biogeosciences Discussions

Interactive comment on "Pyrite Oxidation under initially neutral pH conditions and in the presence of *Acidithiobacillus ferrooxidans* and micromolar hydrogen peroxide" *by* Y. Ma and C. Lin

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Authors Response to the Comments by Reviewer 3

General comments

Reviewer's Comment:

The manuscript "Pyrite Oxidation under initially neutral pH conditions and in the presence of Acidithiobacillus ferrooxidans and micromolar hydrogen peroxide" submitted by Y. Man and C. Lin, is interesting in so far as the studies about this issue are relatively scarce. However, in my opinion, selected pyrite cubes are very large and the surface is low, which affects the results. Thus, the data obtained in the three experimental treatments did not show significant differences between them (Figures 1b, 3, and 4) and do not provide a clear scientific advance.

Authors' Reply:

We thank the reviewer's comments.

Indeed it is challenging to examine the effects of H2O2 at such low levels on pyrite oxidation. The effects were more evident in acidic scenarios (separate experiments) due to the stronger response of A. ferrooxidans to the toxicity of H2O2-induced hydroxyl radical (through Fenton-type reaction). The acidic conditions also allow Fe3+-driven pyrite oxidation to operate.

In contrast under cricumneutral pH conditions, the H2O2-induced footprint is expected to be shallow. So in this study we tried to focus on two aspects with high probability of showing the difference among the control and various treatments: (a) cell attachment and the resulting pyrite surface corrosion and the chemical composition and states on the H2O2-attacked pyrite surfaces using the surface-sensitive XPS technique. We actually conducted experiments using both cubic pyrite and powdered pyrite. As explained below, the use of pyrite cubes has the advantages in terms of achieving our research objective set for this study:

Due to the large specific area of the powdered pyrite, the solution pH dropped sharply from 6.8 to below 3 within hours after the commencement of the experiment for both the control (no added H2O2) and the treatments. In such a case, it is likely that the molecular (dissolved) oxygen played a dominant role in abiotic pyrite oxidation, followed by Fe3+-driven pyrite oxidation. The reaction between powdered pyrite and molecular oxygen may also lead to the introduction of spontaneously generated H2O2 into the reaction systems, as shown in Schoonen et al. (2010). For these reasons, we did not include these data in the manuscript. (Reference: Schoonen, M.A.A.; Harrington, A. D.; Laffers, R.; Strongin, D.R. Role of hydrogen peroxide and hydroxyl radical in

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pyrite oxidation by molecular oxygen. Geochim. Cosmochim. Acta. 2010, 74(17), 4971-4987).

The use of cubic pyrite allowed the maintenance of a circumneutral pH conditions for an extended period and avoid introduce significant amount of spontaneously generated H2O2 into the reaction system. These experimental conditions enhanced the role of the added H2O2 in reaction system. Besides, cubic pyrite has relatively flat and uniform surface than does powdered pyrite, which is an additional advantage over powdered pyrite in terms of showing the H2O2 footprints. Since our focus is not on the aspect of mineral processing, natural pyrite grains are more relevant to our research in acid mine drainage.

We partly agree with the reviewer. Indeed, the difference in the examined parameters among the treatments was not dramatically evident. However, the difference in key parameters we examined is still clearly observable. These include:

(a) The population density of attached cells on the pyrite cube surface was markedly different. No attached cells were observed in T2 and T3; the population density of the attached cells was lower in the control than in T1. This was observed from the entire pyrite cube surface area. Perhaps the resolution of SEM images in Figure 2 is too low to allow a clear comparison. We now provide high-resolution SEM images in the supplementary document to assist in demonstrating the difference.

(b) As shown in Figure 2e and 2f, there was marked difference in the shape, size and orientation pattern of the corrosion pits on the pyrite surface.

(c) There was a clear trend showing that the major XPS peaks (Fe 2p3/2 and S 2p) for the reacted pyrite surfaces shifted to the lower binding energy side with increasing dosage level of H2O2 (Table 1 and Figure 4).

Introduction:

Reviewer's Comment: In page 559, line 22, the concentration range of H2O2 in natural

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environments should be included. A more intense explanation of the importance of the objective of the paper should also be included.

Authors' Reply: We accept the reviewer's suggestion and additional information will be added accordingly.

Materials and methods

Reviewer's Comment: Page 560, lines 12-13, given the importance of the pyrite cube size, the authors must explain the reasons that led them to select this size.

Authors' Reply: The reasons for choosing cubic pyrite rather than powdered pyrite were explained above.

The dimension of the pyrite cubes was not selected on purpose but based on the availability of such pyrite cubes from the available pyrite specimens that met the number requirement (The experiment required over 25 pyrite cubes with similar size).

Reviewer's Comment: Page 561, line 24, the time interval for re-injection of H2O2 should be justified.

Authors' Reply: As explained in the Authors Response to Comments by Reviewer #1, for the pyrite-solution contact experiments involving iron/sulfide-oxidizing bacteria, lengthy experiments were frequently used to create deep footprints to allow adequate characterization of the reacted surfaces of the investigated sulfide minerals. For example: 1 Pisapia et al. 2008, Geomicrobiology Journal, 25(6): 261-273 (experiment length: 22 months) 2 Rojas-Chapana and Tributsch, 2004, FEMS Microbiology Ecology, 47:19-29 (experiment length: 6 months) 3 RodrÄśÌĄguez et al., 2003, Hydrometallurgy, 71: 37-46 (experiment length: 40 days) 4 Jones et al., 2003, Geochimica et Cosmochimica Acta, 67: 955-965 (experiment length: 3 weeks) 5 Edwards et al., 2001, FEMS Microbiology Ecology, 34: 197–206 (experiment length: 4 weeks) 6 Mustin et al. 1992, Applied and Environmental Microbiology, 58: 1175-1182 (experiment length: 55 days)

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Reviewer's Comment: Page 562, line 13, should specify whether the cells counted were dead or alive.

Authors' Reply: Only viable planktonic cells were counted in this study. The direct cell counting was performed using a Neubauer hemocytometer. The cells that showed no sign of motion were not counted as viable cells.

Discussion

Reviewer's Comment: Page 566, lines 14-16, the sentence "The extract mechanism....... is not clear" should be revised.

Authors' Reply: We accept the reviewer's suggestion and changes will be made accordingly.

Reviewer's Comment: Page 567, lines 12-13, the authors suggest that "certain individual cells were able to adapt to high H2O2 conditions by developing H2O2 tolerance", but could be that the survival of planktonic cells were due to the development of intrinsic resistance?

Authors' Reply: We will change the sentence to "certain individual cells were able to adapt to high H2O2 conditions by developing intrinsic resistance/tolerance to H2O2 exposure"

Reviewer's Comment: Page 567, lines 25-27, the authors suggest that iron precipitates were formed, if so, the precipitates at the bottom of the culture flasks should be seen. Were such precipitates observed by the authors?

Authors' Reply: There were trace amounts of precipitates on the bottom of the conical flasks, especially in T3 (the highest-dose treatment). This visible observation will be added to the manuscript.

Reviewer's Comment: In my opinion, more references are needed to support the discussion, especially Section 4.3.

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Authors' Reply: Efforts will be made to increase the number of references.

Conclusions

Reviewer's Comment: In general, the scientific findings are not substantiated enough by the data.

Authors' Reply: As mentioned in the Authors Response to the Comments by Reviewers #1 and #2, we will add additional/new data to enhance the quality of arguments.

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