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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

Anonymous Referee #4

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I agree with the other reviewers that studies on pH neutral (bio)oxidation of pyrite and other sufidic minerals are rare. Maybe, because these investigations are quite difficult to perform, as they require a profound knowledge of both the microbiology and the surface chemistry and, consequently, a strong cooperation of these research disciplines. Nevertheless, considering the significant impact of acid mine drainage and of related environmental problems, this kind of research is highly relevant.

The conclusion drawn in this study is plausible. An attack by the strong oxidant H2O2 likely could prepare the ground for Acidithiobacillus ferrooxidans and related bacteria. Attachment might be facilitated and also the biooxidation process itself could be en-

hanced. However, to my opinion, this conclusion is not justified on the basis of the experimental data shown.

1. Lack of sterile control experiments. Only experiments with addition of bacteria were performed. Consequently, it is really difficult to discriminate between bioleaching activities and the contribution of H2O2 to the pyrite corrosion. In case previous studies on abiotic H2O2 oxidation of pyrite were performed under comparable conditions, results should be discussed in this context. Previous studies applying H2O2 were mentioned in the Introduction but not in the Discussion. On the basis of the presented results, I would say that you have just studied leaching by increasing concentrations of H2O2 in T1 to T3.

2. Viability of bacteria. What is meant with "direct counting"? Just counting cells under a light microscope? This is likely not sufficient to state that the planktonic cells "survive" or even are oxidizing soluble iron and sulfur species. Maybe, you have only counted dead cells. Likewise, we cannot be sure about the viability of the attached cells, as no sterile control experiments were performed.

3. Re-injection of H2O2. The oxidant was re-injected in intervals of 3 to 5 days. What is the half-life of H2O2 under the experimental conditions? Could you explain why you chose the interval? Do you have any idea about the changes of H2O2 during incubation? Is it possible that H2O2 accumulated in the experiments with higher dosage (T2 and T3)?

4. Enhanced attachment in T1. I cannot see in increased attachment when comparing C and T1 in Fig. 2. Is this really significant? I doubt that this conclusion can be drawn without thoroughly counting all cells and considering a much larger surface area than shown in this study.

5. Relevance of the study. A. ferrooxidans is a widespread leaching bacterium. And H2O2 may be produced from many sources (abiotic as well as biological ones). However, as already mentioned by the other reviewers, in Nature an acidophilic bacterium

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is possibly not the initial settler of pyrite surface at pH neutral conditions. More likely, neutrophilic sulfur compound oxidizing bacteria play in important role.

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