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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 #2

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General comments

Attachment of the pyrite oxidizing bacterium Acidithiobacillus ferrooxidans to the pyrite surface was studied at neutral pH in laboratory experiments under the influence of hydrogen peroxide in different concentrations. Effects were evaluated by measuring pH, iron concentrations, numbers of planktonic cells, as well as SEM and XPS measurements of the pyrite surface.

In my opinion the manuscript does not provide any scientific advancement in understanding attachment of A. ferrooxidans to pyrite and its role in the pyrite oxidation process. The manuscript does not fulfill the standard of a scientific publication because of

the following reasons:

1. The data do not allow drawing conclusions about the effect of hydrogen peroxide on cell attachment and pyrite oxidation. The data shown do not provide significant differences for the three experimental treatments with different hydrogen peroxide concentrations and the control, besides that the pH in T3 is lower than for the other treatments presumably due to chemical pyrite oxidation by hydrogen peroxide at the highest concentration applied. Fig. 1b shows a similar decrease of planktonic cell numbers over time for all treatments. Fig. 2 only provide qualitative data, the bars in Fig. 3 are similar for all treatments, error bars are missing, and also the XPS spectra in the supplementary material do not reveal significant differences for the different treatments. The numbers of planktonic cells shown in Fig. 2b are quantitative but do not tell anything about the colonization of the pyrite surface. The decrease of cell numbers over time in all treatments is most likely caused by cell death. A. ferrooxidans is an obligate acidophilic organism (pH maximum at pH 4.5), but cells were exposed to pH > 5 for more than 100 days! No data about the physiological status of the cells is given (e.g. FISH, cultivation), thus it is even unclear if the detected planktonic cells are still alive and active in iron- and sulfur oxidation. As a consequence any statement about the physiological status of the cells are not supported by data, e.g. in the conclusions "The planktonic Acidithiobacillus ferrooxidans were able to survive under the highest H2O2 dosage. . .". Quantitative data for the colonization of the cells on the pyrite surface obtained by fluorescence microscopy after DNA staining or AFM (e.g. Noel et al. 2010 Hydrometallurgy) would have been useful for this study. The iron data given in Table 1 are all lower than 1 mg per L and not worth to be shown. Iron is almost insoluble at oxic conditions above pH 4 and precipitates as iron(hydr)oxide. Soluble S species have not been analyzed (e.g. Schippers and Jorgensen 2002 Geochim. Cosmochim Acta) to backup any conclusions about their role in supporting bacterial growth (e.g. Conclusions).

2. The scientific statements are not supported by the data. The first sentence of the

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abstract is speculative. It has not even shown that the cells oxidize the pyrite, and not at all that "microbial oxidation" is influenced by hydrogen peroxide. Also the second sentence: Colonization of Acidithiobacillus ferrooxidans onto the mineral surface has not been demonstrated (only planktonic cells were counted see 1.). All further sentences in the abstract are speculative as well.

3. The discussion is weak and does not reference to papers on relevant topics such as attachment of A. ferrooxidans to pyrite (e.g. Sand et al. 2001 Hydrometallurgy), the sulfur chemistry and mechanisms of pyrite oxidation (e.g. Sand et al. 2001 Hydrometallurgy; Druschel and Borda 2006 Comment in Geochim. Cosmochim Acta), the role of hydrogen peroxide in pyrite oxidation (e.g. Borda et al. 2003). Instead the discussion proposes a reaction mechanism disconnected from the state-of-the-art and not based on the scientific literature. Beside in the first few lines the entire discussion section does not contain any references.

Interactive comment on Biogeosciences Discuss., 9, 557, 2012.