Biogeosciences Discuss., 9, C1019–C1023, 2012
www.biogeosciences-discuss.net/9/C1019/2012/

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

Y. Ma and C. Lin

chuxia.lin@usq.edu.au

Received and published: 4 May 2012

Authors Response to the Comments by Reviewer #4

Reviewer's Comment: 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 enhanced. However, to my opinion, this conclusion is not justified on the basis of the experimental data shown.

Authors' Reply: We thank the reviewer's comment. As mentioned in the responses to other reviewers' comments, we will provide further experimental data to try to make the arguments more convincing.

Reviewer's Comment: 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.

Authors' Reply: Abiotic experiments were planned. However, it ended up with only powdered pyrite experiment was run. Abiotic experiment was not conducted for cubic pyrite due to insufficient number of pyrite cubes with similar size (it required a total of 50 pyrite cubes for both abiotic and biotic experiment). In the current experiment using polished pyrite plates, we have been able to secure sufficient number of pyrite plates for conducting both abiotic and biotic experiments. These new data will be provided for further discussion.

Reviewer's Comment: 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

BGD

9, C1019–C1023, 2012

the attached cells, as no sterile control experiments were performed.

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.

Reviewer's Comment: 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)?

Authors' Reply: Pre-experiment test showed that H2O2 in the solutions was non-detectable prior to the 72th h after injection (including the highest-dose treatment). The selection of 3 days as the minimum time interval was to avoid the interference of any residual H2O2 from the previous injection on the H2O2 concentration during the subsequent reaction cycle. Due to the practical difficulty in conducting lab work during weekends, a flexible time interval of 3-5 days has to be adopted.

Reviewer's Comment: 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.

Authors' Reply: The comparison was made by observing the entire pyrite cube surface area. Probably 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.

Reviewer's Comment: 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 Na-

BGD

9, C1019–C1023, 2012

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

Authors' Reply: We have addressed this issue in the Authors Response to the Comments by Reviewer #1 (see below):

We agree with the reviewer that it is important to investigate pyrite oxidation by neutrophilic iron/sulfide oxidizing bacteria under circumneutral pH conditions, which is indeed part of our ongoing research efforts.

We investigated Acidithiobacillus ferrooxidans first because this component was in close connection with the parallel component to investigate the pyrite oxidation in acidic scenarios (pH 2), which required acidophilic bacterial strains to be used. We tried to keep the consistency between the two components in terms of the dosage levels of hydrogen peroxide and the bacterial strain used for the experiment.

The interesting work by Mielke et al. (2003) showed that Acidithiobacillus ferrooxidans were able to colonize on pyrite mineral surface at circumneutral pH. This suggests that the pioneer colonizing microbes for grazing on the pyrite surfaces are not exclusively of neutrophilic iron/sulfide oxidizing bacteria. In this work, we examined the colonization of Acidithiobacillus ferrooxidans on the surfaces of pyrite cubes when the reaction system is exposed to intermittent fluxes of H_2O_2 at micromolar levels, which are likely to be encountered in natural environments. This has implications for better understanding the microbially mediated oxidation mechanisms in the real world.

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

Supplementary Figure S1

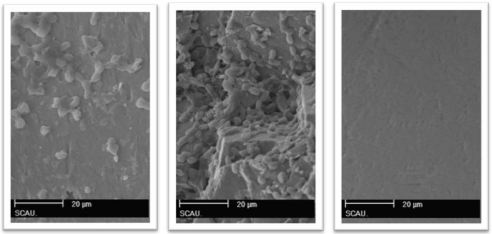

Figure S1 SEM images of the pyrite cube surfaces under different treatments. (a) exposed to *Acidithiobacillus ferrooxidans* only; (b) exposed to *Acidithiobacillus ferrooxidans* and 50 µM H_2O_2; and (c) exposed to *Acidithiobacillus ferrooxidans* and 100 µM H_2O_2. Arrows point to the cells attached to the surfaces of pyrite crystals.

Fig. 1. Supplementary Figure S1

BGD

9, C1019–C1023, 2012