

Pyrite oxidation under initially neutral pH conditions and in the presence of *Acidithiobacillus ferrooxidans* and micromolar hydrogen peroxide

**General comments**

Studies on circumneutral biooxidation of sulfur minerals as pyrite, are rare. Thus, this manuscript represents an opportune research. However, it is not clear why the authors used the acidophilic *Acidithiobacillus ferrooxidans* instead of other chemolithotrophic but circumneutral microorganisms, as *Starkeya novella* (formerly *Thiobacillus novellas*, optimal pH 6.5-8), *Halothiobacillus neapolitanus* (formerly *Thiobacillus neapolitanus*, pH 6.6-7), *Thiobacillus thioparus* and *Thiomonas intermedia* (5-8).

In this research an interfacial characterization of the pyrite was done. Yet, the biooxidation activity is well explained if the interfacial process is carefully analyzed. Thus, I recommended to request to the authors, to include the ESEM-EDX analysis of the pyrite surface after and before the assays, for each trial, *vr. gr.* C, T1, T2 and T3, in order to improve the surface characterization and therefore, the discussion of their results and the conclusion of the overall work. Also, I recommended to the authors a more detailed description of the observed microorganism in the pyrite surface and to discuss the presence of such biofilms in terms of the physical and chemical characteristics of the insoluble species presented on the pyrite surface.

**Specific comments**

*Introduction*

**Page 559, lines 17-18.** This statement comprises the justification of studies like the presented in this manuscript. Please, support it with more references, if possible.

*Materials and methods*

**Section 2.2.** It really worried me: the counted cells were live cells?

**Section 2.3.** Please justify the assays duration, (51 days) as well as the sampled times

*Experiment Results analysis*

**Section 3.1.** After 51 days, the final cell concentration was practically the same in trials T1 to T3, but there were only attached cells in trial T1 and in the control, nevertheless the pyrite surface presented  $S^0$  and  $S_n^{2-}$ . Please, explain such results.

#### *Discussion section*

In my opinion this paper should take more care of the basic fundamentals in order to improve the discussion of the obtained results, specifically regarding:

- (1) About the Fe and S species that has been reported by other researchers in the oxidized pyrite surface and thus species registered *via* XPS in this research. In my opinion, it is also possible that the attachment of cells onto the pyrite surface is deeply influenced by such reduced species, as the registered  $S^0$  and the  $S_n^{2-}$  (**See Page 566, lines 17 and 18**). Please, discuss such possibility.
- (2) About structure, function and development of biofilms of *A. ferrooxidans* in both acidic and circumneutral media.
- (3) The species of Fe(II) or Fe(III) and reduced sulfur species that should be present at the obtained pH and redox potential (Eh) in each trial (C, T1, T2 and T3) and each tested time. **In Page 567, lines 19-20** the authors indicated that planktonic cells only oxidize aqueous Fe(II) and soluble reduced-S species. A reference is needed; and the discussion must be more stressed if the authors inform if such species are present at the pH and Eh registered in the performed assays.
- (4) The role of the EPS in issues as Fe(II)/Fe(III) retention and Fe(II) biooxidation. S Certainly, since 1995 Sand, Gehrker and collaborators have highlighted the importance of biofilms matrix as a reactive space wherein the electrochemical mechanisms/surface reaction takes place, specifically the initial attachment of EPS-complexed Fe ions to the pyrite surface by electrostatic interactions and their acceleration of the dissolution rate of this mineral.
- (5) About the antioxidative response of the microorganism to  $H_2O_2$ ; perhaps about the superoxide-dismutases (SOD) activity. **In Page 567, lines 12-13** the authors suggested that *A. ferrooxidans* developed  $H_2O_2$  tolerance, however such tolerance could be an intrinsic resistance: the SOD is a key enzyme in *A. ferrooxidans* since the typical environment of this microorganism is oxidant.

**Page 567, lines 23-25:** Please, justify why the pyrite cubes of the mentioned dimensions were used in long-term assays. (In my opinion the pyrite weight was quiet enough (*ca.* 36 g)).

**Page 568, lines 2:** Please, inform if in the bottom of each culture flask was observed precipitated of the compounds that may explain the drop in the Fe concentration.

**Section 4.3.** In my opinion, this section needs references, to complete the discussion of the results obtained by XPS.

**Table 1.** Include the species associated to each peak and the corresponding reference, in two new columns.

Figure 3. I recommended present the information showed in Fig. 3 in a Table, in order to focus on the absolute data and thus, to facilitate the lecture to these interesting results.

**Technical corrections**

**Page 566, line 14:** Please, change “extract mechanisms” by “exact mechanism”

**Page 567, lines 19: Please,** use “oxidize” instead “feed”

**Page 567, line 23:** use “source of Fe and/or S” instead “foods”