



## Different isotope and chemical patterns of pyrite oxidation related to lag and exponential growth phases of *Acidithiobacillus ferrooxidans* reveal a microbial growth strategy

Benjamin Brunner<sup>a,\*</sup>, Jae-Young Yu<sup>a,b</sup>, Randall E. Mielke<sup>a</sup>, John A. MacAskill<sup>c</sup>, Stojan Madzunkov<sup>c</sup>, Terry J. McGenity<sup>d</sup>, Max Coleman<sup>a</sup>

<sup>a</sup> Planetary Science and Life Detection Section, Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, Pasadena, CA 91109, USA

<sup>b</sup> Department of Geology, Kangwon National University, Chuncheon, Kangwon-Do 200-701, Republic of Korea

<sup>c</sup> Astrophysics and Space Sciences Section, Jet Propulsion Laboratory/Caltech, 4800 Oak Grove Drive, Pasadena, CA 91109, USA

<sup>d</sup> Department of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, Essex, CO4 3SQ, UK

### ARTICLE INFO

#### Article history:

Received 6 July 2007

Received in revised form 20 February 2008

Accepted 4 March 2008

Available online 18 March 2008

Editor: G.D. Price

#### Keywords:

Acidithiobacillus ferrooxidans  
pyrite oxidation  
growth phases  
oxygen and sulfur isotope  
composition of sulfate  
degassing of sulfur dioxide from acid  
solutions  
sulfite

### ABSTRACT

The solution chemistry during the initial (slow increase of dissolved iron and sulfate) and main stage (rapid increase of dissolved iron and sulfate) of pyrite leaching by *Acidithiobacillus ferrooxidans* (*Af*) at a starting pH of 2.05 shows significant differences. During the initial stage, ferrous iron ( $\text{Fe}^{2+}$ ) is the dominant iron species in solution and the molar ratio of produced sulfate ( $\text{SO}_4^{2-}$ ) and total iron ( $\text{Fe}_{\text{tot}}$ ) is 1.1, thus does not reflect the stoichiometry of pyrite ( $\text{FeS}_2$ ). During the main stage, ferric iron ( $\text{Fe}^{3+}$ ) is the dominant iron species in solution and the  $\text{SO}_4^{2-}:\text{Fe}_{\text{tot}}$  ratio is with 1.9, close to the stoichiometry of  $\text{FeS}_2$ . Another difference between initial and main stage is an initial trend to slightly higher pH values followed by a drop during the main stage to pH 1.84. These observations raise the question if there are different modes of bioleaching of pyrite, and if there are, what those modes imply in terms of leaching mechanisms.

Different oxygen and sulfur isotope trends of sulfate during the initial and main stages of pyrite oxidation confirm that there are two pyrite bioleaching modes. The biochemical reactions during initial stage are best explained by the net reaction  $\text{FeS}_2 + 3\text{O}_2 \Rightarrow \text{Fe}^{2+} + \text{SO}_4^{2-} + \text{SO}_2(\text{g})$ . The degassing of sulfur dioxide ( $\text{SO}_2$ ) acts as sink for sulfur depleted in  $^{34}\text{S}$  compared to pyrite, and is the cause of the  $\text{SO}_4^{2-}:\text{Fe}_{\text{tot}}$  ratio of 1.1 and the near constant pH. During the exponential phase, pyrite sulfur is almost quantitatively converted to sulfate, according to the net reaction  $\text{FeS}_2 + 15/4\text{O}_2 + 1/2\text{H}_2\text{O} \Rightarrow \text{Fe}^{3+} + 2\text{SO}_4^{2-} + \text{H}^+$ . We hypothesize that the transition between the modes of bioleaching of pyrite is due to the impact of the accumulation of ferrous iron, which induces changes in the metabolic activity of *Af* and may act as an inhibitor for the oxidation of sulfur species. This transition defines a fundamental change in the growth strategy of *Af*. A mode, where bacteria gain energy by oxidation of elemental sulfur to sulfite but show little growth is switched into a mode, where bacteria gain a smaller amount of energy by the oxidation of ferrous iron, but induce much faster pyrite leaching rates due to the production of ferric iron.

© 2008 Elsevier B.V. All rights reserved.

### 1. Introduction

Bacteria are important mediators of geochemical reactions. Due to its contribution to acid mine drainage problems, the bacterial oxidation of pyrite by *Acidithiobacillus ferrooxidans* (*Af*) is one of the best-studied examples of such a process. Pyrite oxidation rates are dramatically increased by the role of *Af* in the oxidation of ferrous to ferric iron. While bacterial oxidation of ferrous iron has been studied intensely and is well understood, the initiation of pyrite leaching by *Af* has only been investigated in a few studies (e.g. Mustin et al.; 1992; Yu

et al.; 2001; Mielke et al.; 2003) and characterization of the chemical reactions resulting in sulfur and oxygen isotope fractionations is lacking so far. A deeper understanding of the bacterial mechanisms in the initial stage of pyrite leaching is essential in terms of ecology and evolution of *Af* (survival strategies) and for the evaluation of isotope patterns related to oxidation of pyrite that could indicate presence or absence of life (biomarkers).

During the initial stage of pyrite oxidation in the presence of *Af*, iron is mostly accumulated in its ferrous form,  $\text{Fe}^{2+}$ , and the accumulation rates of iron and sulfate do not correspond to the 2:1 stoichiometry for sulfur and iron in pyrite (e.g. Mustin et al.; 1992; Yu et al.; 2001). In the main stage of pyrite leaching by *Af*, the leaching products are sulfate and ferric iron ( $\text{Fe}^{3+}$ ), and their ratio is close to the 2:1 sulfur–iron stoichiometry of pyrite ( $\text{FeS}_2$ ) (Yu et al.; 2001). The

\* Corresponding author. Currently at Max-Planck-Institute for Marine Microbiology, Celsiusstrasse 1, Bremen 28359, Germany.

E-mail address: [bbrunner@mpi-bremen.de](mailto:bbrunner@mpi-bremen.de) (B. Brunner).

curious discrepancy in the solution chemistry between different stages of pyrite leaching by *Af* leads to the hypothesis that the pyrite oxidation mechanism in the presence of *Af* in the initial stage of pyrite oxidation is different from the one in the main stage.

### 1.1. The Yu et al. (2001) experiments

Yu et al. (2001) carried out batch pyrite leaching experiments with *Af* with open access to air in acidic conditions at a pH of 2.04 and they monitored the extent of evaporation of the media. They observed two stages of pyrite leaching, an initial stage where  $\text{Fe}^{2+}$  and sulfate were the main products, and after approximately 400 h, a second stage with  $\text{Fe}^{3+}$  and sulfate as main products (Fig. 1). In the initial stage, the production of dissolved iron and sulfate was generally low (2.5 mmol  $\text{SO}_4^{2-}$ /L, 1.7 mmol  $\text{Fe}^{2+}$ /L in 381 h), and decreased with time. However, compared to nonbiological (uninoculated) leaching of pyrite under the same conditions, *Af* considerably increases the leaching rate during the initial stage of pyrite leaching (Fig. 1). The second stage of pyrite leaching is marked by strongly increased production rates of iron and sulfate, and therefore, is called the main stage of pyrite leaching. During the initial phase of pyrite leaching, the number of bacteria stayed low and most of the bacteria were attached to the pyrite surface. At the end of the initial leaching stage there is a significant reduction in cell count. During the main stage of pyrite oxidation, the number of bacteria grew dramatically and most bacteria were detached from the pyrite surface (Fig. 1; Table 1).

The change between the modes of pyrite leaching is marked by a change in the ratio of produced sulfate to iron (Fig. 2). During the initial stage, the  $\text{SO}_4^{2-}/\text{Fe}_{\text{tot}}$  ratio was  $1.1 \pm 0.1$ , a behavior that has been called nonstoichiometric, referring to ratios that are not equal to the 2:1 stoichiometry of pyrite ( $\text{FeS}_2$ ). During the main stage, the  $\text{SO}_4^{2-}/\text{Fe}_{\text{tot}}$  ratio was  $1.9 \pm 0.0$ , thus almost stoichiometric. This change is accompanied by a change in the pH of the solution: the pH of the solution stayed constant or slightly increased ( $2.08 \pm 0.04$ ) during the initial

stage and dropped to  $\text{pH } 1.84 \pm 0.04$  in the main stage of pyrite leaching (Fig. 2).

The nonstoichiometric behavior of the  $\text{SO}_4^{2-}/\text{Fe}_{\text{tot}}$  ratio in the solution implies that not as much sulfur as iron is leached from pyrite as one would expect from strict dissolution of  $\text{FeS}_2$ , or that other sulfur species than sulfate (e.g. elemental sulfur  $\text{S}^0$ , sulfite  $\text{SO}_3^{2-}$ , thio-sulfate  $\text{S}_2\text{O}_3^{2-}$ ) are accumulated. Yu et al. (2001) neither detected any elemental sulfur by Soxhlet extraction (detection limit, 10  $\mu\text{mol}$ ), nor dissolved sulfur species besides sulfate by ICP-AES (detection limit, 1 ppm). We, therefore, refer to this undetected sulfur species as “missing sulfur”. The  $\text{SO}_4^{2-}/\text{Fe}_{\text{tot}}$  ratio (Fig. 2) clearly shows that the “missing sulfur” was not converted to sulfate at the beginning of the main stage of pyrite leaching, but remained missing. As indicated by the  $\text{SO}_4^{2-}/\text{Fe}_{\text{tot}}$  ratio of 1.9 during the main stage, “sulfur loss” still continues.

### 1.2. Nonstoichiometric leaching of pyrite in abiotic experiments

Interestingly, nonstoichiometric leaching of pyrite has also been observed in abiotic experiments: Descostes et al. (2004) report  $\text{SO}_4^{2-}/\text{Fe}_{\text{tot}}$  ratios of 1.5 to 1.6 at pH of 1.2 to 2 in abiotic experiments with perchloric and hydrochloric acid and present a data compilation that indicates that the observed ratio depends on the pH of the solution. Descostes et al. (2004) suggest that the “missing sulfate” may have degassed as sulfur dioxide ( $\text{SO}_2$ ).

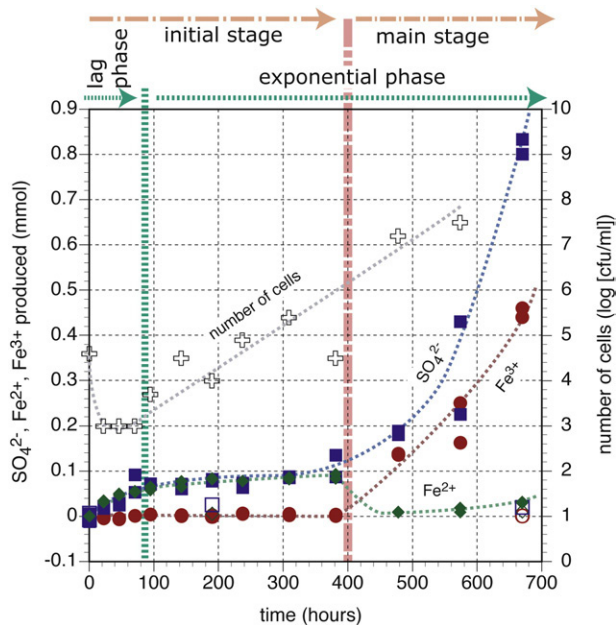
### 1.3. Speciation of sulfite, and potential degassing of sulfur dioxide ( $\text{SO}_2$ )

Sulfite is an important intermediate in biologic oxidation of sulfur compounds (e.g. Vestal and Lundgren, 1971; Eccleston and Kelly, 1978; Pronk et al., 1990; Hirose et al., 1991; Suzuki et al., 1992; Wodara et al., 1997; Masau, 1999; Friedrich et al., 2001; Rohwerder and Sand, 2003; Wakai et al., 2004; Rawlings, 2005; Sugio et al., 2006) and likely to be an important intermediate in the pyrite leaching mechanism. In the absence of an oxidant (e.g.  $\text{Fe}^{3+}$ ), it may accumulate in solution. At a pH of 2, speciation programs (such as visual Minteq®), calculate that 37% of sulfite is present as  $\text{H}_2\text{SO}_3$  (sulfurous acid molecule). However, there is no evidence for the existence of  $\text{H}_2\text{SO}_3$  (Betts and Voss, 1970; Horner and Connick, 2003 and references therein), the most acidic species is sulfur dioxide ( $\text{SO}_2$ ). Thus, 37% of the sulfite species in an acid solution must exist as volatile sulfur dioxide.

### 1.4. Observations from sulfur and oxygen isotope studies

The sulfur in sulfate of pyrite leaching solutions is ultimately derived from pyrite. If pyrite is quantitatively oxidized to sulfate, no sulfur isotope fractionation can occur and the sulfur isotope composition of sulfate reflects that of pyrite. However, if sulfur pools other than sulfate are formed, sulfur isotope fractionation can occur and the actual oxidation mechanism of pyrite sulfur becomes important. Sulfur isotope fractionation may occur during the stepwise oxidation of pyrite sulfur, when existing sulfur species are consumed (e.g. breaking of S–S bond when thiosulfate is transformed into elemental sulfur and sulfite) and new sulfur species are formed (e.g. oxidation of elemental sulfur to sulfite), or also when sulfur isotopes are exchanged (e.g. sulfur isotope exchange between sulfonate and sulfane sulfur of the thiosulfate molecule, Uyama et al., 1985; Chu et al., 2004). Thus, the sulfur isotope composition of sulfate from leaching of pyrite preserves information about the isotope composition of the leached pyrite, as well as information about potential diversion of sulfur species in pools other than sulfate. Commonly, sulfur isotope fractionation related to abiotic and biotic oxidation of sulfur species is relatively small (Taylor et al., 1984; Toran and Harris, 1989; Balci et al., 2007; Pisapia et al., 2007 and references therein).

Under neutral conditions (pH 7) and at ambient temperatures (25 °C), oxygen isotope exchange between sulfate and water is



**Fig. 1.** The dotted lines highlight the trends in the amount of produced  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{SO}_4^{2-}$  and the number of cells. Interestingly, the production of sulfate and ferrous iron almost ceases between the start of the exponential phase of growth and the end of the initial stage of pyrite leaching. The dashed vertical lines mark the transition from lag phase to exponential phase of growth and initial stage to main stage of pyrite leaching. Symbols: crosses: number of cells; filled squares: produced sulfate (*Af*); hollow squares: produced sulfate (uninoculated); filled diamonds: produced  $\text{Fe}^{2+}$  (*Af*); hollow diamonds: produced  $\text{Fe}^{2+}$  (uninoculated); filled circles: produced  $\text{Fe}^{3+}$  (*Af*); hollow circles: produced  $\text{Fe}^{3+}$  (uninoculated).

Download English Version:

<https://daneshyari.com/en/article/6431067>

Download Persian Version:

<https://daneshyari.com/article/6431067>

[Daneshyari.com](https://daneshyari.com)