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Kinetics of Pyrite, Pyrrhotite and Chalcopyrite Dissolution by

*Acidithiobacillus ferrooxidans*

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Kinetics of Pyrite, Pyrrhotite and Chalcopyrite Dissolution by *Acidithiobacillus ferrooxidans*

**Abstract**

The main objective of this study was to investigate the dissolution kinetics of pyrite, pyrrhotite, and chalcopyrite. Crushed minerals were reacted with *A. ferrooxidans* (25°C). The kinetics of dissolution was investigated by monitoring pH and Fe$^{2+}$, Fe$^{3+}$ ion concentrations in the leaching solutions. Pyrite, pyrrhotite and chalcopyrite dissolution by *A. ferrooxidans* were found to be chemically controlled processes. The dissolution rates of the minerals with bacteria increased in the order of pyrrhotite, pyrite, and chalcopyrite. The attached cell numbers to mineral surfaces increased in the same order. *A. ferrooxidans* was found to enhance the dissolution rates of the minerals. The acid-insoluble trait of pyrite and acid-soluble trait of the other two minerals affected the pH changes in the leaching solutions.

**Keywords:** *A. ferrooxidans*, pyrite, pyrrhotite, chalcopyrite, dissolution kinetics
1. Introduction

Over the last few decades, the mining industry has had the growing interest in bioleaching of sulphide minerals as a means of metal recovery. “Bioleaching” is described as biological oxidation of metal sulphides (Rawlings and Silver 1995), and the mobilization of metal cations involving biological oxidation from otherwise insoluble ores (Vera et al. 2013). Bioleaching is a vital alternative to traditional extraction methods because of its low cost, environmental benefits, and broad efficiency for low-grade and recalcitrant ores (Rawlings and Silver 1995). Pyrite (FeS$_2$) and pyrrhotite (Fe$_{1-x}$S) are the examples of sulphide minerals composed entirely of Fe and S, whereas other sulphide minerals such as chalcopyrite (CuFeS$_2$) contain economically valuable metals as well (Bulaev et al. 2012). Although pyrite is not an important economic mineral by itself, many studies on biologically mediated pyrite oxidation have been conducted (Bellenberg et al. 2015; Konhauser et al. 2011; Edwards et al. 2003; Singer and Stumm 1970) because it is commonly associated with valuable minerals such as sphalerite, chalcopyrite, and galena (Chandra and Gerson 2010). Pyrrhotite (Fe$_{1-x}$S) is the second common iron sulphide in nature. Although extracting copper by bioleaching is difficult due to the refractoriness of chalcopyrite, it has been studied intensively because of its high crustal abundance (Brierly and Brierly 2013).

Iron-oxidizing bacteria obtain metabolic energy through the oxidation of iron- and sulfur-bearing minerals. In doing so, they dissolve economically valuable metals (Sand et al. 1995; Ehrlich and Fox 1967). *Acidithiobacillus ferroxidans* (*Acidithiobacillus* was formerly known as “*Thiobacillus*”, Kelly and Wood 2000) was the first to be isolated and is the best-studied iron and/or sulfur-oxidizing bacterium (Vera et al. 2013).

Most studies on bioleaching of sulphide minerals have focused mainly on the effect of mixed bacterial cultures (Romo et al. 2013), mineral morphology (Dong et al. 2013), bacterial attachment (Yang et al. 2014), mineral particle size (Olubambi et al. 2009), pulp density...
(Pradhan et al. 2010), the presence of various metallic ions in solution (Wong et al. 2015), and temperature on the dissolution kinetics of sulphide minerals (Vilcaez et al. 2008). A significant number of individual studies focused on the bioleaching of pyrite (Edwards et al. 1998; Florian et al. 2011), pyrrhotite (Korehi et al. 2014) and chalcopyrite (Zhu et al. 2015).

To date, more studies on chemical leaching kinetics of sulphide minerals were carried out (Perez and Dutrizac 1991; Dreisinger and Abed 2002; Aydogan et al. 2006; Adebayo et al. 2003; Havlik and Kammel 1995) compared to bioleaching kinetics (Pradhan et al. 2010; Abhilash et al. 2013). On the other hand, very few studies compared the bioleaching rates of pyrite, pyrrhotite, and chalcopyrite (Lei et al. 2007). The objective of this study was to investigate the dissolution kinetic mechanisms of pyrite, pyrrhotite, chalcopyrite, and compare the bioleaching rates in the presence of A. ferrooxidans.

2. Materials and Methods

2.1. Bacterial Cultivation

A. ferrooxidans strain ATCC 23270 was purchased from the American Type Culture Collection (ATCC). Cells were cultured in a medium containing solution A, and solution B. Solution A contained (in g/L): (NH₄)₂SO₄ 0.8; MgSO₄·7H₂O 2.0; K₂HPO₄ 0.4; and 0.5% Wolfe’s mineral solution (V/V) (Wolin et al. 1963). Solution B contained 20 g FeSO₄·7H₂O per liter of solution. The ratio in which solutions A and B were mixed for preparing the final medium was 4:1. Solution A was prepared as described above, and the pH was adjusted to 2.3 with H₂SO₄, then sterilized by autoclaving at 121°C for 15 minutes. Solution B was prepared separately and filtered through a 0.2 μm membrane filter (Acrodisc). Solution A and solution B were then aseptically combined. Batch cultures of A. ferrooxidans were grown at 120 rpm on a rotary shaker at 25°C for seven days before harvesting for bioleaching experiments.

2.2. Minerals

The following sulphide minerals were used in the bioleaching experiments: Pyrite
(FeS$_2$; Ward’s, Scientific, Huanzala, Peru), pyrrhotite (Fe$_{1-x}$S, x=0 to 0.2; Ward’s, Scientific, Galax, Virginia, USA), and chalcopyrite (CuFeS$_2$; Ward’s, Scientific, Durango, Mexico). All minerals were crushed using a mortar and pestle and sieved to a grain size of $\leq 355\mu$m. Crushed minerals were cleaned by following three steps: First, they were repeatedly rinsed with distilled water and sonicated in an ultrasonic cleaner for 1-2 minutes to remove fine particles. Second, they were treated with 10% HCl for 2 hours to remove any preexisting oxide layers, then rinsed with 100% ethanol (Koptec, 200 proof) and dried in a shallow layer (Edwards et al. 2001). Finally, 2 grams of the cleaned, crushed minerals were added to each of sterile 250-ml flasks and autoclaved for 8 min.

2.3. Experimental Procedures

Cells of the *A. ferrooxidans* grown in the ferrous sulfate medium for one week in shake culture were harvested by centrifugation at 10,000 rpm for 20 minutes and then washed once by centrifugation at 10,000 rpm for 15 min in filter-sterilized distilled water adjusted to pH 1.5 with H$_2$SO$_4$. Cells were re-suspended in salts medium (solution A) at pH 2.3. Two mL of cell suspension (initial cell number $\approx 6.5 \times 10^6$ cells mL$^{-1}$) was inoculated into 250 mL flasks containing 2 grams of sterile crushed minerals and 35 mL of salts medium (solution A) (Edwards et al. 2001). Duplicate sets for each mineral were prepared. Bioleaching flasks (*A. ferrooxidans* + medium + crushed minerals) and control flasks (medium + crushed minerals) were incubated without shaking at 25°C for 30 days.

2.4. Analytical Techniques

The leaching solutions were analyzed for the concentration of bacteria unattached to mineral particles, total Fe, Fe$^{2+}$, Cu$^{2+}$ ions, and pH. For cell counting, 2µL of culture samples were spread evenly on a 1% agarose-coated fluoroslide printed with 4 mm diameter circles (Emerson and Moyer 2002). After air-drying, the samples were stained with DAPI (200 µg/mL) and fifty fields/circle were counted at 100X magnification using an epifluorescent
Axiostar Plus microscope. From each flask, a 1-mL sample was removed daily, filtered through a 0.2 μm membrane, and pH values were determined using a pH-meter (Thermo Scientific). For dissolved iron measurements, a 1-mL sample was collected from each flask, filtered through a 0.2μm membrane and diluted ten times with 1 mM HCl when their absorbance measurements with UV- spectrophotometer were higher than 1.0. 1-mL of samples were also collected from the chalcopyrite flasks and filtered through a 0.2μm membrane and diluted 100 times with distilled water to measure Cu$^{2+}$ ion concentrations. All ion concentrations were measured using the UV-spectrophotometer (Shimadzu UV-1601). Any decrease in volume due to sampling was adjusted to the original volume by addition of an equivalent volume of sterile solution A.

The original ferrozine method was modified to measure total Fe and Fe$^{2+}$ ion concentrations (Stookey 1970). Ferrozine reagent (2 mM Ferrozine in 50 mM Hepes) was used to determine both total Fe and Fe$^{2+}$ ion concentrations whereas a reducing reagent (6.25 mM hydroxylamine hydrochloride) was used to determine total Fe ion concentrations. The absorbance values at 562 nm were used to calculate iron ion concentrations. Standards containing different known concentrations of FeSO$_4$ in 1mM HCl were prepared. Fe$^{3+}$ concentrations were calculated as the difference between total Fe and Fe$^{2+}$.

To measure Cu$^{2+}$ ion concentrations, 0.1 mM Bathocuprione disulfonic acid (Aldrich) and reducing reagent (0.3 mM hydroxylamine hydrochloride) were used. The method was modified from that of Moffett et al. (1985). Standards containing different known concentrations of CuSO$_4$ in distilled water were prepared. For each sample, a full spectrum was run between 400-800 nm. A peak for Cu$^{2+}$ concentrations can be detected at 484 nm. The area of the peak was measured using the Shimadzu UV Probe Version 2.20 software. Cu$^{2+}$ concentrations in the leaching solutions were calculated by using the peak area.

The XRD patterns and chemical compositions of original pyrite, pyrrhotite and...
chalcopyrite, were characterized by X-ray diffraction (XRD, Philips Panalytical X’Pert-Pro, CuKα radiation), and X-ray fluorescence spectrometry (XRF, Philips Panalytical Minipal 4), respectively.

3. Results and Discussion

Leaching experiments in the presence and absence of A. ferrooxidans were carried out to investigate the dissolution kinetic mechanisms of pyrite, pyrrhotite and chalcopyrite. In the following, we record our findings on pH, Fe²⁺, Fe³⁺, Cu²⁺ concentration variations in the leaching solutions, XRD and XRF analysis of untreated minerals.

The mineral structure is an important factor, which determines the mechanism and chemistry of dissolution. Sand et al. (2001) reported that metal sulphides are degraded through a chemical attack of Fe³⁺ ions and/or protons. Most metal sulphides, including pyrite, pyrrhotite, and chalcopyrite, are semiconductors. In semiconductors, the valence band has the highest energy level and is filled with electrons. In pyrite, the valance bands are derived only from orbitals of metal atoms, whereas the valence bands in pyrrhotite and chalcopyrite are derived from both metal and sulfur orbitals. Thus, the bonding between the metal and sulfur atoms in the pyrite crystal lattice can be broken only by Fe³⁺ ions. In contrast, both Fe³⁺ ions and protons can break metal-sulfur bonds in many other metal sulphides including pyrrhotite and chalcopyrite. Consequently, pyrite is insoluble in acid while the others are acid-soluble (Sand et al. 2001). Schippers and Sand (1999) proposed a thiosulfate mechanism for the oxidation of acid-insoluble metal sulphides and a polysulfide mechanism for the oxidation of acid-soluble metal sulphides. Thus for acid-insoluble pyrite, the reactions of the thiosulfate mechanism are as follows (Eq. 1-2):

$$\text{FeS}_2 + 6\text{Fe}^{3+} + 3\text{H}_2\text{O} \rightarrow \text{S}_2\text{O}_3^{2-} + 7\text{Fe}^{2+} + 6\text{H}^+$$

(1)

$$\text{S}_2\text{O}_3^{2-} + 8\text{Fe}^{3+} + 5\text{H}_2\text{O} \rightarrow 2\text{SO}_4^{2-} + 8\text{Fe}^{2+} + 10\text{H}^+$$

(2)

For acid-soluble metal sulphides (MS) including pyrrhotite and chalcopyrite, the polysulfide
mechanism reaction is as follows (Eq. 3):

$$\text{MS} + \text{Fe}^{3+} + 2\text{H}^+ \rightarrow \text{M}^{2+} + \text{H}_2\text{S}^{4+} + \text{Fe}^{2+}$$  (3)

$\text{Fe}^{3+}$ ions are the most reactive oxidants in the oxidation of sulphide minerals. The dissolution reactions of pyrite, pyrrhotite and chalcopyrite with $\text{Fe}^{3+}$ are shown in equations 4, 5 and 6 (Singer and Stumm 1970; Nicholson and Scharer 1994; Dutrizac and MacDonald 1974).

$$\text{FeS}_2 + 14 \text{Fe}^{3+} + 8\text{H}_2\text{O} \rightarrow 15\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+$$  (4)

$$\text{Fe}_{1-x}\text{S} + (8-2x) \text{Fe}^{3+} + 4\text{H}_2\text{O} \rightarrow (9-3x) \text{Fe}^{2+} + \text{SO}_4^{2-} + 8\text{H}^+$$  (5)

$$\text{CuFeS}_2(s) + 16\text{Fe}^{3+} + 8\text{H}_2\text{O} \rightarrow \text{Cu}^{2+} + 17\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+$$  (6)

$$4\text{Fe}^{2+} + 4\text{H}^+ + \text{O}_2 \rightarrow 4 \text{Fe}^{3+} + 2\text{H}_2\text{O}$$  (Fe-oxidizers)  (7)

$$\text{S}^0 + 3/2 \text{O}_2(\text{aq}) + \text{H}_2\text{O} \rightarrow \text{H}_2\text{SO}_4$$  (S-oxidizers)  (8)

As the most abundant metal sulphides on Earth, pyrite, pyrrhotite and chalcopyrite are well-studied sulphide minerals. However, the oxidation processes of these minerals are complicated and not yet fully understood (Evangelou 1995; Chirita and Rimstidt 2014; Ahmadi et al. 2011). Because the dissolution mechanisms of chalcopyrite in acidic solutions are still under debate, a general theory could not be proposed. Many researchers have studied the efficient factors on dissolution rates and the possible reaction mechanisms (Li et al. 2010). Stott et al. (2003) studied the bioleaching of chalcopyrite by 11 species of acidophilic bacteria and archaea and investigated the effects of microorganisms, temperature and redox potential on the dissolution rates. The effects of acid and ferric iron concentrations, solid/liquid ratio, and synergistic effect of cupric/ferrous ions on chalcopyrite dissolution behavior were also investigated (Antonijevic and Bogdanovic 2003; Hiroyoshi et al. 2004). In another study, neither an increase in ferric iron nor an increase of bacterial concentration could improve the dissolution rates. A limited bacterial activity and a controlled ferric iron concentration were suggested for the improved bioleaching rates (Third et al. 2000). Sasaki et al. (1995) studied
the chemical dissolution of pyrite with $\text{Fe}^{3+}$ ions around pH 2. The results showed that pyrite dissolved nonstoichiometrically in the initial stage, and later a sulfur-rich layer was formed on the mineral surface. Yahya and Johnson (2002) examined the bioleaching of pyrite by two strains of acidophilic bacteria. They discussed the advantages of bioleaching of sulfidic minerals under low pH (<1) and low redox potential. Bhatti et al. (1993) studied the oxidation of pyrrhotite by *A. ferrooxidans*. They observed an acid-consuming step with the formation of elemental sulphur, following by an acid-producing step, which is a result of the formation of solid products. Janzen at al. (2000) studied on the oxidation of 12 well-characterized pyrrhotite samples by ferric iron and oxygen and reported the incomplete oxidation of pyrrhotite with both oxidants. Harries et al. (2013) investigated the dissolution behavior of pyrrhotite samples, which have different crystal structures, and found that the crystal structure strongly influences the mineral dissolution. Plenty of researches were carried out to investigate the effects of crystal structure, oxygen and ferric iron concentrations, temperature and pH on the dissolution of pyrrhotite (Belzile et al. 2004). Although chalcopyrite is refractory to hydrometallurgical methods, it is studied extensively due to its high crustal abundance. The main factors affecting the dissolution rates of chalcopyrite are the concentration of oxidants, the ferric/ferrous ion ratio in the leaching solutions, and the formation of the passivation layer on the mineral surface (Klauber 2008; Li et al. 2010, 2013). Olvera et al. (2014) studied the dissolution of chalcopyrite electrode in a solution composed of $\text{H}_2\text{SO}_4$, $\text{FeSO}_4.\text{H}_2\text{O}$ and $\text{Fe}_2(\text{SO}_4)_3.\text{H}_2\text{O}$ at 25°C. According to the results, dissolution of mineral increased with increasing $\text{Fe}^{3+}$ concentrations and with the addition of FeS$_2$ particles. Zhao et al. (2015a) reported that additional $\text{Cu}^{2+}$ and $\text{Fe}^{2+}$ ions accelerated the dissolution of chalcopyrite initially, but the formation of jarosite inhibited further dissolution. In another study, Zhao et al. (2015b) studied the bioleaching of chalcopyrite with *A. ferrooxidans*. The results showed that bacteria accelerate the copper extraction rate initially.
In the later stage of bioleaching, copper extraction rate decreased due to the formation of jarosite and polysulfide.

Fe$^{3+}$/Fe$^{2+}$ ratio is another important parameter affecting the dissolution of sulphide minerals (Cordoba et al. 2008; Hiroyoshi et al. 2000, 2001, 2004, 2008; Kametani and aoki 1985; Sandstrom et al. 2004). Microorganisms maintain high ratios of ferric to ferrous ion in bioleaching solutions, thus enhance electron transfer between oxidizing agents and the minerals (Wu et al. 2014). To understand the role of bacteria, Khoskhou et al. (2014) studied on the chemical dissolution of chalcopyrite by mimicking the redox conditions of thermophilic bioleaching of the mineral. Surprisingly, copper recoveries were very similar in the presence and the absence of bacteria. Therefore, they concluded that the role of bacteria was only to provide the oxidizing agent for chalcopyrite dissolution, by oxidizing Fe$^{2+}$ to Fe$^{3+}$. Similarly, Third et al. (2000) carried out a series of leaching experiments with and without bacteria and found that ferric/ferrous ratio is more relevant for determining chalcopyrite dissolution rates than the concentration of bacteria in the leaching solution. Petersen and Dixon (2006) studied on bioleaching of a copper-gold concentrate by using mesophilic, moderately thermophilic and extremely thermophilic bacteria and concluded that high temperature and low redox potential supported the dissolution of chalcopyrite. In contrast, the opposite conditions were sufficient for obtaining high dissolution rates of pyrite. Gericke et al. (2010) found that redox control is not necessary for achieving high copper extraction rates at high temperatures around 70°C, but it increases the extraction rates around 45°C.

Attacking agents for dissolution of pyrite are only Fe$^{3+}$ ions (Eq. 1-2), whereas attacking agents for pyrrhotite and chalcopyrite are both Fe$^{3+}$ and H$^+$ (Eq. 3). In our study, the pH values continuously decreased throughout the experiments in pyrite leaching solutions due to its acid-insoluble nature. The pH values in pyrrhotite and chalcopyrite leaching solutions
increased at the initial stage of bioleaching due to their acid-soluble nature and then decreased. Lei et al. (2007) observed significant amounts of jarosite and elemental sulfur during the bio-oxidation of pyrrhotite and chalcopyrite with *A. ferrooxidans* while no such deposition was observed for pyrite. Similarly, in our study, no deposition was observed for pyrite during the experiments. For pyrrhotite and chalcopyrite, some deposits were seen after a week, corresponding to the decrease in pH (Fig. 1).

Because bacteria both accelerate the oxidation of Fe$^{2+}$ and sulphur, more Fe$^{3+}$ ions were involved in dissolution reactions, and more sulfate was produced in bioleaching solutions compared to control solutions (Eq. 4-5). Thus, the pH values in the pyrite and pyrrhotite bioleaching solutions were lower than the corresponding controls (Fig. 1). By contrast, the pH values in chalcopyrite bioleaching solutions were higher than in control solutions during measurements (Fig. 1). Chalcopyrite (bio)leaching processes include complex oxidation-reduction reactions such as the oxidation of mineral, iron, sulphur, and hydrolysis of ferric iron. The pH behavior in chalcopyrite leaching solutions may be a result of combined effect of these reactions. Although chalcopyrite has the lowest %Fe content compared to other minerals (Table II), it is apparent from Fig. 5c that the highest bacterial oxidation of Fe was determined in chalcopyrite bioleaching solutions. Therefore, bacterial oxidation of Fe, an acid-consuming reaction, is very likely to be the dominant reaction in chalcopyrite bioleaching processes and resulted in high pH values of biotic solutions compared to controls. On the other hand, our result is in agreement with that of Zhao et al. (2013). They studied the bioleaching of chalcopyrite with *A. ferrooxidans*, and the pH values in bioleaching system were found to be higher than the controls during the experiments.

Fe$^{2+}$ released from minerals (Eq. 1-3) is cycled between the oxidized and reduced states by bacterial oxidation of Fe$^{2+}$ (Eq. 7) and consumption of Fe$^{3+}$ during mineral dissolution (Eq. 4-6). Because of the bacterial oxidation of Fe$^{2+}$, we observed low
concentrations of Fe$^{2+}$ in the bioleaching solutions (Fig. 2), and low levels of Fe$^{3+}$ in control (abiotic) solutions for each mineral tested (Fig. 3).

The state of iron depends on environmental factors, such as pH. Above pH 3, iron is insoluble, and it precipitates (Bird et al. 2011). Because of that, the concentration of free Fe$^{3+}$ ions, which plays an active role in the dissolution of minerals, decrease in the leaching solutions (Eq. 4-6). Figure 4 shows the Fe$^{3+}$ concentration changes by pH in the bioleaching solutions of the three minerals. Fe$^{3+}$ concentrations decrease with increasing pH in pyrite bioleaching solution, whereas they increase up to pH values 3.0 and 3.4 for pyrrhotite and chalcopyrite respectively, and then decrease. Eq. (7) explains the Fe$^{3+}$ behavior in pyrite and pyrrhotite bioleaching solutions. However, the oxidation of ferrous ion might be affected both by bacteria (Eq. 7) and cupric ions (Eq. 9) in chalcopyrite bioleaching solutions (Cher and Davidson 1955).

\[
Fe^{2+} + Cu^{2+} \leftrightarrow Fe^{3+} + Cu^{1+} \quad (9)
\]

Figure 5 compares the pH, Fe$^{2+}$ and Fe$^{3+}$ concentration changes by time in the bioleaching and control solutions of the three minerals.

Mathematical modeling of fluid-solid systems such as leaching of metals from minerals is crucial to gain insight into the reaction mechanisms and to interpret experimental results. During the leaching processes, many dissolution products were found on the mineral surfaces. Researchers suggested the formation of various leaching products on the chalcopyrite surface including jarosite, chalcocite, covellite, and sulfur (Pradhan et al. 2008; Watling 2006; Karimi et al. 2010; Gericke et al. 2010). When pyrrhotite is exposed to moist air, an over-layer of iron (III) oxyhydroxides form on the mineral surface (Buckley and Woods 1985; Pratt et al. 1994; Mycroft et al. 1995). As the oxidation proceeds, a sub-layer of polysulfides forms on the mineral surface. Bhatti et al. (1993) identified the mineral weathering products (elemental sulfur, K-jarosite, goethite, schwertmannite) during the
bioleaching of pyrrhotite by *A. ferrooxidans*. Sasaki et al. (1995) studied the dissolution of pyrite by $\text{Fe}^{3+}$ ions around pH 2. They observed a S-rich layer on pyrite surface. According to Schippers et al. (1996), more elemental sulfur forms on the mineral surfaces, which are oxidized by polysulfide mechanism (such as pyrrhotite) compared to the minerals oxidized by thiosulfate mechanism.

If the product layers forming on the mineral surfaces are porous and permeable to attacking agents, mineral dissolution process is controlled by chemical reactions. The kinetic equation can be written as follows:

$$
k_c t = 1 - (1-x)^{1/3}
$$

(10)

If the product layers are impermeable to attacking agents, diffusion through the product layer controls the dissolution rate of mineral. The kinetic equation can be written as follows:

$$
k_d t = 1 - (2x/3)-(1-x)^{2/3}
$$

(11)

In these equations, $k_c$ and $k_d$ are the leaching rate constants, and $x$ is the fraction of iron oxidized (Pradhan et al. 2010).

Abhilash et al. (2013) studied on the bioleaching of a low-grade chalcopyrite ore by *A. ferrooxidans*. Their kinetic data was compatible with diffusion-controlled. Pradhan et al. (2010) studied on the bioleaching of complex sulphide minerals including chalcopyrite, sphalerite and galena by *A. ferrooxidans*. The dissolution of minerals showed a good fit with product diffusion model. Natural pyrite samples were leached under various conditions, and the dissolution found to be controlled by surface chemical reactions (Wiersma and Rimstidt 1984; Antonijevic et al. 1997; Dimitrijevic et al. 1999; Dimitrijevic et al. 1996).

Of the two kinetic equations, equation (10) has been found to give a straight line. Figure 6 indicates the plot of $1-(1-x)^{1/3}$ versus bioleaching time of pyrite, pyrrhotite, and chalcopyrite. The rate constants and the $R^2$ values for both equations (Eq. 10-11) are shown in Table I. Based on the $R^2$ values of the plots, the dissolution of pyrite, pyrrhotite and...
chalcopyrite by *A. ferrooxidans* were found to be chemically controlled processes. It is also evident that the bioleaching rate of minerals increases in the order of pyrrhotite, pyrite and chalcopyrite (Table I). On the other hand, we observed very different ferric/ferrous iron ratios for the bioleaching solutions of the three minerals. This might be one of the reasons for various dissolution behaviors.

Chemical analysis of sulphide minerals is shown in Table II. Iron percentage of pyrite, pyrrhotite and chalcopyrite are 53.5, 66.4 and 33.7 (wt %), respectively.

Three main bioleaching mechanisms are generally accepted: indirect, contact, and cooperative mechanisms. For the latter two, bacterial attachment onto the mineral surface is essential. In the indirect mechanism, planktonic cells make Fe$^{3+}$ ions, which are present in the bioleaching solutions, available for mineral dissolution (Li et al. 2013). According to Rodriguez et al. (2003), bioleaching process includes three stages. In the first stage, bacterial cells attach to the mineral surface. In the second phase, bacterial attachment decreases due to surface saturation. Therefore, the concentration of planktonic cells in the leaching solution increases. In the last stage, a balance between planktonic and attached cells is reached.

Yang et al. (2015) studied the early stage attachment of *A. ferrooxidans* at pyrite and chalcopyrite surfaces. They did not observe a significant difference in selectivity of attachment between the minerals. Africa et al. (2013) obtained the higher levels of attachment of *A. ferrooxidans* to pyrite surface compared to chalcopyrite surface. Zhu et al. (2015) studied the relation between bacterial attachment and bioleaching rate of chalcopyrite by *A. ferrooxidans*. They concluded that as the number of attached cells increased, the dissolution rate of mineral increased. Shrihari et al. (1995) studied on bioleaching of pyrite by *T. ferrooxidans* in shake flasks. They concluded that bacteria dissolved the mineral primarily through the direct mechanism.

In the present study, the planktonic cell concentrations in the bioleaching solutions of
each mineral reached stationary phase around the 13th day (Fig. 7). It seems that A. *ferrooxidans* cells were effectively oxidizing the minerals during the first two weeks of incubation (Eq. 7-8). The planktonic cell concentrations in the bioleaching solutions including all minerals dropped on the second day and then increased. This decrease was likely due to the bacterial attachment to the mineral surfaces within two days. It is known that most of the leaching bacteria grow attached on the sulphide mineral surfaces. Schippers et al. (2014) reported that more than 80% of inoculated cells were lost from the bioleaching solution within one day. The second day of our experiments, 86%, 84%, and 80% of inoculated cells were lost from the bioleaching solutions including chalcopyrite, pyrite, and pyrrhotite, respectively. Attached cell numbers were determined by the difference between the initial concentration of cells and the concentration of cells remaining in the solution after two days. The results for chalcopyrite, pyrite, and pyrrhotite were 5.64x10^6, 5.51x10^6, and 5.20x10^6 (cells/mL), respectively. These findings are also consistent with the rate constants of the minerals (Table I).

The oxidation rate of Cu (0.31 mM Cu^{2+} day^{-1}) was almost five times higher than the oxidation rate of Fe (0.054 mM Fe^{3+} day^{-1}) in chalcopyrite bioleaching solution (Fig. 8a). It is likely that some Fe^{3+} precipitated and some underwent further reaction with chalcopyrite. Fe^{3+} and Cu^{2+} ion concentrations in chalcopyrite bioleaching solution increased as the pH increased up to pH 3.4, after which both ion concentrations decreased as the pH increased (Fig. 8b).

The X-ray diffraction (XRD) analysis was conducted for the untreated minerals (Fig. 9). FeS_2 and FeS were the major and the minor phases of pyrite, respectively. The main peaks for chalcopyrite were djurleite (Cu_{1.96}S), anhydrite (CaSO_4), and Cu (FeO_2). Pyrrhotite showed an unidentified peak, which might be an evidence of its amorphous structure.

Although investigating the effect of some other parameters on the dissolution
behaviors of sulphide minerals is out of scope in this paper, they are stated in the following paragraph to gain a broad perspective.

In a relatively early study, pyrite, pyrrhotite and chalcopyrite were subjected to 68% humidity in the air at 52°C to ascertain the nature of the oxidation of these minerals. The results showed that pyrite and chalcopyrite were initially oxidized to ferrous/cuprous thiosulphates, and then further oxidized to ferric/cupric sulphates. Pyrrhotite was oxidized to goethite and elemental sulphur (Steger and Desjardins, 1978). Iron sulphides show different dissolution behaviors due to their structures. Thomas et al. (2000, 2003) studied the acidic dissolution of troilite (FeS), pyrite and pyrrhotite. Pyrite was found to dissolve oxidatively, troilite was found to dissolve nonoxidatively, and pyrrhotite was found to dissolve in both ways. They also reported that the temperature and the supply of oxidizing agents have an impact on the dissolution mechanism of pyrrhotite. For further analyzing the effect of different crystal and electronic structures on dissolution behaviors, Schippers and Sand (1999) selected six different metal sulphides for dissolution experiments. The oxidizing agent was Fe(III) chloride, and the pH was 1.9. As a result, they observed the formation of different sulphur compounds for each of the mineral. Yevenes et al. (2010) studied the dissolution of various chalcopyrite concentrates in chloride solutions with additional cupric ions at 35°C. They reported that the cupric ion concentrations below 0.1 g/L increased the chalcopyrite dissolution rate. Another possible factor affecting the dissolution of minerals is the composition of the exopolymeric layer, a special reaction compartment, where the dissolution reactions take place. It can differ concerning the substrate, thus affect the dissolution rates (Sand et al. 2001).

In summary, our results showed that bioleaching rates of minerals by *A. ferrooxidans* increase in the order of pyrrhotite, pyrite and chalcopyrite. Considering the individual pH values of each of the bioleaching solutions (Fig. 5a), different dissolution behaviors of these
minerals might be explained. Besides, the difference in crystal structures of minerals (Fig. 9), the mineral compositions (Table II), the number of attached cells, the concentration of oxidizing agents (Fig. 5c), ferric to ferrous ion ratio in bioleaching solutions, the (in)solubility of minerals in acid, and the presence of cupric ions in chalcopyrite bioleaching solutions might be responsible for observing different dissolution rates.

4. Conclusion

Aspects of dissolution kinetics of pyrite, pyrrhotite, and chalcopyrite by A. ferrooxidans were studied in 30-day experiments using crushed minerals. Plots of \(1 - (1-x)^{1/3}\) versus bioleaching time has been found to give a straight line for pyrite, pyrrhotite and chalcopyrite (Fig. 6). These results indicate that the dissolution of pyrite, pyrrhotite and chalcopyrite by A. ferrooxidans were chemically controlled processes. From the results in Table I, it can be observed that the dissolution rates of minerals increase in the order of pyrrhotite, pyrite, and chalcopyrite. The attached cell numbers to mineral surfaces increase in the same order. A. ferrooxidans was found to accelerate the dissolution rates of the three minerals. The pH behavior of the leaching solutions, containing the three minerals, was affected by the acid-insoluble nature of pyrite, and the acid-soluble natures of pyrrhotite and chalcopyrite.

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### Table I. The $k_c$ and $k_d$ values and correlation coefficients for each mineral

<table>
<thead>
<tr>
<th>Mineral</th>
<th>$k_c$ (10^{-3} day^{-1})</th>
<th>$R^2$</th>
<th>$k_d$ (10^{-5} day^{-1})</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chalcopyrite</td>
<td>1.6</td>
<td>0.914</td>
<td>9.0</td>
<td>0.787</td>
</tr>
<tr>
<td>Pyrite</td>
<td>0.6</td>
<td>0.904</td>
<td>1.0</td>
<td>0.674</td>
</tr>
<tr>
<td>Pyrrhotite</td>
<td>0.2</td>
<td>0.828</td>
<td>0.2</td>
<td>0.656</td>
</tr>
</tbody>
</table>

### Table II. Chemical analysis of sulphide minerals (wt %)

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Fe</th>
<th>S</th>
<th>Cu</th>
<th>Zn</th>
<th>Si</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chalcopyrite</td>
<td>33.7</td>
<td>21.0</td>
<td>29.3</td>
<td>-</td>
<td>4.9</td>
<td>11.1</td>
</tr>
<tr>
<td>Pyrite</td>
<td>53.5</td>
<td>45.1</td>
<td>0.69</td>
<td>0.67</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pyrrhotite</td>
<td>66.4</td>
<td>24.7</td>
<td>1.90</td>
<td>3.3</td>
<td>3.7</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 1. The changes of pH in the biotic flasks inoculated with A. ferrooxidans and abiotic controls during the leaching of pyrite, pyrrhotite, and chalcopyrite.

Figure 2. The changes of Fe\(^{2+}\) ion concentrations in the biotic flasks inoculated with A. ferrooxidans and abiotic controls during the leaching of pyrite, pyrrhotite, and chalcopyrite.

Figure 3. The changes of Fe\(^{3+}\) ion concentrations in the biotic flasks inoculated with A. ferrooxidans and abiotic controls during the leaching of pyrite, pyrrhotite, and chalcopyrite.

Figure 4. Fe\(^{3+}\) ion concentration changes by pH in the biotic solutions of pyrite, pyrrhotite, and chalcopyrite.

Figure 5. The comparison of (a) pH, (b) Fe\(^{2+}\), and (c) Fe\(^{3+}\) ion concentrations in biotic (data are average of duplicates) and control flasks.

Figure 6. Plot of (1-(1-x)\(^{1/3}\)) versus leaching time for pyrite, pyrrhotite and chalcopyrite.

Figure 7. Free bacterial cell concentrations in pyrite, pyrrhotite and chalcopyrite bioleaching solutions.

Figure 8. Cu\(^{2+}\) and Fe\(^{3+}\) ion concentration changes by time, and by pH in the chalcopyrite biotic and control flasks.

Figure 9. XRD patterns of untreated pyrite, pyrrhotite and chalcopyrite. An unidentified phase is present for pyrrhotite.
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180x33mm (300 x 300 DPI)
Figure 2. The changes of Fe$^{2+}$ ion concentrations in the biotic flasks inoculated with A. ferrooxidans and abiotic controls during the leaching of pyrite, pyrrhotite, and chalcopyrite.
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180x56mm (300 x 300 DPI)
Figure 4. Fe$^{3+}$ ion concentration changes by pH in the biotic solutions of pyrite, pyrrhotite, and chalcopyrite.

99x59mm (300 x 300 DPI)
Figure 5. The comparison of (a) pH, (b) Fe$^{2+}$, and (c) Fe$^{3+}$ ion concentrations in biotic (data are average of duplicates) and control flasks.
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99x60mm (300 x 300 DPI)
Figure 7. Free bacterial cell concentrations in pyrite, pyrrhotite and chalcopyrite bioleaching solutions.

99x72mm (300 x 300 DPI)
Figure 8. Cu$^{2+}$ and Fe$^{3+}$ ion concentration changes by time, and by pH in the chalcopyrite biotic and control flasks.

160x64mm (300 x 300 DPI)
Figure 9. XRD patterns of untreated pyrite, pyrrhotite and chalcopyrite. An unidentified phase is present for pyrrhotite.

113x150mm (300 x 300 DPI)