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Title: Microbiological Redox Potential Control to Improve the Efficiency of Chalcopyrite Bioleaching

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ABSTRACT: The effect of controlling the redox potential (E_h) on chalcopyrite bioleaching kinetics was studied as a new aspect of redox control during chalcopyrite bioleaching, and its mechanism was investigated by employing the "normalized" solution redox potential (E_{normal}) and the reaction kinetics model. Different E_h ranges were established by use of different acidophiles (*Sulfobacillus acidophilus* YTF1; *Sulfobacillus sibiricus* N1; *Acidimicrobium ferrooxidans* ICP; *Acidiplasma* sp. Fv-AP). Cu dissolution was very susceptible to real-time change in E_h during the reaction. It was found that efficiency of bioleaching of chalcopyrite can be effectively evaluated on the basis of E_{normal}, since it is normalized for real-time fluctuations of concentrations of major metal solutes during bioleaching. For steady Cu solubilization during bioleaching at a maximum rate, it was important to maintain a redox potential range of $0 \leq E_{normal} \leq 1$ (-0.35 mV optimal) at the mineral surface by employing a "weak" ion-oxidizer. This led to a copper recovery of > 75%. At higher E_{normal} levels ($E_{normal} > 1$ by "strong" microbial Fe^{2+} oxidation), Cu solubilization was slowed by diffusion through the product film at the mineral surface (< 50% Cu recovery) caused by low reactivity of the chalcopyrite and by secondary passivation of the chalcopyrite surface, mainly by jarosite.

Introduction

The demand for copper by a number of industrialized and economically emerging nations has been increasing steadily in the last decade. Even though chalcopyrite ($CuFeS_2$) ores are generally low-grade and highly refractory, they are an attractive source of copper because they constitute a significant component of porphyry copper deposits [approx. 70% of the world's copper reserves (Sillitoe, 2010)]. For this reason, a number of studies to improve the copper recovery by overcoming the very slow leaching/bioleaching kinetics are being undertaken, which depend on an improved understanding of the mechanism of chalcopyrite leaching.

Although a number of studies of chemical leaching of chalcopyrite using aqueous lixiviants (e.g., H_2SO_4, HCl, $HClO_4$ with and without Fe^{3+}) have been done, no universal agreement on the nature of the chalcopyrite surface has been reached. The presence of elemental sulfur (S^0), disulfide (S_2^{2-}); polysulfide (S_n^{2-}) (metal deficient sulfide), Fe oxyhydroxide (jarosite-like species) have been proposed (Li et al. 2013). The leaching of metal sulfide ores was reported to be inhibited by formation of such passivating surface layers (e.g., Stott et al., 2000; Cordoba et al. 2009; Viramontes-Gamboa et al., 2010), but the nature of the surface layers is being debated. For instance, it was suggested that the presence of porous S^0 layers do not impede the leaching efficiencies significantly (Parker et al. 1981; Antonijevic et al. 1994; Córdoba et al. 2008, 2009). Such diversity in findings

is often caused by differences in experimental conditions (e.g., pH, temperature, solute concentration (Li et al., 2013).

Nonetheless, it is generally understood that one of the most important parameters governing bioleaching is the redox potential of the leaching solution (E_h): From a review of a number of electrochemical, chemical, and biological studies, Viramonetes-Gamboa et al. (2007; 2010) suggested that chalcopyrite leaching with ferric sulfate is in its active state at < 685 mV (SHE), regardless of impurities in the chalcopyrite, or the acidity or the temperature of the reaction. At 685-755 mV (SHE), chalcopyrite is in a bistable system (it could be passive or active depending on how it was brought to that potential). At > 755 mV (SHE), chalcopyrite leaching is in the passive state with a strong passivation effect.

Based on some electrochemical/chemical leaching studies, Hiroyoshi et al. (2000, 2001, 2004, 2008a, b) proposed a mechanism of chalcopyrite dissolution controlled by E_h levels. According to this mechanism, chalcopyrite leaching using ferric sulfate is enhanced by the presence of Fe^{2+} and Cu^{2+}, leading to the formation of intermediate chalcocite (Cu_2S) owing to the low E_h from the presence of Fe^{2+} (1). The oxidation of Cu_2S then yields Cu^{2+} (2):

$$Cu^{I}Fe^{III}S^{-II}{}_2 \text{ (chalcopyrite)} + 3Cu^{2+} + 3Fe^{2+} \rightarrow 2Cu^{I}{}_2S^{-II} \text{ (chalcocite)} + 4Fe^{3+} \quad (1)$$

$\Delta G_1{}^0 = 35.0$ kJ/mol^{-1}

$$Cu_2S \text{ (chalcocite)} + 4Fe^{3+} \rightarrow 2Cu^{2+} + S^0 + 4Fe^{2+} \quad (2)$$

ΔG_2^0 = - 81.1 kJ/mol^{-1}

Copper recoveries are enhanced when $E_{ox} < E_h < E_c$ is satisfied (with an optimal potential of 630 mV (SHE); Hiroyoshi et al. 2000, 2008a), where;

E_c ("critical potential") is the equilibrium redox potential of the formation of the intermediate chalcocite from chalcopyrite $(E_c\,(\mathrm{V}) = E_c^0 + \frac{RT}{4F} \ln \frac{(a_{Cu^{2+}})^3}{a_{Fe^{2+}}})$ (3)

E_{ox} ("oxidation potential") is the equilibrium redox potentials of the subsequent oxidation of chalcocite to Cu^{2+} $(E_{ox}\,(\mathrm{V}) = E_{ox}^0 + \frac{RT}{4F} \ln (a_{Cu^{2+}})^2)$ (4)

Since bioleaching is an economic approach for processing highly refractory chalcopyrite ores/concentrates, it is important to investigate the applicability of the above reactions (1) through (4) to this process. Previously, Gerricke et al. (2010) and Ahmadi et al. (2010, 2011) reported on maximizing Cu extraction from chalcopyrite at 600-650 mV (SHE) by suppression of pyrite oxidation, thereby preventing jarosite formation. The aim of the present study was set to achieve a more comprehensive understanding of the mechanism of chalcopyrite leaching. Furthermore, microbiological E_h control instead of electrochemical/chemical control began to be studied as a new aspect of redox control of chalcopyrite bioleaching. Specific aims of the present study were (i) evaluation of the effect of "microbiological redox control" on chalcopyrite leaching through application of specific Fe-oxidizing microorganisms, and (ii) elucidation of the mechanism of chalcopyrite bioleaching in terms of reaction kinetics under specified low redox

91 potentials by considering the concept of E_{normal}.

92

93 **Materials and Methods**

94 **Microorganisms**

95 Five moderately thermophilic, acidophilic strains were used: (i) Fe-oxidizers; three
96 bacterial species (*Sulfobacillus acidophilus* YTF1; *Sb. sibiricus* N1^T (DSM 17363);
97 *Acidimicrobium ferrooxidans* ICP^T (DSM 10331)) and one archaeal species (*Acidiplasma*
98 sp. Fv-Ap), (ii) S-oxidizer; *Acidithiobacillus* (*At.*) *caldus* KU^T (DSM 8584). The five
99 strains were maintained and pre-grown aerobically at 45°C in heterotrophic basal salts
100 (HBS) medium (Masaki et al. 2015; pH 1.5) supplemented with 0.01% (w v^{-1}) yeast
101 extract (YE). Ten-millimolar Fe^{2+} (as $FeSO_4 \cdot 7H_2O$) and 0.1% (w v^{-1}) S^0 plus trace
102 elements (TE; Masaki et al. 2016) were supplied to Fe-oxidizers and to S-oxidizing *At.*
103 *caldus* KU, respectively. All pH adjustments in this work were made with H_2SO_4.

104

105 **Mineral**

106 Chalcopyrite concentrate (20-106 μm; JX Nippon Mining & Metals) was washed with 1
107 M HNO_3, deionized water and 100% ethanol before freeze-drying overnight. The
108 elemental composition (Table I) was calculated from the ICP measurement after a
109 hydrothermal pre-treatment in aqua regia (5 g L^{-1}) using Ethos Plus computerized

110 microwave labstation (Milestone General; 50 Hz; heated to 220°C in 8°C increments per
111 minute, kept at 220°C for 15 min, and finally allowed to cool to room temperature). The
112 concentrate consisted mainly of chalcopyrite with pyrite as minor constituent (Figure 5).
113
114 **Comparison of Fe^{2+}-oxidizing abilities of different microbial strains**
115 One-hundred milliliter flasks containing 50 mL HBS medium (pH 2.0; + 0.01% S^0; + 10
116 mM Fe^{2+}; +TE; ± 0.01% YE) were inoculated with mixed culture containing *At. caldus*
117 KU (1×10^7 cells mL^{-1}) plus one of the four Fe-oxidizers (*Sb. acidophilus* YTF1, *Sb.*
118 *sibiricus* N1, *Am. ferrooxidans* ICP or *Acidiplasma* sp. Fv-AP; 1×10^7 cells mL^{-1}). The
119 flasks were incubated at 45°C, shaken at 150 rpm. Samples were regularly taken for
120 solution analyses (as described below) (water evaporation was compensated with
121 deionized water prior to sampling). All experiments were conducted in duplicate.
122
123 **Chalcopyrite Bioleaching**
124 Five-hundred milliliter flasks containing 200 mL HBS medium (pH 2.0; + 1% (w v^{-1})
125 chalcopyrite concentrate; + 0.01% S^0; + 5 mM Fe^{2+}; +TE) were inoculated with mixed
126 culture containing *At. caldus* KU (1×10^7 cells mL^{-1}) plus one of the four Fe-oxidizers
127 (1×10^7 cells mL^{-1}). Mixed cultures containing *Am. ferrooxidans* ICP and *Acidiplasma* sp.
128 Fv-Ap were prepared with or without 0.01% YE. The flasks were incubated at 45°C,

129 shaken at 150 rpm. Sterile controls were run in parallel. Samples were regularly taken for
130 solution analyses (as described below) (water evaporation was compensated with
131 deionized water prior to sampling).
132
133 **Characterization of bioleaching residues over time**
134 Mixed cultures containing *Sb. sibiricus* N1 plus *At. caldus* KU were prepared (as
135 described above) in nine-fold to monitor overtime changes in characteristics of
136 bioleached chalcopyrite concentrate residues. Liquid samples were taken for solution
137 analyses prior to collecting the bioleached residues.
138
139 **Solution and Solid Analyses**
140 Cell density was monitored by direct counting (Thoma chamber). Liquid samples were
141 first filtered (0.20 μm) to determine concentrations of Fe^{2+} (*o*-phenanthroline method;
142 Caldwell et al. 1946) and total dissolved Fe/Cu (ICP-AES; SEIKO Vista-MPX). E_h
143 values were measured with an Ag/AgCl reference electrode (with an automatic
144 conversion to SHE) (MM-60R, TOADKK).
145 Solid samples (bioleached residues) were collected by filtration, freeze-dried
146 overnight and analyzed by X-ray diffraction (XRD) (Ultima IV, Rigaku; CuKα 40 mA,
147 40 kV), scanning electron microscope (SEM) (JSM-7001F, JEOL), X-ray photoelectron

148 spectroscopy (XPS) (ESCA 5800, ULVAC-PHI; Al Kα) and electron probe micro

149 analyzer (EPMA) (JEOL JXA-8530F; 10 nA, 20 kV).

150

151 **Results and Discussion**

152 **Solution Redox Potentials (E_h) Exhibited by Different Fe-oxidizing Microbes**

153 To screen the conditions for the next bioleaching test, changes in Fe^{2+} concentration, 10

154 mM initially, and in E_h over time in each mixed culture (*At. caldus* plus one of the four

155 Fe-oxidiaers; i.e. *Sb. acidophilus* YTF1, *Sb. sibiricus* N1, *Am. ferrooxidans* ICP or

156 *Acidiplasma* sp. Fv-AP) were determined. The Effect of 0.01% YE on

157 mixotrophic/heterotrophic Fe^{2+} oxidation was also evaluated.

158 *Am. ferrooxidans* ICP was the strongest Fe-oxidizer of the group, leading to the

159 quick establishment of higher E_h values (> 800 mV) by day 2. Addition of YE slightly

160 enhanced Fe^{2+} oxidation and cell growth (Figure 1b, c). In the mixed culture with *Sb.*

161 *sibiricus* N1, YE addition caused a rapid rise in E_h to ~800 mV but did not affect cell

162 density significantly (Fig. 1c). Fe^{2+} oxidation by *Acidiplasma* sp. Fv-Ap was slower than

163 that by the former two strains (*Am. ferrooxidans* ICP and *Sb. sibiricus* N1), but it

164 generated higher E_h levels (~800 mV) after day 6 (Fig. 1b). In contrast, a constantly low

165 E_h level (< 600 mV) was maintained in *Sulfobacillus* sp. YTF1 cultures with or without

166 YE addition, due to only minor Fe^{2+} oxidation by the strain (Figure 1a).

167 Based on the above results, the subsequent bioleaching tests were conducted by
168 selecting the following conditions: i.e., Mixed cultures containing *At. caldus* KU plus one
169 of the following Fe-oxidizers; *Sb. acidophilus* YTF1 (-YE), *Sb. sibiricus* N1 (-YE),
170 *Acidiplasma* sp. Fv-Ap (±YE) or *Am. ferooxidans* ICP (+YE).

171

172 **Chalcopyrite Bioleaching at Redox Potentials (E_h) Generated by Different**
173 **Fe-oxidizers**

174 Rapid Fe^{2+} oxidation was observed with *Am. ferrooxidans* ICP and *Acidiplasma* sp.
175 Fv-Ap in the presence of YE (Figure 2d), accompanied by an immediate increase in E_h
176 (>800 and 750 mV, respectively; Figure 2b), and an increase in cell density (up to 7×10^8
177 cells mL^{-1}), compared to other cultures (Figure 2f). At the same time, less chalcopyrite
178 dissolution was noted with these two strains (Figure 2a, c) than with the others tested. *Am.*
179 *ferrooxidans* ICP is able to grow autotrophically on Fe^{2+} and heterotrophically on YE
180 (Clark and Norris, 1996). The genus *Acidiplasma* is able to oxidize Fe^{2+} in the presence
181 of trace amount of YE (Golyshina et al. 2009). Therefore, YE promoted strong
182 heterotrophic Fe^{2+} oxidation by the two strains with an immediate rise in E_h. In the
183 absence of YE, a 14-day lag was followed by rapid Fe^{2+} oxidation by *Acidiplasma* sp.
184 Fv-Ap (Fig. 2d), leading to a sudden increase in E_h on day 14 (Fig. 2b). Thus,
185 chalcopyrite dissolution started off rapidly, and then slowed after day 14 (Figure 2a, c), a

186 clear transition in E_h levels from 550-600 mV to ≥ 700 mV (Figure 2b). With *Sb.*
187 *sibiricus* N1, Fe^{2+} oxidation became extensive only after day 29 (Figure 2d), creating a
188 clear transition in E_h levels from 550-600 mV to > 700 mV (Figure 2b). Chalcopyrite
189 dissolution readily progressed until the E_h level came to this transition (Figure 2a, c). *Sb.*
190 *acidophilus* YTF1 showed the weakest Fe^{2+} oxidation throughout (Figure 2d) and E_h
191 values remained stable at around 600 mV (Figure 2b). As a result, dissolution of Cu and
192 Fe progressed continuously for 70 days (Figure 2a, c). Relatively higher pH values
193 (Figure 2e) shown in *Sb. acidophilus* YTF1, *Sb. sibiricus* N1, and *Acidiplasma* sp. Fv-Ap
194 (-YE; only at the beginning) cultures are indicative of greater H^+-consuming chalcopyrite
195 dissolution that counteracting sulfuric acid production by *At. caldus* KU (Figure 2a, c).

196 Microscopic observation of morphological differences between *At. caldus* KU
197 (smaller rods) and *Sb. acidophilus* YTF1 cells (larger and longer rods), or between *At.*
198 *caldus* KU (larger rods) and *Acidisplasma* sp. Fv-Ap cells (smaller irregular cocci)
199 showed that *At. caldus* KU dominated by cell count initially, followed by emergence and
200 gradual increase of respective Fe-oxidizing cells. This observation suggests that
201 autotrophic growth of *At. caldus* KU on S^0 (Hallberg and Lindstörm, 1994) occurred first,
202 producing cell exudates/lysates of organics that supported subsequent growth of
203 mixotrophic/heterotrophic Fe-oxidizers. Since steady growth of *Sb. sibiricus* N1 is only
204 possible by simultaneous utilization of inorganic substrates (e.g., Fe^{2+}, S^0) and organic

205 substrates (e.g., yeast extract) (Melamud et al. 2003), sudden initiation of active Fe^{2+}

206 oxidation by *Sb. sibiricus* N1 on day 36 (Figure 2d) was probably the result of

207 accumulation of the organic substrates. Likewise, the heterotrophic Fe^{2+} oxidation by

208 *Acidiplasma* sp. Fv-Ap on day 14 (Figure 2d) was probably the result of release of

209 organics by the autotrophic organisms. Growth of *Sb. acidophilus* YTF1 was reported to

210 occur both autotrophically (on Fe^{2+}/S^0) and heterotrophically (on yeast extract), although

211 Fe^{2+} oxidation was more rapid and complete in the presence of yeast extract (Norris et al.

212 1996). Unlike *Sb. sibiricus* N1 and *Acidiplasma* sp. Fv-Ap, weak Fe^{2+} oxidation by *Sb.*

213 *acidophilus* YTF1 continued steadily. This difference may be due partly to the metabolic

214 inefficiency in utilizing the organic substrates derived from the autotrophs.

215 The above results indicate that different E_h levels can be created by selected

216 Fe-oxidizers in the bioleaching system. Chalcopyrite dissolution was highly susceptible

217 to the real-time change in E_h and maintaining lower E_h values facilitated a constant Cu

218 solubilization.

219

220 **Correlation Between Cu Recovery and Normalized Solution Redox Potentials**

221 **(E_{normal}) During Bioleaching**

222 Until now, only relatively limited studies have examined the effect of controlling E_h of

223 bioleaching systems: Running an electrochemical bioleaching reactor at 600-630 mV

224 (SHE) resulted in improvement of chalcopyrite leaching when compared to results from
225 conventional bioleaching, chemical leaching and electrochemical leaching. This can be
226 explained by (a) an absence of jarosite formation at the low E_h level, (b) higher cell
227 concentrations achieved by electrochemical Fe^{3+} reduction to Fe^{2+}, and (c) to
228 electro-reduction of chalcopyrite to produce less refractory chalcocite and covellite
229 (Ahmadi et al 2010, 2011). Improved chalcopyrite bioleaching was also observed by
230 controlling E_h at 580 mV (SHE) (Third et al. 2002) or at 620 mV (SHE) (Gericke et al.
231 2010) through arresting the oxygen supply.

232 From previous chemical leaching studies, experimental conditions such as metal
233 ion concentrations, solid/liquid ratios and co-existing minerals were shown to affect the
234 optimal E_h value for Cu extraction (Okamoto 2004, 2005; Hiroyoshi 2008a, 2008b). This
235 could cause misleading interpretation of results obtained from different studies conducted
236 under different conditions. In order to define the optimal E_h value independent of such
237 conditional differences, "the normalized redox potential (E_{normal})" was proposed for a
238 reaction model assuming the formation of intermediate Cu_2S from chalcopyrite, with its
239 optimal range being $0 \leq E_{normal} \leq 1$ (highest copper extraction rate achieved at $E_{normal} \approx$
240 0.43; Okamoto et al. 2004, 2005; Hiroyoshi et al. 2008a, 2008b):

241 $$E_{normal} = (E_h - E_{ox}) / (E_c - E_{ox}) \quad (5)$$

242 A reaction model based on E_{normal} has yet to be discussed in detail for bioleaching

243 studies. We are using the data in Figure 2 of this study to evaluate the effectiveness of the
244 bioleaching reaction based on E_{normal} to reflect real-time concentrations of Fe^{2+} and Cu^{2+},
245 using equations 3, 4, and 5 in Figure 3.

246 With *Acidiplasma* sp. Fv-Ap and *Am. ferrooxidans* ICP (+YE), E_{normal} rapidly rose to
247 > 1 ($E > E_c$) due to prompt microbial Fe^{2+} oxidation. The intermediate Cu_2S does not
248 form in this "passive region" (Hiroyoshi et al. 2008a, 2008b). In fact, chalcopyrite
249 leaching was less effective, resulting in only 49 and 48% final Cu recovery, respectively
250 (Figs. 3d and 3e). In *Sb. acidophilus* YTF1 cultures, on the other hand, E_{normal} values were
251 stable within $0 \leq E_{normal} \leq 1$. The intermediate Cu_2S is formed in this "active region"
252 (Hiroyoshi et al. 2008a, 2008b). In fact, bioleaching under this condition resulted in
253 higher final Cu recovery (75%) (Figure 3a).

254 *Sb. sibiricus* N1 and *Acidiplasma* sp. Fv-Ap cultures (-YE) displayed a transition in
255 the E_{normal} level from $0 \leq E_{normal} \leq 1$ to $E_{normal} > 1$. This clearly corresponded to the trend
256 of Cu extraction behavior: The E_{normal} values stayed within the optimal range ($0 \leq E_{normal}$
257 ≤ 1) for a longer period in the *Sb. sibericus* N1 cultures than in the *Acidiplasma* sp.
258 Fv-Ap cultures, resulting in greater Cu recovery in the former (77%) than in the latter
259 (59%) (Fig 2b, c). In sterile controls, copper leaching slowed as E_{normal} approached 0.
260 This was because $E_{normal} < 0$ ($E_{ox} > E_h$) indicates a "non-reactive region" (Hiroyoshi et al.
261 2008a, b) in which Cu_2S is not oxidized and Cu extraction ceases. The final Cu recovery

262 in sterile controls was 20% (Figure 2f).

263 Overall, the results suggest that efficiency of chalcopyrite bioleaching can be
264 effectively evaluated by incorporating E_{normal} as a valuation basis which is independent of
265 the real-time change in major metal solutes in the system. The relationship between the
266 Cu leaching rate and E_{normal} is summarized in Figure 4. The maximum Cu leaching rate
267 was achieved at around E_{normal} = 0.35. The optimum E_{normal} value of 0.43, originally
268 reported by Hiroyoshi et al. (2008a, 2008b), was shifted to slightly lower value in this
269 study. This difference may have been caused partly by the higher (45°C) temperature
270 used in this study compared to ambient temperature of 30°C (Hiroyoshi et al. 2008a,
271 2008b), facilitating the kinetics of chalcocite-intermediate reactions at even lower E_h
272 levels. Another possible explanation is involvement of microbial reactions in this study,
273 compared to abiotic chemical leaching studies (except for one kind of experiment using a
274 mesophilic Fe-oxidizing bacterium) by Hiroyoshi et al. (2008a, b). Thus, (i) Fe-oxidizing
275 microbes generate an Fe-cycle by which they promote chalcocite/covellite oxidation
276 while simultaneously maintaining lower E_{normal} values. (ii) The S^0, a by product of
277 covellite oxidation, is also solubilized by S^0-oxidizing cells, which shifts the chemical
278 equilibrium in favor of covellite dissolution. The above microbially mediated covellite
279 dissolution processes (i and ii) may also lower the optimal E_{normal} value slightly in
280 bioleaching compared to chemical leaching.

281

282 **Changes in Residues During Bioleaching of Chalcopyrite Concentrate with Mixed**

283 **Cultures Containing *Sb. sibiricus* N1 and *At. caldus* KU**

284 Each flask was incubated until day 2, 7, 12, 21, 26, 31, 38, 55 and 70 to collect all of the

285 bioleached residues remaining in the flask. Cu dissolution and E_{normal} behaviors were

286 reproduced almost identically to results shown in Figure 3b. Thus, the sampling times in

287 this experiment were indicated by overlapping Figure 3b.

288 According to the XRD results, jarosite and S^0 peaks started to appear at day 38

289 (E_{normal} > ~0.6; Figure 3b), accompanied by distinctive color change of the mineral to

290 yellow (Figure 5). XPS peaks of Cu $2p_{3/2}$ / Cu $2p_{1/2}$ (Figure 6a) and S $2p_{3/2}$ / S $2p_{1/2}$

291 (Figure 6b) derived from the original chalcopyrite concentrate were observed in

292 bioleached residues until day 31. Sulfate S 2p peaks emerged at day 38. Together with the

293 XRD results, surface-sensitive XPS analyses indicate formation of passivation layers

294 (mostly jarosite) on the chalcopyrite concentrate surface at around day 38, coinciding

295 with declining Cu dissolution after day 38 (Figure 3b).

296 Hiroyoshi et al. (2004) suggested that oxidation of chalcocite at acidic pHs proceeds

297 according to the following two-step reaction;

298 Cu_2S (chalcocite) → $Cu_{2-x}S + xCu^{2+} + 2xe^-$ $(0 < x \leq 1)$ (6)

299 CuS (covellite) → $Cu^{2+} + S^0 + 2e^-$ (7)

300 Since covellite dissolution is slower than that of chalcocite (Gupta 2003), covellite may
301 be more readily detected during chalcopyrite leaching: In fact, other researchers detected
302 covellite (rather than chalcocite) in bioleached and chemically-leached chalcopyrite
303 residues via Raman spectroscopy (Sasaki et al. 2009) and XRD patterns (Córdoba et al.
304 2008), respectively. In this study, no direct evidence for intermediate chalcocite or
305 covellite was obtained (by XRD, XAFS, EPMA and XPS; data not shown). Since the
306 original chalcopyrite concentrate contained a minor amount of blue-colored covellite (by
307 EPMA observation; data not shown), it was also not possible to visually confirm the
308 secondary formation of covellite. Detection of such intermediates may become more
309 difficult in active low-redox chalcopyrite bioleaching systems compared to the abiotic
310 counterparts, again because of the effects of microbially mediated covellite dissolution (i
311 and ii), as described in the previous section.

312 The findings of this study indicate that low Cu leaching rates at higher E_{normal},
313 especially at $E_{normal} > 1$ (Fig. 4), were caused by the low reactivity of chalcopyrite at high
314 E_h (Hiroyoshi et al. 2004) and by the passivation mainly due to jarosite that occurred on
315 the chalcopyrite surface.

316

317 **Kinetics of Bioleaching of Chalcopyrite Concentrate at Different Redox Potentials**
318 **(E_h)**

319 Mineral dissolution is most frequently interpreted in terms of a shrinking core model.

320 Such fluid-solid reaction may be generally described as follows:

321 $$A_{\text{(fluid)}} + bB_{\text{(solid)}} \rightarrow cC_{\text{(fluid)}} + dD_{\text{(solid)}} \quad (8)$$

322 In this model, the overall reaction proceeds via diffusion through a liquid film (9),

323 diffusion through a product layer (10), and surface chemical reaction (11), one of which

324 steps becomes rate-limiting under certain conditions (Sohn and Wadsworth, 1979):

325 $$X = k_1 t \quad (9)$$

326 $$1 - 3(1 - X)^{2/3} + 2(1 - X) = k_d t \quad (10)$$

327 $$1 - (1 - X)^{1/3} = k_r t \quad (11)$$

328 Where, X; the fraction of B reacted, t; the reaction time, k; the rate constant

329 In chalcopyrite dissolution, the fluid film resistance is considered negligible

330 compared to the effects of diffusion through the product layer or of the chemical

331 reactions at the surface. In fact, no linear relationships were obtained between X and t

332 (data not shown). The calculated R^2 and k values are listed in Table II. Data were plotted

333 linearly where R^2 values of regression analyses > 0.97. Data from day 0 were not

334 plotted to exclude the effect of initial rapid dissolution of covellite, which was a

335 contaminant in the original concentrate.

336 Surface chemical reaction was likely the rate-limiting step until the end in *Sb.*

337 *acidophilus* YTF1 cultures (Figure 7a; Table II), where $0 \leq E_{\text{normal}} \leq 1$ was satisfied

338 throughout the bioleaching period (Figure 3a). A similar trend was found with *Sb.*
339 *sibiricus* N1 cultures, but only until day 44 (Figure 7a; Table II); this was consistent with
340 $0 \leq E_{normal} \leq 1$ having being satisfied until day 44 (Figure 3b).

341 With *Acidiplasma* sp. Fv-Ap (-YE), the initial bioleaching phase (until day 10)
342 appeared to be controlled by a surface chemical reaction, whereas subsequent phases
343 appeared to be controlled by diffusion through the product film (Figure 7b; Table II).
344 This corresponded to the major shift in the E_{normal} level at around day 12 from $0 \leq E_{normal}$
345 ≤ 1 to $E_{normal} > 1$ (Figure 3b). The E_{normal} level immediately jumped to > 1 in *Acidiplasma*
346 sp. Fv-Ap and *Am. ferrooxidans* ICP cultures (+YE) (Figure 3d and 3e, respectively). In
347 this case, chalcopyrite dissolution was controlled by diffusion through product film
348 throughout the bioleaching period (Figure 7b; Table II). E_{normal} having a value greater
349 than 1 appeared to be consistent with diffusion through product film, resulting from
350 passivation from the precipitation of potassium jarosite ($KFe_3(OH)_6(SO_4)_2$) on the surface
351 of the chalcopyrite particles as discussed in the previous section. In sterile controls, the
352 pH values steadily increased owing to the absence of *At. caldus* for production of sulfuric
353 acid (Figure 2e). Ferric precipitation of compounds such as jarosite, detected by XRD
354 analysis (data not shown), resulted therefore in a process consistent with diffusion
355 through product film (Figure 7b).

356 Different conditions used in previous chemical leaching studies produced

357 different results suggesting different kinetic mechanism of chalcopyrite leaching. Thus,
358 whereas chemical leaching with non-Fe^{3+} oxidants resulted in dissolution of chalcopyrite
359 controlled by chemical reaction, chemical leaching with Fe^{3+} proceeded through product
360 layer-controlled chalcopyrite dissolution because of passivation of the chalcopyrite
361 surface by jarosite deposition (Dutriziac et al. 1969, 1981). It should be noted that
362 deposition of secondary S^0 at the chalcopyrite surface did not cause diffusion resistance
363 (Antonijevic et al. 1994, 2004; Adebayo et al. 2003; Mahajan et al. 2007). However, at
364 fixed pH, E_h and temperature, it appears most likely that Fe^{3+} leaching is surface reaction
365 rate-controlled during the initial stage, which is later controlled by surface layer diffusion
366 (Li et al. 2013). Maintaining a lower E_h level (e.g., 600-650 mV (SHE)) during chemical
367 chalcopyrite leaching enabled a reaction controlled by surface chemical reaction in ferric
368 sulfate media (Córdoba et al. 2008).

369 In the case of bioleaching, Fe^{3+} is the essential oxidant in the system. Even though
370 bioleaching is initiated at different E_h levels (500-800 mV (SHE)), rapid microbial Fe^{2+}
371 oxidation can increase the E_h levels over 800 mV (SHE) within a few days (Cordoba et al.
372 2008b). Therefore, chalcopyrite bioleaching is highly susceptible to jarosite formation
373 especially at higher pHs (Sasaki et al. 2009; Sandström et al. 2005), leading to the
374 reaction being controlled by diffusion through product film (Pradhan et al. 2010;
375 Abhilash et al. 2013, 2015; Stott et al. 2000).

376 Consideration of the results of this study and the above-mentioned previous
377 observations leads to the conclusion that the rate-limiting step of chalcopyrite dissolution
378 depends greatly on the E_h value, which fluctuates continually during bioleaching. It was
379 found that efficiency of bioleaching of chalcopyrite could be evaluated on the basis of
380 E_{normal}. Controlling the optimal E_h level (to satisfy $0 \leq E_{normal} \leq 1$; especially at E_{normal} =
381 ~0.35 at 45°C) in bioleaching systems is thus critical for promoting steady Cu
382 solubilization by a surface chemical reaction. This was possible by selecting a suitable
383 Fe-oxidizing microbe. No physicochemical/electrochemical manipulation was needed.
384 In actual bioleaching operations, however, a range of indigenous Fe-oxidizing
385 microbes are present on the mineral surface. In order to microbiologically maintain a low
386 E_h level at the mineral surface, prior separate cultivation of mixed cultures containing a
387 "weak" Fe-oxidizer and subsequent inoculation of the ore would be necessary to make it
388 the dominant Fe-oxidizing species in the system. Inclusion of minerals such as PbS that
389 are inhibitory to some microbes (Vilcáez 2015) is a possible means of selectively
390 favoring the growth of weak Fe-oxidizers or of suppressing the activity of indigenous
391 strong Fe-oxidizers. Acidophilic microbes also display different degrees of sensitivity to
392 flotation agents (Okibe and Johnson 2002), which could be exploited in favoring selective
393 growth of preferred Fe-oxidizers in a leaching operation. Further optimization is ongoing
394 to maximize the controlling effect of low redox potential on bioleaching of chalcopyrite

concentrate. The utility of the findings of this study is also currently under evaluation using low grade (< 1%) chalcopyrite ores.

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558 **Figure Legends**

559 **Figure 1.**

560 Changes in Fe^{2+} concentrations (a) and redox potentials (E_h) (b) during growth (c) of each
561 of four different Fe-oxidizers (*Sb. acidophilus* YTF1 (●, ○) / *Sb. sibiricus* N1 (■,
562 □) / *Acidiplasma* sp. Fv-Ap (▲, △) / *Am. ferooxidans* ICP (◆, ◇)) in mixed culture
563 with *At. caldus* KU. Sterile controls (+, ×) were run in parallel. Cell densities in the
564 samples were determined by counting the cells of *At. caldus* KU and the other
565 Fe-oxidizer with which it was combined. Solid lines indicate addition of 0.01% yeast
566 extract. Broken lines indicate no yeast extract addition. Data points are mean values from
567 duplicate cultures. Error bars depicting averages are not visible in some cases as these
568 were smaller than the data point symbols.

569

570 **Figure 2.**

571 Changes in total soluble Cu concentrations (a), E_h values (b), total soluble Fe
572 concentrations (c), Fe^{2+} concentrations (d), pH values (e) and cell densities (f) during
573 bioleaching of chalcopyrite concentrate. Mixed cultures contained *At. caldus* KU plus one
574 of the following Fe-oxidizers: *Sb. acidophilus* YTF1 (●) / *Sb. sibiricus* N1 (■) /
575 *Acidiplasma* sp. Fv-Ap (▲) / *Acidiplasma* sp. Fv-Ap (+YE) (▼) / *Am. ferooxidans* ICP
576 (+YE) (◆). Sterile controls (×) were run in parallel. Cell densities represented the total

577 of all bacterial cells in a sample. Data points are mean values from duplicate cultures.
578 Error bars depicting averages are not visible in some cases as these were smaller than the
579 data point symbols. "+YE" indicates addition of 0.01% yeast extract.
580
581 **Figure 3.**
582 Relationship between the normalized redox potential (E_{normal}; open symbols) and
583 dissolution of Cu (solid symbols on solid lines) and Fe (solid symbols on broken lines)
584 during bioleaching of chalcopyrite concentrate. Each of four Fe-oxidizing microbes
585 (inoculated at 1×10^7 cells mL^{-1}) was used in mixed cultures with S-oxidizing *At. caldus*
586 KU (inoculated at 1×10^7 cells mL^{-1}): (a) *Sb. acidophilus* YTF1; (b) *Sb. sibiricus* N1; (c)
587 *Acidiplasma* sp. Fv-Ap; (d) *Acidiplasma* sp. Fv-Ap (+YE); (e) *Am. ferooxidans* ICP
588 (+YE). Sterile controls (f) were run in parallel. "+YE" stands for addition of 0.01% yeast
589 extract in the media. Data points are mean values from duplicate cultures. Error bars
590 depicting averages are not visible in some cases as these were smaller than the data point
591 symbols.
592
593 **Figure 4.**
594 Relationship between the Cu leaching rate and the E_{normal} value. The plots originated from
595 mixed culture containing *At. caldus* KU plus one of the following Fe-oxidizers; *Sb.*

596 *acidophilus* YTF1 (●) / *Sb. sibiricus* N1 (■) / *Acidiplasma* sp. Fv-Ap (▲) /

597 *Acidiplasma* sp. Fv-Ap (+YE) (▼) / *Am. ferrooxidans* ICP (+YE) (◆). Sterile controls

598 (×) were also included. A single triangle plot (▲) positioned at exceptionally high Cu

599 leaching rate of 2.2, likely resulted from rapid oxidation of chalcocite/covellite

600 (accumulated before day 14) triggered by a jump in the E_{normal} level at day 14 (Figure 3c).

601

602 **Figure 5.**

603 X-ray diffraction patterns and the color change of original chalcopyrite concentrate and

604 bioleached residues in mixed cultures containing *At. caldus* KU plus *Sb. sibiricus* N1

605 collected on day 2, 7, 12, 21, 26, 31, 38, 55, or 70. C; chalcopyrite (ICSD 2518), P; pyrite

606 (ICSD 633289), J; potassium jarosite (ICSD 166801), S; elemental sulfur (ICSD

607 412326).

608

609 **Figure 6.**

610 XPS spectra of Cu 2p (a) and S 2p (b) of chalcopyrite concentrates bioleached in mixed

611 cultures containing *Sb. sibiricus* N1 and *At. caldus* KU. Samples were collected at day 2,

612 7, 12, 21, 26, 31, 38, and 55. Peak positions corresponding to original chalcopyrite (Cu

613 $2p_{3/2}$ = 932.4 eV, Cu $2p_{1/2}$ = 952.3 eV, S $2p_{3/2}$ = 161.4 eV, S $2p_{1/2}$ = 162.5 eV;

614 Ghahremaninezhad et al. 2013) are indicated by vertical grey bars. Peak positions

615 corresponding to sulfate (S $2p_{3/2}$ = 168.7 eV, S $2p_{1/2}$ = 169.9 eV; Klauber et al. 2001) are
616 indicated by grey broken lines. The doublet S 2p peaks (161.4 eV and 162.5 eV) reflect
617 the occurrence of monosulfide and disulfide (Ghahremaninezhad et al. 2013). The shift
618 (to higher binding energies) of Cu $2p_{3/2}$ and Cu $2p_{1/2}$ peaks originating from chalcopyrite
619 was likely the result of a differential charging effect.
620
621 **Figure 7.**
622 Kinetic modeling of chalcopyrite bioleaching in mixed cultures containing *At. caldus* KU
623 plus the following Fe-oxidizers; *Sb. acidophilus* YTF1 (●) / *Sb. sibiricus* N1 (■) /
624 *Acidiplasma* sp. Fv-Ap (▲) / *Acidiplasma* sp. Fv-Ap (+YE) (▼) / *Am. ferrooxidans* ICP
625 (+YE) (◆). Sterile controls (×) are also included. The rate-limiting step was assumed to be
626 (a) surface chemical reaction or (b) diffusion through product film. Linear lines were
627 drawn where R^2 values were calculated to be > 0.97. Day 0 plots were excluded to
628 remove the effect of initial rapid dissolution of covellite contaminating the original
629 chalcopyrite concentrate.
630
631 **Table 1.**
632 Elemental composition of chalcopyrite concentrate used in this study.
633

634 **Table 2.**

635 R^2 and k values calculated using the kinetic model of surface chemical reaction and

636 diffusion through product film.

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