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The role of bioleaching microorganisms in saline water leaching of chalcopyrite concentrate

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ABSTRACT

In order to tackle the dual challenge of utilizing highly refractory chalcopyrite ($CuFeS_2$) while saving scarce freshwater resources, this study aimed to systematically understand the individual role of chemical lixiviant and bioleaching microorganisms in the complex Fe^{3+}-Cu^{2+}-SO_4^{2-}-Cl^- chalcopyrite leaching system. In general freshwater bioleaching conditions, the E_h level sharply increased, and the "high-E_h-bioleaching" became the major leaching driving force. In this case, the lowest Cu yield was obtained. The chalcopyrite leaching reaction responded differently to different salinity levels. At a low salinity of 0.5% NaCl, chemical Cl^--leaching effect resulted in a higher Cu yield than the fresh-water "high-E_h-bioleaching" system. The growth of tested microbes was observed at 0.5% NaCl, but partial deactivation of microbial Fe-oxidation suppressed the E_h level. Under this condition, synergism between the chemical Cl^--leaching effect and the "low-E_h-bioleaching" effect was found. At a high salinity of 2% NaCl, on the other hand, no active cell growth was observed, and thus pre-grown cells were used to mimic the presence of Cl^--tolerant cells. Chemical Cl^--leaching readily proceeded at 2% NaCl at low E_h, but quickly ceased upon the depletion of H^+. The presence of bioleaching cells somewhat slowed down the speed of chemical Cl^--leaching, but the acid depletion was alleviated by microbial acid generation. Chemical Cl^--leaching, which favors low E_h condition, was the main driving force for chalcopyrite leaching at 2% NaCl. Therefore, the activity of Cl^--tolerant S-oxidizer alone, rather than mixed Fe- and S-oxidizing consortium, was shown to play a critical role in maximizing the chalcopyrite dissolution.

1. Introduction

Chalcopyrite ($CuFeS_2$), a highly refractory primary Cu sulfide mineral, represents an alternative Cu source due to its abundance. Challenges are thus being made towards the realization of its economically-feasible biohydrometallurgical processing. Whereas, due to the shortage of freshwater in major Cu-producing countries such as Chile and Australia, utilization of saline waters (i.e., seawater, groundwater) as an alternative water source for hydrometallurgical processes exists as another challenge to be overcome. The successful combination of the two could bring about a significant benefit to address both problems.

So far, several bioleaching studies attempted to utilize saline waters at different temperature systems. Bevilaqua et al. (2013) tested the effect of NaCl addition (100–200 mM; 0.58–1.2%) on chemical and bacterial leaching of chalcopyrite in shake flasks and stirred tank bioreactors at 26–28 °C, pH 1.6 (using mesophilic *Acidithiobacillus* (*At.*) *ferrooxidans* and *At. thiooxidans*). A synergistic effect of bacteria and NaCl (100 mM, 0.58%) on chalcopyrite dissolution was noted, especially under low E_h conditions (< 650 mV vs. NHE). As for bioleaching using moderately thermophilic microbes, Wang et al. (2012) suggested the effectiveness of NaCl (< 0.5%) to improve copper extraction in mixed consortium (*Leptospirillum ferriphilum*, *At. caldus*, *Ferroplasma thermophilum* and a marine acidophilic halotolerant bacterium *Sulfobacillus* sp. TPY) in shake flask test at 45 °C, pH 2.0. The consortium showed high Fe oxidation, and the culture E_h increased up to around 710 mV (vs. NHE). Bobadilla-Fazzini et al. (2014) also studied the effect of NaCl (85 mM, 0.5%) on chalcopyrite bioleaching by moderately thermophilic *Sulfobacillus* (*Sb.*) *thermosulfidooxidans* in shake flasks at 50 °C, pH 1.6. Cu extraction was increased by the dual action of the bacteria (E_h maintained at ~750–780 mV vs. NHE) and Cl^-. In the case of thermophilic archaeal species, Watling et al. (2016) compared *Metallosphaera (M.) hakonensis*, *Sulfolobus (S.) metallicus* and *Ac. brierleyi* in the chalcopyrite bioleaching test at 60 °C with the addition of 0–0.086 M (0–0.5%) NaCl. However, no evidence of enhanced Cu extraction was obtained in the presence of Cl^-.

The reactions taking place during chalcopyrite bioleaching in general Fe^{3+}-SO_4^{2-} systems are known to be a slow process, especially at

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lower temperatures. Several chemical leaching studies attributed its reason to the passivation of the mineral surface in high E_h conditions, including formation of S^0, metal-deficient sulfides and jarosites (Dutrizac, 1989; Lu et al., 2000b; Córdoba et al., 2009). Electrochemical/chemical leaching studies using Fe^{3+}-SO_4^{2-} solutions by Hiroyoshi et al. (e.g. Hiroyoshi et al., 2008a; Hiroyoshi et al., 2008b) proposed the mechanism to promote chalcopyrite dissolution under the controlled E_h ($E_{ox} < E_h < E_c$), wherein the chalcopyrite leaching is driven by Fe^{2+} and Cu^{2+} ions below the critical potential (E_c), forming intermediate chalcocite (Cu_2S). The oxidation of this intermediate by Fe^{3+} then produces Cu^{2+} at above the oxidation potential (E_{ox}). This theory was shown to be applicable in actual bioleaching situations, as the benefit of "low-E_h bioleaching" was confirmed by E_h-controlled bioleaching studies by using electro-bioreactors (600–630 mV vs. SHE (Ahmadi et al., 2010, 2011)), by arresting the oxygen supply (620 mV vs. SHE (Gericke et al., 2010); 580 mV vs. SHE (Third et al., 2002)) or by using "weak" Fe-oxidizing microorganisms (Masaki et al., 2018a; Christel et al., 2018).

When considering replacing freshwater with saline waters, the effect of co-existing Cl^- ions needs to be taken into account in the above described Fe^{3+}-SO_4^{2-}based bioleaching system. There is a possibility that partial deactivation of microbial Fe-oxidation by Cl^- toxicity could, in turn, benefit the chalcopyrite dissolution as a driving force of "low-E_h bioleaching." Also, as reported by several chemical/electrochemical studies using acidic Cl^- or hybrid Cl^--SO_4^{2-} systems (typically using elevated reagent concentrations and temperatures, compared to bioleaching conditions), the presence of Cl^- can directly improve the dissolution rate of chalcopyrite (Watling, 2014). Wang (2005) summarized the principal chemical leaching reactions in the Fe^{3+}-Cl^- system as [Eq. 1] and [Eq. 2], and the corresponding reactions for $CuCl_2$ attack as [Eq. 3] and [Eq. 4], leaving S^0 as the dominant sulfur compounds (Dutrizac, 1990):

$$CuFeS_2 + 3FeCl_3 = Cu^{I}Cl + 4FeCl_2 + 2S^0 \quad (1)$$

$$CuFeS_2 + 4FeCl_3 = Cu^{II}Cl_2 + 5FeCl_2 + 2S^0 \quad (2)$$

$$CuFeS_2 + 3Cu^{II}Cl_2 = 4Cu^{I}Cl + FeCl_2 + 2S^0 \quad (3)$$

$$S^0 + 6Cu^{II}Cl_2 + 4H_2O = 6Cu^{I}Cl + 6HCl + H_2SO_4 \quad (4)$$

Yoo et al. (2010) suggested through a thermodynamic study that complex Cu^+/Cl^--species become increasingly dominant at elevated Cl^- concentrations (in the order of CuCl, $CuCl_2^-$, $CuCl_3^{2-}$ and $CuCl_4^{3-}$; Lin et al., 1991), leading to an increase in the standard potential between Cu^+ and Cu^{2+} to act as the second Cu^{2+}/Cu^+ redox couple to contribute to sulfide mineral oxidation. Even under the Fe^{3+}-free/depleted Cu^{2+}-Cl^- system, the effectiveness of Cl^- was shown under mild conditions (35 °C, 0.2 M HCl, 0.008 M Cu^{2+}), as represented by [Eq. 5–7] (Miki and Nicol, 2011):

$$CuFeS_2 + 4HCl = Cu^{II}Cl_2 + FeCl_2 + 2H_2S \quad \text{Non-oxidative acid dissolution of } CuFeS_2 \quad (5)$$

(or $CuFeS_2 + 2HCl = CuS + FeCl_2 + H_2S$ [Eq. 5′], followed by oxidation of CuS to Cu^{2+})

$$H_2S + 2Cu^{II}Cl_2 = 2Cu^{I}Cl + S^0 + 2HCl \quad \text{Oxidation of } H_2S \text{ by } Cu^{2+} \text{ (rapid)} \quad (6)$$

$$4Cu^{I}Cl + O_2 + 4HCl = 4Cu^{II}Cl_2 + 2H_2O \quad \text{Oxidation of } Cu^+ \text{ by dissolved } O_2 \text{ (slow)} \quad (7)$$

Owing to the stability of Cu^+/Cl^-- and Cu^{2+}/Cl^--complexes, [Eq. 6] proceeds rapidly in Cl^- media, which explains the faster kinetics observed in Cl^--solutions.

Overall, the introduction of saline waters to chalcopyrite bioleaching would create a highly complex system where the above reactions may simultaneously come into effect to varying degrees, depending on the ion concentration, temperature and type of microbes used. The greatest synergism (between a variety of microbial and chemical reactions) may appear differently under different conditions. Hence, this study aimed to systematically explain the individual effect of chemical lixiviant and bioleaching microorganisms at different salinity levels.

2. Materials and methods

2.1. Minerals

Chalcopyrite concentrate from Chile (20–106 μm; JX Nippon Mining & Metals) was washed with 1 M HNO_3, deionized water and 100% ethanol before freeze-drying overnight. The elemental composition of chalcopyrite concentrate was as follows; S 35%, Cu 33%, Fe 26% (Masaki et al., 2018a).

2.2. Microorganisms and their Cl^- tolerance

Three bacterial strains (*Acidimicrobium* (*Am.*) *ferrooxidans* ICP^T, DSM 10331; *Sulfobacillus* (*Sb.*) *sibiricus* N1^T, DSM 17363; *At. caldus* KU^T, DSM 8584) and an archaeal strain (*Acidiplasma* sp. Fv-Ap) were used in this study. They were routinely cultivated under aerobic condition in 500 mL Erlenmeyer flasks containing 200 mL of heterotrophic basal salts (HBS) medium (0.5 g/L $MgSO_4{\cdot}7H_2O$, 0.45 g/L $(NH_4)SO_4$, 0.05 g/L KCl, 0.05 g/L KH_2PO_4, 0.014 g/L $Ca(NO_3)_2{\cdot}4H_2O$, 0.142 g/L Na_2SO_4; pH 1.5 with H_2SO_4). For *Am. ferrooxidans* ICP and *Acidiplasma* Fv-Ap, 0.02% (*w/v*) yeast extract plus 10 mM Fe^{2+} (as $FeSO_4{\cdot}7H_2O$) were added. For *Sb. sibiricus* N1, 0.02% yeast extract, 10 mM Fe^{2+} plus 0.02% (w/v) S^0 were added. For *At. caldus* KU, 0.02% S^0 plus 200 μL trace elements stock solution (Masaki et al., 2018b) were added. Flasks were incubated at 45 °C, shaken at 150 rpm.

Microbial Cl^- tolerance test was conducted by monitoring cell growth of each strain at different NaCl concentrations (0, 0.1, 0.5, 1, 2 or 3% (i.e., 0, 0.017, 0.085, 0.17, 0.34 or 0.51 M)).

2.3. Saline water bioleaching test

2.3.1. Effect of various initial NaCl concentrations (0, 0.5, 1, 2 or 3%) in growing cultures

Mixed cultures containing all four Fe- and S-oxidizing strains were prepared by harvesting (10,000 rpm for 10 min at 4 °C) and washing (dilute H_2SO_4 water at pH 1.5) each pre-grown strain, prior to inoculation into 200 mL HBS medium containing 1% (*w/v*) chalcopyrite concentrate, 5 mM Fe^{2+} and 0.01% S^0 (pH 1.5 with H_2SO_4; 500 mL Erlenmeyer flasks). The initial cell density of each strain was set to 1×10^7 cells/mL (i.e. 4×10^7 cells/mL in total). Sterile control cultures were also set-up in parallel. The initial NaCl concentration was set to 0%, 0.5%, 1%, 2% or 3%. Flasks were incubated shaken at 45 °C and 150 rpm.

2.3.2. Effect of the midway addition of 2% NaCl in pre-grown cultures

Mixed cultures of all four strains, as well as pure cultures of S-oxidizing *At. caldus* KU were prepared as described in 2.3.1. After inoculation, cells were allowed to grow without NaCl until the early stationary phase for 9 days. On day 9, NaCl was added to 2% final concentration into the pre-grown cultures. Sterile control cultures were also set-up in parallel. Flasks were incubated shaken at 45 °C and 150 rpm.

Additionally, a separate experiment was conducted by replacing 2% NaCl with an artificial seawater mix (Marine art SF-1; Osaka Yakken). The artificial seawater mix was added on day 10 after the pre-growth of the cells. The total amount of Cl^- originating from the artificial seawater mix was set to be equivalent to that from 2% NaCl.

Samples were regularly withdrawn to monitor pH, E_h (vs. SHE), cell density and concentrations of total Cu, total Fe and Fe^{2+}. Flasks were incubated shaken at 45 °C and 150 rpm.

2.4. Effect of varying initial E_h (E_{ini}) on chemical Cl^--leaching of chalcopyrite concentrate

Abiotic tests were carried out in 500 mL Erlenmeyer flasks containing 200 mL pure water (pH 1.5 with H_2SO_4) and 1% chalcopyrite concentrate. The initial NaCl concentration was fixed to 3%. To create a range of E_{ini} values (550, 590, 670, 720 or 740 mV), Fe^{2+} and Fe^{3+} (as $Fe_2(SO_4)_2 \cdot nH_2O$) were added at different initial ratios (the total Fe concentration was set to 20 mM). Flasks were incubated shaken at 45 °C and 150 rpm.

2.5. Liquid analyses

Cell density was monitored by direct counting (Thoma chamber). Liquid samples were filtered through 0.20 μm membranes to determine concentrations of total Fe and Fe^{2+} (o-phenanthroline method; Caldwell and Adams, 1946) and total Cu (ICP-OES; Optima 8300DV; PerkinElmer). E_h values were measured with an Ag/AgCl reference electrode (with automatic conversion to SHE) (MM-43X, TOADKK).

3. Results and discussion

3.1. Microbial Cl^- tolerance

Prior to bio- and chemical leaching tests, Cl^- tolerance of the four strains was tested by monitoring the cell growth in the presence of different concentrations of NaCl (0, 0.1, 0.5, 1, 2 or 3%). Fig. 1 shows that the minimum inhibitory concentration (MIC) of NaCl was 0.5% for *Am. ferrooxidans* ICP (Fig. 1a), 2% for *Acidiplasma* Fv-Ap (Fig. 1b), 1% for *Sb. sibiricus* N1 (Fig. 1c) and 3% for *At. caldus* KU (Fig. 1d).

The average seawater salinity is about 3.5% (mainly NaCl). Therefore, active cell growth of these bioleaching strains cannot be expected unless lower strength saline waters are used or the seawater is diluted prior to use for the biohydrometallurgical process. Rea et al. (2015) also reported the NaCl tolerance of several mesophilic and moderately thermophilic Fe- and S-oxidizers (*Leptospirillum* spp., *Ferroplasma acidiphilum*, *Acidimicrobium* spp., *At. ferrooxidans*, *Sb. thermosulfidooxidans*, *At. caldus*) and found that pure cultures of common bioleaching strains do not tolerate sea water salinity and Fe-oxidizers are more salt-sensitive to S-oxidizers. The authors have also mentioned that salt tolerance becomes less at higher temperatures. Watling et al. (2016) concluded that known species of thermoacidophiles (*Acidianus brierleyi*, *M. hakonensis* and *S. metallicus*) would not be active in hot seawater bioleaching systems.

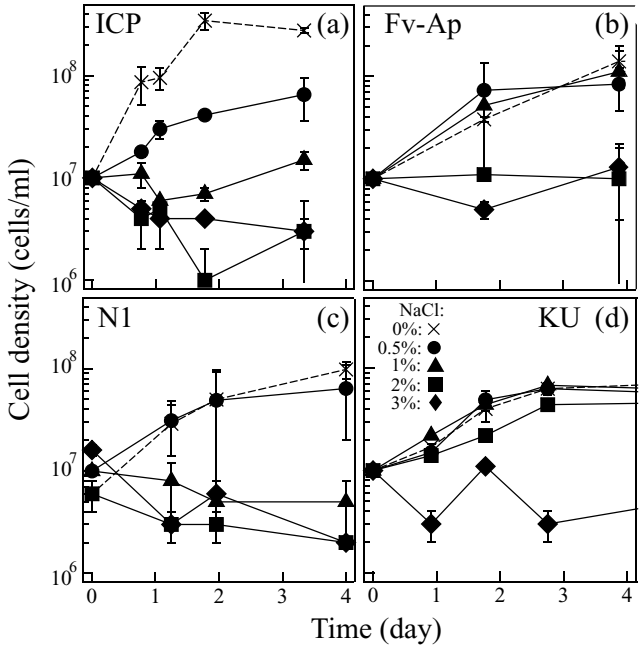

Fig. 1. Cl^- tolerance of *Am. ferrooxidans* ICP (a), *Acidiplasma* sp. Fv-Ap (b), *Sb. sibiricus* N1 (c) and *At. caldus* KU (d). Cells were grown in the presence of 0% (×), 0.5% (●), 1% (▲), 2% (■) or 3% (◆) NaCl.

3.2. The effect of mixed Fe- and S-oxidizing cultures on saline water leaching at lower salinity (0.5% NaCl)

3.2.1. Abiotic control cultures

Elevating the NaCl dose from 0 to 3% clearly resulted in the increasingly greater chemical Cl^--leaching of chalcopyrite (29% Cu at 0% NaCl, 54–56% Cu at 1–3% NaCl on day 30, Fig. 2a). Since the E_h level remained consistently low at around 550 mV (Fig. 2b) without any detectable amount of Fe^{3+} ions throughout the experiment (data not shown), Cu dissolution in abiotic cultures likely progressed mostly independent of the Fe^{3+} oxidant through [Eq. 5] to [Eq. 7]. The increased NaCl dose seems to have promoted Fe^{3+} precipitation after day 5, likely as natrojarosite and $Fe(OH)_3$ (Fig. 2c), by immediately scavenging fresh Fe^{2+} oxidation product: This reaction halted a pH increase temporally (day 5–10) by mineral acidity produced via jarosite formation ($3Fe^{3+} + 2\ SO_4^{2-} + 4\ 6H_2O + M^+ = MFe_3(SO_4)_2(OH)_6 + 6H^+$ (M = K^+, Na^+ and NH_4^+), but then continuous H^+ consumption based on [Eq. 7] further raised pH to >4, eventually stopping Cu dissolution (Fig. 2a). Carneiro and Leão (2007) also reported that although NaCl favored natrojarosite precipitation to reduce the total Fe concentration during leaching, chalcopyrite dissolution in Fe^{3+}-SO_4^{2-} media was promoted by the addition of NaCl. It was suggested that Cl^- ions contribute to the sulfide mineral dissolution not only by adding the second Cu^{2+}/Cu^+ redox couple but also by increasing the surface area, crystallinity and porosity of the product layer that allow the diffusion of reactants through the product film (Lu et al., 2000a; Carneiro and Leão, 2007).

3.2.2. Inoculated Fe- and S-oxidizing mixed cultures

The NaCl dose of ≥1% resulted in no active cell growth, and thus bioleaching reactions did not proceed. The cell density in NaCl-free mixed cultures readily increased to reach about 3×10^8 cells/mL, whereas that at the lowest NaCl concentration tested (0.5%) was almost comparable except for a few-days lag time at the beginning (data not shown). Following the active cell growth in NaCl-free mixed cultures, the E_h level sharply rose to exceed 800 mV (Fig. 2b), resulting in the slowest Cu dissolution (29% on day 30), compared even to the NaCl-free abiotic counterpart (Fig. 2a). At 0.5% NaCl, microbial Fe^{2+} oxidation was partially deactivated, and the E_h level was suppressed (< 760 mV; Fig. 2b). In addition to some chemical Cl^--leaching effect caused by the presence of 0.5% NaCl, the E_h-controlling effect owing to the toxicity of Cl^-, in turn, led to a higher Cu dissolution compared to the NaCl-free system (Fig. 2a). A greater Cu dissolution observed in 0.5% NaCl-mixed cultures (45% Cu on day 30) than in 0.5% NaCl-abiotic cultures (39% Cu on day 30) indeed suggests the existence of synergism between chemical Cl^--leaching and "low-E_h bioleaching" at this low salinity (0.5% NaCl).

However, the effectiveness of higher salinity (> 1% NaCl) without microbial activity easily exceeded the synergism mentioned above (observed at 0.5% NaCl; Fig. 2a). This observation poses the question of whether or not the future emergence (by isolation or acclimation) of salt-tolerant bioleaching microorganisms would still add any extra benefit to the process using higher salinity (> 1% NaCl). Therefore, to simulate active microbial Fe^{2+} oxidation (i.e., high E_h condition) in such higher-salinity waters, chemical Cl^--leaching tests were set-up at 2% NaCl with varied initial E_h levels (E_{ini} = 550–740 mV). As shown in

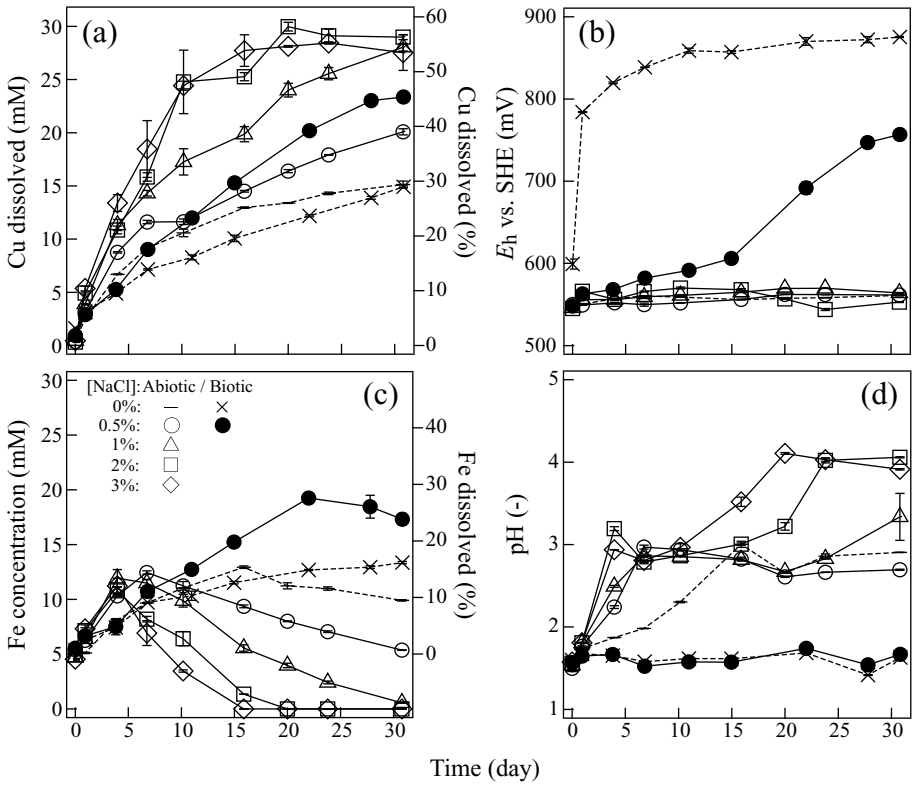

Fig. 2. Comparison of chemical Cl^--leaching (−, ○, △, □, ◇) and bioleaching (×, ●) of chalcopyrite concentrate using different strengths of NaCl solution: 0% (−, ×), 0.5% (○, ●), 1% (△), 2% (□), 3% (◇). Changes in the total Cu concentration (a), E_h (b), total Fe concentration (c) and pH (d) are shown.

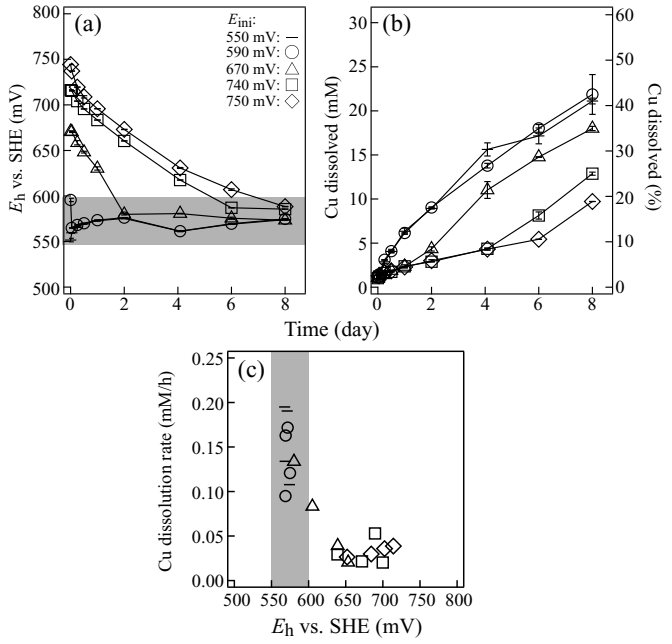

Fig. 3. Effect of initial E_h (E_{ini}) on chemical Cl^--leaching efficiency of chalcopyrite concentrate in 3% NaCl solution: E_{ini} was adjusted to 550 mV (×), 590 mV (○), 670 mV (△), 740 mV (□) or 750 mV (◇) using a total of 20 mM Fe of different Fe^{2+}/Fe^{3+} ratios. Changes in E_h values (a), total Cu concentrations (b) and the relationship between the Cu leaching rate and E_h (c) are shown. The shaded area in (a) indicates the E_h range where Cu dissolution was mainly facilitated. Cu dissolution rates in (c) were calculated using the data from (b) (from day 0 to 4, to avoid the possible effect caused by secondary mineral passivation in the later stage).

Fig. 3, chemical Cl^--leaching of chalcopyrite readily proceeded at lower E_{ini} of 550 mV and 590 mV (Fig. 3ab). In contrast, Cu dissolution stagnated at higher E_{ini} of >670 mV, until the E_h level fell into the range of <600 mV, at which point rapid Cu dissolution was boosted (Fig. 3ab). Fig. 3c indicates that chemical Cl^--leaching of chalcopyrite favors lower E_h level at around 550–600 mV. Therefore, it can be said that introducing active microbial Fe^{2+} oxidation (to raise E_h) to a high-salinity chalcopyrite leaching system may rather hinder the Cu dissolution efficiency. The reason for the above observation may be attributed to the contribution of the second Cu^{2+}/Cu^+ redox couple to the mineral dissolution, which becomes significant, especially in the low redox potential (E_h) condition wherein Cu^+/Cl^--complexes can exist stably (Bonan et al., 1981). Ruiz et al. (2011) also reported in their chemical leaching study of chalcopyrite concentrate in H_2SO_4–NaCl solutions that the evolution of E_h by the addition of Fe^{3+} in the system negatively affected the leaching rate.

3.3. The effect of salt-tolerant S-oxidizing microbes on chemical Cl^--leaching of chalcopyrite

The results from the previous section posed a question; it there any advantage of adding E_h-raising Fe-oxidizing microbes to purely chemical Cl^--leaching reaction at higher salinity? Since the bioleaching microorganisms (tested here) did not grow successfully in the chalcopyrite bioleaching condition containing ≥1% NaCl, cells were first pre-grown without NaCl to imitate the situation of the presence of salt-tolerate cells in the system.

During the NaCl-free pre-growth period (day 0–9), usual cell growth was observed to reach the late exponential phase in all cases (Fig. 4f). The Cu dissolution behavior was relatively similar in all cases during this phase (Fig. 4a), indicating that the activity of Fe-oxidizing cells raising the E_h level did not merit the chalcopyrite dissolution, as was explained by Hiroyoshi et al. (e.g., Hiroyoshi et al., 2008a; Hiroyoshi et al., 2008b).

After the NaCl addition (at 2%) on day 9, the most rapid initial Cu dissolution rate (between day 9–21) was observed in cell-free controls,

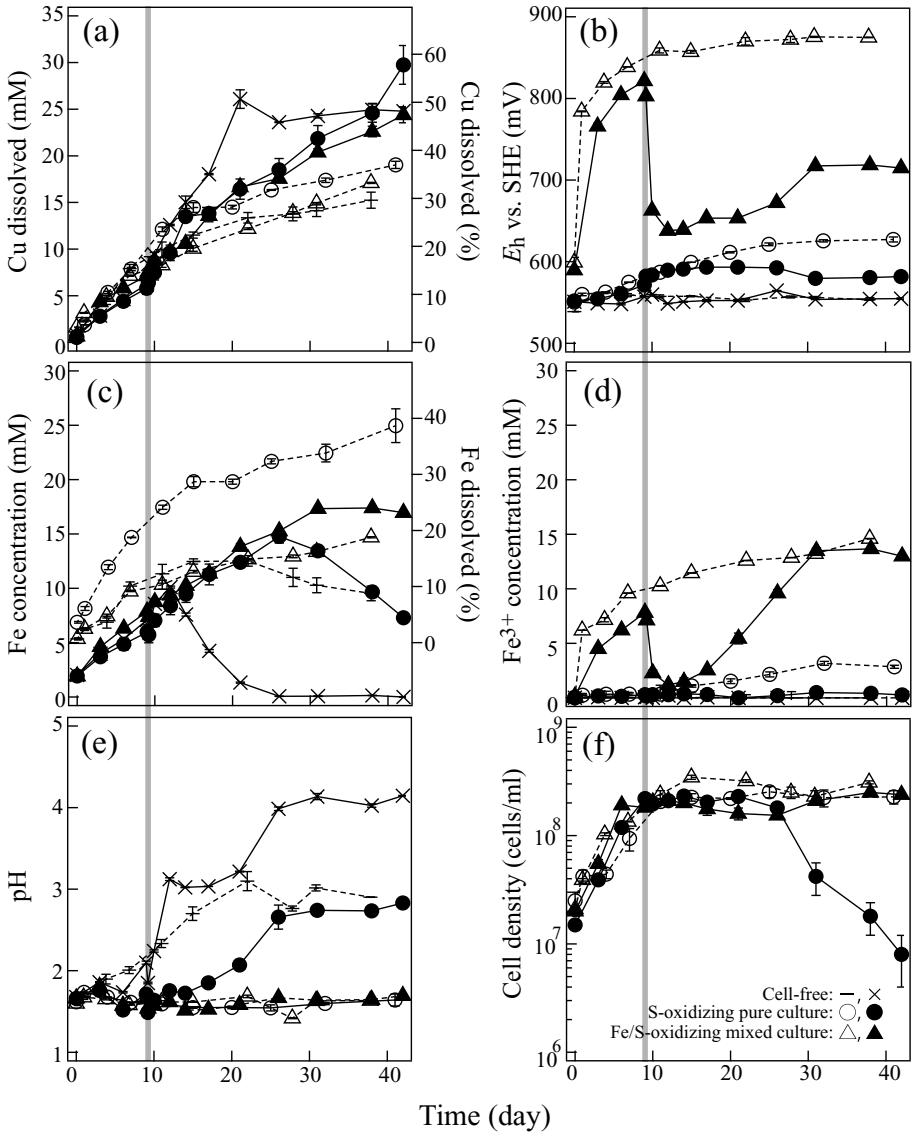

Fig. 4. Comparison of saline water bioleaching of chalcopyrite concentrate in S-oxidizing pure cultures (○,●), Fe/S-oxidizing mixed cultures (△, ▲) and cell-free control cultures (−, ×). Cells were first pre-grown without NaCl for 9 days. Grey vertical lines indicate the timing of NaCl addition on day 9 to adjust the culture salinity to 2% (●, ▲, ×). NaCl-free controls were set-up in parallel (○, △, −). Changes in the total Cu concentration (a), E_h (b), total Fe concentration (c), Fe^{2+} concentration (d), pH (e) and cell density (f) are shown.

which constantly maintained the lowest E_h level at ~550 mV (Fig. 4a, b). Since the amount of Fe^{3+} ions were only negligible throughout the experiment (Fig. 4d), Cu dissolution in cell-free cultures likely progressed mostly independent of the Fe^{3+} oxidant via [Eq. 5–7]. However, the accompanying pH jump up to >4 in cell-free cultures (Fig. 4e) triggered Fe^{3+}-mineral precipitation (Fig. 4c), and Cu dissolution halted after that (Fig. 4a).

In Fe- and S-oxidizing mixed cultures, the NaCl addition on day 9 caused an instant E_h fall from >800 mV to ~630 mV (Fig. 4b). Nonetheless, the cell density (Fig. 4f) and activity persisted to gradually recover the E_h level to over 700 mV towards the later-stage (Fig. 4b), while maintaining the culture acidity due to microbial S oxidation (Fig. 4e). The Cu dissolution rate after day 9 was slower but more continuous in mixed cultures than in cell-free controls, owing to H^+ availability in the culture to drive the reactions [Eq. 5–7], and the final Cu yield on day 42 in the former (48%) was comparable to that in the latter (47%) (Fig. 4a).

In pure cultures containing only S-oxidizing cells, the E_h values remained low (< 600 mV: Fig. 4b) without any detectable amount of Fe^{3+} ions (Fig. 4d) throughout the experiment due to the lack of Fe-oxidizers. As was discussed in the previous section 3.2, the E_h values in pure cultures were constantly within the optimal range to promote chemical Cl^--leaching of chalcopyrite. In fact, Cu dissolution in pure cultures progressed continuously and eventually exceeded that in mixed cultures and cell-free controls (58% Cu on day 42; Fig. 4a). The *At. caldus* cell density in pure cultures declined towards the end of the experiment (Fig. 4f), which was consistent with the gradual pH increase (up to <3.0) towards the end of the experiment (Fig. 4e). Such cell decline accompanied by pH increase was not observed in mixed cultures (note that *At. caldus* KU should be the main acid-producer in this test due to its MIC for NaCl; Fig. 1). This implies that the S-oxidizer survives against salinity better in the mixed consortium than in pure cultures. In other words, mixed microbial consortia would be advantageous to support the better survival of S-oxidizers (for culture acidification purpose to support continuous chemical Cl^--leaching of chalcopyrite). Nonetheless, active microbial Fe^{2+} oxidation did not add any positive, since high E_h condition hinders the overall reaction when chemical Cl^--leaching functions as the major leaching driving force in the system.

Overall, when using higher salinity waters, the chemical Cl^--leaching becomes the major driving force of chalcopyrite dissolution. Therefore, the introduction of microbial activity needs to be designed to support this chemical reaction. Searching and eventually introducing active Fe^{2+} oxidation by halophilic Fe-oxidizers would raise the E_h even higher than in this test (by non-halophilic strains), which could discourage the chemical Cl^--leaching reaction. While exploiting

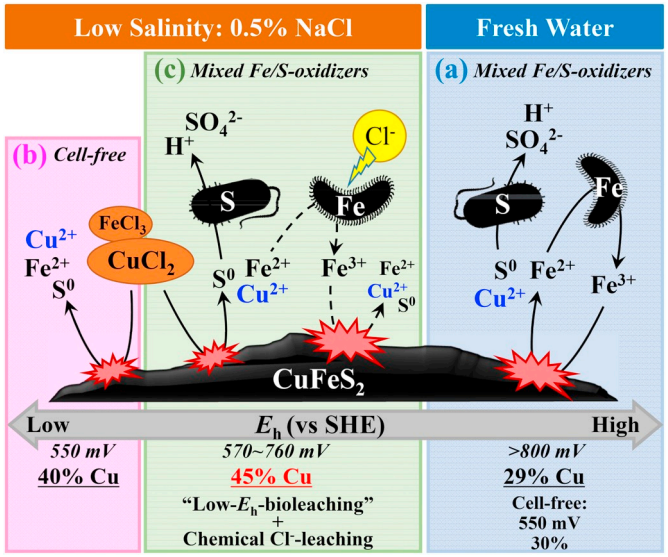

Fig. 5. Schematic summary depicting the chalcopyrite dissolution reaction in the lower-salinity condition (0.5% NaCl). Note that the Cu yields in Fig. 5 and Fig. 6 cannot be compared directly, as the experimental set-ups are different.

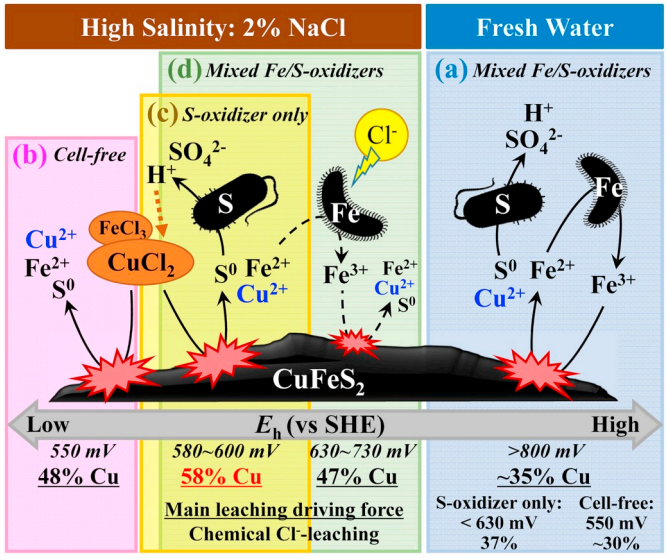

Fig. 6. Schematic summary depicting the chalcopyrite dissolution reaction in the higher-salinity condition (2% NaCl). Note that the Cu yields in Fig. 5 and Fig. 6 cannot be compared directly, as the experimental set-ups are different.

halophilic S-oxidizers would be able to maintain the optimal low E_h range to sustain the proton-consuming chemical Cl^--leaching reaction of chalcopyrite. The use of artificial seawater mix resulted in similar results (Supplemental Fig. 1). The Cu dissolution in S-oxidizing pure cultures was more efficient than that in Fe- and S- oxidizing mixed cultures.

Efforts are being made by researchers to explore more halotolerant or halophilic Fe-oxidizers for the purpose of saline water bioleaching. However, such Fe-oxidizers would even further increase the E_h level, which would exceed the optimal E_h range for chemical Cl^--leaching. Rather, exploring halotolerant/halophilic S-oxidizers would sustain and promote the chemical Cl^--leaching reaction of chalcopyrite by keeping the lower E_h range while providing the necessary acidity to the reactions according to [Eq. 5–7]. Therefore, saline water bioleaching should be carefully designed depending on the salt concentration to be used. The activity of conventional bioleaching microorganisms (both Fe- and S-oxidizers) would provide additional benefit when using low salinity waters. On the other hand, when higher salinity waters (such as seawater) are to be used, our attention should be directed to exploit the utility of S-oxidizers.

The commercial significance of seawater (containing ~0.5 M Cl^-) for the purpose of chemical Cl^--leaching of chalcopyrite has been implicated by several studies (Watling, 2014): Lu et al. (2000a) suggested that Cl^- concentrations of >0.5 M did not further improve the leaching rate. Hernández et al. (2015) investigated the effect of seawater-based acidic media in chemical leaching of the chalcopyrite ore at 45 °C and reported that the addition of extra NaCl declined the copper extraction.

In the case of saline water bioleaching, its utility of seawater would come into light provided that a robust salt-tolerant/halophilic S-oxidizer becomes available as an H^+ provider.

4. Conclusions

The findings of this study were schematically summarized in Figs. 5 and 6:

When NaCl-free freshwater media were used for bioleaching, the E_h level sharply increased to >800 mV, and the "high-E_h-bioleaching" became the major leaching mechanism. In such cases, the lowest Cu yields were obtained, as shown in Figs. 5a and 6a. The response of bioleaching reaction to saline water was shown differently in the case of lower (0.5%: Fig. 5) or higher (2%; Fig. 6) salinity:

At lower salinity of 0.5% NaCl, the cell-free chemical Cl^--leaching effect led to a greater Cu yield than the fresh-water "high-E_h-bioleaching" system (Fig. 5b). This low salinity allowed microbial growth, but partial deactivation of Fe-oxidation suppressed the E_h level, wherein synergism between the chemical Cl^--leaching and the "low-E_h-bioleaching" was observed (Fig. 5c).

At higher salinity of 2% NaCl, no active cell growth of tested strains was observed, and thus pre-grown cells were used to mimic the existence of Cl^--tolerant cells. Cell-free chemical Cl^--leaching readily proceeded at low E_h, but quickly ceased upon the depletion of H^+ (Fig. 6b). The existence of bioleaching cells added a positive effect in preventing this acid depletion (Fig. 6c, d). Since chemical Cl^--leaching, which favors low E_h condition, became the main driving force for chalcopyrite leaching at this high salinity, the activity of Cl^--tolerant S-oxidizer alone (Fig. 6c), rather than mixed Fe- and S-oxidizing consortium (Fig. 6d) was shown to play a key role in maximizing the reaction.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.hydromet.2020.105397.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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