Respirometry studies of bioleaching of low grade chalcopyrite ore using six acidophilic strains

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Abstract

Respirometry was used to study the growth and activity of six pure cultures of acidophilic bioleaching strains grown on a concentration series of low grade chalcopyrite ores under various pH and nutrient conditions. *Sulfolobus metallicus*, *Acidithiobacillus ferrooxidans*, *Acidianus brierleyi* and *Leptospirillum ferriphilum* were able to grow on a very low grade ore (equivalent to 0.1% Cu content). However, the two sulphur-oxidizing bacteria *Acidithiobacillus caldus* and *Acidithiobacillus thiooxidans* grew poorly on low grade ore. Growth rates of all strains, except for perhaps *Sulfolobus metallicus* at highest ore grades, displayed growth that was limited by substrate availability on this low grade ore (0.5% Cu content in the ore). The decrease in solution pH from 3.0 to 1.0 enhanced both the cell growth and Cu dissolution. The addition of Fe^{2+} or S^0 as energy substrates facilitated growth and Cu dissolution by the six strains grown on low grade chalcopyrite to some extent.

Keywords: Bioleaching, Biotechnology, Sulphide ores

1. Introduction

The ore grades for various commodities show long-term declining trends over time (Mudd, 2007) which poses severe challenges to the mining industry since low-grade ores are not always amenable to conventional flotation and smelting practices (Brierley, 2008). In particular, most of the largest Cu deposits in the world are low grade "porphyry" deposits. The prevalent form of Cu in these deposits is chalcopyrite ($CuFeS_2$) (Brierley, 2008). In addition, mining of metals invariably produces large amounts of waste rock (Cu-rich tailings) which are generally piled in large dumps on the land surface. Bioleaching has potential for extracting Cu from these substantial low-grade ore and Cu-rich waste rock dumps, since the low and slow recoveries are countered by the low processing costs (Schnell, 1997). However, bioleaching may not always be economic when dealing with very low grade chalcopyrite ore. Mine-site operators may be able to discern an ore grade cut-off beneath which processing of ore becomes uneconomic, but currently, there is no clear understanding of the minimum ore grade required for successful bioleaching of chalcopyrite ore (Plumb *et al*., 2008 a). Although many studies described the bioleaching of chalcopyrite (see the review of Pradhan *et al*., 2008), there is a paucity of information on the ability of leaching microorganisms to grow on low grade chalcopyrite ore. In addition, the ore grades vary widely even among the low-grade chalcopyrite ores. For instance, the chalcopyrite ore from the Kennecott Utah Copper operation in North America is a low grade ore with about 0.5% Cu content, while the average grade of the waste rock dump of Dexing Copper mine has an average 0.08% Cu content (Wu *et al*., 2009), and the waste ore in Yongping Copper Mine, Jiangxi Province of China, has an average Cu content of 0.4% (Zhang and Sun, 2009). The content of mineral sulphides

and reduced iron and sulphur in the ore is important for bioleaching. For lithotrophic bioleaching acidophiles, ferrous iron, reduced sulphur species and mineral sulphides act as the energy sources for growth.

Plumb *et al*. (2008 a) demonstrated the relatively slow growth of 11 bioleaching strains and inability of the strains to achieve high cell densities on low grade chalcopyrite ore. However, the effect of low ore grades on microbial growth kinetics has not been previously studied, in part due to an inability to quantify the small differences in growth kinetics on a series of low-grade ores. Cell counting provides a useful way to monitor the total cell numbers during incubation (Plumb *et al*., 2008 a). However, cell counting is prone to bias caused by adsorption of cells to mineral particles and it is hard to discern and compare microbial growth when the concentration of accessible growth substrate is extremely low. Therefore, a more accurate, reproducible and robust quantitative method is required in order to understand the role of bioleaching microorganisms in bioleaching of low-grade ores. Respirometric methods, in which the headspace concentrations of oxygen and carbon dioxide are measured, have been used for many years to assess the microbial activity of mainly heterotrophic microorganisms. Several investigations have successfully applied respirometry to evaluate the activity of iron- and/or sulphide-oxidizing microorganisms (Sampson and Blake, 1999; Harahuc *et al*., 2000; du Plessis *et al*., 2001; Petersen *et al*., 2009).

The objectives of this research were to quantify the growth kinetics and activity of pure cultures of six bioleaching strains when grown on low grade chalcopyrite ore at a

range of different ore grades by using respirometry, and to evaluate the effects of pH and nutrient availability on microbial growth and activity when the ore grade was low.

2. Materials and methods

2.1. Microbial strains and routine culture conditions

The six microbial strains used in this study were *Acidithiobacillus ferrooxidans* DSM 584, *Acidianus brierleyi* DSM 1651^T and *Sulfolobus metallicus* DSM 6482^T, which oxidise both Fe^{2+} and S^0; *Leptospirillum ferriphilum* ATCC 49881^T, which oxidises Fe^{2+} only; *A. thiooxidans* DSM 14887^T and *A. caldus* DSM 8584^T, which oxidise S^0 only. The *A. ferrooxidans* and *A. thiooxidans* were incubated at 28°C, *L. ferriphilum* and *A. caldus* were incubated at 37°C, *A. brierleyi* and *S. metallicus* were incubated at 65°C. All these strains were grown on the same 9K medium (K_2HPO_4 0.5 g, $MgSO_4.7H_2O$ 0.5 g, $(NH_4)_2SO_4$ 3.0 g, KCl 0.1 g, $Ca(NO_3)_2$) supplemented with either ferrous iron or elemental sulphur as reported previously (Franzmann *et al*., 2005).

2.2. Low grade ores

Low grade chalcopyrite ore used in these experiments was obtained from Rio Tinto. Based on QEMSCAN analysis the ore contained (wt%): chalcopyrite ($CuFeS_2$) 1.3%, pyrite (FeS_2) 0.2%, with other Cu sulphides bornite (Cu_5FeS_2), covellite (CuS) and chalcocite (Cu_2S) only present at less than 0.05% in the ore. The elemental composition of the ore, as analysed by ICP-AES, was (wt %): Cu 0.5, Fe 3.0, S 0.7,

S^{2-} 0.4, Na 1.2, K 5.4, Mg 2.9, Ca 1.4, Al 6.7, Si 24.9. Chalcopyrite in the ore was encapsulated within gangue minerals (Plumb *et al*., 2008a). Therefore, continued acid (18 M H_2SO_4) addition was necessary to maintain the desired test pH. Quartz used in this study was bought from Cook Industrial Minerals Pty Ltd. Quartz was used with the low grade chalcopyrite to dilute the chalcopyrite to a series of even lower grades. Both the chalcopyrite ore and quartz were ground and sieved and the particle size fractions used in this study were < 53 µm.

2.3. Experimental setup and analyses

Reactor vessels (300 mL Schott Duran bottles) containing 100 mL of 9K medium supplemented with 10% weight:volume (wt:vol) different grades of chalcopyrite ore as sole energy source were used in this study. The bottles were heated from the base and stirred with magnetic stirrers as shown in Figure 1. Six different grades of chalcopyrite ore (0, 0.1, 0.2, 0.3, 0.4 and 0.5% Cu content) were used to test the effect of ore grade on microbial growth kinetics at pH 1.8. The microbial growth kinetics at different pH conditions (pH 1.0, 1.8 and 3.0) were also investigated with the highest ore grade (0.5% Cu). To test the effect of additional energy substrates on cell growth and Cu leaching from the low grade ore, either 20 g ferrous sulphate was added for *A. ferrooxidans* and *L. ferriphilum* or 0.5 g elemental sulphur was added for the rest of test strains supplemented with 10% wt:vol chalcopyrite ore (0.5% Cu content). Abiotic leaching of 10% wt:vol chalcopyrite ore (0.5% Cu content) at pH 1.8 was conducted at 28°C, 37°C and 65°C with two replicate flasks at each temperature.

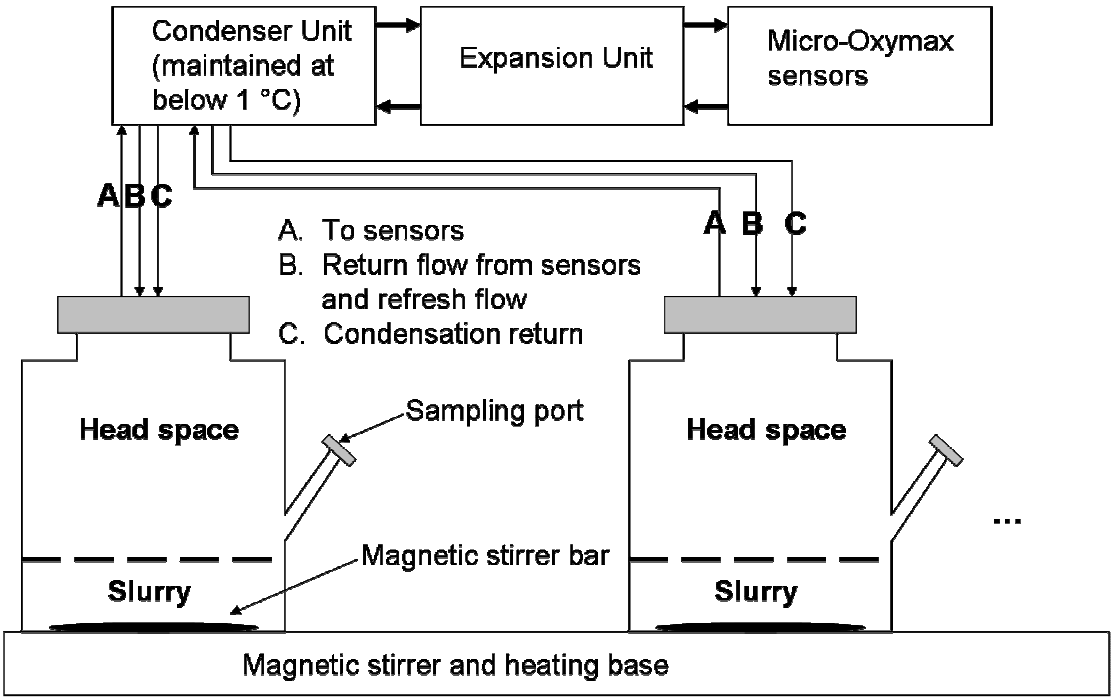


Figure 1. Experimental setup to quantify the growth and activity of microorganisms by respirometry during the bioleaching of a range of different ore grades under a variety of operating conditions.

Respirometry was used in this study to evaluate both the microbial growth (based on CO_2 consumption) and activity (based on O_2 consumption). The Micro-Oxymax respirometer (Columbus Instruments, Ohio, USA) was equipped with an oxygen sensor (C/N CiTiceL®, City Technology Ltd, UK. Range from 19.3% to 21.5%) and a carbon dioxide sensor (Gascard II®, Edinburgh Instruments Ltd, UK. Range from 0% to 0.9%). Oxygen and carbon dioxide concentrations in the headspace of the respirometer reactor vessels were measured at 126 min intervals. The air in the headspace was replenished periodically. The threshold for air replenishment was set at 0.25%, so that the air in the bottles was replenished automatically if the O_2 or CO_2 level rose 0.25% above or fell 0.25% below the starting levels.

Prior to inoculation, cells of all the six strains were collected by centrifugation at 10109 g for 20 min and then washed twice using 9K medium in order to prevent carry-over of Fe^{2+} and S^0 from the stock cultures. The inoculum was adjusted to provide initial cell numbers of approximately 2×10^7 cells/mL (except *A. thiooxidans* which had an initial cell number of 8×10^6 cells/mL). Cell numbers were monitored using a Thoma counting chamber and a phase contrast microscope (OLYMPUS CX41RF, Olympus Corporation, Japan). Samples were collected from the cultures in a microcentrifuge tube, vortexed briefly and then centrifuged at low speed (approximate 700 g for 5 min) to remove ore particles prior to counting (Plumb *et al*., 2008 a).

The Fe^{2+} concentration was measured using the potassium dichromate titration method as reported previously (Plumb *et al*., 2008 a). Cu concentration was determined using atomic absorption spectroscopy (Varian SpectrAA-40, Varian, Inc., USA).

2.4. Stoichiometry

The chemical leaching of chalcopyrite by either oxygen or ferric iron can be represented by reactions (1) and (2) (Pradhan *et al*., 2008; Cordoba *et al*., 2008):

$$CuFeS_2 + 4H^+ + O_2 \rightarrow Cu^{2+} + Fe^{2+} + 2S^0 + 2H_2O \quad (1)$$

$$CuFeS_2 + 4Fe^{3+} \rightarrow Cu^{2+} + 5Fe^{2+} + 2S^0 \quad (2)$$

The oxidation of Fe^{2+} and S^0 and formation of jarosite can be represented by reactions (3), (4) (Pradhan *et al*., 2008; Plumb *et al*., 2008 b) and (5) (van Aswegen *et al*., 2007), respectively:

$$4Fe^{2+} + O_2 + 4H^+ \xrightarrow{\text{Microbial and / or Chemical}} 4Fe^{3+} + 2H_2O \quad (3)$$

$$S^0 + 3/2O_2 + H_2O \xrightarrow{\text{Microbial}} SO_4^{2-} + 2H^+ \quad (4)$$

$$3Fe_2(SO_4)_3 + 12H_2O + M_2SO_4 \rightarrow 2MFe_3(SO_4)_2(OH)_6 + 6H_2SO_4 \quad (5)$$

Where M^+ is K^+, Na^+, NH_4^+ or H_3O^+

The CO_2 uptake and biomass synthesis by autotrophic microorganisms can be represented by the following reaction (6) where molar ratios of the elements C, H, O and N present are normalised to the number of moles of carbon, (McCarty, 1975; Blight and Ralph, 2008).

$$HCO_3^- + 0.25NH_4^+ + 4.86H^+ + 4.11e^- \rightarrow CH_{1.70}O_{0.42}N_{0.25} + 2.58H_2O \quad (6)$$

Using calcite as an example, carbon dioxide may be released from carbonate rocks in the ore as shown in reactions (7), (8), and (9).

$$CaCO_3 \rightarrow Ca^{2+} + CO_3^{2-} \quad (7)$$

$$CO_3^{2-} + H^+ \leftrightarrow HCO_3^- \quad (8)$$

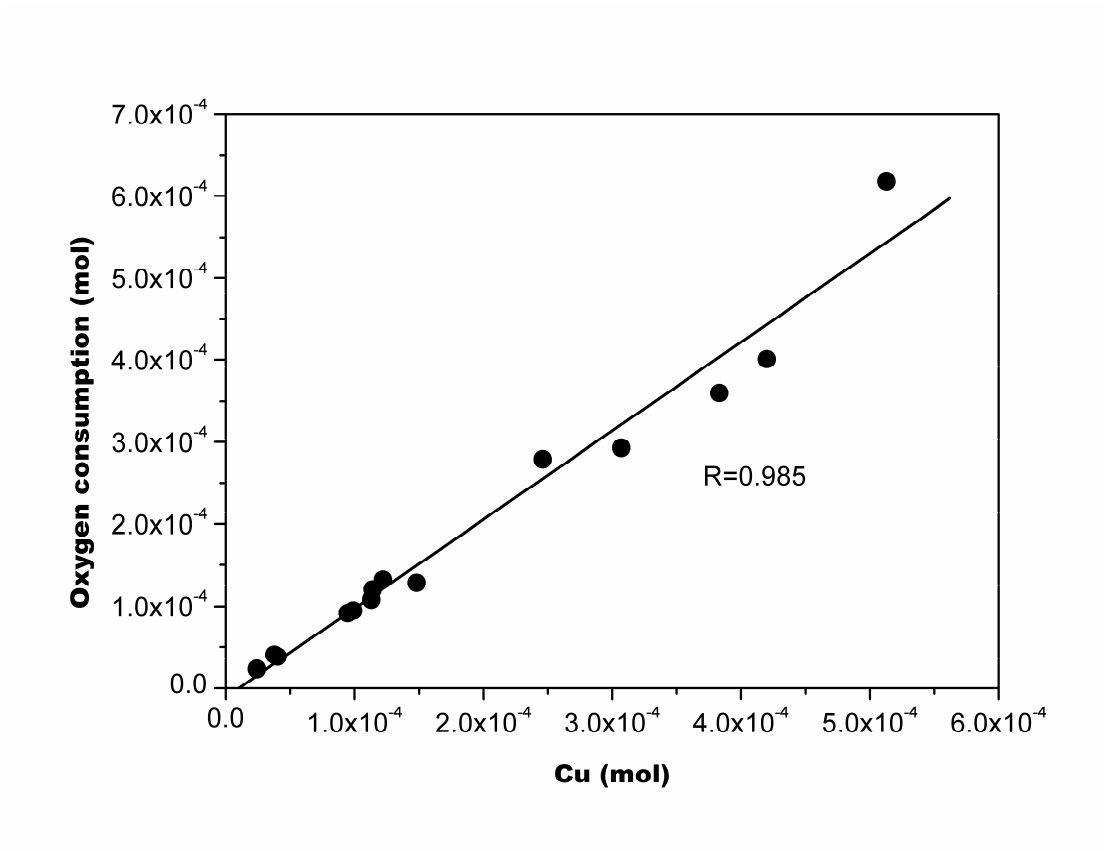
$$HCO_3^- + H^+ \leftrightarrow CO_2 + H_2O \quad (9)$$

Therefore, although the carbon dioxide consumption is mainly caused by microbial CO_2 uptake and cell synthesis (Reaction 6), the release of carbonate from the ore (Reaction 7) and solution pH (Reactions 8 and 9) also affect the observed carbon dioxide consumption. The observed oxygen consumption is a sum of oxygen consumed for ore oxidation (Reaction 1), chemical oxidation of ferrous iron (Reaction 3) and microbial oxidation of ferrous iron and/or sulphur (Reactions 3 and 4).

3. Results and discussion

3.1. Oxygen consumption

The correlation between oxygen consumption and the amount of leached Cu during abiotic leaching of chalcopyrite is presented in Figure 2. A linear correlation was found between oxygen consumption and Cu leached. The observed molar ratio of oxygen consumption to leached Cu was close to approximately 1:1, which corresponded well with reaction stoichiometry (reaction 1).



215

216 Figure 2. Plot of oxygen consumption and leached Cu during abiotic leaching of 10% w/v

217 chalcopyrite ore at pH 1.8.

218

219 Oxygen consumption rate (r_{O_2}) has been used as an indicator of the rate of mineral

220 ore leaching (Boon *et al*., 1995), and microbial oxidation activity. When linked to

221 conversion factors for microbial yield from ferrous iron ($Y_{Fe^{2+}}$), the oxygen

222 consumption rate has also been used to describe microbial growth during bioleaching

223 processes (Crundwell, 1995; Boon and Heijnen, 1998; Breed *et al*., 1999). However,

224 in this study, the correlation between oxygen consumption rate and microbial growth

225 rate was not good (data not shown).

226

227 **3.2. CO_2 consumption**

228

The growth kinetics of autotrophic bioleaching microorganisms were analysed by measuring the consumption of CO_2 during low grade chalcopyrite bioleaching in the respirometry assay. The CO_2 consumption rates were consistent with the growth rates determined by cell counting as shown in Figure 3 for *Sulfolobus metallicus* as an example. Therefore, the carbon dioxide consumption rate can be used as an indicator for microbial growth even with the very low grade chalcopyrite. The results were also in agreement with previous reports where carbon dioxide utilization rate could be used to estimate the bacterial growth rate (Boon *et al*., 1994; Breed *et al*., 1999).

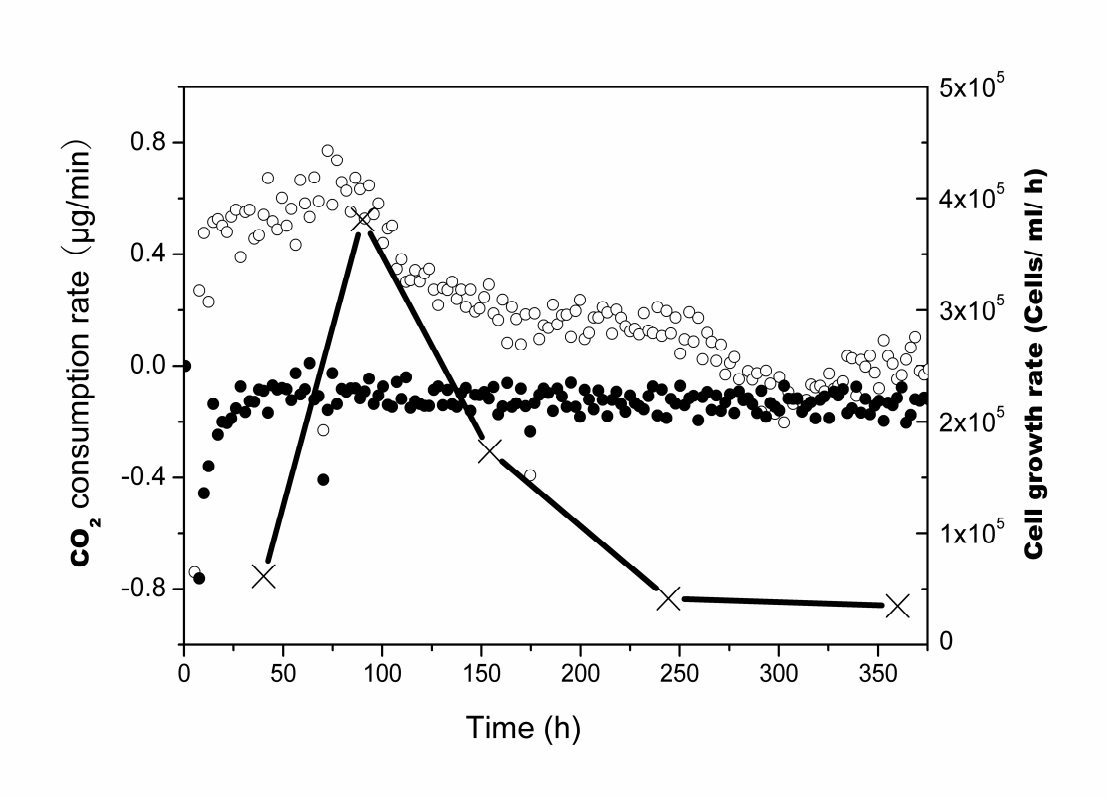


Figure 3. Carbon dioxide consumption rate (○) and cell growth rate (×) of *Sulfolobus metallicus* DSM 6482^T culture with 10% w/v chalcopyrite ore at pH 1.8 at 65°C. The CO_2 consumption of, or production in the abiotic control (●) with 10% w/v chalcopyrite ore at pH 1.8 at 65°C is shown for a comparison.

In contrast to the earlier studies which used mineral concentrate, low-grade chalcopyrite ore was used in this study. Therefore the effects of carbonate released from the gangue minerals (Reaction 7) on the solution pH value (Reactions 8 and 9) and on the observed carbon dioxide consumption rate may have been greater in this study. The carbon dioxide consumption rate in the abiotic control bottle with low grade chalcopyrite remained slightly negative (near -0.1 µg/min, i.e. net CO_2 production) during most of the experiment which indicated slow and stable release of CO_2 from the ore. Therefore, the effect of carbonate release from the ore on measurement of carbon dioxide consumption by leaching microorganisms was negligible during most of the experiment. Temporarily higher CO_2 release rates were observed during the initial 20 hours after the ore was exposed to acidic leaching solution, and after each addition of sulphuric acid which was added to maintain a stable solution pH. The pH of the leaching solutions fluctuated between 1.8 and 3.4, and had to be readjusted repeatedly to the desired value.

3.3. Effect of ore grade on microbial growth kinetics

A dilution series of low grade chalcopyrite ore was used to test the effect of ore grade on microbial growth kinetics. Figure 4 shows the effects of ore grade on microbial growth measured as maximum CO_2 consumption rate. For all the strains the maximum carbon dioxide consumption rate increased with elevated chalcopyrite ore grade, which, as expected, reflects a positive correlation between ore grade and microbial growth rate. However, for some strains the effect of ore grade on microbial

growth was stronger than for others. Growth rates of all strains, except for perhaps *Sulfolobus metallicus* at highest ore grades, displayed growth that was limited by substrate availability on this low grade chalcopyrite ore (0.5% Cu content).

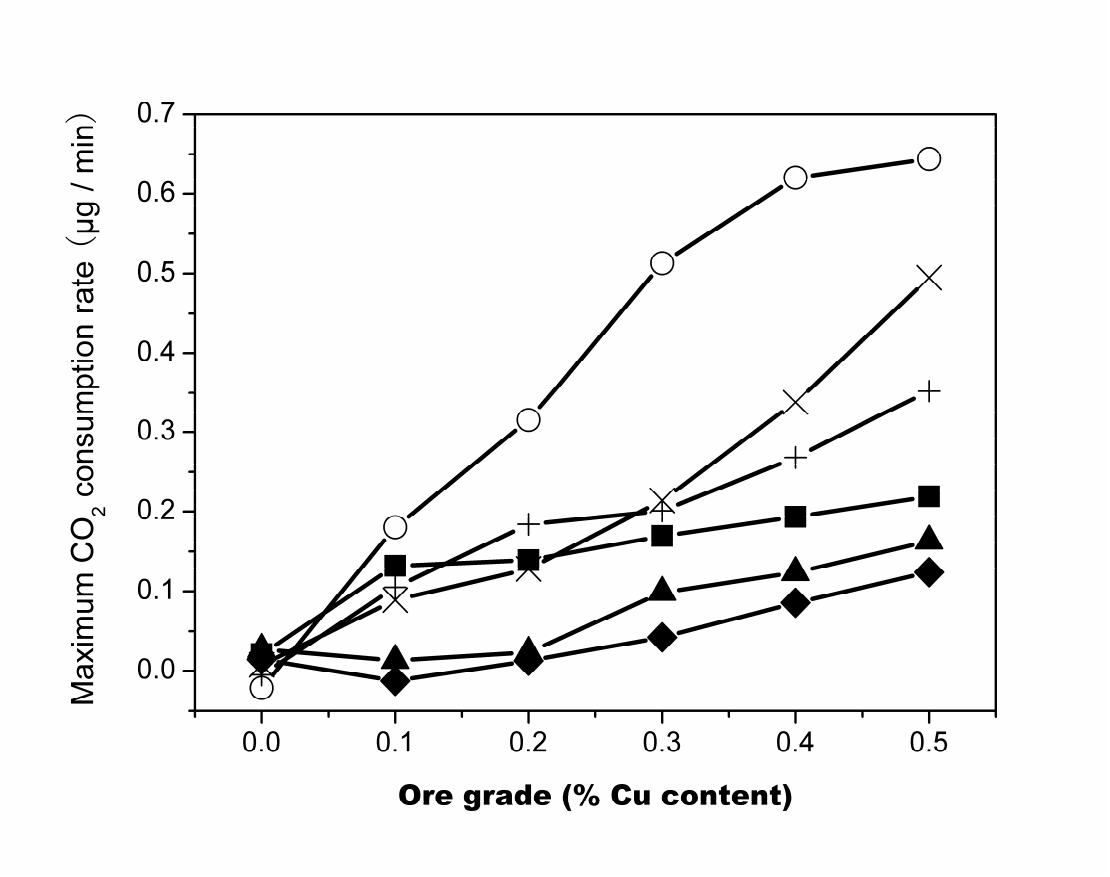


Figure 4. The effect of low grade chalcopyrite ore grade on microbial growth measured as maximum CO_2 consumption rate. In order to obtain even lower ore grades, quartz was mixed with the chalcopyrite ore. The ore grades were represented as Cu content in the ore mixture. The pH of leaching solutions were all set to pH 1.8 and controlled periodically during experiment. Meaning of symbols: ○ *Sulfolobus metallicus* DSM 6482^T at 65°C; × *Acidithiobacillus ferrooxidans* DSM 584 at 28°C; + *Acidianus brierleyi* DSM 1651^T at 65°C; ■ *Leptospirillum ferriphilum* ATCC 49881^T at 37°C; ▲ *Acidithiobacillus thiooxidans* DSM 14887^T at 28°C; ♦ *Acidithiobacillus caldus* DSM 8584^T at 37°C.

283

284 The effect of ore grade was smallest on the maximum CO_2 consumption of *L.*
285 *ferriphilum*. For this strain the maximum CO_2 consumption rate with 0.5% Cu content
286 was only 1.65 times higher than with 0.1% Cu content. Highest maximum CO_2
287 consumption rates were obtained for thermophilic *S. metallicus* for all ore grades
288 tested. With *S. metallicus*, the effect of ore grade on CO_2 consumption rates was also
289 more profound (largest difference between CO_2 consumption rates with 0.1% Cu
290 content versus 0.5% Cu content) when compared to other strains.

291

292 The relatively high chemical leaching rate of chalcopyrite at 65°C may have enhanced
293 the growth of *S. metallicus* by generating relatively high concentrations of both
294 ferrous iron and reduced sulphur compounds as growth substrates. *S. metallicus* has
295 high affinity for ferrous iron (Norris, 1992) and bioleaching of chalcopyrite is
296 enhanced at thermophilic temperatures (Stott *et al*., 2003). Another thermophilic
297 strain *A. brierleyi* also grew actively on the low grade chalcopyrite ore. Although its
298 affinity for ferrous iron is also greater than that of *A. ferrooxidans* (Norris, 1992), the
299 maximum CO_2 consumption rate was lower than that of *A. ferrooxidans* when the ore
300 grade was higher than 0.3% Cu content in the ore.

301

302 The growth of *A. ferrooxidans* was strongly affected by the ore grade. The maximum
303 CO_2 consumption rate rose noticeably with an increase in ore grade. *A. ferrooxidans*
304 had the second highest growth rate at higher ore grades (0.3-0.5% Cu content),
305 whereas at low ore grades (0.1-0.2% Cu content), both *A. brierleyi* and *L. ferriphilum*
306 grew faster than *A. ferrooxidans*. It has generally been considered that *A. ferrooxidans*
307 has a faster growth rate than *L. ferriphilum* during the initial stages of a bioleach when

the redox potential is low (Rawlings *et al*., 1999). However, *L. ferriphilum* has a higher affinity for Fe^{2+} than *A. ferrooxidans* (apparent Michaelis Menten constant K_m 0.3 mM for Fe^{2+} oxidation by *L. ferriphilum* versus 1.3 mM for *A. ferrooxidans*) and is inhibited less by Fe^{3+} (competitive inhibition constant K_i 43 mM for *L. ferriphilum* versus 3.1 mM for *A. ferrooxidans*) (Norris *et al*., 1988). Despite the fact that *L. ferriphilum* has high affinity for Fe^{2+} and was not much affected by the ore grade, its maximum growth rates were lower than those of *A. ferrooxidans* with ore grades of 0.3-0.5% Cu content. It may be due to the lesser energy obtained from oxidising Fe^{2+} when compared with S^0, so that biomass growth (CO_2 uptake) would be less for *L. ferriphilum* than the S^0 oxidisers or mixed oxidisers. It may also be due partly to the lower tolerance of *L. ferriphilum* to dissolved Cu or formation of passivating S^0 layers as *L. ferriphilum* is unable to oxidise sulphur or sulphur compounds (Bosecker, 1997).

The maximum CO_2 consumption of two sulphur-oxidizing bacteria *A. caldus* and *A. thiooxidans* correlated positively with ore grade, but the overall growth rates of these strains were very slow on the low grade ore. With ore grades of 0.1-0.2% Cu content, the maximum CO_2 consumption was nearly zero. Also the increase of cell numbers was nonexistent or very small throughout the two week incubation period. Although Plumb *et al*. (Plumb *et al*., 2008 a) demonstrated that the pure cultures of both *A. caldus* and *A. thiooxidans* could grow on 5 % w/v crude chalcopyrite ore (equivalent to the 0.25% Cu content in this study), the present study showed that the two strains did not grow well in pure culture with ore grades of 0.1-0.2% Cu content.

In this study, the ore was the sole accessible energy source for these bioleaching strains at the beginning of the experiment since nearly all of the carry-over Fe^{2+} and

S^0 were removed from stock cultures of each strain prior to inoculation. Most of the tested strains have the capability to grow on chalcopyrite ore even at 0.1% Cu content in the ore. The relationship between microbial growth rate and the concentration of limiting nutrient has been extensively described (e.g., Shehata and Marr, 1971; Ferenci, 1999). In the case of bioleaching, mineral particles are not the directly accessible substrates for microorganisms; only the ferrous iron and sulphur compounds dissolved from the mineral particles can be utilized by microorganisms directly (Hansford and Vargas, 2001; Rohwerder *et al*., 2003). Thus, mineral leaching kinetics also contributes to the effect of ore grades on microbial growth.

3.4. Effect of pH on microbial growth and ore leaching

Although the CO_2 consumption rate indicated microbial growth (Figure 3), it could not be used to compare growth in response to pH changes because of the effect of different pH on CO_2 concentrations. Thus, the growth rates calculated from cell counting data were used to compare the growth of the tested strains at different pH conditions. The growth rates of test strains on chalcopyrite ore with 0.5% Cu content at different pH values are presented in Figure 5.

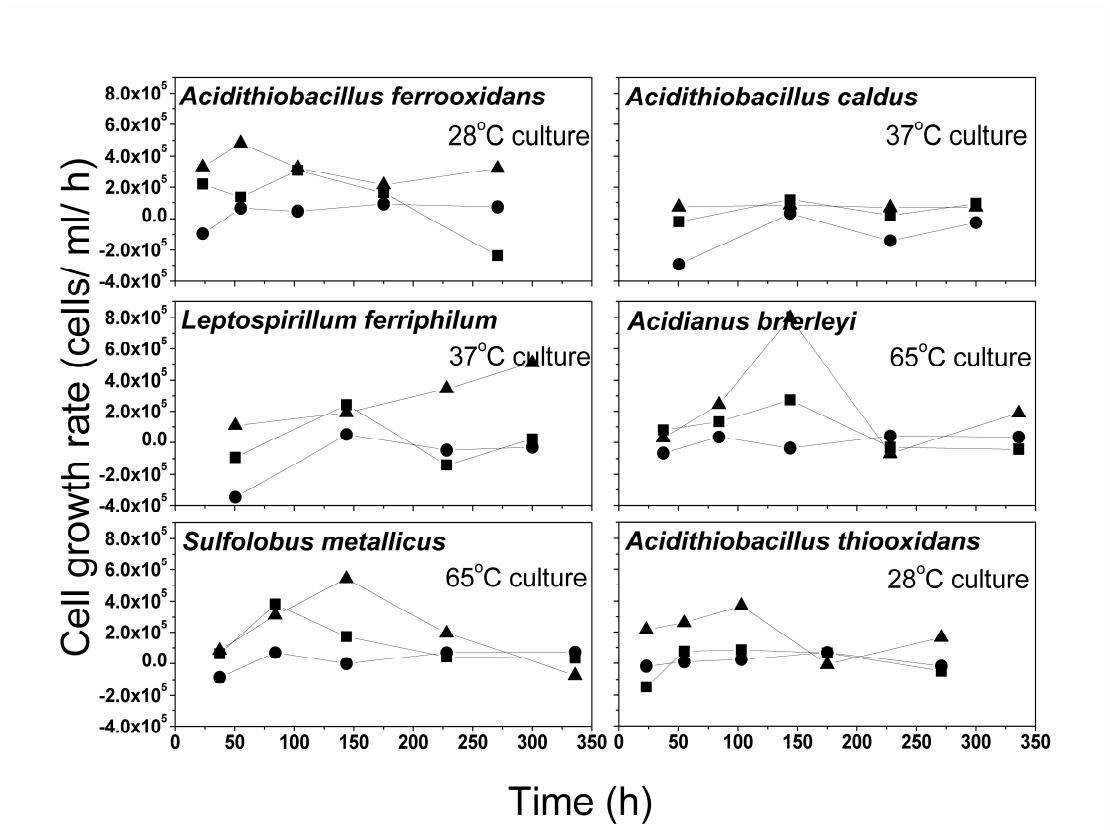
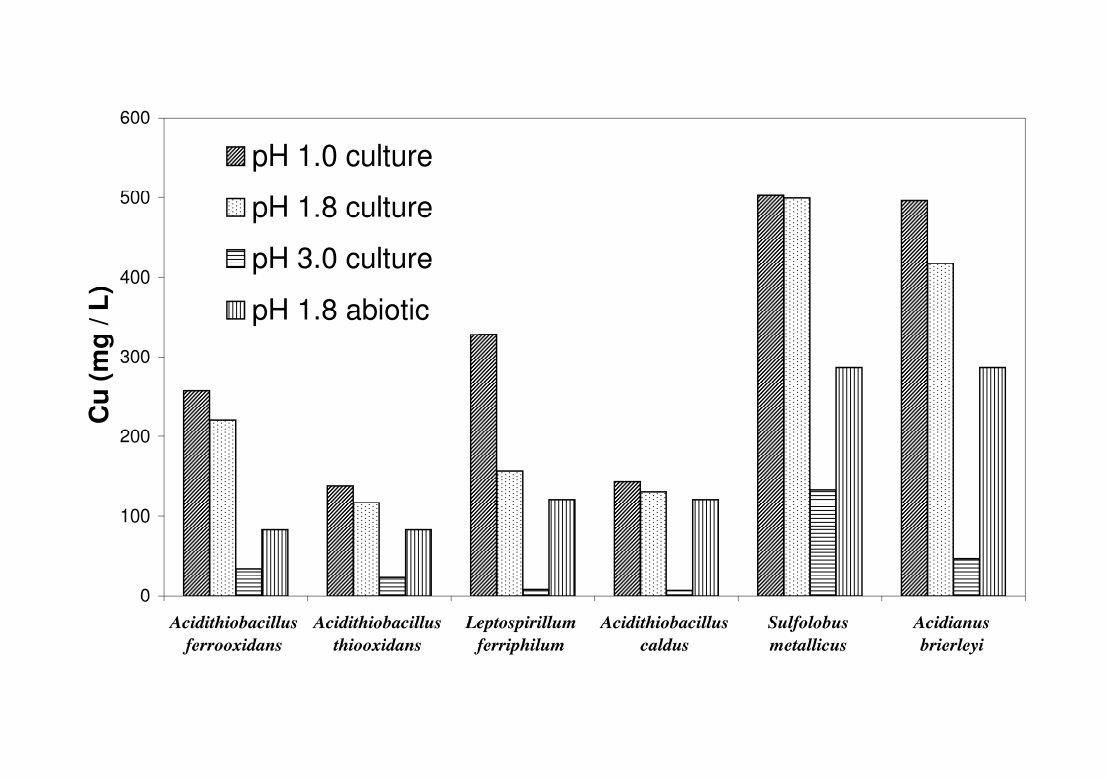


Figure 5. The effects of pH on the cell growth rate of the six strains grown on chalcopyrite ore with 0.5% Cu content. Different symbols represent various target pH conditions: ▲ pH 1.0, ■ pH 1.8 and ● pH 3.0.

All test strains grew poorly at the target pH 3.0 with cell numbers decreasing during the first 50 hours of incubation. Because the low grade chalcopyrite ore used in this experiment was acid-consuming, the leaching solution pH increased to around pH 5 within one day after inoculation at pH 3.0. Therefore, this rapid pH increase probably resulted in cell death. Compared to the other strains tested, cell numbers of *L. ferriphilum* and *A. caldus* decreased most at the commencement of the experiment. After 96 h, the cell count was only 5% and 6% of that originally inoculated with *L. ferriphilum* and *A. caldus*, respectively. Although a subsequent increase in cell

numbers was observed for all the strains grown at pH 3.0, the growth rates were relatively low when compared with the growth rates at lower initial pH values.

Lower pH (pH 1.0) favoured the growth of all test strains on the low grade chalcopyrite ore. Given the fact that the optimum pH values of growth for the six test strains on iron or sulphur are generally at a higher (pH 1.5–2.0 for *A. thiooxidans* and *A. caldus*, pH 1.0–1.5 for *A. brierleyi* (Plumb *et al*., 2008 b), pH 1.1–1.5 for *L. ferriphilum* (Hawkes *et al*., 2005), pH 1.3–1.7 for *S. metallicus* (Rawlings, 2002), pH 3.0 for *A. ferrooxidans* (Ehrlich and Newman, 2008)), the acceleration of growth rate may have been facilitated by the increased dissolution of chalcopyrite in the more acidic leaching solution. This would provide more available substrates (Fe^{2+} and S^0) for the microbial growth and would lead to the consumption of more protons at the mineral surface by the acid consuming ore. The highest growth rates for all tested strains were observed at pH 1.0. The thermophilic strain *A. brierleyi* grew particularly well at pH 1.0 when compared to higher pH values, which was in good agreement with the preference of *A. brierleyi* to grow at the lower pH (Plumb *et al*., 2008 b). The effect of pH on the growth rate of sulphur-oxidizing *A. caldus* was not as clear as for the other strains.



387

388 Figure 6. Dissolution of Cu after 14 days of bioleaching of low grade chalcopyrite (0.5% Cu
389 content) with pure cultures at different pH conditions and during abiotic leaching at pH 1.8.

390

391

392 Figure 6 shows the dissolved Cu concentrations in the inoculated and uninoculated
393 bottles at different target pH values after 14 days. The pH of the leaching solution
394 significantly affected the efficiency of bioleaching of the low grade chalcopyrite ore.
395 Highest Cu dissolution occurred at pH 1.0 for all test strains. In addition, the Cu
396 dissolution from bioleaching of chalcopyrite ore at pH 3.0 was extremely low when
397 compared with the other pH conditions, and even less than the amount of Cu leached
398 under abiotic conditions at pH 1.8. Although lower pH enhanced the Cu leaching, the
399 effect of pH was different for each of the tested strains. Bioleaching of this low grade
400 chalcopyrite ore by *L. ferriphilum* was strongly affected by the pH of the leaching
401 solution. The Cu dissolution at pH 1.0 showed an approximately two-fold increase

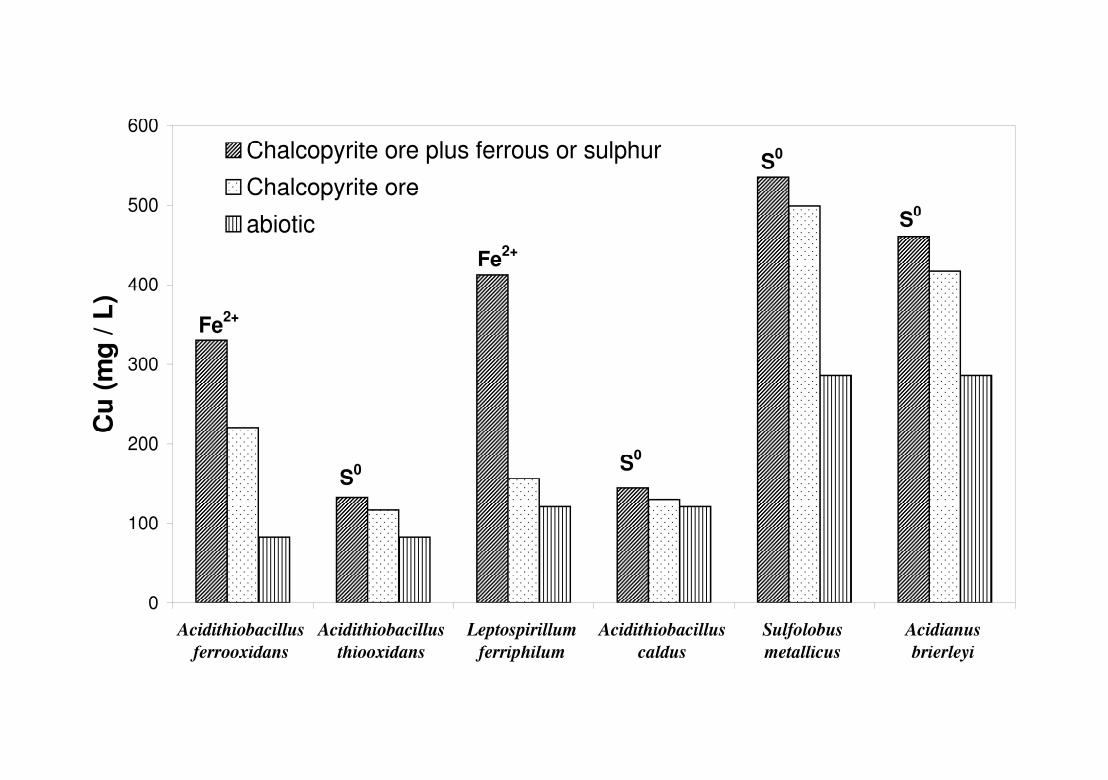
when compared to that at pH 1.8, and the Cu dissolution at pH 1.8 was nearly 20 times greater than at pH 3.0. In contrast, the increase of pH from 1.0 to 1.8 did not have a great effect on Cu leaching by *S. metallicus* and by sulphur-oxidizing *A. caldus* and *A. thiooxidans*.

Abiotic leaching of chalcopyrite ore was performed at pH 1.8 as a control experiment. The enhancement of chalcopyrite leaching by *A. ferrooxidans* when grown at the same pH was most profound, with Cu leaching at 2.7 times greater than abiotic leaching. The contribution of the two thermophilic strains *A. brierleyi* and *S. metallicus* to facilitate Cu dissolution were obvious although the enhancement was not as great as with *A. ferrooxidans*, given the relatively higher rate of abiotic Cu leaching at 65°C. Bioleaching using *L. ferriphilum* at pH 1.8 was not very effective when compared to abiotic leaching, which may be due to the lower pH growth optimum of this species. The two sulphur-oxidizing bacteria *A. caldus* and *A. thiooxidans* played a minor role in bioleaching of low grade chalcopyrite ore, possibly due to their inability to oxidise Fe^{2+}. There were only 1.1 and 1.4-fold increases in the Cu extraction for *A. caldus* and *A. thiooxidans* compared to that obtained for abiotic leaching.

3.5. Effect of additional energy substrates

As the low grade chalcopyrite ore is probably a poor substrate for microbial growth, some bottles with the ore grade of 0.5% Cu content were supplemented with additional energy substrates to test their effects on cell growth and Cu leaching. The

addition of growth substrates enhanced the CO_2 consumption rates of all test strains when grown on chalcopyrite ore (Table 1). The maximum CO_2 consumption rates of the two sulphur-oxidizing bacteria *A. caldus* and *A. thiooxidans* were 10 times higher with additional elemental sulphur than rates obtained with chalcopyrite ore as the sole growth substrate. However, the additional S^0 substrate only slightly increased the Cu leaching with these two strains or with the two thermophilic strains *A. brierleyi* and *S. metallicus* when compared to the leaching of chalcopyrite ore without sulphur supplementation (Figure 7). Nevertheless, the addition of extra S^0 substrate enhanced the generation of acid by these sulphur-oxidizing microorganisms and thus greatly reduced the need of sulphuric acid addition with a decrease of 61% for *A. caldus*, 67% for *A. thiooxidans*, 44% for *A. brierleyi* and 60% for *S. metallicus*, respectively. The addition of ferrous sulphate enhanced both microbial growth and Cu leaching by *A. ferrooxidans* and *L. ferriphilum*. The Cu leaching by *L. ferriphilum* increased by 2.6-fold when ferrous iron was added to the culture when compared to leaching of the ore without iron supplementation.



443

444 Figure 7. Effect of extra growth substrates on the Cu dissolution after 14 days of leaching of
445 low grade chalcopyrite ore by pure cultures of test strains or by abiotic leaching at pH 1.8.

446

447 Table 1. The effect of additional energy substrates on the maximum CO_2 consumption rates
448 by pure cultures at target pH 1.8.

Maximum CO_2 consumption rates (µg/min)	*A. ferrooxidans*	*A. thiooxidans*	*L. ferriphilum*	*A. caldus*	*S. metallicus*	*A. brierleyi*
Low grade chalcopyrite ore	0.50	0.16	0.22	0.12	0.64	0.35
Low grade chalcopyrite ore plus additional substrate	1.15	1.69	0.67	1.63	0.75	0.43

Although both the cell growth and Cu extraction were improved by the addition of ferrous iron as an energy source, the higher iron concentrations in the leachate may hinder the recovery of Cu from solution in industrial settings. Moreover, the addition of the iron and the removal of the extra iron in downstream solution processing would increase the operational costs (Jergensen, 1999). Therefore, the economic feasibility of the addition of ferrous sulphate to enhance the microbial growth and Cu dissolution from the low grade chalcopyrite ore would need to be carefully evaluated.

4. Conclusions

The results of this study suggested that the observed CO_2 consumption indicated the subtle differences in growth kinetics of autotrophic bioleaching strains grown on low grade ore. Due to the paucity of accessible energy sources when cells were grown on low grade ore, the effect of limited substrate on microbial growth was observed for all test strains. Growth of *S. metallicus*, *A. ferrooxidans*, *A. brierleyi* and *L. ferriphilum* could be detected even at the lowest ore grade tested (0.1 % Cu in the ore mix), although the growth rate was very low. The minimum grade of ore which still supported the growth of *A. caldus* and *A. thiooxidans* was 0.3% Cu content in the ore. A decrease in solution pH increased cell growth and Cu solubilisation from low grade chalcopyrite ore. The addition of sulphur as an extra substrate resulted in slightly increased Cu extraction. Although the addition of ferrous sulphate facilitated Cu dissolution, the addition may complicate the downstream Cu recovery and may not economically feasible.

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