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Two-Step Oxidation of Refractory Gold Concentrates with Different Microbial Communities

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Bio-oxidation is an effective technology for treatment of refractory gold concentrates. However, the unsatisfactory oxidation rate and long residence time, which cause a lower cyanide leaching rate and gold recovery, are key factors that restrict the application of traditional bio-oxidation technology. In this study, the oxidation rate of refractory gold concentrates and the adaption of microorganisms were analyzed to evaluate a newly developed two-step pretreatment process, which includes a high temperature chemical oxidation step and a subsequent bio-oxidation step. The oxidation rate and recovery rate of gold were improved significantly after the two-step process. The results showed that the highest oxidation rate of sulfide sulfur could reach to 99.01 % with an extreme thermophile microbial community when the pulp density was 5%. Accordingly, the recovery rate of gold was elevated to 92.51%. Meanwhile, the results revealed that moderate thermophiles performed better than acidophilic mesophiles and extreme thermophiles, whose oxidation rates declined drastically when the pulp density was increased to 10% and 15%. The oxidation rates of sulfide sulfur with moderate thermophiles were 93.94% and 65.73% when the pulp density was increased to 10% and 15%, respectively. All these results indicated that the two-step pretreatment increased the oxidation rate of refractory gold concentrates and is a potential technology to pretreat the refractory sample. Meanwhile, owing to the sensitivity of the microbial community under different pulp density levels, the optimization of microbial community in bio-oxidation is necessary in industry.

Keywords: Bio-oxidation, mesophiles, moderate thermophiles, extreme thermophiles, two-step process

Introduction

Bio-oxidation of refractory gold-bearing sulfide concentrates prior to cyanidation as a pretreatment step has proven to be economically competitive and environmentally friendly compared with conventional methods [7, 8, 11, 26]. As a promising technology, bio-oxidation operations have been commissioned in a number of countries [33, 35]. However, the rate of bio-oxidation is slow, often lasting 4−6 days. Such long residence time causes excessive operational cost, so it is desirable to enhance the rate of bio-oxidation. The main available processes of intensifying bio-oxidation for refractory gold are to develop new technology approaches and optimize the microbial community [16, 38].

It has been reported that the chemical oxidation kinetics of pyrite via ferric ion (Eqs. (1)−(2)) was greatly influenced by temperature [6, 24, 31]. The reaction rate increases as temperature rises, and it would be relatively faster at high temperature (80−100ºC). However, these temperatures would be detrimental to the mixed cultures. The regeneration of ferric ion would be influenced. In addition, the treatment capacity of chemical oxidation was limited, and the oxidation level could be satisfactory only at lower pulp density [6, 21].

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 2SO_4^{2-} + 15Fe^{2+} + 16H^+ \quad (1)$$

$$FeS_2 + 2Fe^{3+} \rightarrow 3Fe^{2+} + 2S^0 \quad (2)$$

A novel two-step approach can overcome these disadvantages described above. This approach comprised a high-temperature ferric ion oxidation step (chemical oxidation step) and a subsequent bio-oxidation step (biological oxidation step). In the chemical oxidation step, the reaction temperature can be elevated to 80ºC, which would improve the oxidation rate significantly (Eqs. (1)−(2)). In the biological leaching step, ferric ion is regenerated and the sulfide is oxidized (Eqs. (1)−(4)). By applying physical separation of the chemical oxidation and biological oxidation steps, favorable conditions for both the chemical oxidation process and microbial activity can be created [18, 28].

$$4Fe^{2+} + 4H^+ + O_2 \xrightarrow{\text{microbes}} 4Fe^{3+} + H_2O \quad (3)$$

$$2S^0 + 3O_2 + 2H_2O \xrightarrow{\text{microbes}} 2H_2SO_4 \quad (4)$$

Because the oxidation reactions of refractory gold concentrates are exothermic, the most promising temperature of this technological process is 40−55ºC. A mixed culture of moderate thermophiles dominates at these conditions [28]. The BIOX process developed by Gencor, operated at 40–45ºC with moderate thermophiles, is used by most of the stirred tank operations [12]. Mesophilies such as *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, and *Leptospirilum ferriphilum* are the most commonly used microbes in the process of bioleaching [15]. In addition, the toxic effect of iron in the bio-oxidation process would become obvious with the accumulation of iron ion especially under high pulp density. Ciftci and Akcil [11] have previously reported that mesophiles exhibited greater iron tolerance than moderate thermophiles and extreme thermophiles. Extreme thermophiles, with their metabolic activity at high temperatures up to 65ºC, have great potential to enhance the kinetic of bio-oxidation at a pulp density lower than 5%.

However, the effect of microbial community composition and pulp density on the bio-oxidation efficiency of refractory gold concentrate via a two-step approach was not investigated before. In this study, bio-oxidation experiments were carried out using a mixed culture of meophiles, moderate thermophiles, and extreme thermophiles on refractory gold concentrates. The oxidation efficiency and performance of the bacterial/archaeal cultures were investigated under different pulp densities via a two-step approach.

Materials and Methods

Microbial Cultures

The microbial cultures utilized in this study were mixed cultures of mesophilic (MC1), moderately thermophilic (MC2), and extremely thermophilic microbes (MC3), respectively. Mixed cultures were prepared from pure strains with equal proportion. The composition of these mixed cultures and cultural conditions are listed in Table 1.

The mixed cultures were adapted to the gold concentrate by serially culturing the microorganisms to the solid particles of the concentrate, with the gradually increasing pulp densities from 5% to 15% (w/v) replacing the energy source. The mixed cultures of mesophilic, moderately thermophilic, and extremely thermophilic microbes were cultured at 30ºC, 45ºC, and 65ºC, respectively. The adaptation procedure was carried out on a rotary incubator

Table 1. Iron and sulfur oxidizing microorganisms used in the experiment.

	Temperature (ºC)	Organism	Growth substance	pH value
Mesophiles (MC1)	30	*Acidithiobacillus ferrooxidans* (F1)	S and Fe(II) oxidation	2.0
		Acidithiobacillus thiooxidans (A01)	S oxidation	
		Leptospirilum ferriphilum (YSK)	Fe oxidation	
Moderate thermophiles (MC2)	45	*Sulfobacillus thermosulfidooxidans* (YN22)	Mixotroph S, Fe(II) oxidation	1.6
		Acidithiobacillus caldus (S1)	S oxidation	
		Ferroplasma thermophilum (L1)	Mixotroph Fe oxidation	
Extreme thermophiles (MC3)	65	*Metallosphaera sedula* (JCM9185)	S oxidation	1.6
		Sulfobacillus metallicus (JCM9184)	Mixotroph S, Fe(II) oxidation	
		Acidianus manzaensis (YN25)	Mixotroph S, Fe(II) oxidation	

shaker with a stirring speed of 170 rpm, and the medium used in the experiment was 9K basal salt medium supplemented with 0.02% (w/v) yeast extract for the moderately thermophilic and extremely thermophilic microbes. The solution pH was adjusted to 2.0 for the mesophilic mixed culture and to 1.6 for the other two mixed cultures with 10 N H_2SO_4.

Concentrates

The gold concentrate was supplied by Axi Gold factory in Xinjiang Province of China. Mineralogical and chemical analyses showed that the concentrate contained pyrite (58.77%) as the major sulfide phase with a chemical composition of Au 55.5 g/t; Fe 23.68 %; As 2.25 %; S^0 trace; and S_s 24.53 %. The mode of occurrence of gold is listed in Table 2.

Reactor and Experimental Design

In the two-step approach, the concentrate was first leached by ferric-ion-containing solution (collected from the industrial bioreactors of Axi Gold Company Ltd.) prior to bio-oxidation. Chemical oxidation was conducted in a 1 L beaker that contained 500 ml of ferric-ion-containing solution under the conditions of 400 rpm and 80ºC by a thermostat. The concentrate was loaded and mixed with the ferric-ion-containing solution at pulp densities of 5%, 10%, and 15 % (w/v). The concentration of ferric ion in the solution was 20 g/l, at pH 1.25. When the concentration of ferric ion and the pH were steady, the residues were filtrated and washed twice with distilled water (pH 1.5) and then subjected to the biological leaching step.

The biological leaching step was carried out in 1 L reactors with a working volume of 500 ml containing 450 ml of 9K basal medium and 50 ml of inoculum with residues of chemical oxidation as the solid phase. In addition, 0.02% yeast extract was supplemented for the moderately thermophilic and extremely thermophilic mixed cultures. A control experiment was also carried out without chemical oxidation at pulp densities of 5%, 10%, and 15% (w/v) using three mixed cultures. All other conditions were the same as described above at the same time. The residence time in each group was 7 days. The experiments were conducted at 30ºC, 45ºC, and 65ºC with a stirring speed of 400 rpm, at the aeration rate of 4 min^{-1}. Samples were taken every 24 h to determine total soluble iron, ferrous ion, Eh, number of microbes.

Analytical Method

The concentration of planktonic microorganisms in the solution was determined by a direct count using an optical microscope at 100× magnification (Olympus, Japan).

The pH values were measured at room temperature with a pH meter (PHSJ-4A) calibrated with a low pH buffer. The redox potential was monitored with a platinum electrode against the Hg/HgCl reference electrode. The total dissolved iron concentrations in the solution were determined spectrophotometrically with 5-sulfosalicylic acid as an indicator [23]. The ferrous ion concentrations in solution were measured by titration with potassium dichromate ($K_2Cr_2O_7$), and the ferric ion concentration was determined by subtraction of the total iron concentration and ferrous ion concentration. The distributions of particle size before and after chemical oxidation at pulp desity 10% (w/v) were investigated by Laser Particle Size Analyzers (Mastersizer 2000). The morphological changes of the concentrate after chemical oxidation were analyzed by SEM (FEI Quanta-200), and the compositions of concentrate before and after chemical oxidation were carried out with EDX and XPS.

The content of gold in the solid phase was determined with a fire assay. Cyanide leaching of the oxidized residues and the untreated concentrate was carried out for the gold extraction. The cyanidation tests were conducted in 250 ml Erlenmeyer flasks at a working volume of 50 ml, with 30% (w/v) pulp density, in an orbital shaker at 25ºC with a rotary speed of 170 rpm for 48 h. The pH of the cyanidation pulp was 10.2−10.5 (adjusted with NaOH). The initial concentration of NaCN was 20 kg/ton.

Calculation Formula

The oxidation level of sulfide sulfur, O_i, was calculated by using Eq. (5), where X_0,X_i are the contents of sulfide sulfur in the initial gold concentrate and undissolved leach residues after the one-step or two-step process in wt%, and W_0,W_i are the weights of initial feed and undissolved residues.

$$\%O_i = \frac{W_0X_0 - W_iX_i}{W_0X_0} \times 100 \quad (5)$$

Results and Discussion

Chemical Oxidation Step

In the chemical oxidation step, the concentrates were first leached by a ferric-ion-containing solution to remove the most easily oxidized sulfides. Under the parameters mentioned above, chemical oxidation occurred within 6 h, for the concentrations of ferric ion and pH in the solution decreased to a steady value within 6 h (Fig. 1). The temperature dependency of pyrite oxidation kinetics has been well investigated, and the leaching kinetics could be improved via elevating the leaching temperature [4, 6]. The

Table 2. Mode of occurrence of gold.

Occurrence	Exposed gold	Gold wrapped in sulfide	Gold wrapped in other minerals	Total
Content (g/t)	28.50	24.50	2.52	55.52
Occupancy (%)	51.33	44.13	4.54	100

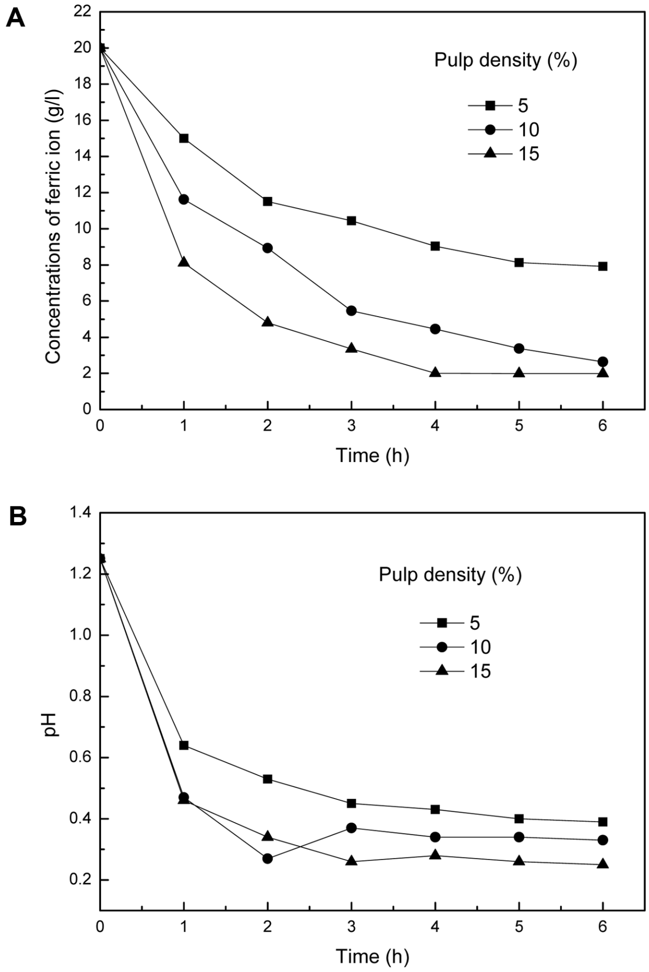

Fig. 1. Variation of the concentration of Fe^{3+} (**A**) and pH value (**B**) in the solution during the chemical oxidation step.

The concentrate of different pulp densities was oxidized with ferric-ion- containing solution for 6 h at 80°C. The volume of the ferric-ion-containing solution was 500 ml and the initial concentration of ferric ion was 20 g/l at different pulp densities, respectively.

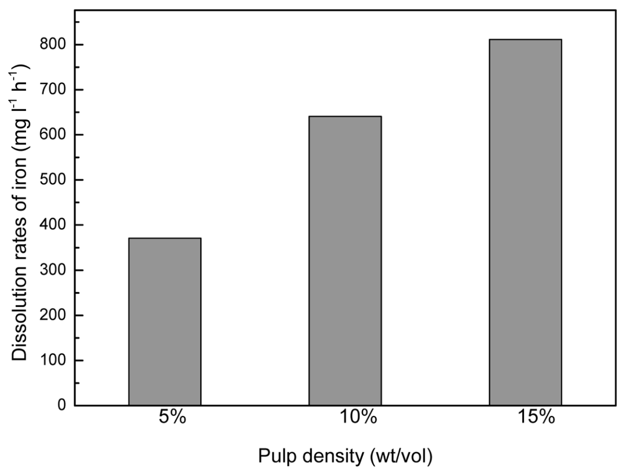

Fig. 2. Dissolution rates of iron after chemical oxidation under different pulp densities.

The figure is representative of three independent experiments. The pulp densities were 5%, 10%, and 15%, respectively.

dissolution rates (Fig. 2) of iron were 370, 641, and 811 mg l^{-1} h^{-1} at 5%, 10%, and 15%, respectively; these results were much higher than those obtained via bio-oxidation [11].

The compositions of the leaching residues are shown in Table 3. The oxidation levels of sulfur declined when the pulp density was elevated. For instance, the oxidation level of sulfide sulfur was 15.28% at 5% pulp density, and the value dropped to 5.69% at 15% pulp density. That was probably because the concentration of ferric ion was limited and most of the sulfide minerals at 15% pulp density was not oxidized.

The SEM images of the gold concentrate and chemical oxidation residues at 10% pulp density revealed that some new minerals formed in the chemical oxidation process (Fig. 3). The results of EDX also indicated that the stoichiometric ratio of iron and sulfur on the surface was close to 1:2, while the ratio declined to 1:2.64 after chemical oxidation (Fig. S1). All these results suggested that the crystal lattice structure was partly disrupted, and a sulfur-rich layer may be formed in the chemical oxidation stage [10, 20, 39]. XPS analysis was conducted to determine the exact composition of these compounds (Fig. S2). The results showed that a small amount of sulfate and element sulfur existed on the surface of the gold concentrate, and the amount of sulfate and element sulfur increased to 31.45% and 24.79% after chemical oxidation. Based on the comparision with peaks of elemental sulfur and results in

Table 3. Analysis of the solid phase after chemical oxidation.

Pulp density (%, w/v)	Residue yield (%)	Content (wt%)		Oxidation levels of sulfide sulfur (wt%)	Recovery rate of gold (%)
		Fe	S_s		
5	83.50	23.03	24.35	15.28	53.91
10	85.35	23.24	26.26	10.16	51.60
15	88.10	23.81	26.26	5.69	51.50

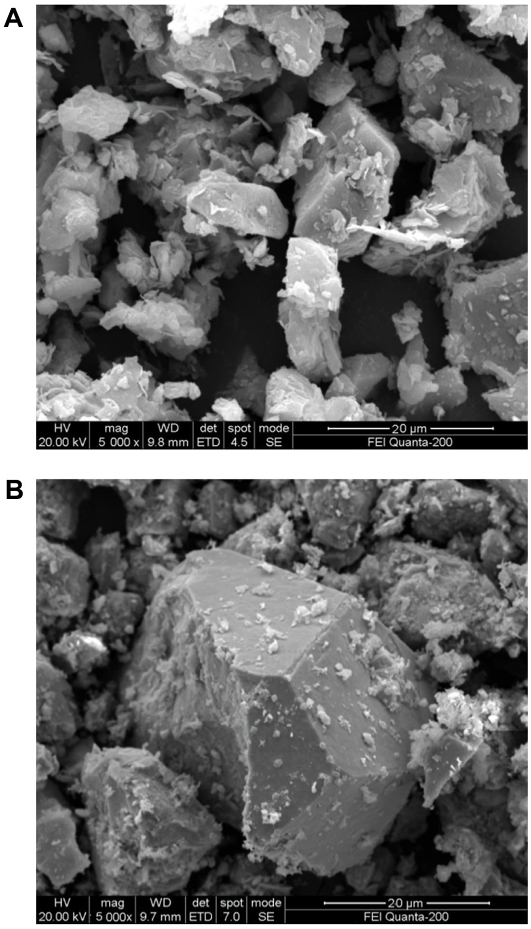

Fig. 3. SEM images of Axi gold concentrate and chemical oxidation residues.

(**A**) Gold concentrate before chemical oxidation. (**B**) Chemical oxidation residues of the 10% pulp density group.

the literature, the binding energy in the 163.75−164.25 eV range was assigned to elemental sulfur S_8 [9, 17], which was the intermediate product in the process of pyrite bio-oxidation. In addition, the surface area mean particle size (D[3,2]) decreased from 11.121 µm to 7.851 µm, and the volume average particle diameter (D[4,3]) diminished from 65.023 µm to 47.337 µm after chemical oxidation. The specific surface area increased from 0.540 m^2/g to 0.764 m^2/g, which implied that the reaction rates would be improved in the subsequent biological oxidation and cyanide leaching of gold with chemically leached gold concentrate [22].

However, the content of pyrite in the untreated concentration was 58.77%, as only a small part of the sulfide was oxidized in the chemical oxidation stage even at 5% pulp density. The recovery rate of gold was only 53.91% after chemical oxidation (Table 3). Even though the oxidizing agent was adequate, the leaching rates decreased significantly after several hours, probably because the formation of the sulfur-rich layer hindered the access of ferric ion to the surface of the minerals. Thus, it was not economical to carry out chemical oxidation with leaching rates similar to those in the one-step biological oxidation process under elevated temperature. Zhong [40] investigated the chemical oxidation of pyrite under pulp density of 1% (w/v) with sufficient ferric ion, and the dissolution rate of pyrite reached 100% after 7 days. The research of lglesias and Carran [21] revealed that the dissolution rate of arsenic was only 25% with 1% pulp density at 70°C after 24 h. Consequently, in the system of chemical oxidation, a short-term process that required minimal power input for the maintainance of temperature might be the most efficient. An additional biological leaching was needed to further improve the oxidation levels of the concentrate.

Biological Oxidation Step

Biological oxidation in the one-step process. The oxidation levels of sulfide sulfur after the one-step process are listed in Table 4. It was stated that the adaptation criterion was a ratio of ferric ion to ferrous ion of higher than 1.0, which indicated an efficient ferrous ion bio-oxidation [3]. According to the criterion, except for the cases of 10% and 15% pulp density with MC3, the mixed cultures used in this experiment could adapt to different pulp densities in the experiment. It has been reported by many researchers that the most efficient microbial community in the bio-oxidation process should contain microorganisms that could oxidize ferrous ion and reduce inorganic sulfur via a synergy mechanism [25, 32, 36]. The mixed cultures used in this experiment were constructed on the basis of the synergy principle, where the ferrous ion oxidizers can convert the ferrous ion into ferric ion, which can react with the sulfidic complexes as an oxidizing agent. The sulfur oxidizing microorganisms can react with the sulfur generated in the bio-oxidation process (Eqs. (1)−(4)).

The optimum mixed culture to pretreat Axi gold concentrate was MC2 under different pulp densities in this experiment. The highest dissolution rates of iron were obtained with MC2 under different pulp densities, and the dissolution rates of iron increased with elevated pulp density using MC2. However, the dissolution rates of iron using the other two mixed cultures increased at first and then decreased with elevated pulp density (Fig. 4). The highest oxidation levels of sulfur were 94.6% at 5% pulp density obtained with MC2. The bio-oxidation efficiency declined considerably with increasing pulp density with

Table 4. Analysis of the solid phase after the one-step process using three mixed cultures.

Bacteria/archaea	Pulp density (% w/v)	$C_{Fe^{3+}}/C_{Fe^{2+}}$	Residue yield (%)	Content (wt%) Fe	Content (wt%) S_s	Oxidation level of sulfide sulfur (wt%)	Recovery rate of gold (%)
MC1	5	15.85	54.00	7.68	6.16	86.44	90.30
	10	14.22	63.31	15.99	14.72	62.01	76.81
	15	3.43	77.04	21.78	20.12	36.81	62.32
MC2	5	20.52	50.82	5.65	2.66	94.49	92.32
	10	16.46	59.25	13.20	11.25	72.83	83.43
	15	4.10	67.34	17.60	16.52	54.65	75.82
MC3	5	1.90	55.21	8.16	8.89	80.00	87.52
	10	0.63	68.11	17.79	19.07	47.05	70.72
	15	0.48	80.70	21.82	22.60	25.65	57.23

$C_{Fe^{3+}}/C_{Fe^{2+}}$, concentration ratio of ferric ion and ferrous ion.

Table 5. Redox potentials of the solution after the one-step and two-step processes.

Initial pulp density (%)	Redox potential (mV) MC1 One-step	MC1 Two-step	MC2 One-step	MC2 Two-step	MC3 One-step	MC3 Two-step
5	550	602	588	633	533	613
10	520	581	530	594	426	487
15	471	523	490	533	410	443

mixed culture MC2, and the oxidation levels of sulfide sulfur decreased to 54.65% at 15% pulp density. Accordingly, the recovery rate of gold declined from 92.32% to 75.82% when the pulp density was elevated from 5% to 15% with MC2.

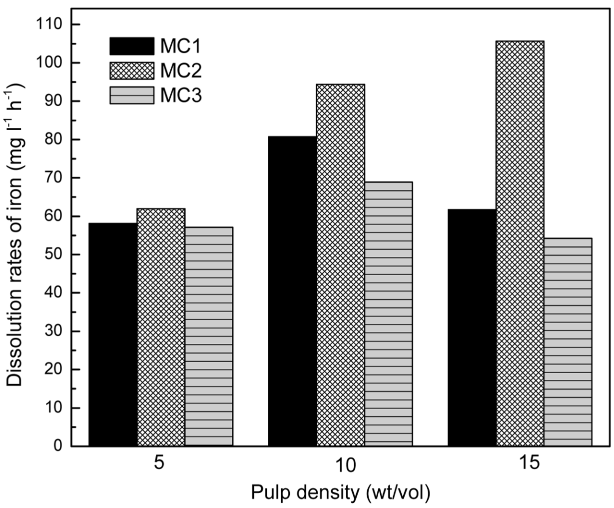

Fig. 4. Dissolution rates of iron after the one-step process using mixed cultures.

MC1, mesophilic mixed cultures; MC2, moderately thermophilic mixed cultures; and MC3, extremely thermophilic mixed cultures.

The decline of the concentration of the microorganisms in the solution was nonsignificant (Table 6), and the concentration ratio of ferric ion to ferrous ion was 3.43, but the redox potential declined from 550 mV to 471 mV (Table 5), which indicated that the metabolic activity of microorganisms was influenced by the elevated pulp density to some extent.

It has been reported that *At. ferrooxidans* could maintain its metabolic activity and oxidize ferrous ion at elevated ferrous ion concentration (30 g/l) [30]. Ciftci and Akcil [11] have pointed out that the growth rates and bio-oxidation efficiency of moderate thermophiles were negatively influenced when the iron concentration in the solution reached 12 g/l. Although the iron concentration in the solution with 15% pulp density (w/v) with MC2 was as high as 17 g/l, the growth rate of microorganisms was not inhibited, and the microbial concentration reached 5.5×10^8 cells/ml, approximately the same microbial concentration as with MC1. In addition, the redox potentials of MC1 and MC2 were roughly the same during the bio-oxidation process (Table 5), so the oxidation levels with MC2 were higher due to the higher temperature. The advantage of mesophiles might become obvious when elevating the pulp density to a higher level [11].

It has been stated that the mixed culture of extreme

Table 6. Concentration of microorganisms in the solution after the one-step and two-step processes.

Initial pulp density (%)	Microbial concentration ($\times 10^8$ cells/ml)					
	MC1		MC2		MC3	
	One-step	Two-step	One-step	Two-step	One-step	Two-step
5	7.0	7.8	5.8	7.5	0.8	1.2
10	6.6	7.2	5.4	6.7	0.7	0.8
15	6.0	6.6	5.5	6.1	0.3	0.5

Table 7. Analysis of the solid phase after the two-step process using three mixed cultures.

Bacteria/archaea	Pulp density (%, w/v)	$C_{Fe^{3+}}/C_{Fe^{2+}}$	Residue yield (%)	Content (wt%)		Oxidation level of sulfide sulfur (wt%)	Recovery rate of gold (%)
				Fe	S_s		
MC1	5	25.51	43.22	3.85	3.85	93.21	92.51
	10	23.57	52.01	7.65	6.77	85.65	89.99
	15	8.17	68.71	17.08	17.78	50.20	72.32
MC2	5	31.52	40.82	2.40	0.93	98.45	92.72
	10	26.46	44.81	4.15	3.32	93.94	92.43
	15	11.27	58.22	14.30	14.41	65.73	78.82
MC3	5	16.39	34.70	2.22	0.70	99.01	92.22
	10	1.03	54.09	12.72	13.03	71.27	80.72
	15	0.67	74.18	18.93	19.40	41.33	67.23

$C_{Fe^{3+}}/C_{Fe^{2+}}$, concentration ratio of ferric ion and ferrous ion.

thermophiles has some advantages over other mixed culture, such as faster chemical oxidation kinetics, decreasing the cost of cooling, and a more complete oxidation [2, 12]. However, the bio-oxidation efficiencies with MC3 were not the highest at different pulp densities. The highest concentration of MC3 was only 0.8×10^8 cells/ml at 5% pulp density (Table 6). The concentration ratio of ferric ion to ferrous ion and the redox potential were only 1.90 and 533 mV, the recovery rate of gold was 87.52%. It has been previously reported that the growth rates of extreme thermophiles were much lower than that of mesophiles and moderate thermophiles [30]. The reason for the lower growth rates of extreme thermophiles could be due to insufficient oxygen and carbon dioxide supplement at elevated temperatures [1, 3]. In addition, the cell walls of extreme thermophiles were not as rigid as those of mesophiles and moderate thermophiles, which resulted in the disruption of cell walls because of shear stress caused by stirring [27, 29]. The negative effect of gas shortage and shear stress would become more serious at higher pulp density [30]. The concentration ratio of ferric ion and ferrous ion decreased from 1.90 to 0.48, and the redox potential declined from 533 mV to 410 mV when the pulp density was elevated to 15%, and the gold recovery rate decreased by 30.29%. All these results revealed that the activity of MC3 was inhibited seriously with elevated pulp density.

Biological oxidation in the two-step process. The oxidation efficiencies were improved significantly via a two-step process with the three mixed cultures. The oxidation levels of sulfide sulfur for the experiments with 5% pulp density (w/v) using different mixed cultures (MC1, MC2, and MC3) as well as 10% pulp density (w/v) with MC2 were found to be above 90% (Table 7). In the one-step process, only the oxidation level of sulfide sulfur with 5% pulp density using MC2 was found to be above 90%. The dissolution rates of iron in the two-step process were higher than those with the same initial pulp density of the one-step process (Fig. 5). The observation in this study revealed that, in all experiments with different pulp densities, the oxidation levels of sulfide had a positive correlation with the dissolution rates of iron. It was feasible to detect the oxidation efficiency via measuring the dissolution rates of iron. The higher oxidation efficiency in the two-step process could be caused by the following reasons. First, the pulp densities decreased to 4.18%, 8.54%, and 13.22%, respectively, after chemical oxidation with different initial pulp densities. Consequently, the negative

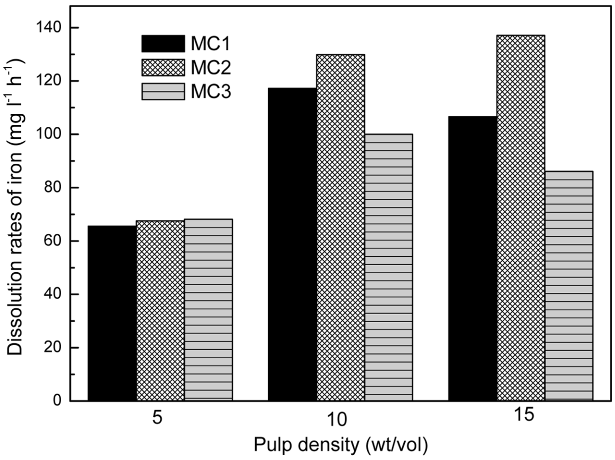

Fig. 5. Dissolution rates of iron after the two-step process using three mixed cultures.

MC1, mesophilic mixed cultures; MC2, moderately thermophilic mixed cultures, and MC3, extremely thermphilic mixed cultures.

effect of high pulp density was diminished partially after chemical oxidation [14, 34]. Second, the specific surface area increased after chemical oxidation, which would increase the leaching rates. Third, chemical oxidation with ferric-ion-containing solution could cleave the bonds in the crystal lattice of the surface structure in sulfide, which would promote the solubilization of pyrite. Although the formation of a sulfur-rich layer would impede the oxidation of sulfide, it could be oxidized by the sulfur-oxidizing microorganisms. As a result, the oxidation efficiency of Axi gold concentrate with two-step chemical-biological oxidation was elevated significantly.

In the two-step process, the highest oxidation efficiency was observed in the experiment with 5% pulp density using MC3. The oxidation level of sulfide sulfur was elevated to 99.01%, and the recovery rate of gold was 92.22%. The concentration ratio of ferric ion to ferrous ion was elevated from 1.90 to 16.39, and the redox potential increased from 533 mV to 613 mV, which indicated the activity of MC3 was improved significantly. In the biological leaching step, redox potential and temperature would influence the leaching kinetics significantly [4, 20, 24]. Although the redox potentials in MC2 group were higher than those in MC3 group, the difference of redox potential values at 5% pulp density between MC2 and MC3 was not significant. In this case, the advantage of high temperature overcame that of higher redox potential, and thus the oxidation performance using extreme thermophiles at 5% pulp density was better. However, the lowest oxidation efficiency was also observed with the same mixed culture with 15% pulp density (w/v) (Table 7). The reason for this trend could be due to high sensitivity of extreme thermophiles to high pulp density, as a number of papers have published the poor performance of extreme thermophiles to high pulp density [3, 5, 11, 13, 37]. Although the pulp density decreased after chemical oxidation, the solid content was still too high (13.22% (w/v)) for these microbes. The cell concentration of MC3 decreased from 1.2×10^8 cells/ml at 5% pulp density to 0.5×10^8 cells/ml at 15% pulp density in the two-step process, and the concentration ratio of ferric ion to ferrous ion declined from 16.39 to 0.67. It suggested that the extreme thermophiles would perform better during the bio-oxidation only if the pulp density was lower than 5%.

A decreasing tendency in the oxidation levels of sulfide sulfur was observed with increasing pulp density (Table 7). However, this trend was more obvious in the experiment with mixed cultures of mesophiles and extreme thermophiles. The highest oxidation efficiencies were observed in the two-step process with 10% and 15% pulp densities with MC2. The oxidation levels of sulfide sulfur were improved to 93.94% and 65.73%, and the recovery rates of gold were elevated to 92.43% and 78.82% accordingly. Working with flasks, He *et al.* [19] found that the dissolution rate of iron with moderate thermophiles was higher than that with mesophiles. Ciftci and Akcil [11] observed that the sulfide oxidation of gold concentrate with moderate thermophiles was best at 10% pulp density. The redox potentials in the MC2 group after the two-step process were higher than those in the MC1 group. In addition, the temperature in the MC2 group was higher than in the MC1 group, and as a result, the oxidation efficiency was higher in the MC2 group.

Cyanidation Leaching

The recovery rates of gold from the oxidized residues by cyanide leaching were used as a criterion to determine the oxidation efficiency. It was obvious that the recovery rate of gold was low in the untreated concentrate (48.1%), whereas the recovery rates were elevated significantly after the two-step process. The dependence of gold recovery rates from the concentrate on the sulfide sulfur oxidation level is shown in Fig. 6. The data presented in Fig. 6 demonstrate that the gold recovery rates from the refractory gold concentrate were linearly dependent on the oxidation levels of sulfide sulfur in the concentrate. The regression coefficient was greater than 0.98, which suggested that the oxidation levels of sulfide sulfur is the most important factor that influences the oxidation efficiency. However, the recovery rate of gold was less than 93%, even though the oxidation level of sulfide sulfur was 99.01% with the

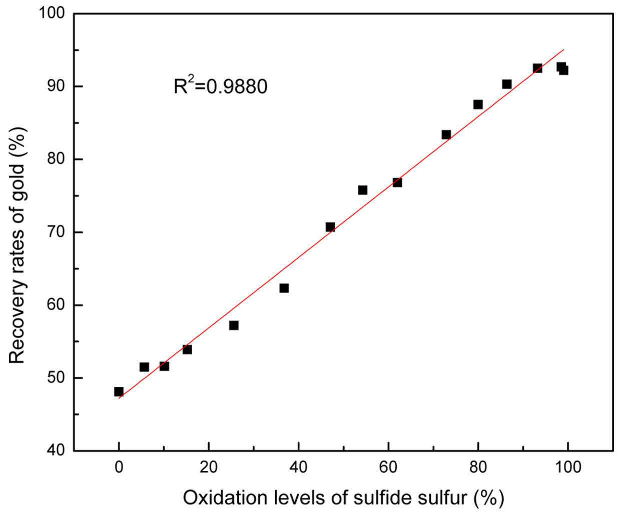

Fig. 6. The dependence of gold recovery from Axi refractory gold concentrate on the oxidation level of sulfide sulfur.
The residues obtained from different pulp densities after one-step and two-step processes were subjected to cyanide leaching. The regression coefficient was greater than 0.98, which meant the gold recovery rate was linearly dependent on the sulfide sulfur oxidation levels.

extremely thermophilic culture. It might because some gold particles were still wrapped within minerals, like silicate, which were insoluble in the conditions used in this experiment [15].

In conclusion, the oxidation levels of sulfide sulfur decreased with increasing solids contents during the bio-oxidation process using the one-step or two-step approach. In addition, the concentrations of all the investigated types of mixed cultures decreased with increasing pulp densities. The adverse effect of increasing the solids ratios in the bio-oxidation process could be attributed to the limited availability of O_2, CO_2, and nutrients; the toxic effect of increasing concentrations of metal ion on the microbial growth; the decrease of the number of microbes-to-solid ratio; and the inefficient mass transfer in the system, together with mechanical damage to microorganisms due to high shear force caused by the increasing solids ratios.

The mixed culture of mesophiles, moderate thermophiles, and extreme thermophiles showed high bio-oxidation performance in the bio-oxidation process of refractory gold concentrate. The greatest degree of sulfide oxidation during the one-step process occurred in the presence of moderate thermophiles at 5% pulp density, and the oxidation level of sulfide sulfur was 94.49%. Most acidophilic microorganisms, including mesophiles and moderate thermophiles, have a rigid cell wall with a relatively high shear resistance. On the contrary, the extreme thermophiles often lack rigid walls, which would make them more sensitive to shear force even at lower pulp density.

The two-step process is a promising method to improve the bio-oxidation kinetics. After a two-step process, oxidation efficiencies of refractory gold concentrate at different pulp densities using the three mixed cultures were all elevated. The oxidation efficiency carried out at 5% pulp density using mixed extreme thermophiles resulted in the best performance compared with the other mixed cultures. The oxidation level of sulfide sulfur was 99.01%. All the results indicated that the advantage of extreme thermophiles can be observed at low pulp density (<5%). However, the performance of mixed extreme thermophilic culture decreased as the pulp density was elevated because of the adverse effect of shear force. Moderate thermophiles performed better at 10% and 15% pulp densities. The values were elevated to 93.94% and 65.73%, respectively.

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References

1. Acevedo F, Gentina J, Valencia P. 2004. Optimization of pulp density and particle size in the biooxidation of a pyritic gold concentrate by *Sulfolobus metallicus*. *World J. Microbiol. Biotechnol*. **20:** 865-869.
2. Ahmadi A, Schaffie M, Petersen J, Schippers A, Ranjbar M. 2011. Conventional and electrochemical bioleaching of chalcopyrite concentrates by moderately thermophilic bacteria at high pulp density. *Hydrometallurgy* **106:** 84-92.
3. Astudillo C, Acevedo F. 2008. Adaptation of *Sulfolobus metallicus* to high pulp densities in the biooxidation of a flotation gold concentrate. *Hydrometallurgy* **92:** 11-15.
4. Basson P, Gericke M, Grewar TL, Dew DW, Nicol MJ. 2013. The effect of sulphate ions and temperature on the leaching of pyrite. III. Bioleaching. *Hydrometallurgy* **133:** 176-181.
5. Boogerd F, Bos P, Kuenen J, Heijnen J, Van der Lans R. 1990. Oxygen and carbon dioxide mass transfer and the aerobic, autotrophic cultivation of moderate and extreme thermophiles: a case study related to the microbial desulfurization of coal. *Biotechnol. Bioeng*. **35:** 1111-1119.
6. Breed AW, Harrison STL, Hansford GS. 1997. A preliminary investigation of the ferric leaching of a pyrite/arsenopyrite flotation concentrate. *Miner. Eng*. **10:** 1023-1030.
7. Brierley C. 2010. Biohydrometallurgical prospects. *Hydrometallurgy* **104:** 324-328.
8. Brierley CL, Brierley JA. 2013. Progress in bioleaching: part

B: applications of microbial processes by the minerals industries. *Appl. Microbiol. Biotechnol*. **97:** 7543-7552.
9. Cai Y, Pan Y, Xue J, Sun Q, Su G, Li X. 2009. Comparative XPS study between experimentally and naturally weathered pyrites. *Appl. Surface Sci*. **255:** 8750-8760.
10. Chernyshova IV. 2003. An in situ FTIR study of galena and pyrite oxidation in aqueous solution. *J. Electroanal. Chem*. **558:** 83-98.
11. Ciftci H, Akcil A. 2010. Effect of biooxidation conditions on cyanide consumption and gold recovery from a refractory gold concentrate. *Hydrometallurgy* **104:** 142-149.
12. Ciftci H, Akcil A. 2013. Biohydrometallurgy in Turkish gold mining: first shake flask and bioreactor studies. *Miner. Eng*. **46-47:** 25-33.
13. Clark D, Norris P. 1996. Oxidation of mineral sulphides by thermophilic microorganisms. *Miner. Eng*. **9:** 1119-1125.
14. Deveci H. 2002. Effect of solids on viability of acidophilic bacteria. *Miner. Eng*. **15:** 1181-1189.
15. Deveci H, Jordan MA, Powell N, Alp I. 2008. Effect of salinity and acidity on bioleaching activity of mesophilic and extremely thermophilic bacteria. *Trans. Nonferrous Metals Soc. China* **18:** 714-721.
16. Dopson M, Lindström E. 2004. Analysis of community composition during moderately thermophilic bioleaching of pyrite, arsenical pyrite, and chalcopyrite. *Microb. Ecol*. **48:** 19-28.
17. Fantauzzi M, Licheri C, Atzei D, Loi G, Elsener B, Rossi G, Rossi A. 2011. Arsenopyrite and pyrite bioleaching: evidence from XPS, XRD and ICP techniques. *Anal. Bioanal. Chem*. **401:** 2237-2248.
18. Fomchenko NyV, Muravyov MI, Kondrat'eva TF. 2010. Two-stage bacterial–chemical oxidation of refractory gold-bearing sulfidic concentrates. *Hydrometallurgy* **101:** 28-34.
19. He Z, Yin Z, Wang X, Zhong H, Sun W. 2012. Microbial community changes during the process of pyrite bioleaching. *Hydrometallurgy* **125-126:** 81-89.
20. Holmes PR, Crundwell FK. 2000. The kinetics of the oxidation of pyrite by ferric ions and dissolved oxygen: an electrochemical study. *Geochim. Cosmochim. Acta* **64:** 263-274.
21. Iglesias N, Carranza F. 1996. Treatment of a gold bearing arsenopyrite concentrate by ferric sulphate leaching. *Miner. Eng*. **9:** 317-330.
22. Kaksonen AH, Perrot F, Morris C, Rea S, Benvie B, Austin P, Hackl R. 2014. Evaluation of submerged bio-oxidation concept for refractory gold ores. *Hydrometallurgy* **141:** 117-125.
23. Karamanev D, Nikolov L, Mamatarkova V. 2002. Rapid simultaneous quantitative determination of ferric and ferrous ions in drainage waters and similar solutions. *Miner. Eng*. **15:** 341-346.
24. Li Q, Li D, Qian F. 2009. Pre-oxidation of high-sulfur and high-arsenic refractory gold concentrate by ozone and ferric ions in acidic media. *Hydrometallurgy* **97:** 61-66.
25. Liu H, Yin H, Dai Y, Dai Z, Liu Y, Li Q, *et al*. 2011. The co-culture of *Acidithiobacillus ferrooxidans* and *Acidiphilium acidophilum* enhances the growth, iron oxidation, and CO_2 fixation. *Arch. Microbiol*. **193:** 857-866.
26. Ma S, Luo W, Mo W, Su X, Liu P, Yang J. 2010. Removal of arsenic and sulfur from a refractory gold concentrate by microwave heating. *Miner. Eng*. **23:** 61-63.
27. Mikkelsen D, Kappler U, Webb RI, Rasch R, Mcewan AG, Sly LI. 2007. Visualisation of pyrite leaching by selected thermophilic archaea: nature of microorganism-ore interactions during bioleaching. *Hydrometallurgy* **88:** 143-153.
28. Muravyov MI, Bulaev AG. 2013. Two-step oxidation of a refractory gold-bearing sulfidic concentrate and the effect of organic nutrients on its biooxidation. *Miner. Eng*. **45:** 108-114.
29. Nemati M, Harrison STL. 1999. Effects of solid particles on thermophilic bioleaching of sulphide minerals. *Process Metall.* **9:** 473-482.
30. Nemati M, Harrison STL. 2000. Effect of solid loading on thermophilic bioleaching of sulfide minerals. *J. Chem. Technol. Biotechnol*. **75:** 526-532.
31. Nicol M, Miki H, Basson P. 2013. The effects of sulphate ions and temperature on the leaching of pyrite. 2. Dissolution rates. *Hydrometallurgy* **133:** 182-187.
32. Okibe N, Johnson DB. 2004. Biooxidation of pyrite by defined mixed cultures of moderately thermophilic acidophiles in pH-controlled bioreactors: significance of microbial interactions. *Biotechnol. Bioeng*. **87:** 574-583.
33. Rawlings DE. 2002. Heavy metal mining using microbes 1. *Annu. Rev. Microbiol*. **56:** 65-91.
34. Rawlings DE. 2005. Characteristics and adaptability of iron- and sulfur-oxidizing microorganisms used for the recovery of metals from minerals and their concentrates. *Microb. Cell Fact.* **4:** 54-56.
35. Rawlings DE, Dew D, du Plessis C. 2003. Biomineralization of metal-containing ores and concentrates. *Trends Biotechnol*. **21:** 38-44.
36. Rohwerder T, Gehrke T, Kinzler K, Sand W. 2003. Bioleaching review part A: progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation. *Appl. Microbiol. Biotechnol*. **63:** 239-248.
37. Sandström Å, Petersson S. 1997. Bioleaching of a complex sulphide ore with moderate thermophilic and extreme thermophilic microorganisms. *Hydrometallurgy* **46:** 181-190.
38. Shiers D, Ralph D, Watling H. 2010. A comparative study of substrate utilisation by *Sulfobacillus* species in mixed ferrous ion and tetrathionate growth medium. *Hydrometallurgy* **104:** 363-369.
39. Xia JL, Yang Y, He H, Zhao XJ, Liang CL, Zheng L, *et al*. 2010. Surface analysis of sulfur speciation on pyrite bioleached by extreme thermophile *Acidianus manzaensis* using Raman and XANES spectroscopy. *Hydrometallurgy* **100:** 129-135.
40. Zhong SP. 2015. Leaching kinetics of gold bearing pyrite in H_2SO_4–$Fe_2(SO4)_3$ system. *Trans. Nonferrous Metals Soc. China* **25:** 3461-3466.