EXTRACTION OF GOLD FROM IRON PYRITE ORE BY ACID LEACHING AND BIOLEACHING

Khin Cho Lin[1], San Nwe Zin[2], Daw Hla Ngwe[3]

Abstract

In this study, acid leaching and bioleaching of iron pyrite ore collected from Sakhangyi gold mining site, Moehti Moemi mining area, Yamethin Township, Mandalay Region were conducted. In acid leaching of iron pyrite ore from Sakhangyi (SKG) gold mining site, aqua regia and di- isobutyl ketone have been used as extraction solvents. In bioleaching process, a bioleaching bacterium, *Acidithiobacillus ferrooxidans* was used. *A. ferrooxidans* was cultured on selected 9 K medium. Leaching of iron pyrite ore sample was done under different leaching parameters (time, pH, ore size and temperature). The leachant has been characterized by atomic absorption spectroscopy. According to acid leaching of iron pyrite ore, Au content of iron pyrite ore was 1.2 ppm, after extracting with DIBK (AAS). The optimum conditions for bioleaching process using *A. ferrooxidans* were found to be 1.5 of pH, 90 ^{o}C, 250 µm ore size after 6 days bioleaching time to get the highest gold content (6.25 ppm).

Keywords: Acid leaching, bioleaching, *Acidithiobacillus ferrooxidans*, gold, Atomic absorption spectroscopy

Introduction

Microbial processes have been developing to assist in the commercial recovery of gold from refractory ores. Iron and sulphur oxidizing acidophilic bacteria are able to oxidized certain sulfide ores containing encapsulated particles of elemental gold. Bio-oxidation of gold ores may be a less costly, less polluting alternative to other oxidation pretreatments such as roasting and pressure oxidation (Sen, 2015). Recently, bio-oxidation of gold ores has been implemented as a commercial process, and is under study worldwide for further application to refractory gold ores. Sulphide-oxidizing bacteria degrade the sulfide matrix surrounding gold, but do not leach the gold itself (Olson, 1994).

Bioleaching, the conversion of an insoluble metal into a soluble form by biological oxidation. Metals for which this technique is mainly employed for recovery includes, copper, cobalt, nickel, iron, sulphur, zinc and uranium. For recovery of gold and silver the activity of leaching bacteria is applied only to remove interfering metal sulphide from ores bearing the precious metals prior to cyanidation treatment. The application of bacterial leaching to metal recovery from mineral ores has progressed steadily in the last 20 years (Rohwerder *et al*., 2003). Bioleaching has improved the efficiency of the mineral procession industry by lowering of the overall capital and procession costs and by diminishing environmental concerns associated with the pollution derived from emission of smelting operations (Quatrini and Holmes, 2005).

Metal sulphides are oxidized by certain bacteria, forming soluble metal sulphates and sulphuric acid. Iron pyrite and arseno pyrite ores are prominent minerals in refractory gold ores and are readily bio-oxidized. The most commonly studied pyrite oxidizing bacteria are thermophilic *Acidithiobacillus ferrooxidans*, that oxidizes ferrous iron or reduced sulphur

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compounds. Moderately thermophilic pyrite oxidizing bacteria are found in mine spoils containing pyrite material that are undergoing weathering (Olson, 1994). It thrives in the pH range of 1.5 to 6, temperature in the range of 28-95 °C (Natal'ya and Muravyov, 2010). The present study, two methods of leaching: acid leaching and bioleaching were used for extraction of gold (Ahmad *et al*., 2015). In acid leaching based on aqua regia was used for sample digestion and di-isobutyl ketone (DIBK) was used as extraction solvent (Raju, 2005).

Materials and Methods

Collection and Preparation of Samples

Soil and ore samples, for this study, were selectively chosen and collected from Sakhangyi (SKG) gold mining site, Moehti Moemi, Yamethin township approximately 150 kilometers southeast of Mandalay at latitude 20° 21′ 35″ (N) and longitude 96° 24′ 47″(E). The collected soil sample is dried in air, ground and sieved through 200 mesh size and pH of the soil sample was determined. The iron pyrite ore sample was grounded, powdered (200-250 μm), and prepared by cone and quartering (Figure 1). Removal of silica from this iron pyrite ore sample was carried out by using hydrofluoric acid (Zahan *et al*., 2017).

(a) (b) (c) (d)

Figure 1 Cone and quartering method for iron pyrite ore
(a) Gathered material into a cone and flatten
(b) Divided into quarters
(c) Opposite quarters taken for mixing and forming
(d) The reduced sample

Isolation of and Identification *A. ferrooxidans*

A. ferrooxidans was cultured on selected 9 K medium, which mixed with solution A and solution B (Silverman and Ludgren, 1959). Solution A was prepared by adding 0.3 g of $(NH_4)_2SO_4$, 0.01 g of KCl, 0.05 g of K_2HPO_4, 0.05 g of $(MgSO_4)$ $7H_2O$ and 0.001 g of $Ca(NO_3)_2$ to 100 mL of distilled water. Solution B was prepared by dissolving 2.2 g of $FeSO_4$ in 50 mL of distilled water. This two solutions were mixed and adjusted (pH 1.5) with 10 N sulphuric acid and sterilized in autoclave (Silverman and Ludgren, 1959). Soil sample containing 9 K medium was placed on a rotary shaker with 200 rpm for 8 days. After 8 days, bacteria were taken with a sterilized inoculation loop from conical flask (9 K medium) and streaked in glucose yeast beef (GYB) medium plate by streak plate method. Then, it was incubated in an incubator at 37 ^oC for 7 days. After 7 days, single colonies of *A. ferrooxidans* species were grown on glucose yeast beef plate. Next, single colonies of *A. ferrooxidans* species were transferred to nutrient agar medium for culturing the pure colonies (Figure 2) (Silverman and Ludgren, 1959). The bacteria were identified by morphological examinations, gram staining and twelve biochemical tests

(Cruickshank, 1968**).** The selected bacteria were also characterized by phenotypic and genotypic identification (Grigorii *et al*., 2003).

Figure 2 Culture medium of isolated *A. ferrooxidans*

Extraction of Gold by Acid Leaching of Iron Pyrite Ore

Iron pyrite ore sample (10 g) was transferred into a porcelain crucible and roasted for 1 h in a muffle furnace at 650 °C. The roasted samples were transferred into a glass beaker (400 mL) and freshly prepared aqua regia (3:1) was added to stabilize $AuCl_3$ complex, during evaporation on a hot plate for at least 1 h. This solution was cooled, filtered and followed by discarding the residue. 18 mL of Sorenson's salt solution (250 mL distilled water and 560 g $Na_2HPO_4.\ 2H_2O$), 5 mL of 1 % Aiquot 336 (methyl trioctyl ammonium chloride) and 5 mL of D.I.B.K (di-isobutyl ketone) were added to this clear solution. This solution was allowed to separate and upper layer of D.I.B.K phase adsorbed the gold. This phase was collected for determination of gold by AAS (Raju, 2005).

Extraction of Gold by Bioleaching of Iron Pyrite Ore

(1) Effect of leaching time

9 K basal medium (50 mL each) was placed in each of six conical flasks and pH was adjusted to 1.5 with 10 N H_2SO_4 and sterilized at 121°C for 20 min. One loop each of the 7 days old cultured (*A. ferrooxidans*) and 5 g of iron pyrite ore (250 µm) were added into each of six conical flasks. Then, the conical flasks were shaken on a shaker at 200 rpm and 30 °C for 2, 4, 6, 8, 10 and 12 days. Liquid samples (20 mL each) were withdrawn and gold contents were measured by atomic absorption spectroscopy.

(2) Effect of pH

9 K basal medium was prepared accordingly with initial pH values of 1, 1.5, 2.0, 2.5, 3.0 and 3.5 with 10 N H_2SO_4 and sterilized 121 °C for 20 min. One loop each of the 7 days old cultured (*A. ferrooxidans*) and 5 g of iron pyrite ore (250 µm) were added into each of six conical flasks. Then, the conical flasks were shaken on a shaker at 200 rpm, 30 °C for 6 days. Liquid samples (20 mL each) were withdrawn centrifuged and gold contents were measured by atomic absorption spectroscopy.

(3) Effect of ore size

9 K basal medium (50 mL) was placed in each of three conical flasks and pH 1.5 was adjusted with 10 N H_2SO_4 and sterilized 121 °C for 20 min. One loop each of the 7 days old cultured (*A. ferrooxidans*) and 5 g of iron pyrite ore (150 µm, 250 µm, 350 µm) were added into each of three conical flasks. Then, the conical flasks were shaken on a shaker at 200 rpm, 30 °C for 6 days. Liquid samples (20 mL each) were withdrawn centrifuged and gold contents were measured by atomic absorption spectroscopy.

(4) Effect of leaching temperature

9 K basal medium (50 mL) was placed in each of four conical flasks and pH 1.5 was adjusted with 10 N H_2SO_4 and sterilized 121 °C for 20 min. One loop each of the 7 days old cultured (*A. ferrooxidans*) and 5 g of iron pyrite ore (250 µm) were added into each of the four conical flasks. Then, the conical flasks were shaken on a shaker at 200 rpm (30 °C, 60 °C, 90 °C, 120 °C) for 6 days. Liquid samples (20 mL each) were withdrawn, centrifuged and gold contents were measured by atomic absorption spectroscopy.

Determination of Gold Content at Optimum Conditions

The experimental procedure was carried out by using 9 K medium (50 mL) with initial pH 1.5 in a 50 mL of conical flask and sterilized at 121 °C for 20 min. One loop each of the 7 days old cultured (*A. ferrooxidans*) and 5 g of iron pyrite ore (250 µm) were added into a conical flask. Then, the conical flask was shaken on a shaker with 200 rpm all 90 °C for 6 days. Liquid sample (20 mL each) was withdrawn and gold content was measured by atomic absorption spectroscopy.

Results and Discussion

In this study**,** the soil and ore samples were collected from Sakhangyi (SKG) gold mining site, Moehti Moemi, Yamethin Township, Mandalay Region (Figure 3). The SKG soil sample was found to be moderately acidic (pH 5.7). *A. ferrooxidans* was isolated from this soil using 9 K basal salt medium. It was also identified by phenotypic and genotypic characterization (16S rDNA amplification by PCR) (Khin Cho Lin *et al*., 2018). According to phenotypic identification, *A. ferrooxidans* was gram negative, single rode shape and motile. According to genotypic identification, amplification conditions were optimized using genomic DNA from pure culture of isolated bacteria. The extraction of genomic DNA can be done for molecular characterization and detection of sequencing for identification of *A. ferrooxidan.* 16S rDNA amplification by PCR gave a few bands after gel electrophoresis. Each each band is corresponded in size to the expected product of 118 bp.

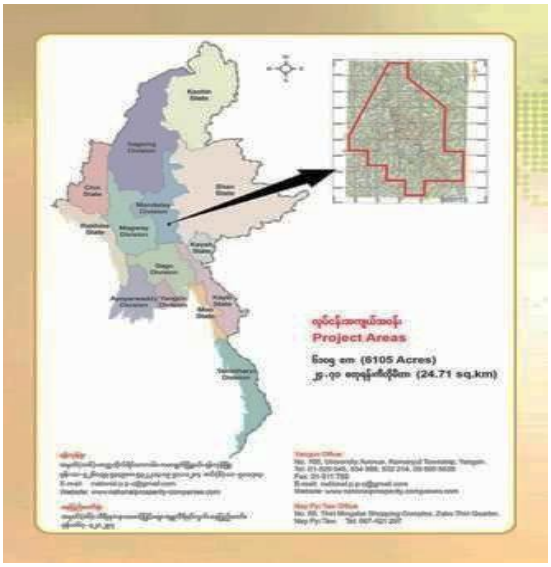

Figure 3 Location of Moehti Moemi Gold Mine in Yamethin Township, Mandalay Region

Gold Extracted from Iron Pyrite Ore by Acid Leaching Method

From the results of extraction of gold from iron pyrite ore by acid leaching method, the gold content 1.2 ppm was detected by AAS method. The amount of gold content obtained from acid leaching was significantly lower than that obtain from bioleaching. This is due to the low grade iron pyrite ore. Acid leaching is the suitable extraction method for high grade ores while bioleaching is the most effective method for low grade ore.

Gold Extracted from Iron Pyrite Ore by Bioleaching Method

In this method, various parameters such as effect of leaching time, effect of pH, effect of ore size and effect of temperature on the gold extraction were studied.

(1) Effect of leaching time

The amount of gold by bioleaching process was conducted with by varying leaching time. The highest gold content was 3.25 ppm after 6 days of leaching time. Therefore, leaching efficiency by the microorganism is dependent on the growth rate of organism. The more increase the leaching time, the less growth of microorganism and decrease the gold content (Table 1 and Figure 4) were observed.

Table 1 Effect of Leaching Time on Gold Content Extracted from Iron Pyrite Ore with *A. ferrooxidans* (AAS)

No.	Leaching time (day)	Au Content (ppm)
1	2	0.02
2	4	0.02
3	6	3.25
4	8	0.02
5	10	0.01
6	12	0.01

pH = 1.5, amount of Iron Pyrite Ore = 5 g, temperature = 30 ^{o}C, ore size = 250 µm

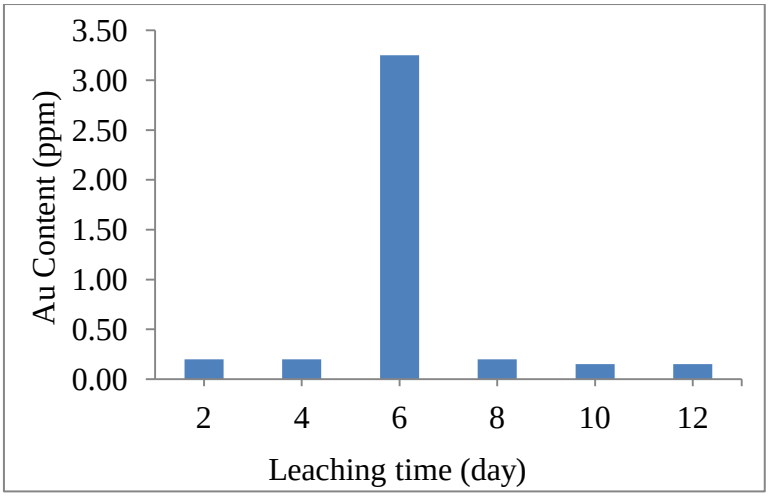

Figure 4 Effect of leaching time on gold content extracted from iron pyrite ore with *A. ferrooxidans* (AAS)

(2) Effect of pH

The experiment was carried out within the pH range of 1 to 3.5. At pH 1.5 the maximum content of gold was 0.02 ppm (Table 2 and Figure 5). Therefore, the control of acidity is important in leaching, because an acid environment must be maintained to keep ferric iron and other metals in solution.

Table 2 Effect of pH on Gold Content Leached from Iron Pyrite Ore with *A. ferrooxidans* (AAS)

No.	pH	Au Content (ppm)
1	1.0	0.01
2	1.5	0.02
3	2.0	0.01
4	2.5	0.01
5	3.0	0.01
6	3.5	0.01

amount of Iron Pyrite Ore = 5 g, temperature = 30 °C, ore size = 250 µm, leaching time = 6 days

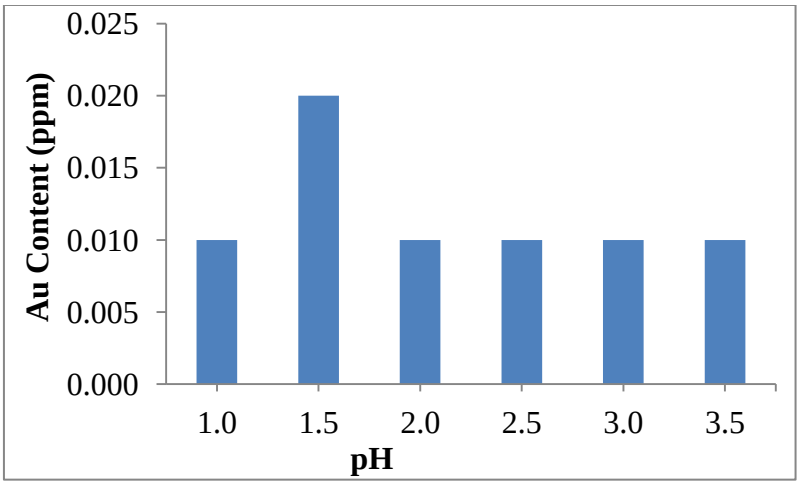

Figure 5 Effect of pH on gold content leached from iron pyrite ore with *A. ferrooxidans* (AAS)

(3) Effect of ore size

The effect of particle size of iron pyrite ore powder on bioleaching process is shown in Table 3 and Figure 6. The nature of substrate is a key role in bacterial leaching process. In this study different particle sizes of 150 µm, 250 µm and 350 µm were used for bioleaching. It was observed that powdered iron pyrite ore with 250 µm gave the highest leaching of gold (0.02 ppm). The rate of leaching of metals is essentially limited by the accessible surface of the minerals and can be enhanced by grinding the minerals of the pieces of ore to small grains.

Table 3 Effect of Ore Size on Gold Content Leached from Iron Pyrite Ore with *A. ferrooxidans* (AAS)

No.	Ore size (µm)	Au Content (ppm) (AAS)
1	150	0.01
2	250	0.02
3	350	0.01

amount of Iron Pyrite Ore = 5 g, temperature = 30 °C, leaching time = 6 days, pH = 1.5

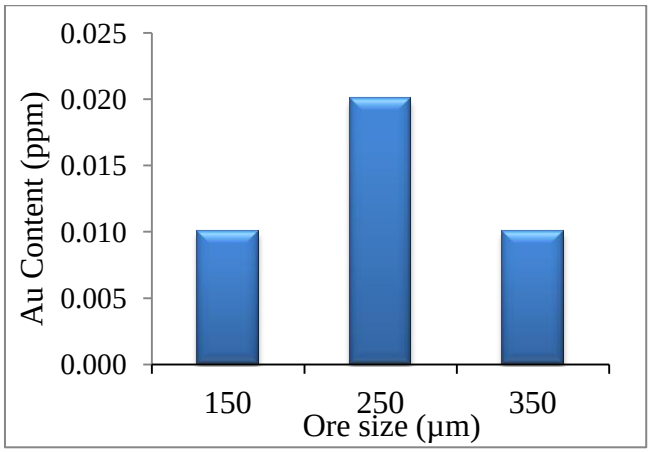

Figure 6 Effect of ore size on gold content leached from iron pyrite ore with *A. ferrooxidans* (AAS)

(4) Effect of Temperature

The effect of incubation temperature on leaching of iron pyrite ore powder is shown in Table 4 and Figure 7. In this study different incubation temperatures (30 °C, 60 °C, 90 °C, 120 °C) were used for bioleaching. It was observed that the highest amount of gold content was 0.02 ppm at 90 °C. It may be due to that although *A. ferrooxidans* is thermophilic it could not survive at high temperature > 90 °C. So, metal leaching property of the bacteria may decrease.

Table 4 Effect of Temperature on Gold Content Leached from Iron Pyrite Ore with *A. ferrooxidans* (AAS)

No.	Temperature (°C)	Au content (ppm) (AAS)
1	30	0.01
2	60	0.01
3	90	0.02
4	120	0.01

amount of Iron Pyrite Ore = 5 g, leaching time = 6 days, pH = 1.5, ore size = 250 µm

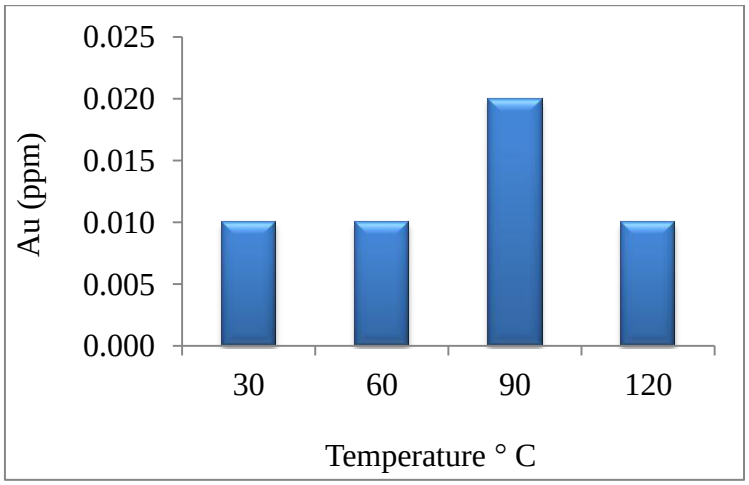

Figure 7 Effect of temperature on gold content leached from iron pyrite ore with *A. ferrooxidans* (AAS)

In gold extraction from iron pyrite ore by bioleaching method, the optimum conditions were found to be pH 1.5, leaching time 6 days, ore size 250 µm and temperature 90 °C giving 6.25 ppm of gold content.

Conclusion

In this study, acid leaching and bioleaching of iron pyrite ore collected from Sakhangyi (SKG) gold mining site, Moehti Moemi, Yamethin township have been revealed. In acid leaching process, aqua regia was used for sample digestion and extraction with D.I.B.K. The amount of gold content extracted by acid leaching was 1.2 ppm. In bioleaching process, *Acidithiobacillus ferrooxidans* was cultured on selected 9 K basal medium and used for leaching of iron pyrite ore powder. Most soluble content of gold was observed to be 3.25 ppm after 6 days of bioleaching. The optimum conditions for bioleaching process using *A. ferrooxidans* were found to be 1.5 of pH, 90 °C, 250 µm ore size after 6 days bioleaching time to get the highest gold content (6.25 ppm).

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References

Ahmad, H. Z., Kham, A. and Raza, A. (2015). " Extraction of Metals from Electronic Waste". *Global Journal of Technology and Optimization,* vol.1, pp. 5-8.

Cruickshank, R. (1968). *Medical Microbiology*. Edinburgh: 11th ed., E. and S. Livingstone Ltd., pp. 125-128.

Grigorii, L., Tat'yana, P. and Tamara, F. (2003). "Phylogenetic Heterogenetity of the Species *Acidithiobacillus ferrooxidans". International Journal of Systematic and Evolutionary Microbiology*, vol. 53, pp. 113-119.

Khin Cho Lin, San Nwe Zin and Daw Hla Ngwe. (2019). "Isolation and Genotypic Identification of *Acidithiobacillus ferrooxidans*". *J. Myanmar Academy of Arts and Science*, vol. XVII, No. 1A, pp. 591-602.

Natal'ya, V. and Muravyov, M. (2010). "Two-stage Bacterial-chemical Oxidation of Refractory Gold-bearing Sulphide Concentrates". *Hydrometallurgy*, vol. 101 (1-2), pp. 28-34.

Olson, J. G. (1994). "Microbial Oxidation of Gold Ores and Gold Bioleaching". *FEMS Microbial Letters*, vol. 119**,** pp**.** 1-6.

Quatrini, R. and Holmes, D. S. (2005). "Genomic Insights into the Iron Uptake Mechanisms of the Biomining Microorganism *A. ferrooxidans*". *Journal of Industrial Microbiol Biotechnol*, vol. 32, pp. 606-614.

Raju, S. V. P. (2005). "Comparison of Different Extraction Methods to Determine Gold in Geological Samples". *Journal of Scientific and Industrial Research*, vol. 65, pp. 65-67.

Rohwerder, T., Gehrke, T. and Kinzler, K. (2003). "Bioleaching Review Part A". *Applied Microbiology and Biotechnology*, vol. 63(3), pp. 239-248.

Sen, C. (2015). "Bioleaching of Gold: An Alternative Green Mining Technology for 21st Century". *Microbiology World*, vol. 2 (3), pp. 13-14.

Silverman, M. P. and Lundgren, D. G. (1985). "Study on the Chemolithotrophic Iron Bacterium *Thiobacillus ferrooxidans*: An Improved Medium and a Harvestion Procedure for Securing High Cell Yields". *J. Bacteriol*, vol. 77, pp. 642-674.

Zahan, S., Tanmi, T. and Rahman, S. (2017). "Extraction of High Purity SiO_2 from Raw Quartz". *Imperial Journal of Interdiscilpinary Research (IJIR)*, vol. 2, pp. 2001-2004.