Recovery of Gold by a Mixed Culture Comprising
*Thiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* and *Thiobacillus thiooxidans*,
*Leptospirillum ferrooxidans*

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**Abstract**—The effects of a mixed culture comprising *Thiobacillus ferrooxidans* (TF), *Leptospirillum ferrooxidans* (LF) and *Thiobacillus thiooxidans* (TT), *Leptospirillum ferrooxidans* on gold extraction were studied using the shake-flask technique. The iron oxidizing bacteria, TF and LF were grown in 9K medium whilst the sulfur oxidizing bacteria, TT was grown in the TT medium supplemented with elemental sulfur. Adaptation of the bacteria was carried out in increasing concentrations of the ore, to a final concentration of 10%. Flasks containing 10% ore comprising a mixture of TF-LF and TT-LF were monitored for the following parameters; Eh, pH, %Au recovered and %Fe solubilized. The amounts of gold recovered on day 5 was slightly higher with the TF-LF combination than the TT-LF combination i.e 89% and 84% respectively. Iron solubilization was also higher with the TF-LF combination. The results seems to suggest that the TF-LF mixture is a better combination for the recovery of gold from the refractory ores used.

1. Introduction

Mineral exploration and mining is one of the major contributors for economic development [1]. Amongst the most sought after minerals are petroleum, copper and gold. However, continuous mining leads to the depletion of good grade ores and the abundance of low-grade or refractory ores.

Low-grade or refractory ore is one which contains copper and iron in a reactive form. These elements form stable complexes with cyanide and compete successfully with gold for available cyanide ions in the leach solution. Thus, excessive quantities of cyanide are consumed and this process may become uneconomical [2]. An ore can also be classified as refractory ore due to its high sulfur and carbon content.

One solution to deal with refractory ores is by roasting or biooxidation. In the former process, the ores are roasted at 600°C, thus converting sulfur to oxides. The individual particles lose internal volume and become more porous, making it accessible to cyanide. Roasting however, incurs a lot more cost and an alternative process has to be sought. Biooxidation offers an attractive solution to the problem. In biooxidation, bacteria is used to degrade the mineral sulfides usually in the forms of pyrite and arsenopyrite, exposing the gold for subsequent cyanidation. Bacteria used for biooxidation includes *Thiothricus ferrooxidans* (TF), *Thiobacillus thiooxidans* (TT) and *Leptospirillum ferrooxidans* (LF). However, biooxidation can be a slow process, but can be solved by adapting the bacteria to the ores prior to biooxidation [3].

In this study, biooxidation of refractory gold ores was attempted using two different combinations of bacteria, TF-LF and TT-LF. The effectiveness of the biooxidation process will be assessed by the amounts of Fe solubilized and gold recovered.

2. Experimental

**Materials**

*Bacteria*. *Thiobacillus thiooxidans* (TT) and *Leptospirillum ferrooxidans* (LF) was obtained from the Deutsche Sammlung Mikroorganismen, DSMZ, Germany. *Thiobacillus ferrooxidans* (TF) was isolated from a local gold mine [4] and sent to DSMZ, Germany for identification. TF and LF was maintained in the 9K medium [5] while TT was grown in the TT medium [6].

*Ore sample*. The refractory ore used in this study was obtained from the Bau Gold Mine in Sarawak. The size of ore used in this study was 75 μm.

*Bacterial adaptation*. Adaptation of TF and LF was carried out as follows; TF and LF previously grown in the 9K medium was serially subcultured into basal salts medium containing 0.5% to 5% w/v ores. TT was adapted by serially subculturing TT grown in the TT medium into basal salts containing 0.5% to 5% w/v ores.

A mixed culture comprising TF-LF and TT-LF was prepared by mixing 5% v/v of each culture in basal salts containing 5% w/v ore. The mixed culture was serially subcultured into basal salts medium containing 6% to 10% w/v ores. This mixture was used as a starter culture in the biooxidation experiment.

**Procedures**

*Biooxidation*. The ore used in this study was mixed using the coning and quartering method to ensure homogeneity of the ore [7]. The ores (10 g) was transferred into 8, 1 L Erlenmeyer flasks containing 100 mL of basal salts medium. The adapted mixed culture of bacteria (10% v/v) was added into 4 of the Erlenmeyer flasks. The remaining 4 flasks acted as control. All 8 flasks were placed in an orbital shaker (Certomat, B.Braun) at 200 rpm, 30°C for 0, 5, 10 and 15 days. On the specified days, Eh and pH readings were taken and
Fe and Au analyses carried out for the respective flasks. Samples for Fe and Au analyses were filtered using a 0.45 μm filter paper (Whatman); the filtrate was collected in eppendorf tubes while the pellet was washed with 4 M HCl and left to dry. Weight of the dried pellet was recorded.

Au analysis. The dried weighed sample was placed in a silica crucible and roasted at 600°C for 2 hours. Upon cooling, the roasted sample was digested in aqua regia, HCl:HNO₃ (1:3) at 70-80°C for 90 minutes. The cooled mixture was then transferred into a 250 mL volumetric flask and the volume made up to the mark with deionized water. Aliquot, 50 mL was pipetted into a separating funnel and the gold was extracted with 10 mL 1% w/v aliquot 336/DIBK. The organic layer was further rinsed with 50 mL 10% v/v HNO₃ while the liquid phase was discarded. Gold analysis was carried out on the DIBK layer using Atomic Absorption Spectrometer (Philips PU-9100X).

Fe analysis. Aliquot, 0.05 mL from the biooxidation experiments was transferred into a 5 mL volumetric flask. The volume was made up to the mark using 1% v/v HNO₃. The Fe content was measured using AAS.

3. Results and discussion

Adaptation

In this study, adaptation of the bacteria was carried out for two main reasons; firstly to adapt the bacteria to utilize Fe from the ores (solid form) compared to soluble Fe(II) supplied in the 9K medium. This is mostly applied to the iron oxidizers, TF and LF. In the case of the sulfur oxidizer, TT adaptation was carried out in basal salts medium supplemented with ore, which serves as the sulfur source. Since the medium used for biooxidation was basal salts, TT normally grown in TT medium had to be adapted to grow in this new medium. The second reason is to increase the rate of bacterial activity [8]. For the TF-LF combination (both iron-oxidizers), the growth period was 1-2 days compared to 3-4 days for the unadapted bacteria. The growth period for TT-LF was longer i.e. between 2-5 days. This is to be expected as the bacteria used are very different, LF an iron oxidizer and TT a sulfur oxidizer.

Figure 1 shows the Eh and pH profiles of the mixed cultures during the adaptation process.

![Figure 1. pH and Eh profiles for mixed-cultures during adaptation on the refractory gold ores](image)

For the TF-LF mixture, pH values was in the range of 1.95 - 2.20. This shows the presence of Fe²⁺ in favour of Fe³⁺. The decrease in Eh values can be explained using the following equation [9].

\[
Eh = 0.771 + 0.059 \log \left[ \frac{[Fe^{3+}]}{[Fe^{2+}]} \right]
\]

Since Fe²⁺ predominates, very little Fe³⁺ is present thereby decreasing the Eh values.

Eh values are also important in predicting the dominant bacterial species during biooxidation. From the Eh profiles obtained, it can be concluded that LF is the dominant species compared to TF. This is in agreement with the findings of Rawlings et al. [10]. LF was found to have a higher affinity for Fe²⁺ than TF with values of Km for Fe²⁺ being 0.25mM and 1.34mM respectively. LF is also less inhibited by
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Fe³⁺, the Kᵢ value for Fe³⁺ is 42 mM compared to 3.1 mM for TF.

The pH-Eh profile for TT-LF mixture is not as clear as for the TF-LF mixture. This is to be expected as the two bacteria present have very different properties, hence different metabolism leading to different pH and Eh profiles. One interesting feature is that the pH value of this mixture did not exceed 2, supporting the dominance of LF over TT.

Biooxidation

The pH and Eh profile of the mixed-cultures during the biooxidation process is shown in Figure 2.

Figure 2. pH and Eh values for mixed-cultures during the biooxidation process.

In general, higher pH values were recorded i.e. 3.7 for the TF-LF mixture and 4.0 for the TT-LF mixture. This high pH value could indicate the presence of jarosites, KFe₃(SO₄)₂(OH)₆ i.e. a ferric ion precipitate which can cover the ore surface, preventing further leaching from occurring. High jarosite levels also produce an additional disposal problem [11]. The Eh values did not vary much during the biooxidation process, probably indicating the presence of Fe³⁺.

Au analysis

To assess the performance of the TF-LF and TT-LF mixtures, day 5 (Figure 3) was chosen as the point of comparison.

Figure 3. %Au extracted during the biooxidation process.

On this day both bacteria recorded a gold recovery of 89% and 84% respectively. This probably indicates that the mixed culture has reached its optimum condition in the biooxidation process [12]. A point to note is that the highest recovery of gold for the TF-LF mixture was 98%. However, in this study time is crucial and the combination of bacteria which gave the highest amount of gold in the shortest possible time is preferred. It is also worth mentioning that the ores in the experiments were not sterilized. Hence, endogenous bacteria present in the ore could proliferate in the basal salts medium, leading to slightly lower gold recoveries than the flask containing the mixed culture (Figure 3).

Fe analysis

The Fe solubilization profile of the mixed culture and the control is shown in Figure 4.

Figure 4. % Fe solubilized during the biooxidation process.
The amounts of Fe solubilized is higher in the TF-LF mixture on Day 0, around 15% compared to 9% for the control. This finding is in agreement with that obtained by previous work [13]. A slightly different trend was observed in the TT-LF mixture. More Fe is solubilized in the control flask (10%) as compared to the flask containing bacteria (7.5%) on Day 0. This can be explained by the use of basal salts as the growth medium which would benefit LF compared to TT. The early stage of biooxidation process is more of an adaptation period of TT in the basal salts medium and the metabolism of S from the ore. This might affect the Fe oxidation by LF. As biooxidation progresses, more of the S is being oxidized, hence exposing Fe. This agrees with the findings of Lawrence, R.W. [12] that S minerals are normally found on the outer layer of refractory ores compared to Fe. Also the presence of endogenous iron-oxidizing bacteria in the control could explain the higher rate of Fe solubilization. The better performance of iron oxidizers (TF-LF) compared to Fe-S oxidizers (TT-LF) is also supported by Lawrence, R.W. [12].

4. Conclusions

Based on the results obtained, it can be concluded that the TF-LF mixture was better than the TT-LF mixture in the biooxidation of refractory gold ores. The amount of gold extracted for the TF-LF mixture was 85-98% while for TT-LF mixture was between 60-85%. During this period, the maximum amount of Fe solubilized was 9% for the TF-LF mixture and 8% for TT-LF. The pH and Eh values were constant for TF-LF but not for the case of TT-LF mixture throughout the biooxidation process. However, from the Eh values obtained, it can be predicted that LF is the dominant species compared to TF and TT.

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References

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