The present work was submitted to the Faculty of Engineering

Bioleaching of Sulfide Ores by a Pure Culture of *Acidithiobacillus Thiooxidans*

Bachelor Thesis

by

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Statutory Declaration

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I hereby affirm in lieu of an oath that I provided the submitted bachelor thesis

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I did not use any sources other than those stated. In case that the work is additionally submitted on a data medium, I declare that the written and the electronic form are completely identical. The work was not submitted in the same or similar form to any examination authority.

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Table of Contents

List of Figures

List of Tables

Abstract

Throughout the world, the reserves of metal ore deposits are dwindling, research into the extraction of metals from low grade deposits are becoming increasingly important. Low concentrations of Cu, Fe were analyzed in the Oyu Tolgoi Southwest deposit's Phase 4B ore, which is unsuitable to be extracted through flotation method. Bacterial cells have been shown to be capable of converting metals form solid to liquid phase in the bioleaching process. Particle size, pH, and pulp density are the most significant characteristics in bioleaching; thus, we focused our research on improving the solid-liquid phase ratio. The microorganisms *Acidithiobacillus thiooxidans*, both acidophilic and mesophilic, were used in this study. AAS analysis was used to determine the metal recovery in the leachate, while the residual metal concentrations in the insoluble form were determined through calculations. The solid-liquid phase ratios of 1:2, 1:4, 1:6 was investigated. The maximum Cu (0.5%) was observed at solid liquid phase ratio of 1:6, according to atomic absorption spectrometry (AAS) data.

Acknowledgement

I would like to express my sincerest gratitude to Ms. Munkhzaya as she was there to assist and guide me through this study. I would also like to thank KhanLab LLC. for their assistance.

1 Introduction

1.1 Background

In the current day and age, as much as the beneficiation, extraction methods, and mining sector as a whole is evolving ever so greatly, so does the rapid depletion of high-grade ore reserves. Additionally, environmental protection regulations have been getting increasingly strict.

Thus, methods that are feasible for low-grade ores with low cost and more importantly, methods that do not require environmentally harming substances are becoming highly attractive options.

Biometallurgy is the perfect solution for the previously mentioned problems. More traditional leaching methods have the disadvantage of leaving many environmentally damaging footprints, and the inability to reach the insides of solids resulting in incomplete leaching which is economically disadvantageous. Neutralizing said leachates requires many steps as well as capital. Extraction of metals from ores that have complex content and properties is ideal for this technology.

Therefore, to further develop this recovery with no excess rejects, environmentally friendly, economically feasible, and low maintenance cost method, scientists all over the globe have been actively studying this subject and have been exercising it since the 1970s.

1.2 Biometallurgy

Biometallurgy is a field of study for biotechnological processes involving the interactions between microorganisms and metals or metal-bearing minerals. In Biometallurgy, Biomining and Bioremediation have been the two most attractive branches in the field, which are also applied on a large scale worldwide.

The simple procedure of biohydrometallurgy goes as follows: solubilizing the insoluble sulfidic content of a metal-bearing mineral with the assistance of microorganisms, and separation of pure metals from the pregnant solution. Biometallurgy consists of three main branches.

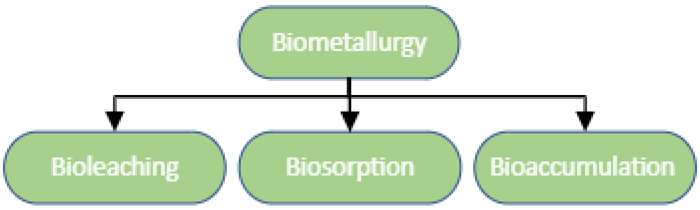


Figure 1. Main branches of biometallurgy

Bioleaching is the process of biological conversion of an insoluble sulfidic compound into a water-soluble sulfidic form with the help of a microorganism, further extraction of pure metal from bioleaching has a very straightforward theory. A general schematic of a bioleaching process is shown here.

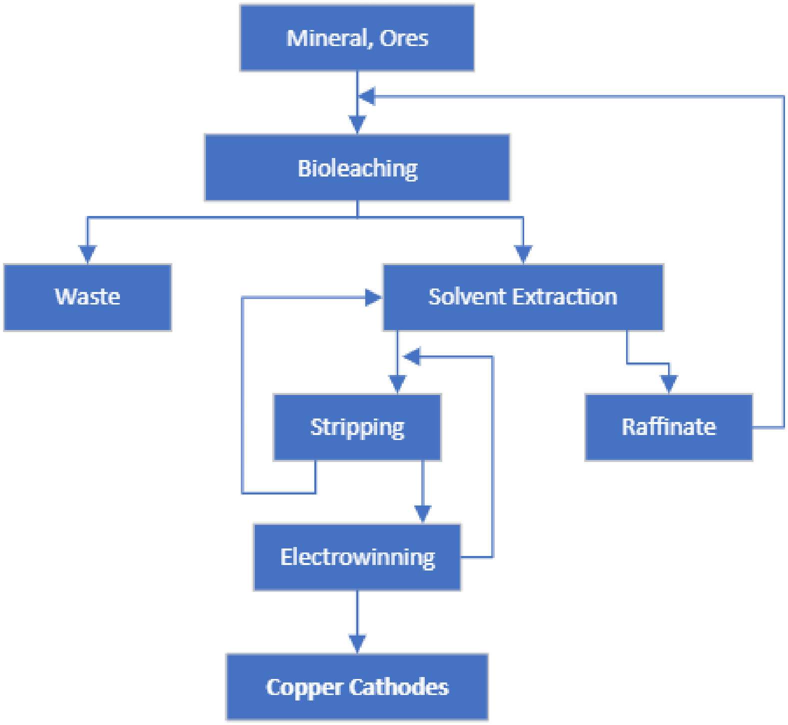


Figure 2. General schematic of bioleaching process

Biometallurgy advantages include:

- Large processing factories with massive pieces of machinery are unnecessary
- Minerals with complex composition can be leached
- Economically feasible
- Environmentally friendly
- Rehabilitation potential for waste solution

1.2.1 Basis of Biohydrometallurgy Process

In 1947, scientists led by Hinkel, Colmer, and Temple (1) have first suggested that Thiobacillus microorganisms could be used as biocatalysts in the production of metals by separation after the discovery that inside the sulfide deposits and mine drainage contained living organisms of thiobacillus, through the result of a research of microbiological analysis, it was shown that the microorganisms could accelerate the dissolution and oxidation of sulfides.

An American scientist G.D. Sylvia has mentioned a few fundamentals of bioleaching:

- Bioleaching can only influence the sulfidic compound of a deposit.
- Mineral bioleaching occurs as a result of chemical reactions.
- Metal recovery can only be determined only by the amount of solute
- The dissolved metal must have formed a compound easy to separate.

Biohydrometallurgy consists of three steps (2).

- Bacterial leaching of mineral ores
- Separation of metals from solutions by traditional methods
- Recovery of the waste solution

The basis of the science of biometallurgical production.

- Industrially important acidithiobacillus bacteria, in particular, strongly oxidizes any sulfide compound to sulfate form.
- The most suitable solution medium for mineral leaching is pH=2.8-3.5 and the temperature at which the bacteria live and remain active as well as biometallurgy production conditions for continuous operation.

1.2.2 Physicochemical Basis of Bioleaching

The process of oxidation of sulfuric mineral by Acidithiobacillus bacteria runs by two methods, direct and indirect.

- ➢ Direct
    - When there is physical contact between the bacterial cell and the mineral sulfide surface, bacteria use their enzymes to act on minerals, and oxidation to sulfate occurs. These enzymes perform as several biocatalysts steps in redox reactions.

      In this process, pyrite is oxidized to iron (III) sulfate according to the following reactions (3):

$$4FeS_2 + 14O_2 + 4H_2O \rightarrow 4FeSO_4 + 4H_2SO_4 \quad \text{(Eq.1)}$$

$$4FeSO_4 + O_2 + 2H_2SO_4 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O \quad \text{(Eq.2)}$$

The direct bacterial oxidation of pyrite is best summarized by the reaction:

$$4FeS_2 + 15O_2 + 2H_2O \rightarrow 2Fe_2(SO_4)_3 + 2H_2SO_4 \quad \text{(Eq.3)}$$

A study by Torma (4) have shown that the following non-iron metal sulfides can be oxidized by T.ferrooxidans indirect interaction: covellite (CuS), chalcocite (Cu2S), sphalerite (ZnS), galena (PbS), molybdenite (MoS_2), stibnite (Sb_2S_3), cobaltite (CoS), millerite (NiS).

Therefore, direct bacterial leaching can be described according to the following reaction:

$$MeS + 2O_2 \rightarrow MeSO \quad \text{(MeS- Metal Sulfide)} \quad \text{(Eq.4)}$$

- Indirect
  - In indirect bioleaching the bacteria generate a lixiviant which chemically oxidizes the sulfide mineral. In acid solution, this lixiviant is ferric iron, and metal solubilization can be described according to the following reaction (4):

$$MeS + Fe_2(SO_4)_4 \rightarrow MeSO_4 + 2FeSO_4 + S^{\circ} \quad \text{(Eq.5)}$$

A. thiooxidans in bioleaching consists of creating favorable acid conditions for the growth of ferrous iron-oxidizing bacteria such as T. ferrooxidans and L. ferrooxidans.

A familiar example of an indirect bioleaching process of uranium extraction from ores, albeit insoluble tetravalent uranium is oxidized to the water-soluble hexavalent stage of uranium:

$$U(IV)O_2 + Fe_2(SO_4) \rightarrow U(VI)O_2SO_4 + 2FeSO_4 \quad \text{(Eq.6)}$$

The process of bioleaching in nature begins and takes place with the dissolution of pyrite. Pyrite is the most abundantly scattered sulfidic mineral. Although it does not dissolve in the presence of water, it does dissolve during mining operations when in contact with air and becomes soluble with oxygens in the air as well as water. Pyrite formation, as well as dissolving steps, are shown below:

$$S + FeS \rightarrow FeS_2 \quad \text{(Eq.7)}$$

$$FeS_2 + 3.5O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \quad \text{(Eq.8)}$$

The resulting Fe^{2+} ions are oxidized by bacteria to form strong oxidants such as Fe^{3+} and sulfidic acid.

$$Fe^{2+}\ bacteria \rightarrow Fe^{3+} \quad \text{(Eq.9)}$$

Resulting Fe^{3+} oxidizes pyrite and other sulfide minerals to form Fe^{2+} ions, which are oxidized again by bacteria.

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad \text{(Eq.10)}$$

1.2.3 Mechanism of dissolution of sulfide mineral by microorganisms

One of the foundations for the successful development of biometallurgical production is to study in detail, how sulfide minerals of non-ferrous metals are oxidized by microorganisms and how they dissolve in biologically active solutions under their influence.

The physicochemical properties of the mineral are altered by the continuous accumulation of microorganisms on the surface of solids and thus the chemical composition changes. The mineral electrode’s potential decreases during this change, and the redox potential increases. Therefore, the solid-liquid phase system undergoes an oxidation-reduction reaction in which the mineral rapidly oxidizes and loses electrons.

1.2.4 Factors affecting mineral bioleaching

Proper determination of mineral composition, the concentration of elements in the biosolution, and bacterial cell number per unit volume furthermore adapting bacteria to its environment, as well as grinding of mineral, solid-liquid ratio, and the optimum choice of temperature are significant to the test result. The following are affecting factors in bioleaching:

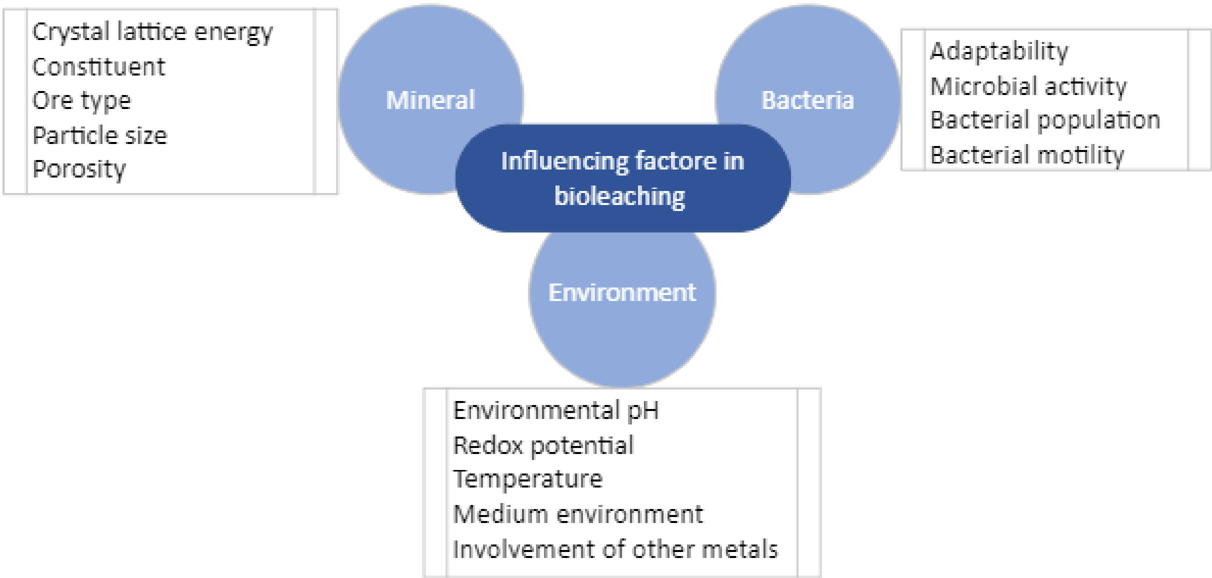


Figure 3. Factors affecting in bioleaching

- **Lattice energy**. A measure of the stability of a sulfide mineral is the energy of the crystal lattice energy, which is the energy used to break the crystals. The lattice energy is determined by the strength of the bond between the atoms and ions, and low-energy sulfides oxidize more rapidly.

- **Sulfide mineral chemical potential**. The chemical potential of sulfide minerals has a great influence on oxidation speed. This represents their extent of oxidation. A gas, Fe(II), Fe(III) and non-ferrous metal dissolved in the electrode potential of a sulfide mineral as well as the redox potential of the environment containing hydrogen ions have considerable influence on bioleachability.

  It is appropriate to use electrode potentials measured in a specific environment to assess mineral strength. The higher the potential of the sulfide electrode, the greater its acceptor (acidic) properties and the stronger it is with low-potential minerals.

- **Temperature impact.** Bioleaching is highly dependent on the environment's temperature. For example, chalcopyrite 20-30℃, chalcocite 25℃, and arsenopyrite 30℃ are the optimum recovery conditions for bioleaching. Furthermore, for non-ferrous sulfidic metals, 20-35℃ is considered ideal.

➔ **Solid-liquid phase ratio.** For any type of metallurgical leaching solid-liquid ratio is one of the most important segments. If the solid-liquid phase ratio is smaller than necessary, the content of metal ions in the solution increases, which makes the medium for microorganisms inhabitable, basically putting a halt to the leaching process. However, when the solid-liquid ratio is higher than necessary, because the non-ferrous metal ions contained in a small portion of the solid phase in the solution, shift in smaller amounts, it cannot be determined accurately by chemical methods.

➔ **Mineral comminution.** The importance of comminution is to reduce the average size of the mineral to increase the surface area of solids, increasing the rate of oxidation. The optimum size reduction for bioleaching is between 0.05 - 0.1mm. Meanwhile, the reduction size is higher, in this case, the amount of liquid phase used in bioleaching is determined by the solid weight ratio. Yet, when the comminution size is lower than 0.05 - 0.1mm, the volume of the liquid phase is determined by the sum of the mineral surface area. In 1970, French scientist Rosin-Rammler generated an equation for the determination of mineral surface by the rate of comminution and the weight of solid.

➔ **Media.** Microorganism cell division requires the use of specific substances. The 9K medium turns toxic if the content of major salts in the medium exceeds a certain level. Water occupies a special place in the growth of microorganisms and more than 20% of the solution must be water for growth, if the medium contains little to no water the microorganisms are unable to populate. Growth media are divided into artificial and non-artificial, and the physical properties are divided into liquid and solid. Under natural conditions the growth and reproduction of microorganisms are slow, and the new number of cells produced and dying cell numbers are the same, this explains why the composition of microorganisms in the environment is stable. In bioleaching, the acidity of the media has a great effect. Microorganisms that act as bioleaching catalysts in an acidic environment can influence mineral leaching mechanisms.

➔ **Inoculation size.** Most microorganisms agglomerate on the surface of the solution, making it difficult to determine the number of microorganisms during bioleaching. The main parameters for determining the number of bacteria are microbial biomass and metabolic activity. It is considered to be of industrial importance when the inoculation size is 10^5-10^6 cells/ml.

Adverse factors affecting Acidithiobacillus bacteria:

- **Effect of anions.** Some anions contained in water, released by the biodegradation of minerals, harm the life of sulfide oxidizing bacteria. This is because they change the electrical potential of their membranes and make them more sensitive to acids. Among the harmful anions Cl^-, Br^-, NO_3^-, and SCN^- are particularly destructive. For example, when the concentration of thiocyanate in a bacterial solution reaches 1 mg/L, the activity of bacteria that oxidize sulfur is completely halted.

- **Metals.** Although metals are extracted from sulfide minerals by bioleaching, metals, and metalloids in the form of cations in solution adversely affect bacterial activity.

1.3 Microbiology

Microbiological research began in the seventeenth century with the invention of the simple microscope. The science of microbiology is a single-celled invisible to the naked eye, visible only under a microscope, but the study of a large number of living organisms. Microbes differ from macrobic cells in their remarkable ability to carry out all the vital processes of life, such as growth, reproduction, and energy exchange, independently of their similarities and differences. The field of modern biotechnology is biogeotechnology. Biogeotechnology is the study of chemical element distribution and the influence of geological microorganisms on it.

1.3.1 Industrially important microorganisms

The use of microorganisms for industrial purposes has a long history, and industrially important microorganisms are those that are utilized in the manufacturing of goods that use the properties of the microbial's own cells to reproduce and the internal functions of the microorganisms. Microbes are being used to make a variety of drugs, chemicals, and foods. Thionic bacteria are one of the most significant microorganisms for manufacturing.

1.3.2 Acidithiobacillus genus bacteria

The most widely used microorganisms in the production of hydrometallurgy are Acidithiobacillus bacteria, which are sulfur-oxidizing bacteria. Which oxidizes about 30 types of sulfide minerals.

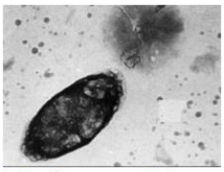
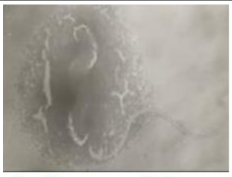
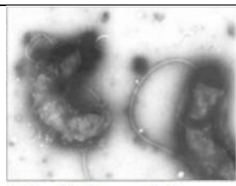
Acidithiobacillus bacteria are single-cavity prokaryotes that breathe oxygen and live in sulfuric environments and are important in biotechnology because they oxidize Fe (II) to Fe (III) and sulfur alone to sulfuric acid. Its most active species are *A. ferrooxidans*, *A. caldus,* and *A. thiooxidans*.

1.3.3 Mongolian thionic bacterial study

The study of the possibility of chemical and biological enrichment of industrial waste and poor ore is of great importance for the comprehensive use of the deposit, the separation of some metals and rare elements, and the development of technological procedures.

In our country, research in this area began in the mid-1980s, and in mid-1988, D. Sereedorj, a microbiologist at the Erdenet Mining Corporation, isolated a new strain of *Acidithiobacillus ferrooxidans* from groundwater in a copper-molybdenum deposit. Furthermore, Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans, and Leptospirillum ferrooxidansa bacterium that oxidizes iron and sulfur were isolated from groundwater at the Erdenet stockpile copper-molybdenum deposit was discovered in 1987. and morphological and physiological studies were conducted in the research laboratory of non-ferrous metals and rare elements of the National University of Mongolia (5) and a biogeotechnological study was started in Mongolia (S.Davaasuren 2012). Morphology and physiological characteristics of these bacteria are shown in Table 1.

Table 1. Thionic bacterial characteristics

Bacterial Properties			 ***Th.ferrooxidans***	 ***L.ferrooxidans***	 ***Th.thiooxidans***
Morphology	Color		Gram Positive	Gram Negative	Gram Negative
	Shape		Bacillus	Bacillus, Spirillus, Coccus	Bacillus
	Size (µm)		0.6×1.35	0.8×1.28	0.4×1.35
Physiology	Respiratory system		Aerobic	Aerobic	Aerobic
	Living environment	pH	1.5-2.5	1.8-2.5	2.5-3.0
		T, °C	30-40	30-32	30-37
	Oxidizing substrate		Fe^{2+}, S^{2-}	Fe^{2+}, FeS_2	S^0, $S_2O_3^{2-}$, $S_4O_6^{2-}$

1.3.4 Acidithiobacillus thiooxidans

Waksman and Joffe isolated and described the sulfur-oxidizing bacterium Acidithiobacillus (At.) thiooxidans (then known as Thiobacillus thiooxidans) in 1921, making it the first severely acidophilic microbe to be isolated and characterized. Figure 4. shows the electron microscope image of *At. Thiooxidans*.

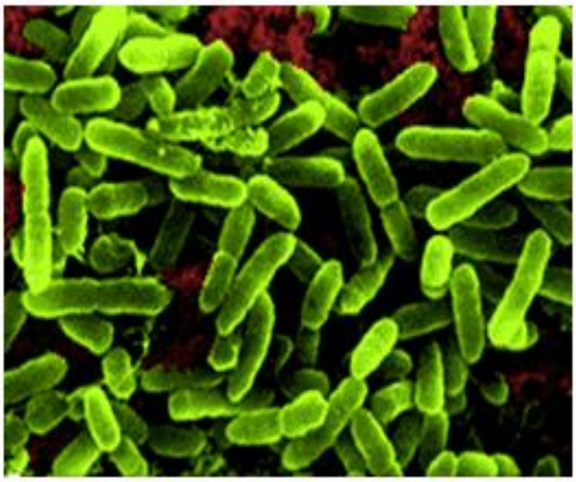


Figure 4. Electron microscope image of *At. Thiooxidans*.

Other sulfur-oxidizing bacteria were identified from water draining a coal mine a few years later, and they possessed the unusual (at the time) ability to oxidize ferrous iron to ferric. As a result, (Acidi)thiobacillus ferrooxidans have become the most researched acidophilic microbes. Both of these early isolates are autotrophic chemolithotrophs, meaning they rely on inorganic electron donors and fixed carbon dioxide for their growth. The average cell length of *A. thiooxidans* is less than 1 µm, while the average diameter is less than 0.5 m (6), with a temperature range of 10-37°C and ideal temperatures of 28-30°C, it is mesophilic. The organism is a Gram-negative, rod-shaped bacterium that obtains energy by metabolizing sulfur. Acidophilic bacteria thrive in settings with a pH range of 2.0 to 3.0. (7)**.**The microorganism is frequently discovered in cave biofilms and may have a role in cave system creation (8). The fundamental nutrients for metabolism are oxygen, carbon, and nitrogen. *A. thiooxidans* is an obligate aerobe that gets its energy from inorganic sulfur and uses oxygen as an electron acceptor and utilizes it to create sulfuric acid. To satisfy its carbon requirements, the organism fixes atmospheric carbon dioxide (9). Their development is slowed by the presence of carbonates, which make the medium alkaline. To help with growth, small quantities of nitrogen are gathered from ammonium salts, such as sulfate (6). Its oxidizing substrates include S^0, S^{2-}, $S_2O_3{}^{2-}$, $SO_3{}^{2-}$, $S_4O_6{}^{2-}$.

Table 2. Morphology and physiology of *Acidithiobacillus thiooxidans*

<table>
<tr><td colspan="4">Acidithiobacillus thiooxidans</td></tr>
<tr><td rowspan="4">Morphology</td><td>Color</td><td colspan="2">Colorless, Gram-Negative</td></tr>
<tr><td>Shape</td><td colspan="2">Thick rod with rounded ends</td></tr>
<tr><td>Movement</td><td colspan="2">Single polar flagellum</td></tr>
<tr><td>Size</td><td colspan="2">0.5 - 0.6 × 0.85 - 1.0 μm</td></tr>
<tr><td rowspan="3">Physiology</td><td>Respiration</td><td colspan="2">Aerobic</td></tr>
<tr><td rowspan="2">Optimum condition</td><td>pH</td><td>1.7 to 3.3</td></tr>
<tr><td>Temperature</td><td>25 - 38°C</td></tr>
</table>

1.4 Oyu-Tolgoi copper-gold deposit

1.4.1 Oyu-Tolgoi copper-gold deposit location

The Oyu Tolgoi copper-gold-molybdenum porphyry deposits are found in Mongolia's South Gobi Desert (43°01′40″N, 106°51′34″E), about 550 kilometers south of Ulaanbaatar and 80 kilometers north of the Chinese border. They are the world's largest group of high-grade Paleozoic porphyry deposits currently known (10).

1.4.2 Deposit exploration status

Along the Oyu Tolgoi district, porphyry Cu-Au deposit and mineral occurrences are found in a 26 km long, N-NWE-trending belt. The Oyu Tolgoi deposits are partially exposed, especially at the South deposit, where tiny pits and stone tools found which imply Bronze age exploitation for copper or blue-green colored secondary copper mineral extraction for paint. Despite these Bronze age workings, the Oyu Tolgoi area was not investigated by trenching or drilling throughout the Soviet era in Mongolia (before 1991), Only a Mo geochemical anomaly was discovered near the Central deposit during joint Mongolian and Russian geochemical research (11).

BHP (Magma Copper Ltd.) geologists are credited with discovering porphyry Cu deposit at Oyu Tolgoi in September 1996 and conducting the initial exploration between 1997 and 1999 (12). BHP carried out geochemical and geophysical surveys as well as 23 diamond drill holes, resulting in the discovery of the North, Central, and South mineralized zones, for which Perelló(12) estimated a total in situ resource of 438 Mt at 0.52 percent Cu and 0.25 g/t Au. Hugo Dummett, the greatest deposit, lies hidden behind more than 800 meters of unmineralized rock, and was not discovered by BHP.

Ivanhoe Mines Mongolia Inc. (IMMI) commenced exploration in May 2000. The focus of exploration during 2000-2001 was a supergene chalcocite zone at Central deposit, three diamond holes drilled near the end of the reverse circulation program in late 2001 marked a turning point in exploration; OTRCD150, drilled between two shallow BHP holes on the western side of the South deposit, intersected 508 m of 0.81% Cu and 1.17 g/t Au, starting below 70 m, and was the discovery hole for the Southwest deposit. A final hole conducted to the north of the IP target in September 2002, uncovered 638m at 1.6% Cu, commencing at 230m deep; this finding would subsequently be known as the Hugo Dummett South deposit. The Heruga deposit (13) was identified in 2007, again targeting an IP anomaly. The finding of Heruga to the south bolstered the geologic notion of deposits aligning along an N-NE trend, and it also spurred exploration of the 3 km gap between the Heruga and Southwest deposits, where continuous drilling has yielded further mineralization.(13)

1.4.3 Open pit Phase 4B ore

The Oyu Tolgoi project has five deposits with a strike length of nearly 6.5 kilometers. They are known as South Oyu, Southwest Oyu, Central Oyu, Hugo South and Hugo North in order from south to north. Two open pits will be used to mine the first three deposits. In this study, Oyu Tolgoi's Southwest Oyu deposits' Phase 4B Low-Grade ore was studied.

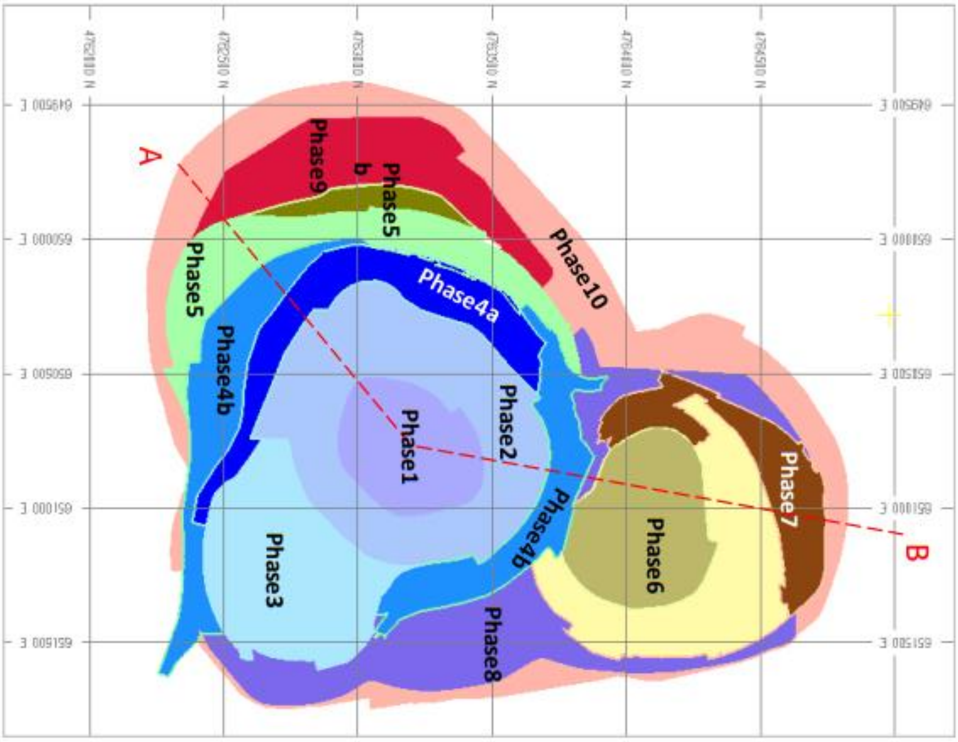


Figure 5. OT open pit mine design

1.4.4 Objectives and Significance of the Study

The objective of this study is to investigate the bacterial leaching of unbalanced sulfidic ow-grade ore of Oyu-Tolgoi's deposit by the pure culture of Acidithiobacillus thiooxidans.

Target:

- Determine the content of primary and secondary elements in the unbalanced ore
- Obtain pure culture of Acidithiobacillus thiooxidans and further monitor and optimization of bacterial growth conditions.
- Sulfidic low-grade copper ore bioleaching under laboratory conditions, to obtain the optimum solid-liquid ratio.

The novelty of the study:

- Brought in and cultivated the first pure strain of *At. Thiooxidans* in Mongolia.

1.5 Mining scale bioleaching process

Bacterial bioleaching tests at the mining scale are carried out in four main areas, these include:

I. Designing of bioreactors for culturing and accumulating Acidithiobacillus bacteria

II. Recovery of waste solution

III. Selection of chemical catalysts to accelerate the bioleaching process

IV. Mathematical modeling to determine the appropriate values of the main parameters of the bioleaching of unbalanced ore with a given grade.

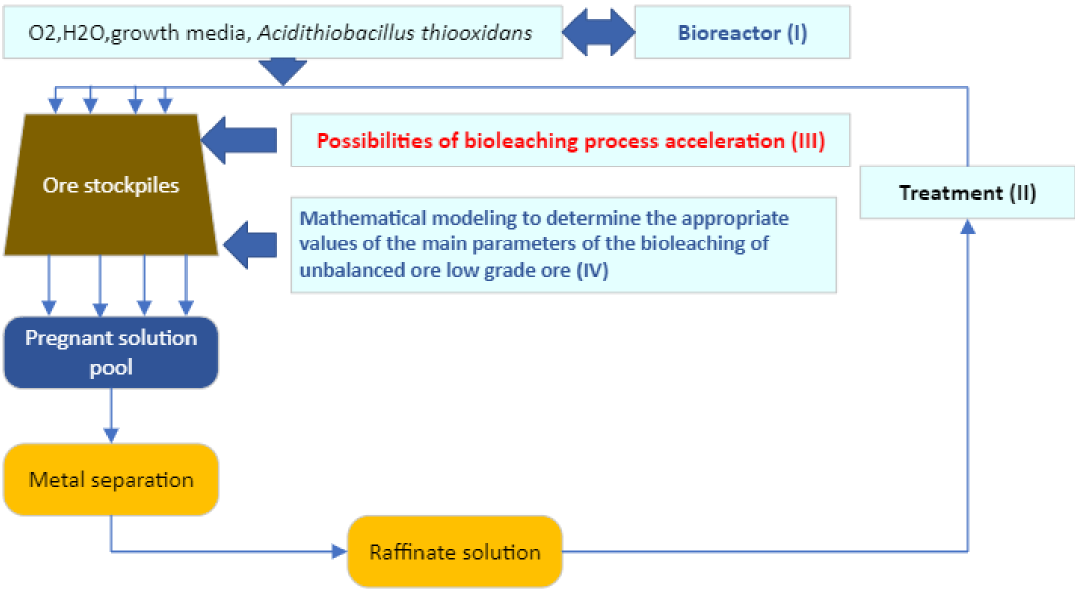


Figure 6. Schematic of the bioleaching process on the ore deposit

1.5.1 Model of bioreactors for culturing and accumulation of *A. thiooxidans*

The biohydrometallurgy industry has been developing rapidly in recent years, with the improvement of leaching technology for poor and oxidized ores. Relating to that, several issues need to be addressed, such as how to cultivate, how to accelerate the cultivation process, and where to store large quantities of biosolutions that contain microorganisms used in bioleaching. These problems are being solved by bioreactors.

The main principle of operation of the bioreactor is to introduce the bacterial culture medium into the tank with a constant flow, and the enriched biosolution is also continuously discharged from the tank and used in the bioleaching process.

The bioreactor consists of several parts, such as the main body (a tank containing bacteria and provided with conditions for growth), nutrition input, steam input (which, in addition to allowing air to pass through the solution evenly, reduce the size of the air bubbles by enlarging the area of oxygen and reducing the diffusion path), impeller (responsible for evenly distributing the medium and homogenizing the bacterial solution), pump (responsible for regulating the flow of media and bacterial solution), control system (responsible for monitoring the entire process in the bioreactor, i.e. pH, redox potential, the temperature of the bacterial solution) and they shall meet the following requirements as a basis:

- Keeping large amounts of biomass.
- Good mixing to ensure that the additives and nutrient medium are evenly distributed.
- Keeping constant temperature.
- Must be sterile and clean.

1.5.2 Possibilities of acceleration of bioleaching process

For the treatment of sulfide minerals, bioleaching is an intriguing option (ores and concentrates). It does, however, have a significant drawback: its sluggish kinetics which restricted its wider commercial applicability. Significant efforts have been undertaken in recent years to optimize the process in all aspects, including biological, microbiological, and metallurgical. Various metal ions are used as chemical catalysts. For examples: Sn^{2+}, Ag^{+}, Co^{2+}, Bi^{0}, Ni^{2+}, Mn^{2+}, Zn^{2+}.

The bioleaching of chemical catalysts for the silver catalyst is as follows:

$$CuFeS_2 + 4Ag^+ \rightarrow 2Ag_2S \downarrow + Cu^{2+} + Fe^{2+} Ag_2S + 2Fe^{3+} \rightarrow 2Ag^+ + 2Fe^{2+} + S^0 \qquad \text{(Eq.11)}$$

The poor ores' reaction involving chemical catalyst is described by chalcopyrite, the worst soluble mineral in copper, which is combined with mineral sulfur to accelerate bioleaching.

Catalysts are used in very small quantities in bioleaching acceleration tests because they are not economically viable. Researchers in other countries have found that it is possible to accelerate bioleaching using chemical catalysts by 2-4 times.

2 State of the Art

The science behind the biological conversion of the insoluble metal compound into a water-soluble form, the Bio Hydrometallurgical field, has been studied since the early 21st century and has been making tremendous progress ever since.

A brief history of Biohydrometallurgy

- In 1922, the first scientific paper relating to the leachability of bioleaching from sulfidic minerals was published. (14)

- In 1947, Colmer, Tempel, and Hinkel first extracted sulfur oxidizing microorganism *Thiobacillus ferrooxidans*, not only does it oxidizes sulfur in an aqueous solution, in an acidic environment, oxidizes iron (II) ions and forms iron (III) ions to dissolve sulfide minerals. (1)

- In 1958, the first patent for the usage of Thiobacillus was given, which later was applied to an industrial scale factory in Bingham, Maine, USA. (15)

- In 1964, Russians tested the method of oxidizing copper-bearing minerals while it was underground, in the copper-producing factory for the first time.

Now, In the biohydrometallurgy field, copper, zinc, uranium as well as precious metals, and heavy metals are being extracted by the methods of heap leaching and underground mining methods such as in-situ leaching. These technologies have been applied on an industrial scale in countries such as the USA, Canada, Russia, and Bulgaria, as well as countries with low-grade ores, and for recycling metal-bearing landfills. After all, this technology is 1.5 to 2.0 times more cost-efficient than the conventional methods (5), which is why copper produced by microorganisms accounts for 5% of copper production globally.

In our country, biohydrometallurgy field of science has attracted many studies since 1980s. The local strain of *A. ferrooxidans* and accompanying *Acidithiobacillus* were first discovered and isolated from the Tsagaan Suvarga deposit of copper and molybdenum. From the groundwater of the open pit mine of Erdenet Mining Corporation, pure strains of:

- In 1987, Thiobacillus ferrooxidans
- In 1992, the bacteria Thiobacillus thiooxidans, Leptospirillum ferrooxidans

were isolated and their morphology and physiology were studied in the Laboratory of Combined Biogeotechnology and Environmental Chemistry of the National University of Mongolia (5). Researches are still underway to leach minerals using these bacteria.

3 Methodology

3.1 Monitoring or experiment procedure

The process of bio-oxidation with the help of microorganisms can be expressed by the reaction equation:

$$Fe^{2+} + 1/4O_2 + H^+ \xrightarrow{Bacteria\ (A.t)} Fe^{3+} + 1/2H_2O \qquad \text{(Eq.12)}$$

$$S^0 + 3/2O_2 + H_2O \xrightarrow{Bacteria\ (A.t)} SO_4^{2-} + 2H^+ \qquad \text{(Eq.13)}$$

The solution's pH changes due to the consumption and production of H^+ ions. The pH value drops due to the production of H^+, according to (Eq.13). However, the depletion described in (Eq.12) persists on chalcopyrite bioleaching and Fe^{3+} ion regeneration. Hence according to this theory, when the depletion and production of H^+ ions are in dynamic equilibrium, the pH value remains virtually constant in the range of 1.6 - 2.

As a result, the pH of the solution, the amount of copper transferred to the solution, and factors such as solutions redox potential were selected as the key bioleaching control parameters, and a 25-day bioleaching test was performed on the Oyu-Tolgoi's Low-Grade Phase 4B non-balanced ore.

Figure… shows the main factors to control during the bioleaching process.

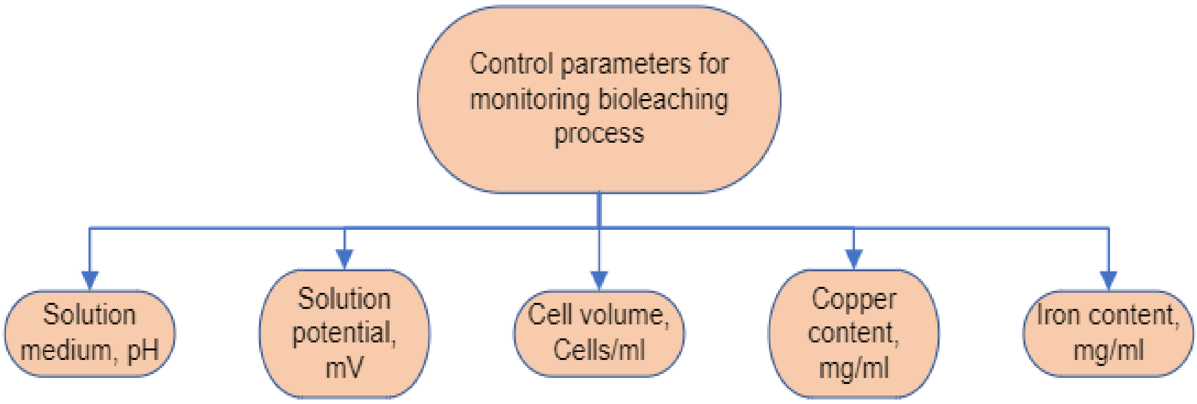


Figure 7. Bioleaching process control factors

3.1.1 Determination of pH and Redox potential of the solution

The pH and redox potential of the solution were measured with a SARTORIUS PB-10 pH/mV meter.

Figure 8. SARTORIUS PB-10 pH meter

pH=7.01 and pH=4.62 solutions were used for pH/mV meter calibration.

Figure 9. Buffer solutions used in pH meter calibration

3.1.2 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) method for the determination of copper (Cu).

The components elements (atoms) of an analytical sample are stimulated when plasma energy is applied from the outside. Emission rays (spectrum rays) are released when the excited atoms return to a low energy location, and the emission rays that correspond to the photon wavelength are measured. The strength of the photon rays determines the element type, and the position of the photon rays determines the content of each element. The working principle of ICP-OES is shown in Figure 10. (16)

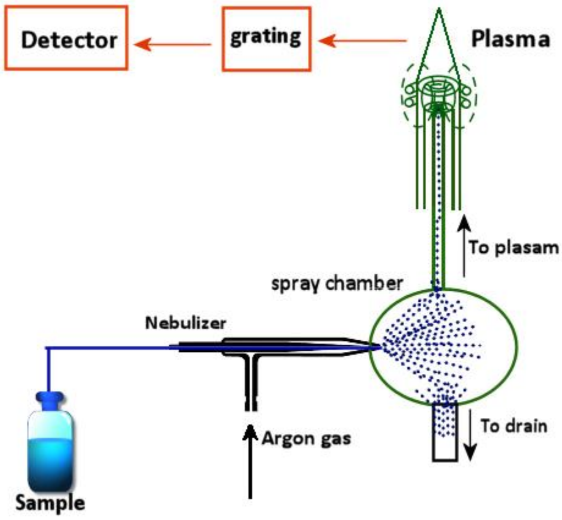


Figure 10. ICP-OES Schematic diagram

To make plasma, argon gas is fed into the torch coil, and a high-frequency electric current is passed through the work coil at the torch tube's tip. Then, argon gas is ionized and plasma is generated using the electromagnetic field established in the torch tube by the high frequency current. This plasma has a high electron density and temperature (10000K), which is used in the sample's excitation-emission. The operation steps of ICP-OES is shown in the following figure..:

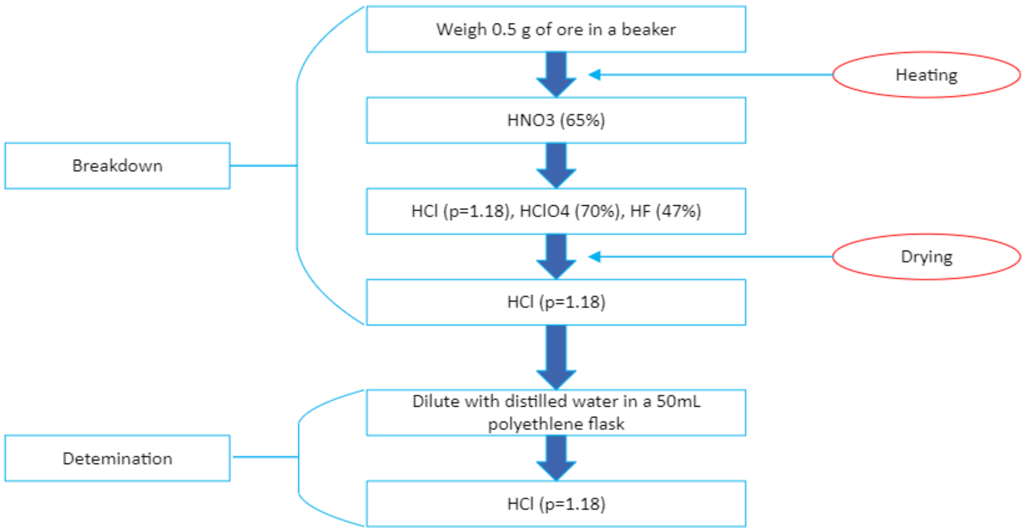


Figure 11. ICP-OES method for the determination of copper content

This instrument determines the content of elements stored in ore samples, metal alloys, soil water, salt and mineral samples. Plasma measures the spectrum of light emitted at thermal equilibrium. The elements that can be determine by induction coupling plasma spectrometry are shown. (Green: non-detectable elements)

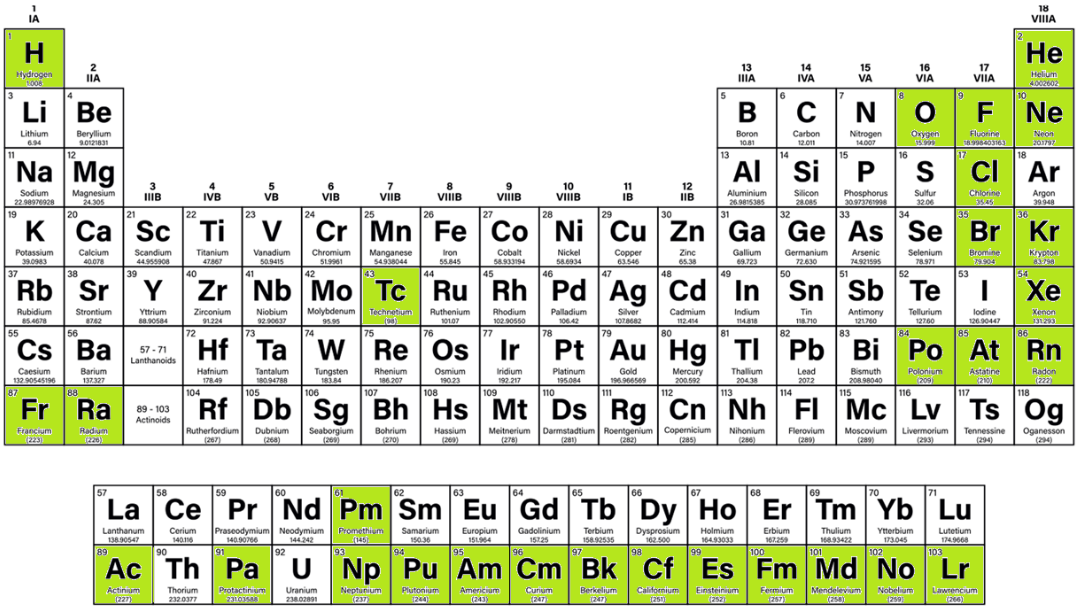


Figure 12. Elements detected by ICP spectrometry (White)

3.1.3 Atomic Absorption Spectroscopy method

The AAS's principle are similar to ICP, their main difference is AAS measures sequentially while ICP measures simultaneously. The device repeats the test separately for each element you analyze in an AAS, and each hollow cathode lamp emits its light, and the absorption is measured for a single element in each run.

The atomic absorption spectrophotometry method is based on the atomization of the test element at 2300℃ in an air-acetylene flame and the measurement of the UV absorbed by the atom and the visible light.

The Beer-Bouguer-Lambert law is expressed by the following formula.

$$A = lg\frac{J_0}{J_H} = \varepsilon \times l \times C$$

J_0 – the intensity of the reflected light

J_H – the intensity of the transmitted light

ε – atomic absorption coefficient

l – the thickness of the atomic vapor layer

C – the number of concentration of atoms in the vapor layer

Working principle of AAS is shown in Figure 13. (17)

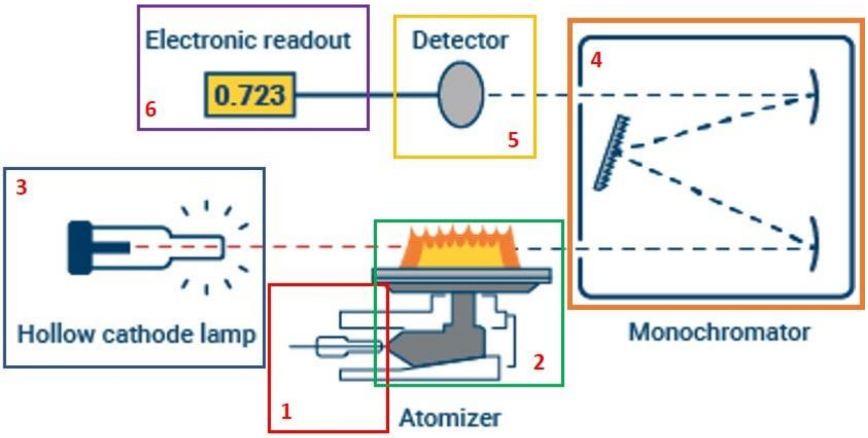


Figure 13. AAS working principle schematic diagram

3.2 Bacteria

In this experiment, A. thiooxidans strain of ATCC19377 was used, ordered by Zanaspex Co., Ltd. from American Type Culture Collection (ATCC) which collects, stores and distributes standard reference microorganisms, cell lines and other materials for research and development (18)**.** Prior to bioleaching, the bacteria were cultured in a culture medium at a constant temperature in a rotary shaker.

3.2.1 Bacterial growth

For the cultivation of *A. thiooxidans* under laboratory conditions, the culture medium is added on the bacterial solution. A. thiooxidans bacteria can thrive in an acidic environment (pH1.7-3.3), its primary energy source is elemental sulfur, and they are cultured in Thiobacillus medium (ATCC medium #125) containing the following ingredients:

Table 3. Waksman growth medium

	500mL	**250mL**	**100mL**
$(NH_4)_2SO_2$ (g)	0.1	0.05	0.12
KH_2PO_4 (g)	1.965	0.9825	0.393
$MgSO_4$ (g)	0.25	0.125	0.05
$CaCl_2$ (g)	0.063	0.0315	0.0126
S (g)	2.5	1.25	0.5

Thiobacillus medium /pH=4/

- $(NH_4)_2SO_4$
- KH_2PO_4
- $MgSO_4$
- $CaCl_2$
- S

After preparing the growth medium incubation starts only after sterilizing the medium in an autoclave under 1 atmospheric pressure, for 20 minutes as well as 121℃.

Sterilization: 1 atm

20 mins

121℃

The liquid medium of bacterial suspension has a ratio of 1:10. After sterilization the culture is ready for incubation after adding bacteria. Bacteria was incubated in a rotary shaker (Biobase BJPX-100B) for 14 days under conditions of:

- T=30℃
- Aerobic
- 150 rpm

Thereafter, the cultivated bacteria were stored in a refrigerator with the temperature range of 2-8℃ then was directly used for the experiment.

During the incubation, in its initial state was transparent medium will start to grow white dots floating on top of the liquid solution. Then with time, yellow sulfur colloid particles begin to precipitate and accumulate at the bottom of the solution container.

Due to high temperature and aeration rate it took only 7 days for the growth medium to reach pH value of 1.5 from its initial medium pH of 4. This indicates that in its optimal growth rate the bacterial consumption rate increases, resulting in more production of sulfuric acid. The conversion of thiosulfate to sulfate still has a long way to go in terms of speed and time. A high oxygen concentration should be given to speed up conversion process of the thiosulfate to sulfuric acid (19).

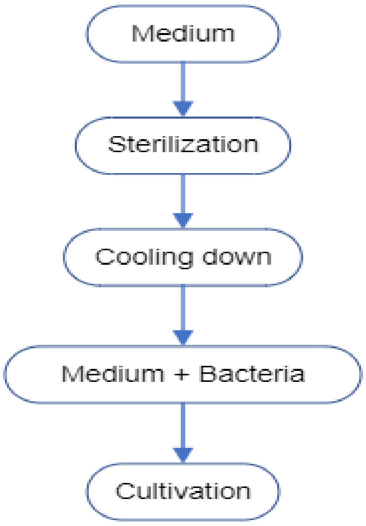


Figure 14. General scheme of cultivation of *At. thiooxidans*

3.2.2 Determination of the bacterial number

The bacterial culture of Acidithiobacillus thiooxidans was incubated for 14 days in a rotary shaker at constant temperature (30℃) and rotary speed (150 rpm). The number of cultivated bacterial cells was determined by the Direct Microscopy Count (DMC) method. After preparing a 2×2 cm square on the microscope glass slide. Add 1 drop of the bacterial suspension solution. Distribute it evenly on the surface inside the square and dry the solution using burners. Because *A. thiooxidans* possesses no color fuchsine dye was used to dye the bacterial cells. The microscope used in the experiment had an ocular lens of x15 and an objective lens of x100. As a consequence, the bacterial cells were counted at x1500 times magnification with an objective lens area of 0.02 mm^2. As determined by the ratio of total surface and the objective lens area, the bacterial cell count was 3.262×10^4 cells/ml.

Table 4. Bacterial cell count

	Number of counts															
Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Average
1	4	3	10	5	17	24	10	4	4	18	40	19	24	35	19	15.733
2	38	15	11	9	14	11	18	10	17	11	19	11	16	13	16	15.266
3	23	23	29	23	5	7	8	13	11	18	37	11	6	38	17	17.933
																16.311

$$C = \frac{a * S * n}{s * V}$$

C- Cell count

$S = 400mm^2$ (Area of the square)

$s = 0.02mm^2$ (Objective lens area)

$V = 0.01ml$ (10mm^3 suspension volume)

n – degree of dilution

$$\boldsymbol{C = \frac{16.31 * 400 * 10^0}{0.02 * 10} = 3.262 * 10^4\ Cells/ml}$$

4 Experiment procedure

4.1 Sample ore preparation

Several factors influence the process of dissolving metals in solution, including microorganisms characteristics, chemical reactions (precipitation formation), and the degree of friction in the suspension (20). As well as pulp density, pH, stirring frequency (rpm), temperature, nutrient concentration, oxygen content, and total bioleaching duration and finally particle size, are all factors to consider. These parameters have a considerable impact on the overall performance of metals transitioning to the liquid phase, and it is critical to explore their impact in order to obtain the best metal yield. The particle size of -75 µm was chosen after the study of bioleaching by Rouchalova, as it yielded best result from particle size ranges of <40 µm, 40-71 µm, 71-100 µm, 100-200 µm. According to Deveci's (21) research, particles that were <45 µm, no longer had a significant effect on the recovery of metals.

The sample was crushed to -6mm using 5E-JCA jaw crusher, further crushed to -2mm size using LMRC100 roll crusher. Following the standard work procedure of grinding in rod mill by Oyu-Tolgoi, series procedures to grind the sample in order to achieve a targeted percent of passing at a given size fraction takes place.

Pre-start preparations:

1. Open the mill cover, and raise the mill to a 90-degree angle
2. Check the rods in the mill if they are aligned parallel to the length of the mill, (no cross-crossing) and tightly placed next to each other
3. Put 1kg ore sample into the mill
4. Measure 600ml of tap water (for 62.5% solids) and pour it into the mill
5. Close up the mill with a lid and tighten the bolt to ensure that the lid is secured to the mill
6. Let down the mill into the horizontal position and close the cover
7. Adjust the grinding time

Grinding:

a. Setting and turning on the timer will simultaneously start the mill
b. The mill stops automatically when the required grind time has been reached

c. Open the mill cover, raise the mill to a 90-degree angle, loosen the bolt of the lid and remove the lid

d. Wash the rods using spray water and remove them from the mill

e. Tilt the mill down and pour the slurry into the container

f. Clean the mill thoroughly using spray water

To achieve, P100 (particle size at which 100% of the material will pass when screened) total of 5 grinding tests were performed. From which, a result of 45 minutes of grinding time was determined to obtain P100 of 1kg sample.

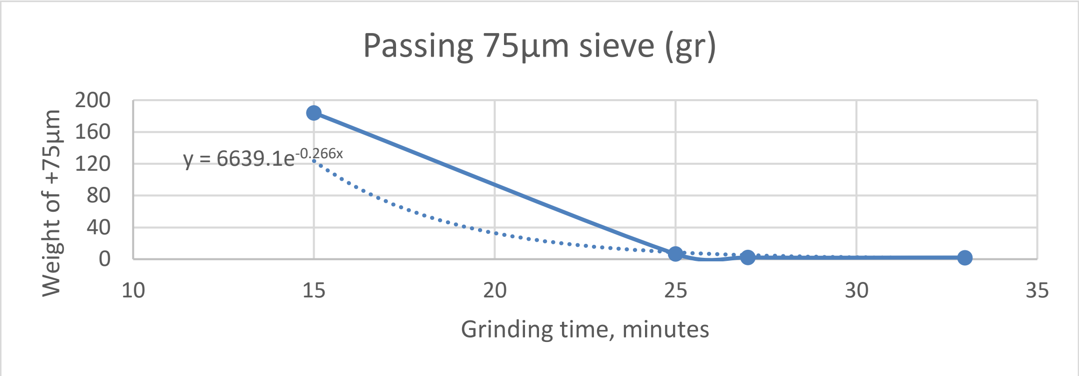


Figure 15. Determination of grinding time for P100 -0.75 micrometer

The sample was dried using an ERIEZ filter press and in a drying oven at 150℃ for 20 hours to eliminate any possibility of other microorganisms inhabiting the sample. Additional samples for ICP-OES analysis, 50mL of Oyu-Tolgoi's low-grade sulfuric ore were prepared in a ROCKLABS Benchtop Ring Mill for 8 minutes.

The bioleaching experiment was carried out in nine, 250mL Erlenmeyer flasks, which are divided into three batches of 1:2, 1:4, and 1:6 solid-liquid ratio samples.

Table 5. Solid-Liquid phase ratio

	1:2	1:4	1:6
Solid (g)	50	25	16.6
Liquid (ml)	100	100	100

5 Results and Discussion

1. The amount of copper dissolved during the bioleaching of sulfide ore was determined in the laboratory of KhanLab LLC by the atomic absorption spectroscopy method.
   The end result of any metallurgical process is expressed in metal recovery, Figure 16. shows the copper content of the pregnant solution after 25 days of bioleaching.

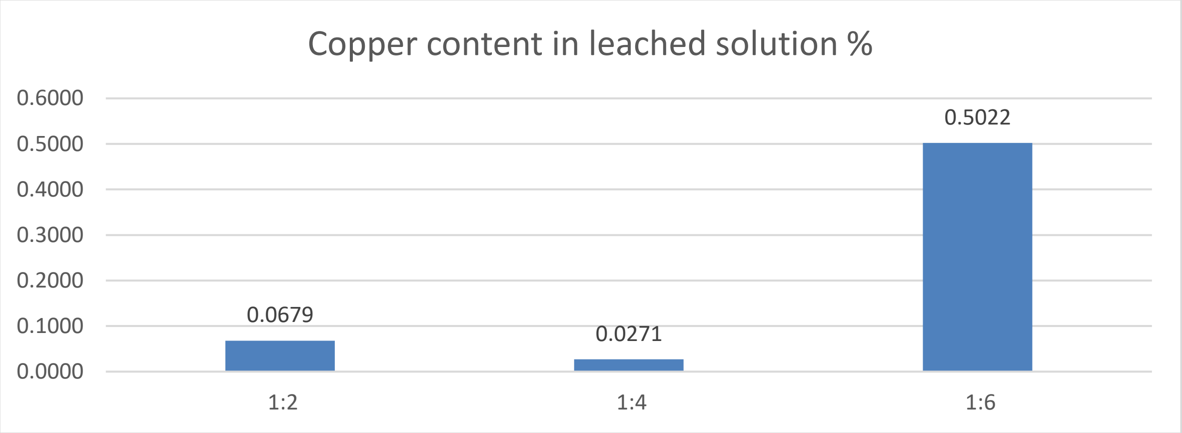


Figure 16. Copper content in the leached solution by percentage

According to the copper content in the leached solution after bioleaching, the copper recovery is 0.068% at 1:2 solid-liquid ratio, 0.027% at 1:4 solid-liquid ratio, 0.5% at 1:6 solid-liquid ratio. Depending on the solid-liquid phase ratio of the ore, bioleaching is slow at 1:2, 1:4 ratios. This is due to the fact that the small particles of ore contained in the solution form a phase-separating layer and weaken the interaction between bacteria and minerals. The low copper recovery after 25 days of bioleaching indicates that the bioleaching process is slow in this case.

Table 6. shows the result if the determination of copper content in the sample solution by the AAS method.

Table 6. Result of post-bioleaching copper recovery

Solid-liquid phase ratio	Copper content in the ore pre-bioleaching, gr	Copper content in the ore after bioleaching, gr	Copper recovery %
1:2	0.1105	0.110425	0.0679
1:4	0.05525	0.055235	0.0271
1:6	0.0368407	0.0366557	0.5022

2. The mineral composition as well as ICP result data sent by Oyu-Tolgoi, regarding Phase 4B ore.

Table 7. Mineral composition of Phase 4B ore

Number	Category	Mineral	Chemistry equation
1	**Ore minerals**	Clinoclase	$Cu_3AsO_4(OH)_3$
		Pseudomalachite	$Cu_5(PO_4)_2(OH)_4$
		Tennantite	$Cu_6[Cu_4(Fe,Zn)_2]As_4S_{13}$
		Calaverite	$AuTe_2$
		Pyrite	FeS_2
2	Non-ore minerals	Quartz	SiO_2
		Birnessite	$Na_{0.7}Ca_{0.3}Mn_7O_{14} \cdot 2.8H_2O$.
		Corundum	Al_2O_3
		Gismondine	$CaAl_2Si_2O_8 \cdot 4(H_2O)$
		Muscovite	$KAl_2(AlSi_3O_{10})(F,OH)_2$

From the data it is indicated that the Oyu Tolgoi Southwest Phase 4B ore contained compounds that are ineligible in flotation process.

Table 8. ICP result of Phase 4B (done by Oyu Tolgoi)

Sample Description	Cu, %	Au, ppm	Fe,%	As,ppm	S, %	F	Soluble Cu
ST-2-LG Phase 4B	0.22	0.56	3.98	5	2.87	880	0.005

When determining the chemical element content of Phase 4B ore sample used in this study by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES), the main ore element was copper (0.221%) and iron (3.96%). The result of the chemical analysis is summarized in the Table 9.

Table 9. ICP-OES result of the sample ore

Element, %					
Ca	Mg	K	Na	Fe	S
2.3	1.01	1.91	3.41	3.96	2.8
ELEMENT, mg/kg					
As	Ba*	Be	Bi	Cd	Co
14.84	388	<5.00	<10.0	1.16	14.63
Cr	Cu	Ga	Ge	La	Li
27.14	2210	18.97	<5.0	8.53	14.33
Mn	Mo	Nb	Ni	P	Pb
1136	5.76	16.56	8.16	845.3	9.98
Re	Sb	Sc	Sn*	Sr*	Ta
<5.0	<10.0	5.53	<50.0	560.7	<5.0
Te	Th	Ti	Tl	V	W*
<5.0	<10.0	2230	<5.0	142.8	<50.0
Y	Yb	Zn	Zr*	Ag	
13.84	<5.0	157.2	50.96	<1.0	
Explanation: *- Not completely decomposed. /Semi-numeric value/					

From the three results mentioned above, the ICP-OES results of Phase 4B sample used in this study matches to the one sent from Oyu Tolgoi, therefore, it is safe to assume that the mineral composition within the sample used in this study are the same to the mineral composition in the Table 7.

Another assumption on the result of this study is related to the inability of At. Thiooxidans to oxidize pyrite. As seen in the Table 7 and Table 9 it is assumed that the most of the sulfur content existing within the sample are connected to pyrite, and because of that the microorganisms were unable to oxidize enough sulfur to sustain continues activity (*At.*

thiooxidans primary energy source) from the pyrite to produce sulfuric acid, ultimately resulting in complete inactivity.

Furthermore, most of the copper content within the sample are associated to the mineral components such as clinoclase, pseudomalachite, and tennantite. Where the initial dissolved copper content results are presumed to be from the dissolution of tennantite compound.

3. In theory, free sulfuric acid is formed in solution as a result of bioleaching. The process of bioleaching can be monitored by measuring the pH of the solution during bio-oxidation, based on the facts that iron (III) ions are poorly soluble at ambient pH>2.5, and copper, the main raw metal, is also well ionized in aqueous solution at low pH medium. The test proceeded with 3 batches of 1:2, 1:4, 1:6 solid-liquid ratio solutions.

   Figure 17. shows the pH value of the solution during the bioleaching of sulfide ore in Oyu-Tolgo's low grade deposit.

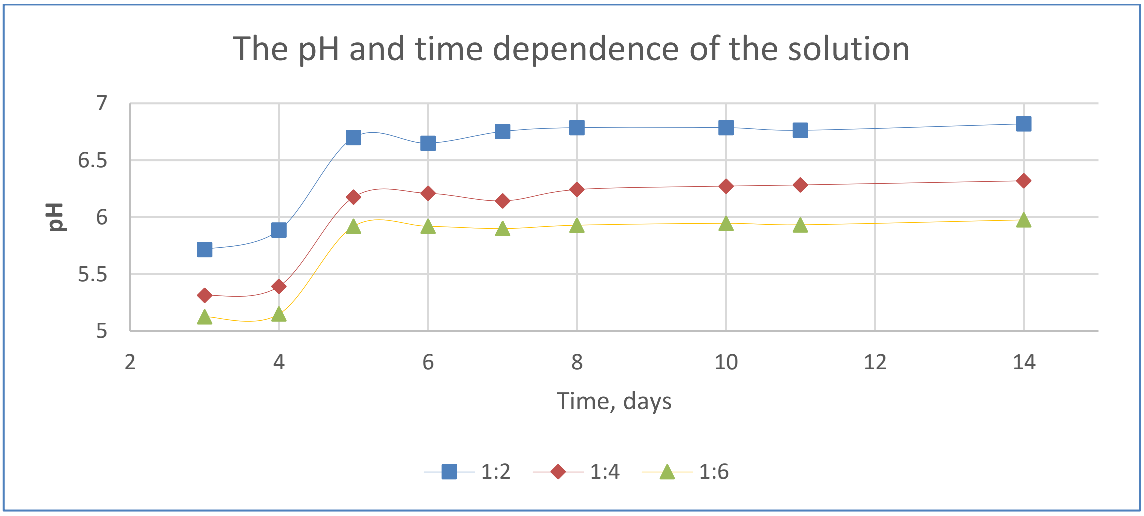


Figure 17. The pH and time dependence of the solution

It can be shown in Figure 17. that the general pattern of pH during bioleaching of sulfide ore has increased significantly in the first 3 days after its initial value of pH 1.5. The increase in pH is due to the lack of formation of free sulfuric acid in the solution as a result of the bioleaching process, and the increase in pH indicates that the bioleaching process is unable to proceed normally. *Acidithiobacillus thiooxidans* is a slightly thermophilic and mesophilic microorganism which live and thrive with a pH of 1.7 to 3.3 environment (6).

Depending on the solid-liquid ratio of the ore, the pH of the Oyu-Tolgoi's low grade sulfide ore bioleaching solution increased to pH = 1.5 - 6.82 at 1:2 ratio, pH = 1.5 - 6.32 at 1:4 ratio, pH = 1.5 - 5.97 at 1:6 ratio. The increase in pH during bioleaching is due to the inadequate oxidation of sulfur in mineral samples to form free sulfuric acid in the solution. Alternatively, due to the lack of an initial energy source, the sulfuric compounds in the mineral sample may have required more energy from microorganisms to dissolve.

Because the microorganisms had been inactive for a prolonged time of 14 days, sulfuric acid (98% concentrated) was supplied in to the sample in the hopes of reactivating the microorganisms by creating optimal acidic environment of pH = 2 – 2.8.
Figure 18. shows the pH value of the solutions after adding sulfuric acid.

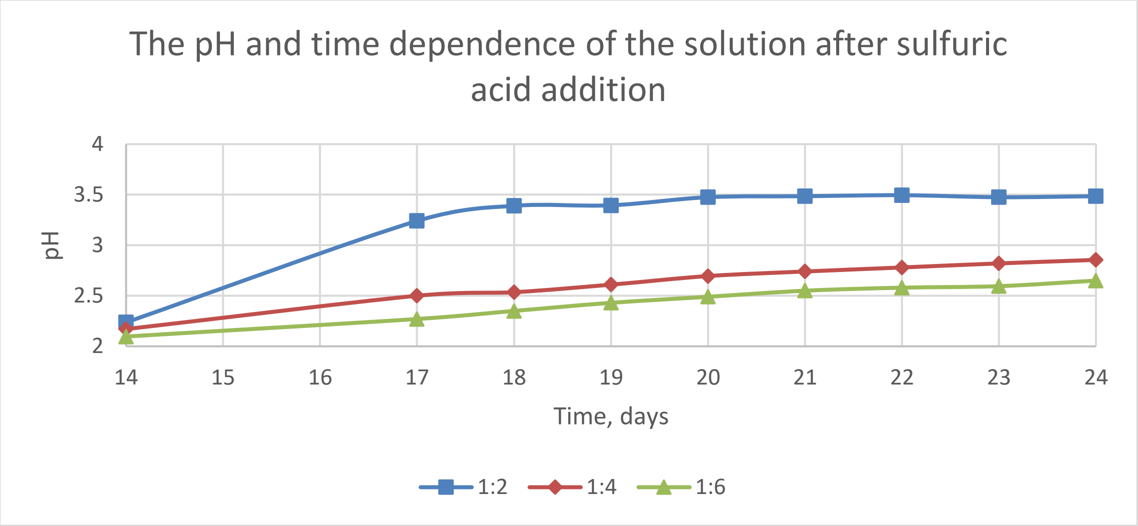


Figure 18. The pH and time dependence of the solution after sulfuric acid addiction

After the addition of sulfuric acid into the sample solution, the pH of the solution increased to pH = 2.2 – 3.48 at 1:2 ratio, pH = 2.17 – 2.85 at 1:4 ratio, and pH = 2.1 – 2.65 at 1:6 ratio was observed in the next 10 days of the test. However, in three samples of total of nine samples, repeated increase in pH was observed, namely in a batch #2 of 1:2 ratio, batch #3 of 1:4 ratio, and batch #3 of 1:6 ratio increases in pH of 2.78 to 6.3, 3.13 to 5.49, 2.8 to 5 respectively.
Solution redox potential is important for bioleaching. This is due to the entire leaching process is an oxidation-reduction process that results in the conversion of the ore into a useful metal solution. Figure 19. shows the changes in redox potential of the Oyu-Tolgoi's low grade sulfidic ore bio-oxidation solution.

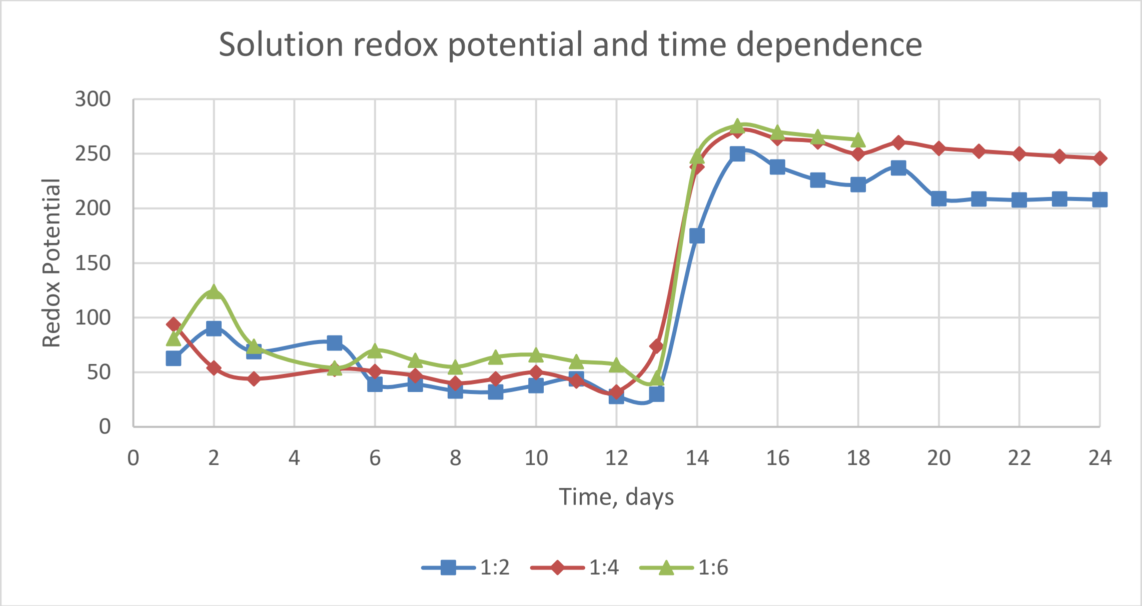


Figure 19. Redox potential and time dependence of the solution

From the graph above, it can be seen that the decrease in value of redox potential during bio-oxidation indication that the process of oxidation-reduction in the system, is not taking place. Except for the fact, that redox potential spikes when sulfuric acid was added into the samples.

According to the result of redox potential of sample solutions decreased to 63 – 30 mV at 1:2 ratio, 94 – 32 mV at 1:4 ratio, 81 – 45 mV at 1:6 ratio, in 14 days before sulfuric acid addition. After the additional sulfuric acid, the pH of the samples spiked, and then again proceeded to drop to 289 – 208.5 mV at 1:2 ratio, 292 – 245.9 mV at 1:4 ratio, 292 – 258 mV at 1:6 ratios in 10 days.

4. The number of cells after bioleaching could not be determined because no bacterial activity was observed from the post bioleaching solution. It is assumed that the bacterial population of $\mathbf{3.262 * 10^4}$ ***Cells/ml*** was too low for the amount of sample to continue the process of oxidation in the sample's environment, as the other studies indicated the optimum inoculation size ranged from 10^6 - 10^8 Cells/ml. On the first 3 days of bioleaching test, slight decrease in pH were observed before complete halt in oxidation process and the pH of the solution started to gradually increase. It is concluded that, after 3 days of said oxidation process, the activity of microorganisms has ceased completely, resulting in expiration of all bacterial population within the samples.

5. It is suspected that, the unsatisfactory result of this study was decided during the bacterial growth stage, where the steps of cultivation of microorganisms was not followed strictly to the instructions given by ATCC. For glycerol strains, inoculation requirement for liquid

medium was 1:10 ratio of bacteria liquid – growth media liquid. In spite of that, a total of 1mL bacteria liquid was divided to 0.25mL and was put into 250mL growth media solution, where bacteria liquid – growth media liquid ratio was 1:1000. Furthermore, after cryopreservation (which the state the bacteria liquid was in before inoculation), the strains are in a dormant state, and growth may be delayed during resuscitation and cultivation. At this time, longer cultivation time is required. Which also was not followed during the actual growth step (cultivated for 4 days as opposed to recommended 14 days of cultivation).

6. The bioleaching of the Oyu Tolgoi Southwest Phase 4B low-grade non-balance ore sample study was proceeded in the following conditions: at ore size -0.75μm, at ambient temperature range of 22.5℃ - 23.5℃, solid:liquid ratio of 1:2, 1:4, 1:6, at a constant 160rpm, and the inoculation size of 10^4 Cells/ml of *At. Thiooxidans*. After 25 days, depending on the solid liquid phase ratio, copper recovery was found to be 1:2 – 0.068%, at 1:4 – 0.027%, at 1:6 – 0.5%. It is concluded that the bioleaching process proceeds at a necessary rate when the solid liquid phase ratio is 1:6, nevertheless it is presumed that these recovery values are the result of the first 3 days of bioleaching process.

6 Conclusion

In this study, the bioleaching process by the pure strain of *At. Thiooxidans* was investigated and yielded an unsatisfactory copper recovery rate in the process, results can be summarized as follows:

- The mineral composition of the ore contained an unsuitable mineral to be oxidized and leached by the pure strain of *At. Thiooxidans,* during the bioleaching study continued for 25 days. It is presumed that, most of the sulfur content in the sample are part of the pyrite bond, which is the only metal sulfide *At. Thiooxidans* is unable to oxidize. Consequently, the *At. Thiooxidans* culture within the sample, with the lack of energy source and not enough free sulfur element to produce sulfuric acid went dormant for a prolonged time and ultimately died off. Furthermore, the ore sample contained mineral composition unsuited for flotation beneficiation method.
- However, it is right to mention, even on the unsatisfactory copper content recovery, solid-liquid phase ratio of 1:6 yielded better than ratios of 1:2, 1:4. The sample with the solid liquid phase ratio 1:4 yielded the lowest recovery rate. It is assumed that, on this specific ratio the layer separating layer were more severe than the other two ratios, thus reducing the interaction between bacteria and minerals.
- The increase in pH during bioleaching is due to the inadequate oxidation of sulfur in mineral samples to form free sulfuric acid in the solution. Alternatively, due to the lack of an initial energy source, the sulfuric compounds in the mineral sample may have required more energy from microorganisms to dissolve.
- The number of bacterial cells post-bioleaching could not be determined as there was no indication of activity within the solution. The reasons are mentioned above.
- The other factor which has effects on the bioleachability of the sample were all within the acceptable range. The main setbacks during this study happened within the mineral composition of the mineral, inaccuracy during bacterial growth period, inoculation size of the sample, alternatively it could have been a strain related problem. The above mentioned, suggests that further research is needed for this strain of bacteria under strict monitoring.

In conclusion, to choose the most suitable microorganisms for bioleaching for any mineral ore, the mineral composition of the ore must be studied extensively for optimum results.

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8 Appendices

Appendix #1

Table 10. pH meter result

15/4	1:2	1:4	1:6	mV	1:2	1:4	1:6
	5.48	4.91	4.42		63	94	81
16/4	1:2	1:4	1:6	mV	1:2	1:4	1:6
	5.01	5.62	5.15		90	54	124
17/4	1:2	1:4	1:6	mV	1:2	1:4	1:6
	5.37	5.8	5.28		69	44	74
18/4	1:2	1:4	1:6				
#1	5.7	5.3	5.13				
#2	5.64	5.3	5.1				
#3	5.81	5.34	5.15				
19/4	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	5.84	5.37	5.15	Average	77	53	54
#2	5.88	5.4	5.16				
#3	5.94	5.41	5.14				
20/4	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	6.69	6.16	5.9	Average	39	51	70
#2	6.72	6.18	5.93				
#3	6.69	6.19	5.93				
21/4	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	6.57	6.18	5.9	Average	39	47	61
#2	6.71	6.22	5.9				
#3	6.67	6.23	5.96				
22/4	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	6.62	6.14	5.87	Average	33	40	55
#2	6.8	6.23	5.9				
#3	6.84	6.06	5.93				
23/4	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	6.74	6.21	5.92	Average	32	44	64
#2	6.79	6.28	5.92				
#3	6.83	6.24	5.95				
25/4	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	6.73	6.24	5.93	Average	38	50	66
#2	6.8	6.31	5.93				
#3	6.83	6.27	5.98				
26/4	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	6.67	6.26	5.94	Average	44	42	60
#2	6.8	6.35	5.91				
#3	6.82	6.24	5.95				
29/4	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	6.7	6.3	5.95	Average	28	32	57
#2	6.87	6.35	6.02				
#3	6.89	6.31	5.96				

29/4 after sulfuric acid	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	1.9	1.4	1.63	Average	248	268	274
#2	2.78	1.98	1.86				
#3	2.03	3.13	2.8				
2/5	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	2.8	1.83	2.06	Average	175	238	248
#2	5.6	3.17	2.48				
#3	3.68	5.26	4.7				
3/5	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	2.96	1.91	2.13	Average	289	292	292
#2	6.11	3.16	2.57				
#3	3.82	5.33	4.77				
4/5	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	3	2	2.22	#1	237.3	296.4	283.7
#2	6.18	3.22	2.64	#2	45.2	224	259.3
#3	3.79	5.54	4.83	#3			
5/5	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	3.1	2.09	2.29	#1	231.5	291.3	279.3
#2	6.16	3.3	2.69	#2	48	219	255
#3	3.85	5.48	4.92	#3	186.8	90	123.4
6/5	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	3.13	2.12	2.33	#1	230	289	276.5
#2	6.23	3.36	2.77	#2	44	216	251
#3	3.84	5.46	4.95	#3	187.3	91.3	121.7
7/5	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	3.14	2.18	2.36	#1	228.8	285.6	274.7
#2	5.77	3.38	2.8	#2	41.5	214.4	249.8
#3	3.85	5.38	4.97	#3	186.7	89	120.4
8/5	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	3.15	2.22	2.39	#1	228	283	273
#2	6.35	3.42	2.8	#2	38.3	212.5	249.3
#3	3.8	5.44	4.94	#3	189.6	91	121
9/5	1:2	1:4	1:6	mV	1:2	1:4	1:6
#1	3.16	2.26	2.45	#1	227.2	280.5	269.5
#2	6.3	3.45	2.85	#2	37.5	211.3	246.5
#3	3.81	5.49	5	#3	188.9	89.7	119

Appendix #2

Table 11. AAS analysis result

	Sample ID	Aliquot	Diluted	Conc (sample)
1:2	1	1	5	0.15
1:4	2	1	5	0.03
1:6	3	1	5	0.37
	blank	1	1	-0.01
	chk1.0ppm	1	1	0.96
	chk1.0ppm	1	1	0.98

Appendix #3

Figure 22. 5E-JCA jaw crusher to comminution size of -6mm

Figure 21. Roll crusher to comminution size of -2mm

Figure 20. Rod miller used in this study for comminution size of -75 micrometer

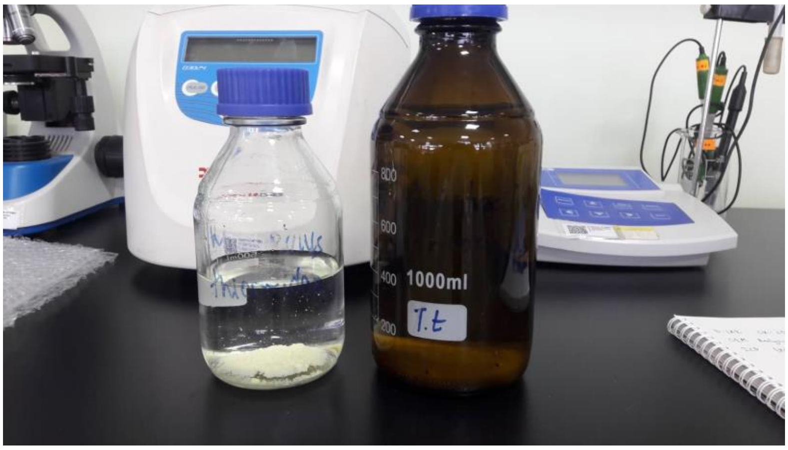

Figure 25. Bacterial medium used in the bioleaching directly

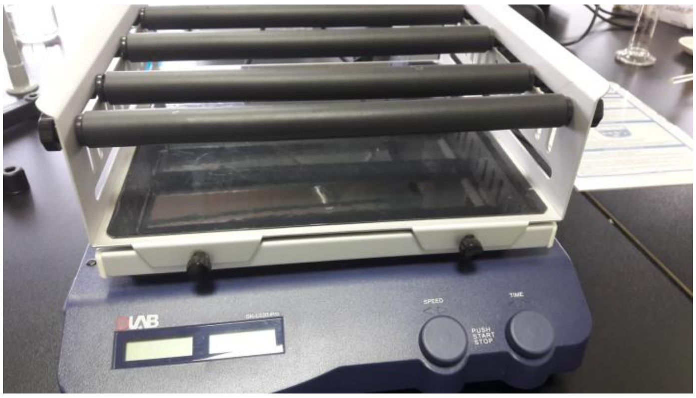

Figure 24. Linear shaker used in bioleaching study

Figure 23. All 9 samples were prepared and put on an linear shaker at 150 rpm