

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Bio Hydrometallurgical Technology, Application and Process Enhancement

*Mulugeta Sisay Cheru*

## Abstract

The review is in general try to see some of the major microorganism involved in bioleaching process and studied by different scholars, identify the mechanics and techniques employed to bioleach minerals and factor that enhance or to inhibit the leaching process of microorganism with major reaction taking while bioleaching. Here the methodology and different leaching technique presented with their respected pros and cons, which are commonly employed and reasons behind with justifiable evidences were presented. The values and bioleaching sulfide mineral (copper), precious metal (gold) and radioactive element (uranium) were discussed with some the known producers in the world and finally some highlight given on industrial application of bioleaching.

**Keywords:** bioleaching, leaching techniques, biooxidation, radioactive, pretreatment

## 1. Introduction

Bioleaching is the extraction of metals from ores using the principal components water, air and microorganism [1]. It is the extraction or mobilization of valuable (target) metal from the ore, can also be defined as a process of recovering metals from low grade ore [2, 3], with regard to solubility, bioleaching can be defined as a process of recovering soluble one from insoluble impurities after dissolving sulfide metal as soluble salt in a solution [4] that results toxics and heavy metals removed. It is isolation of metals from their ores, concentrates and mineral wastes under the influence of microorganisms leading to dissolution of metal solutions of leach liquor containing metals [5], followed by solvent extraction, stripping, ion exchange, electro wining to get pure metal.

Both bioleaching and biooxidation leads to recovery target mineral; but there is technical difference between the two technologies. Bioleaching refers to the use of bacteria, the common Thiobacillus Ferrooxidans and other bacterial as a leachant to leach sulfide minerals where the target elements remains in the solution during oxidation process, after the metal recovery the solid left behind regarded as residue and in the contrary biooxidation discard the solution after having metal values in solid phase [6, 7] microorganism also engaged in removal of radionuclides and leaching of metal that are regarded as toxic in some cases and good for bioremediation of soil, the process stops radio nucleation that result the removal of stability of target elements [7].

Bioleaching has been used for a long period of time without regarded as microbial leaching process; it has been used as early as 1000 BC when a man from metal laden recovered copper from a water, passes through copper ore deposit [8]. It was in 1556 at the mine located in Spain at Rio Tinto (Rd River) mine, slurry containing very high concentration of ferric ions leached due to the action of microorganisms [4]. Copper was precipitated from the solution obtained from this river, the very first bio mineralization process was copper dissolution, then the process continued to be developed in countries like Norway, Germany and English at different era of time, in the year 1947 heap and dump leaching was practiced that leads to the development of bacterial bioleaching process [9].

The gram-negative chemolithotroph bacterial, *Thiobacillus Ferrooxidans* was first cultured and isolated from mine water by Colmer and Hinkel [9]. *Thiobacillus Ferrooxidans* is rod shaped ranging in diameter from 0.3 to 0.8 micrometers ( $\mu\text{m}$ ), in length from 0.9 to 2  $\mu\text{m}$ , 0.5  $\mu\text{m}$  in width in which its movement is due to a single polar flagellum [10]. Since now this bacteria is the most studied. These bacteria were able to oxidize sulfur to sulfuric acid and ferrous to ferric in acidic environment where pH value is less than 5 [7, 10, 11]. From this point onwards the technology of bioleaching has shown tremendous growth, especially industrial coppers production, which makes annualized world copper production reach up 10% from 0.2%. It was in Chile the first industrial scale copper bioleaching plant was established in 1980 using *Thiobacillus* bacteria [12] large-scale production begins and bioleaching taken as main manufacturing process as any convection techniques in Chile 1984 [13]. Among the many microorganism involved, bacteria (autotrophic and heterotrophic), fungi and yeasts can be mentioned. The bacterium has these calcification based on their species as *Thiobacillus Ferrooxidans*, *Leptospirillum Ferrooxidans*, *Thiobacillus Thiooxidans*, *Sulfolobus*, but there are many classifications based on different characteristics reveled by the organisms.

Acidophilic *Thiobacillus* species are used to leach refractory elements like gold, they generally characterized as aerobic, acidophilic, and autotrophic used to leach sulfide minerals (copper, nickel, zinc and soon). Most common bacteria involved in bioleaching are *Acidithiobacillus Ferrooxidans* (*Thiobacillus Ferrooxidans*), *Acidithiobacillus Thiooxidans*, *Leptospirillum Ferrooxidans*, *Sulpholobus* Spp, *Sulpholobus Thermosulphidoxidans* and *Sulpholobus Brierleyi*. *Acidithiobacillus Ferrooxidans* is most vital one, which was named and characterized in 1951. Most common fungi are *Aspergillus Niger* and *Penicillium Simplicissimum*. The efficiency of bioleaching depends up on physiological requirement and capability of *Thiobacillus* to oxidize ferrous ion ( $\text{Fe}^{2+}$ ) and sulfur (S). There are five main species of *Thiobacillus*, these are *Thiobacillus Thioparus*, *Thiobacillus Dentrificans*, *Thiobacillus Thiooxidans*, *Thiobacillus Intermedius*, and *Thiobacillus Ferrooxidans*. On the bases of pH values for growth genus *Thiobacillus* can be divided into two groups, those that can grow only in neutral pH values are T. *Thioparus* and T. *Dentrificans*. The second *Thiobacillus* species those grow at lower pH value are T. *Thiooxidans*, *T. intermedius*, and T. *Ferrooxidans*.

Study of different scholars at the inceptions shows the capability of bacteria (genus *Thiobacillus*) to oxidize sulfur compounds to sulfuric acid; it can oxidize also range of sulfur compounds ( $\text{S}^{2-}$ ,  $\text{S}^0$ ,  $\text{S}_2\text{O}_4$ ,  $\text{S}_2\text{O}_4^{2-}$ ,  $\text{SO}_4^{2-}$ ) [11], followed by separation process of the iron and the bacteria *Acidithiobacillus Ferrooxidans* (*Thiobacillus Ferrooxidans*) from the solution. A. *Ferrooxidans* is found in drainage waters and it is commonly identified as pyrite oxidizer [14]. The bacterial (acidophile) obtain energy from inorganic sources, it grows in acidic medium that fixes carbon to the bacteria itself. Most economically important metals like iron, copper, gold, and uranium can be easily extracted by using acidophilic and chemo-litho-autotrophic microorganism. *Acidithiobacillus Ferrooxidans* is chemoautotrophic microorganism or acidophilic.

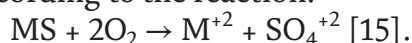


**Figure 1.**  
*Image of bioleaching bacterial [4].*

Let see the ecology, physiology, availability and genetics of microorganism involved in bioleaching. There are three basic principles for microorganism to leach and mobilize target metals from ore concentrate – redox reaction, formation of organic and inorganic acid and finally the excretion of complexing agent (**Figure 1**) [4].

Here is a generalized reaction used to express biological oxidation of sulfide mineral.

$MS + 2O_2 \rightarrow MSO_4$ , where M is bivalent metal and reaction below show a metal sulfide directly oxidize by *Acidithiobacillus Ferrooxidans* to soluble metal sulfate according to the reaction.

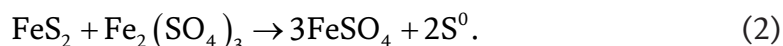


## 2. Mechanisms of bioleaching

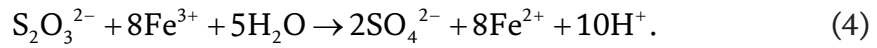
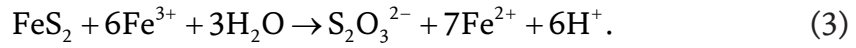
The two majorly known mechanism in bacterial leaching are direct mechanism (involves physical contact of the organism with the insoluble sulphide) or hypothesized enzymatic reaction taking place between an attached cell and the underlying mineral surface which is independent of indirect mechanisms and it is where reduced sulfur dissolution takes place [16], it is only the direct attack of the bacteria can lead to leaching. Check the following reactions.



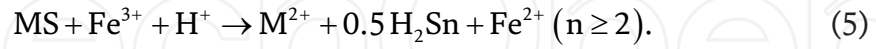
Indirect (involves the ferric-ferrous cycle) or it is a mechanism of sulfide oxidation involves non-specific oxidation of surfaces by  $Fe^{3+}$  that is generated by microorganisms that oxidize iron or oxidation of mineral by ferric ions [16]. The attached cells of bacterial oxidize the surface using either of the two mechanisms [9, 11, 14]. The reaction below shows oxidation of iron.



Minerals are broken due to the attack to their constituents, that results energy production for the microorganism. This energy production or oxidation passes through intermediates reaction processes. Two mechanisms have been proposed for the oxidation, viz. thiosulphate mechanism and polysulfide mechanism. Thiosulfate mechanism includes acid-insoluble metal sulfides like pyrite ( $FeS_2$ ) and molybdenite ( $MoS_2$ ) and polysulfide mechanism includes acid-soluble metal sulfides like chalcopyrite ( $CuFeS_2$ ) or galena ( $PbS$ ) [15]. In thiosulfate mechanism, the attack of ferric ion on acid insoluble metal sulfides brings about solubilization via thiosulfate as an intermediate and sulfates as end product. The breaking reaction shown below.



In polysulfide mechanism, a combined attack of ferric ion and protons on acid-soluble metal sulfides causes the solubilization with sulfur as an intermediate in its elemental form which can be oxidized to sulfate by sulfur-oxidizing microbes that the reaction is shown below [17].



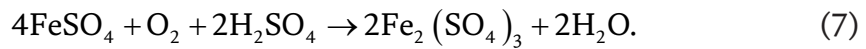
$0.125 \text{S}_8 + 1.5\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 2\text{H}^+$  the reaction show the production of sulfuric acid results hydrogen (proton) for attacking mineral.

Fe (II) is re-oxidized to Fe (III) by iron oxidizing organisms (chemotrophic bacteria), the role of microorganisms in solubilization.

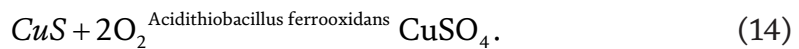
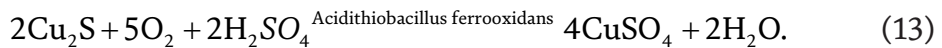
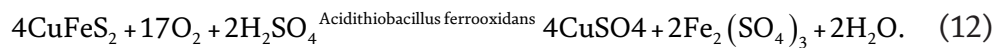
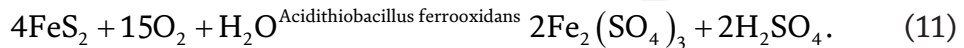
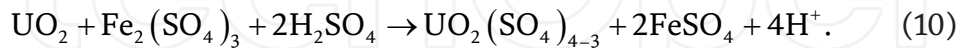
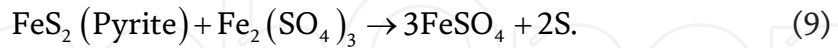
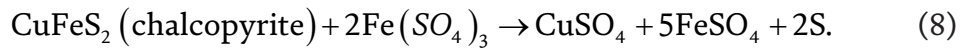
$2\text{Fe}^{2+} + 0.5\text{O}_2 + 2\text{H}^+ \rightarrow 2\text{Fe}^{3+} + \text{H}_2\text{O}$  this reaction keep iron in ferric state that oxidize mineral.

The process of chemical attack takes place on a substrate or the mineral surface where the bacteria forms a composite and attach itself as firm as possible in order to increases maturity that finally detached and dispersed into the solution.

An important reaction mediated by *Acidithiobacillus Ferrooxidans* is:

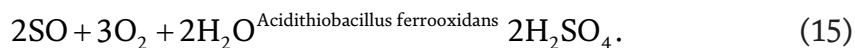


Strong oxidizing agent, ferric sulfate that basically used to dissolve metal sulfide minerals, and leaching brought about by ferric sulphate is termed indirect leaching due to the absence of both oxygen and viable bacterial. Check the following leaching mechanism of reaction on several minerals.

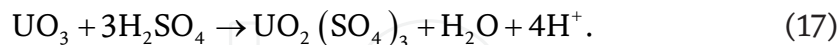
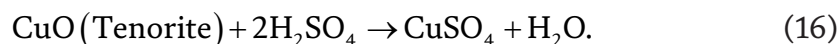


*Acidithiobacillus Ferrooxidans* can convert elemental sulfur generated by indirect leaching to sulfuric acid –.





This sulfuric acid maintains the pH value at levels, which is favorable to the growth of bacteria and also helps for effective leaching of oxide minerals:



Chemolithotrophic (uses carbon for the synthesis of new cell material) bacteria can be categorized based on response to temperature as mesophiles, moderate thermoacidophiles and extreme thermoacidophiles.

Mesophiles-grows at a temperature values ranges (28°C -37°C) where Thiobacillus Ferrooxidans is able use the inorganic substrate to draw energy by oxidizing Fe (II) to Fe (III) and sulfur to sulfide and sulfate. The other mesophiles is Leptospirillum Ferrooxidans that use Thiobacillus Ferrooxidans to effect the oxidation of sulfur to sulfate. Moderate thermoacidophie-temperature values ranges (40–50°C), Sulfolobus Thermosulfidooxidans is common one, which oxidize both sulfur and iron from energy production. This category includes Archaea and Eubacteria, and most of gram-positive microorganisms are included here. Extreme thermoacidophiles-temperature ranges 60–80°C, genera Sulfolobus, Acidianus, Metallosphaera and Sulfurococcus are in this category, [11, 18, 19]. Thermal value some time extends above the limitation values, it is due to exothermal reaction which is above the maximum growth temperature of microorganism, some micro-organism genus like Archaea withstand thermal values up to 90° [19, 20].

This category is formed by closely related species that can act together with a common name given Sulfolobus. Sulfolobus Acidocaldarius, Sulfolobus Sofataricus, Sulfolobus Brierley, and Sulfolobus Ambioalous that can oxidize Fe (II) to Fe (III) and sulfur to sulfate. Aspergillus Niger and Penicillium Simplicissimum are both used to leach sulfide minerals like copper with mobilization rate of 65% and aluminum, nickel, lead and zinc by more than 95% mobilization rate. Thiobacillus and Leptospirillum are characterized by the oxidation of sulphide minerals in acidic environment and temperature values less than 35°C, with regard to area of application these two are mostly used in dump and tank leaching of metal from sulphide based mixed ores [20, 21]. The other group of genus Sulphobacillus used under the same areas of application but relatively higher temperature up to 60°C, the temperature reaches up to 90°C in case of genera Sulpholobus and Acidianus, Organotrophic microorganisms like yeast, fungi and algae which destruct sulphide mineral and aluminum silicate, facilitate bio sorption of metals that solubilize gold, these microorganism uses carbonate and silicate ore for the extraction of metals and selective gold extraction from ore floatation and metal solution.

## 2.1 Autotrophic and heterotrophic leaching

The two bacterial leaching namely autotrophic and heterotrophic leaching has their distinct characteristics while bioleaching process takes place, in case of autotrophic bioleaching (effective on sulfide minerals) there are two proposed mechanism of Acidithiobacillus Ferrooxidans action on sulfide minerals, first the mechanism, that the bacterial oxidize ferrous ion to ferric ion in which the bulk solution where the mineral is leached counted as indirect, this mechanism which is

indirect oxidation of ferrous ions to ferric ions is exopolymeric process, both takes place on the layer where the mineral is leached. The second proposed mechanism, does not require ferrous or ferric ions, the bacteria directly oxidize the minerals by biological means having direct contact mechanism of reaction. Autotrophic leaching uses both *Thiobacillus Ferrooxidans* and *Thiobacillus Thiooxidans* to leach sulfide mineral and studies shows combining the two bacterial results an increase in selectivity and rate of leaching efficiency while leaching of nickel sulfide. From the heterotrophic genus of bacteria *Thiobacillus* and *Pseudomonas* are those used to leach non-sulfide minerals and from the genus of fungi *Penicillium* and *Aspergillus* (heterotrophic fungi) are those used in leaching process, a study shows 55–60% leaching rate for nickel and cobalt, some other studies indicates that 95% and 92% leaching rate achieved while using pretreated *Aspergillus Niger* by ultrasound for 14 and 20 days respectively which increase its stability [4, 11, 20, 22] (**Table 1**).

Heterotrophic fungi *Aspergillus* and *Penicillium* species combined to leach low-grade nickel-cobalt oxide ores, low-grade laterite ores and spudumene (aluminosilicate), these aluminosilicate (spudumene) also leached by heterotrophic yeasts (*Rhodotorula rubra*), *Aspergillus Niger* used to leach zinc and nickel silicate [11]. Bacterial leaching can be generalized in three mechanism redoxolysis, acidolysis, complexolysis, and in case fungal leaching bioaccumulation is important mechanism. To solubilize rock phosphorous, *Aspergillus Niger* has been used in many occasions due to the production of organic acids with low molecular weight and phosphorous is basically essential micronutrients for the growth of bio organism, these microorganism convert insoluble phosphate to soluble, the two filamentous fungi used in phosphate leaching are *Aspergillus Niger* and some *Penicillium*, the metabolic fungal reaction produces organic acid that result the formation of acidolysis, complex and chelate [22].

The second group of bacterial genus is *Leptospirillum*, which is categorized in moderate thermophilic bacteria that can only oxidize ferrous ions; it is dominate iron oxidizer, which is referred as *Leptospirillum Ferrooxidans* (L. *Ferrooxidans*). Oxidation process takes place under strong acidity and temperature up to 30°C, L. *Ferrooxidans* has high affinity to  $\text{Fe}^{2+}$  and low affinity to  $\text{Fe}^{3+}$  which results a working condition of high  $\text{Fe}^{3+}/\text{Fe}^{2+}$  ratio, when redox potential is low, L. *Ferrooxidans* has low growth rate at the initial stage of a mixed batch culture, a native strain of *Leptospirillum Ferrooxidans* used to leach zinc from low grade sulfide complex from La Silvita and La Resbalosa (Patagonia Argentina) [23]. The leach liquor itself has been a place where microorganism found, higher amount of

Microorganism/ both autotrophic and heterotrophic	Ore sample
<i>Aspergillus Niger</i> , <i>Hyphomicrobium</i>	Flourapatite (phosphorus ore)
<i>Pseudomonas Oryzihabitans</i>	Magnesite, Dolomite (magnesium ore)
<i>Bacillus Licheniformis</i>	Silica
<i>Thiobacillus Ferrooxidans</i> , <i>Acidianus Brierleyi</i> , <i>Sulfobacillus</i> , <i>Thermosul Fidooxidans</i> , <i>Sulfolobus Rivotincti</i>	Chalcopyrite (Low and high grade), Pyrite, Covellite
<i>Penicillium Simplicissimum</i> , <i>Penicillium Verruculosum</i> , <i>Aspergillus Niger</i> , <i>Acidithiobacillus Ferrooxidans</i>	Iron ore, Hematite, Zinc and nickel Silicates
<i>Thiobacillus Thiooxidans</i>	Pyrrhotite
<i>Thiobacillus Caldas</i>	Arsenopyrite
<i>Metallosphaera Sedula</i> , <i>Sulfolobus Metallicus</i> (BC),	Pyrite
<i>Paenibacillus polymyxa</i>	Bauxite (low grade)

**Table 1.**  
Some of microorganism and leachable ore [4, 11].

*Leptospirillum Ferriphilum* were in a leach liquor, in a study conducted to leach the effect of pH on the bioleaching of a low-grade, black schist ore from Finland using *Acidithiobacillus Ferrooxidans* and *Leptospirillum Ferrooxidans* as extractant [24]. The bacteria can relatively resist high concentration of uranium, molybdenum, and silver, this is due to its affinity towards ferrous ions or resistivity to refractory elements, but it cannot oxidize sulfur or any sulfur related compounds. By combining it with other sulfur-oxidizing acidophiles, sulfur-oxidizing process can be achieved; these are *T. Calvus*, *T. Ferrooxidans*, or *T. Thiooxidans*, to oxidize sulphidic gold concentrate a mixed culture of *Thiobacillus* and *Leptospirillum* has been used [11].

The third group thermophilic bacteria mainly characterized by oxidation of iron to assure growth chemolithotrophically, some are facultative autotrophs that require synergetic effect of other microorganism to like yeasts extract, cysteine, or glutathione. Among the microorganism in this group *Sulfolobus* species is the major one, these organism categorized as moderate thermophilic at an average values of temperature 40°C -60°C and the second group is extreme thermophilic at an average values of temperature 65–85°C. One of the moderate thermophilic gram positive bacteria, *Sulfolobus Thermosulfidooxidans* is facultative autotrophs in which its growth stimulated by yeasts extract, where the presence of CO<sub>2</sub>, weight and volume ratio (w/v) are factor to facilitate and inhibit growth. From of extreme thermophilic *Sulfolobus Acidocaldarius* and *Acidianus Brierleyi* are those in genera Archaeobacteria, among the other four genera *Sulfolobus*, *Acidianus*, *Metallosphaera*, and *Sulfurococcus* act aerobically and categorized in extreme thermophilic acidophilic bacterial which oxidizes ferrous and elemental sulfur and sulphide based minerals. These bacteria grows under all conditions (auto, mixo, heterotrophic) depending on the yeast extract ratio (w/v), found in facultative chemolithotrophic species act in acidic medium and temperature value can be up to 90°C [11].

All the major concepts of bioleaching have been discussed, so what are factors affecting rate of bioleaching and leaching efficiency, the major factors can be summarized as microbiological, mineralogical and physiochemistry factors. A physiochemistry factor includes temperature, pH, redox potential, oxygen content, carbon dioxide content which facilitate mineral oxidation required for cell growth, mass transfer, light, surface tension which mean that the topography of mineral surface that indicate the rate adsorption and crystal structure which has direct relation on the rate of reaction. Microbiological factors includes microbial diversity that is the distinct nature of micro organisms with regard to range of unicellular organisms, variety of microorganism found in an environment suitable for bioleaching, these includes bacteria, fungi, algae, flagellates, and those found in microbial biocenosis, the other microbiological factors are population diversity, metal tolerance, spatial distribution microorganism and adaptation ability of microorganism. The third major factor is the nature of mineral processed, characteristics like grain size which affect rate of dissolution, porosity related to rate of chemical attack and digestion rate, hydrophobicity is another physiochemistry factor to determine the rate leaching, hydrophobicity is differentiating whether the elements are water hating or loving while floatation takes place. Process is the other major factor affecting leaching efficiency, techniques where bioleaching process takes place (heap, dump, in situ) which we will be discussed below, pulp density is the variable which results variation on dissolution rate, a study shows that dissolution metal increases while pulp density increases but it is based on (w/v) ratio that is between 5 and 20%, the other factor is concentration of target mineral, this can inhibit the growth of microorganism, that cause a limitation of pulp density usage [25]. Stirring speed is also another factor affecting rate of dissolution and geometry of the heap during heap leaching process, the other major factor is the presence of fluoride released from the ore sample, which inhibits the process of bioleaching, and when the release decreases the rate of inhibition eventually reduced.



Besides leaching process microorganisms are used for bioremediation of mining sites, treatment of gangue, tailing, and mineral wastes from the industry, contamination of sediments due heavy metals and soil from toxicities, sewage sludge can cured by microorganism in which the process is called bioremediation [26].

### **3. Bacterial leaching techniques**

The successful bioleaching process is characterize by the intimacy of microorganisms to a mineral surface, strong attachment result high rate of oxidation and dissolution on a substrate (mineral surface), this is achieved by the rate success of bio film formation. In general leaching techniques are two – Percolation leaching – a solution infiltrate through a fixed mineral location, and agitation leaching - mineral bearing ore stirred by a solution but while working in large scale, percolation leaching is usually chosen [7]. The principal commercial methods are aerated stirred-tanks, in situ, dump, heap, vat, bench scale, tank, column, reactor leaching are among the many. It was dump bioleaching process taken as the first commercial bioleaching in 1950 used to leach copper from sulfide minerals, since then bioleaching bloomed by copper oxide heap leaching, industrial microbial leaching process applied for sulfidic gold and bioheap commercial leaching of copper ore (chalcocite and covellite). The high production of bio heap leaching of copper in 1980 established at Lo Aguirre mine in Chile processing 16,000 tones ore/per year at the inception [27], these wipe the way that led to Chile's industrial bio copper production in large scale especially from the year 1984 [28].

#### **3.1 Stirred-tank biooxidation**

Aerated, stirred-tank bioreactors, used in mineral concentrate feeds, involve a series of stages that can have lots of tanks connected in parallel depending on the retention of the concentrate [7] a study conducted to check Na-chloride can possibly enhances the chemical and bacterial leaching of chalcopyrite uses three bioreactors engaged with inoculum of the bacteria [29]. Other tanks needed for value adding purposes which are usually single tanks might be connected in series, since these tanks subject to chemical attack, air, heat and sulfide mineral, they should be relatively resistant to corrosion, chemical attack, and soon, in order to have these character tanks can be lined with rubber, galvanized, or other corrosion protection method like using sacrificial anode or using high grade material like stainless steel, aluminum or copper.

Temperature maintained at optimum level by cooling coil or some time tanks are equipped with water jackets depending on the required temperature by the bacteria, these values can be conditioned based of the mineral to be leached, and sometimes the chemical used to enhance the leaching process [29]. Several tanks can be continuously arranged, named as continuous stirred tank reactor (CSTR), as per the above it can be followed by a series of small equal sized reactors [16]. Example of bioleaching of sulfidic gold concentrates, that the discharge from the final stage is subjected to water washing and solid/liquid separation in thickeners. Even though there is less power consumption basically used for agitator and blower, it has linear relationship with the amount of sulfide -sulfure which is required to oxidize and recover the target metal from the parent ore, rate of recovery depends up on the metal grade also.

The main advantages of these tank over other conventional methods like pressure autoclave, roasting, smelting, calcination and soon are; it has low capital and operational cost, relatively less construction period, less complicated requiring less skilled man power and most importantly it is environmental friendly. In general

Australia, Chile, USA, Brazil and South Africa are among those countries involved in bio oxidation by stirred tank [7, 16].

### 3.2 Dump leaching

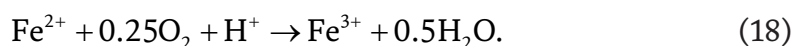
Dump (run of mine) [18] leaching involves uncrushed waste rock and low grade ore is piled up or changing a pit to dump by blasting it. Conventional methods would be very expensive to process these type of ores samples, except dump leaching, dump can be very huge, containing in excess of 10 million tons of waste rock, up to 60 m deep [7, 30].

In order to digest some of unwanted minerals like silicate and to promote the growth of acidophilic microorganism, acid water solution is spread on the top surface, the acid water solution percolate through the dump, the more acidic the environment the more growth of microorganisms that oxidize minerals to be recovered. The pregnant leach liquor or acid run-off is collected at the bottom of the dump, from where it is pumped to a recovery station. After collection the process followed by solvent extraction, electro wining for the metal production but dump aeration is vital for the microorganism to growth, tailing from solvent extraction recycled on the top of dump. Escondida mine found Chile is the biggest bio dump leach in world [26, 30].

### 3.3 Heap leaching

Heap leaching (crushed and agglomerated) [18] is composed of air, acid and microorganism where commutation takes place on rock samples to turn it to smaller particles which increases the surface area for acid digestions and conditioning it to microorganisms, particle should not be very fine and should be piled allowing a simplifies aeration pipe placed to facilitate air flow. To improve drainage of the mineral containing solution from the bottom of the ore, conditioned ore is spread on specially engineered pads (lined with high-density polyethylene (HDPE)), which consist of perforated plastic drain lines and air also supplied to optimize the growth of microorganism [7]. Heap can be large up to kilometer long, but commonly less than 500 m wide and 10 m long, the size and height of a heap depends up on air (for bacterial to grow) water, acid, heat generated due to the process and its dissipation [31]. Heap surface should be permeable enough for the sulfuric acid to infiltrate and dissolves iron to ferric solution producing ferric ion that react with copper sulfide results ferrous ion and copper solutions. Acidithiobacillus Ferrooxidans oxidize iron where the bacterial can be inoculated and works by attaching itself to ore, with having free movement. After collecting PLS (pregnant leach solution), then solvent extraction is followed where the target mineral recovered and formed into cathodes.

This aerobic bacteria works only in the presence of oxygen in the heap, those bacteria consume it from the solution where oxygen is in liquid phase. This process enhances the conversion of ferrous to ferric ions as per the reaction below.



Heap some time can be crushed 19 mm with rotating drum with acidified water [29] aeration can be conducted using low pressure fans those directing air through piping on the pad [26]. It is clear that heap leaching requires the preparation of the ore, primarily size reduction, so as to maximize mineral-lixiviant interaction and lay of an impermeable base to prevent lixiviant loss and pollution of water bodies. Heap leaching basically used to leach low-grade ore of copper and zinc, even in the case of copper grade level can be (0.2–2%). To have an effective heap leaching

process a mathematical model has been developed by taking heat, mass transfer, liquid, gas flow and chemical process in to consideration [31]. Heap also employed to bioleach silicate mineral, in a study where two microorganism were tested 'Ferroplasma acidarmanus or the common Acidithiobacillus ferrooxidans against the amenity of silicate minerals. Beside oxidation process energy was generated from flat plate solar energy collectors where heap is designed by HeapSim, heap bioleach simulation tool was used to simulate the heap and process occurring in the heap, even calculating the copper output [32].

### **3.4 In situ bioleaching**

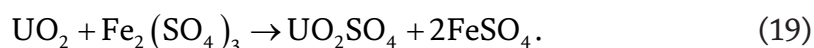
In situ leaching requires making the ore permeable for a solution and air to be circulated through the ore body. It does not require metal containing material to be removed from the ground [18]. It employs a method of recovering target minerals from the leach solution. The acid solution percolates until it reaches to impermeable layer. In situ includes recovery of minerals from the intact ore. The resulting metal-enriched solutions are recovered through wells drilled below the ore body. In case of in situ leaching the main concern is pollution of ground water, with this regard there are three types of ore bodies generally considered for in situ leaching: surface deposits above the water table, surface deposits below the water table and deep deposits below the water table. It is burden materials, establish permeability allowing air to pass in which metal bearing solution collected in the sumps [7]. It is combined with mineral recovery operation time and again to pull out the minerals from recovered fluid or pregnant solution or leachant. Acidified leach solutions, applied to the top surface of the entire ore zone, infiltrate through the fragmented ore due to the blast. The leaching bacteria become established and facilitate metal extraction. Metal-rich solutions or large volume of solution is circulated with the aid of gravity flow and pumped and recovered in sumps then again pumped to the surface for metal recovery, the returning fluids to the extraction operation are known as "barren solution". Metal recovery depends on two major things first the bacteria used (Acidithiobacillus Ferrooxidans) and permeability of the ore-body, which can be increased by fragmenting of ores in place, called "rubbleizing". Due to the ground water pollution this leaching process becomes less used and less popular [18] on the contrary it has been said that it is a best substitute for open pit and shaft mining operation, basically when in situ leaching is applied, no gangue or tailing is byproduct, it also called green mining or mine of the future [33].

## **4. Bioleaching of some elements**

### **4.1 Bioleaching of uranium**

Recent study shows that elements like uranium, copper, gold, zinc and other elements are commercial focus of bioleaching and biooxidation [34]. Many studies indicate microbial leaching is more important in low-grade ore, ore sample collected from Mianhuakeng uranium mine located in northern Guangdong province in China, leached by heap, by mixed microorganism of Acidithiobacillus Ferrooxidans and Leptospirillum Ferriphilum with 88.3% leaching efficiency [35]. Uranium leaching takes places by indirect mechanism, as Acidithiobacillus Ferrooxidans does not directly interact with uranium minerals. The role of Acidithiobacillus Ferrooxidans in uranium leaching is the best example of the indirect mechanism. Bacterial activity is limited to oxidation of pyrite and ferrous iron. The process involves periodic spraying or flooding of worked-out stops and tunnels of underground mines with

lixiviant [4]. The pH of lixiviant was optimized during the bioleaching of uranium from low grade Indian silicate-apatite ore with 0.024% of  $U_3O_8$ . This study uses *Acidithiobacillus Ferrooxidans* for leaching and biochemically generated ferric ions as an oxidant, optimizing particle, pulp density and redox potential results 98% uranium bioleaching. In this indirect bioleaching of uranium, the bacteria generate ferric sulfate and pyrite is oxidized by a lixiviant, within acidic environment the oxidations of ferrous ion to ferric ions process executed by the bacteria is faster than chemical oxidation [36]. In case of uranium bioleaching the main drawback is to oxidize uranium (IV) since it is insoluble but on this bioleaching process when ferrous sulfate produced in the process, then re-oxidized to ferric sulfate which enzymatically oxidizes uranium (IV) to uranium (VI) by the energy produced by this reaction. A case study in India at Jaduguda mines proves that use of biogenic ferric sulfate produced by the strain which was then used for efficient uranium extraction and cause no harm to the environment, while extracting uranium, use of reduced  $MnO_2$  in Bacfox process to generate biogenic ferric sulfate, results passed air saturated ferrous sulfate solution over *Acidithiobacillus Ferrooxidans* which is absorbed on solid surface [36]. Since the permeability of the ore surface is a factor, the above study uses a process called “rubbleizing” that increases fragmenting of ore in place which can be applied in the extraction of sulfide mineral, gold and uranium. While isolating the bacteria from mine water, the isolation media and  $H_2SO_4$  consumption during isolation, pH variation and temperature were determinate factors, the microbial cell count and the growth of (*A. Ferrooxidans*) determines by rate of oxidation of iron from  $Fe^{2+}$  to  $Fe^{3+}$ , so while leaching if the amount of  $Fe^{2+}$  decreases means the bacteria is using it as energy source to convert it to  $Fe^{3+}$ , uranium bioleaching depends on the synergic effects  $Fe^{3+}$  and proton produced by the bacterial [37] that process uses either of the two energy sources for growth iron or sulfur. The reaction of making insoluble uranium to soluble form is as follows [38].



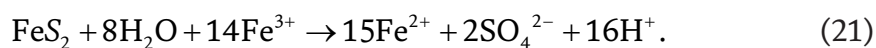
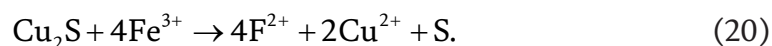
Studies indicate that microbial cell count and pulp density ranges 5–30% (w/v), particle size  $<75 \mu m$  has brought an optimum ore leaching but it should be clear that each ore has its own distinct behavior and no size fits all, meaning results indicated here might be different for another ore sample due to ore elemental composition, crystal structure, grade, topography and surface tension.

## 4.2 Bioleaching of copper

The ore is loaded on a water-resistant surface or ore is piled on an impermeable surface until a dump of suitable dimension forms. After leveling the top, then spraying a leach solution onto the dump is followed [4]. These dumps are a habitat of heterogeneous microorganisms. Dumps can have variety of particle sizes, where the bacterial annexation, which is anaerobic (microaerophilic), thermophilic begins from the top.

Dump leaching is used to pretreat low-grade, refractory-sulfidic gold ores and to leach copper from chalcocite ores while ore grade is low with values ranging between 0.1–0.5%. Copper can be obtained from ore rocks from the mound then washed with dilute  $H_2SO_4$  to facilitate the oxidation process of mineral by acidophiles, which is followed by cementation process where copper is precipitated from the drainage with scrap iron since it is a primary iron oxidizing process [39]. Check the leaching process of copper sulfide chalcocite ( $Cu_2S$ ), which occurs with pyrite ( $FeS_2$ ), leaching is due to ferric ion reacts with copper sulfide mineral processes ferrous and copper ions in solution.





In these regions indirect leaching by ferric sulphate also prevails. The exterior of the dump is at ambient temperature and undergoes changes in temperature reflecting seasonal and diurnal fluctuations. Many different microorganisms have been isolated from copper dumps, some of which have been studied in the laboratory. These include a variety of mesophilic, aerobic iron and sulfur oxidizing microorganisms; thermophilic iron and sulfur oxidizing microorganisms; and anaerobic sulphate reducing bacteria. In copper leaching the concentration of target metal by itself is an important variable, copper concentration (100–300 mM range) is values cause difficulty for the microorganism to operate, selecting the microorganism is one of the mechanisms of copper resistant, *Acidithiobacillus Ferrooxidans* can resist copper concentration and strong acidic environment [40]. *Thiobacillus Ferrooxidans* was the main product observed after a culture study, from an ore or leach solution for the identification of composition of bacterial population and incase of low ferrous ions, it was *Leptospirillum Ferrooxidans* was observed, the study shows that utilization of ferrous iron as energy source is dominated by the previous bacteria as the culture shows. *Pseudomonas aeruginosa*, where heterotrophic bacteria produce various organic acids in an appropriate culture medium is used in copper leaching [41]. The addition of salt in bioleaching of copper resulted process enhancement, after designing the bioreactor the bioleaching of copper was enhanced in both stirred tank or shack flask by adding sodium chloride in leach solution, increasing the dissolution of  $\text{Fe}^{3+}$  that eventually reduces precipitation [29] addition of some elements might result inhibition of bioleaching process, fluorine in solution increase the viscosity of leach liquor that result inhibition of bioleaching [42]. It is important to understand the microbiology, which is responsible or identify a means to study bulk activity of microorganism, these features are oxygen uptake in solid and liquid samples, redox potential, pH, ferrous iron concentration and temperature. Microbial leaching has also direct relation with enrichment and culture from solution of ores. *Acidithiobacillus Thiooxidans*, *Acidithiobacillus Ferrooxidans*, and *Leptospirillum Ferrooxidans* have been cultured where the process run at an ambient temperature and the strain of bacterial related to the microorganism mentioned here [27, 43]. Leach solutions enriched with copper exit at the base of the dump and are conveyed to a central recovery facility. In most large-scale operations the leach solution, copper-bearing solution pumped into large cementation units containing iron scrapings for cementation and then electrolysis followed [4]. It was in Chile and Australia the commercial bio heap leaching of copper started mass production. And the first bioleach heap copper extraction plant is in China [44]. The copper extracted percentage can be calculated as,

$$E = \text{Copper content in the solution/copper content in the sample} \times 100\% \quad [41].$$

### 4.3 Bioleaching of gold

Acidophilic bacteria are able to oxidize gold containing sulphidic ore, such a process can be ameliorated by conventional process of cyanidation, these basically reduces the complexation by increasing the capability of microorganisms to reach to the target metal. Certain sulphidic ores containing encapsulated particles of elemental gold, resulting in improved accessibility of gold to complexation by leaching agents such as cyanide. Relative to other conventional process and pre-treatments like roasting, smelting and pressure oxidation, bio-oxidation demands

less cost and no harm to nature [7]. Though it is under study a commercial bio-oxidation and bio heap leaching of gold prior cyanide extraction. It is the bacteria, *Acidithiobacillus Ferrooxidans* used to oxidize the sulphide matrix for gold recovery. Prior to extraction, gold ore must be bio-oxidized by the bacteria. In this process refractory sulphidic gold ores contain mainly two types of sulphides: pyrite and arsenopyrite where silver ion was used as a catalyst in acidic environment. Since gold is usually finely disseminated in the sulphide matrix, the objective of biooxidation of refractory gold ores is to break the sulphide matrix by dissolution of pyrite and arsenopyrite and extract 95% of iron and arsenic, the residue of both filtered through a vacuum pump. The consumption of cyanide is much higher while biooxidation, the study suggested that using thiourea instead of cyanide is much less toxic but since the process requires high consumption of thiourea cost increases steadily, consumption of thiourea reduced by using different agents like  $\text{SO}_2$ , bisulfite, cystine, cystine with oxygen during extraction process [45].

## 5. Industrial application

The mesophilic tank leaching is the most common bioleaching process in the world; thermophilic tank is favored while the temperature is high, among such tanks BioCop™ well known. In order to have effective thermophilic tank the following are basic requirements, microbial catalyzed reaction which is needed to facilitate metal dissolution by microbial oxidizing of ferrous iron to ferric iron, initial solubilization of ferrous ion takes place using acid solution, oxidation of mineral sulfide takes place by the combination effects of ferric iron and acid solution followed by oxidation of reduced sulfur to sulfate by microorganisms. Reactor configuration is the other factor where the six equal size continuous reactor, three arranged in parallel considered as primary reactors, and the other three arranged in series considered as secondary reactors, in this case reactors are considered as a large continuous stirred tank supplied with aeration and agitation. The other factors are oxygen, carbon dioxide, pulp density and finally even though the operational cost is much less plant location, construction material, blower or compressor to supply oxygen to the microbes, high power agitator in case of oxygen plant for oxygen dispersal in the reactor. Growth of industries results the demand of metals in very high quantity and likely go further in the years to come. This brings diminution of high grade ore with effluents and solid wastes that needs to be treated to recover the important elements and protect the environment.

Regarding to environment biohydrometallurgy is a vital process, the fact that bio-process is conducted without the presence of toxic chemical and relatively required low cost makes it most needed. The direct implication of microorganisms in the reduction of uranium is of considerable interest because of its potential application in bio remediation of contaminated sites, in pretreating radioactive wastes, bioleaching is becoming a promising technology.

IntechOpen

IntechOpen

### **Author details**

Mulugeta Sisay Cheru

Institution- Jimma Institute of Technology, Jimma, Ethiopia

\*Address all correspondence to: mulugetajimma07@gmail.com

### **IntechOpen**

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

## References

- [1] B.P Gilbertson, (2013) Creating Value Through Innovation Biotechnology in Mining. Mineral Processing and Extractive Metallurgy, 109(2), pp 61-67.
- [2] K. Bosecker, (2001) Microbial Leaching in Environmental Clean-Up Programmes. Hydrometallurgy, 59(2-3), pp 245-248.
- [3] K. Bosecker, (1997) Bioleaching: Metal Solubilization by Microorganism. FEMS Microbiology Reviews, 20(3-4), pp 591-604.
- [4] J.D.S.D Mishra, (2007) Bioleaching of Lateritic Nickel Ore Using Chemolithotrophic Micro Organisms (Acidithiobacillus Ferrooxidans). A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Bachelor of Technology. National Institute of Technology, Rourkela, India.
- [5] M.A Faramarzi, M.M Manzari, H Brandle, (2020) Bioleaching of Metals from Wastes and Low Grade Sources by HCN-forming Microorganism. Hydrometallurgy, 191, pp 105-228.
- [6] Y. Songrong, X Jiyuan, Q. Guanzhou, H. Yeuhua, (2002) Research and Application of Bioleaching and Biooxidation Technology in China. Minerals Engineering, 15(5), pp 361-363.
- [7] D.E Rawlings, (1997) Biomining: Theory, Microbes and Industrial Processes, Springer-Verlag Berlin Heidelberg, 1st Edition, New York.
- [8] H.L Ehrich, (2002) Geomicrobiology, Revised and Expanded. Marcel Dekker Publisher, 4th Edition, New York.
- [9] A.D Agate, (1996) Recent Advances in Microbial Mining. World journal of Microbiology and Biotechnology, 12, pp 487-495.
- [10] A.E Yüce, H. M Tarkan, M Dogan, (2006) Effect of Bacterial Conditioning and The Flotation of Copper Ore and Concentrate. African Journal of Biotechnology, 5(5), pp 448-452.
- [11] W Jonglertjunya, (2003) Bioleaching of chalcopryrite, A Thesis Submitted to the University of Birmingham for the Degree of Doctor of Philosophy, United Kingdom.
- [12] C.S Demergasso, P.A.P Galleguillos, L.V.G Escudero, V.J.A Zepeda, D Castillo, E.O Casamayor, (2005) Molecular Characterization of Microbial Populations in a Low-Grade Copper Ore Bioleaching Test Heap. Hydrometallurgy, 80, pp 241-253.
- [13] O Pizarro, E Jedlicki, O Orellana, J Romero, R.T. Espejo, (1996) Bacterial Populations in Samples of Bioleached Copper Ore as Revealed by Analysis of DNA Obtained Before and After Cultivation. Applied and Environmental Microbiology, 62(4), pp 1323-1328.
- [14] K.J Edwards, B Hu, R.J Hamers, J.F Banfeld, (2001) A New Look at Microbial Leaching Patterns on Sulphide Minerals. FEMS Microbiology Ecology, 34, pp 197-206.
- [15] A. Schippers, W.Sand, (1999) Bacterial Leaching of Metal Sulfides Proceeds by Two Indirect Mechanisms via Thiosulfate or via Polysulfides and Sulfur. Appl Environ Microbiol, 65(1), pp 319-321.
- [16] R Gonzalez, J.C Gentina, F Acevedo, (2004) Biooxidation of a Gold Concentrate in a Continuous Stirred Tank Reactor: Mathematical Model and Optimal Configuration. Biochemical Engineering Journal, 19, pp 33-42



- [17] T Das, S Ayyappan, G.R Chaudhury, (1999) Factors Affecting Bioleaching Kinetics of Sulfide Ores Using Acidophilic Micro-Organism. *Biomaterials*, 12, pp 1-10.
- [18] D.E Rawlings, B.D Johnson, (2007) *Biomining*. Springer-Verlag Berlin Heidelberg, 1st Edition, New York.
- [19] A.K Halinen, N Rahunen, A.H Kaksonen, J.A Puhakka, (2009) Heap Bioleaching of a Complex Sulfide Ore: Part II. Effect of Temperature on Base Metal Extraction and Bacterial Compositions, *Hydrometallurgy* 98, pp 101-107.
- [20] M Jafari, H Abdollahi, S.Z Shafaei, M. Gharabaghi, H Jafari, A Akcil, S Panda, (2018) Acidophilic Bioleaching: A Review on the Process and Effect of Organic-Inorganic Reagents and Materials on its Efficiency. *Mineral Processing and Extractive Metallurgy Review*, 40(2), pp 87-107.
- [21] J.A Diaz, J Serrano, E Leiva, (2018) Bioleaching of Arsenic-Bearing Copper Ores. *Minerals*, 8(5), pp 215.
- [22] B.M Goebel, E Stackebrandt, (1994) *Applied and Environmental Microbiology Cultural and Phylogenetic Analysis of Mixed Microbial Populations Found in Natural and Commercial Bioleaching Environments*, 60(5), pp 1614-1621.
- [23] A. Giaveno, L. Lavalle, P. Chiacchiarini, E. Donati, (2007) Bioleaching of Zinc from Low-Grade Complex Sulfide Ores in an Airlift by Isolated *Leptospirillum ferrooxidans*. *Hydrometallurgy*, 89, pp 117-126.
- [24] A.K Halinen, N Rahunen, A.H Kaksonen, J.A Puhakka, (2009) Heap Bioleaching of a Complex Sulfide Ore Part I: Effect of pH on Metal Extraction and Microbial Composition in pH Controlled Columns. *Hydrometallurgy*, 98, pp 92-100.
- [25] H.N Bhatti, F Anjum, S Saeed, K.U Rehman, (2009) Bioleaching of Rock Phosphate by *Aspergillus Niger*. *Asian Journal of Chemistry*, 21(8), pp 6029-6040.
- [26] A.J Francis, (2006) *Microbial Transformations of Radionuclides and Environmental Restoration Through Bioremediation*, for Presentation at the Symposium on "Emerging Trends in Separation Science and Technology" SESTEC 2006 Bhabha Atomic Research Center (BARC), Trombay, Mumbai, India.
- [27] J.C Gentina, F Acevedo, (2016) Copper Bioleaching in Chile. *Minerals*, 6(23), pp 1-9.
- [28] J.C Gentina, F Acevedo, (2013) Application of Bioleaching to Copper Mining in Chile. *Electronic Journal of Biotechnology*, 16(3), pp 16.
- [29] D Bevilaqua, H Lahti, P.H Suegama, O.J Garcia, A.V Benedetti, J.A Puhakka, O.H Tuovinen, (2013) Effect of Na-chloride on the Bioleaching of a Chalcopyrite Concentrate in Shake Flasks and Stirred Tank Bioreactors. *Hydrometallurgy*, 138, pp 1-13.
- [30] C. L Brierley, (2008) How will Biomining be Applied in Future?. *Trans.Nonferrous Met Soc China*, 18, pp 1303-1310.
- [31] M.J Leahy, M.R Davidson, M.P Schwarz, (2005) A Two-Dimensional CFD Model for Heap Bioleaching of Chalcocite. *Anziam.J* 46(E), pp C439-C457.
- [32] C Murray, W Platzer, J Petersen, (2017) Potential for Solar Thermal Energy in the Heap Bioleaching of Chalcopyrite in Chilean Copper Mining, *Minerals Engineering*, 100, pp 75-82.
- [33] M Haschke, J Ahmadian, L Zeidler, T Hubrig, (2016) In-Situ Recovery of Critical Technology Elements. *Procedia Engineering*, 138, pp 248-257.

- [34] A.H Kaksonen, N.J Boxall, Y Gumulya, H.N Khaleque, C Morris, T Bohu, K Y Cheng, K Usher, A.M Lakaniemi, (2018) Recent Progress in Biohydrometallurgy and Microbial Characterization. Hydromet, DOI: 10.1016/j.hydromet.2018.06.018.
- [35] X Wang, Y Liu, Z Sun, J Li, L Chai, X Min, Y Guo, P Li, Z Zhou, (2017) Heap Bioleaching of Uranium from Low-Grade Granite-Type Ore by Mixed Acidophilic Microbes. J Radioanal Nucl Chem, DOI: 10.1007/s10967-017-5406-8.
- [36] Abhilash, S Singh, K.D Mehta, V Kumar, B.D Pandey, V.M Pandey, (2009) Dissolution of Uranium from Silicate-Apatite Ore by Acidithiobacillus Ferrooxidans. Hydrometallurgy, 95, pp 70-75.
- [37] Abhilash, B.D Pandey, (2013) Microbially Assisted Leaching of Uranium – A Review. Mineral Processing and Extractive Metallurgy Review, 34(2), pp 81-113.
- [38] Abhilash, K.D Mehta, V Kumar, B.D Pandey, P.K Tamrakar, (2011) Bioleaching -An Alternate Uranium Ore Processing Technology for India. Energy Procedia, 7, pp 158-162.
- [39] N Pradhan, K.C Nathsarma, K.S Rao, L.B Sukla, B.K Mishra, (2008) Heap Bioleaching of Chalcopyrite: A Review. Minerals Engineering, 21, pp 355-365.
- [40] C.M Bussenius, C.A Navarro, C.A Jerez, (2017) Microbial Copper Resistance: Importance in Biohydrometallurgy. Microb Biotechnol, 10(2), pp 279-295.
- [41] M.A. Shabani, M. Irannajad, A.R. Azadmehr, and M. Meshkini, (2013) Bioleaching of Copper Oxide Ore by *Pseudomonas aeruginosa*. International Journal of Minerals, Metallurgy and Materials, 20(12), pp 1130.
- [42] M Dopson, L Lövgren, D Boström, (2009) Silicate Mineral Dissolution in the Presence of Acidophilic Microorganisms: Implications for Heap Bioleaching. Hydrometallurgy, 96, pp 288-293.
- [43] D.E Rawlings, (2005) Characteristics and Adaptability of Iron- and Sulfur-Oxidizing Microorganisms Used for the Recovery of Metals from Minerals and Their Concentrates. Microbial Cell Factories, 4(13), pp 1-15.
- [44] L Xingyu, C Bowei, W Jiankang, R Renman, (2010) Leptospirillum Forms a Minor Portion of the Population in Zijinshan Commercial Non-Aeration Copper Bioleaching Heap Identified by 16S rRNA Clone Libraries and Real-Time PCR. Hydrometallurgy, 104, pp 399-403.
- [45] T Deng, M Liao, (2002) Gold Recovery Enhancement from a Refractory Flotation Concentrate by Sequential Bioleaching and Thiourea Leach. Hydrometallurgy, 63, pp 249-255.