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Microbial Leaching of Uranium Ore

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1. Introduction
This chapter is a review of the microbiological leaching of uranium ores. Microbiological leaching has been used as an alternative approach to conventional hydrometallurgical methods of uranium's extraction. In the microbiological leaching process, iron-oxidizing bacteria oxidize pyritic phase to ferric iron and sulfuric acid, and uranium is dissolved from the ore due to sulfuric acid attack. If uranium in the ore material is reduced state and involves tetravalent form and a redox reaction is involved whereby uranium oxidized to the hexavalent form. Future sustainable development requires measures to be taken to reduce the dependence on non-renewable raw materials and the demand for primary resources. New resources for metals must be developed with the help of novel technologies. In addition, improvement of previously existed mining techniques can be resulted in metal recovery by the sources that have not been of economical interest up to today. The metal-winning processes based on the activity of microorganisms offer a possibility to obtain metals from mineral resources which are not accessible by conventional mining. Generally bioleaching is a process described as being “dissolution of metals from their mineral source by certainly and naturally occurring microorganisms” or “use of microorganisms to transform elements so that the elements could be extracted from a material when water is filtered through it”. However, there are some slight differences in definition: Usually, “bioleaching” is described as the conversion of solid metal values into their water soluble forms using microorganisms. Bacterial leaching is the extraction of metals from their ores using microorganisms. The capital costs are low compared to those for a smelter. Environmental pollution caused by mineral processing is a serious problem and on the other hand, microorganisms play crucial roles in biogeochemical cycling of toxic metals and radionuclides. Recent progresses have been made to understand metal–microbe interactions and new applications of these processes to the detoxification of metal and radionuclide contamination have been developed. It also suggests an opportunity to reduce of environmental and air pollution by sulphur dioxide.

2. Historical review
One of the initial reports in which leaching might have been involved in mobilization of metals is given by the Roman writer Plinius Secundus. In his works on natural sciences, Plinius describes how copper minerals are derived by means of utilizing a leaching process. In cold weather during the winter the sludge freezes to the hardness of pumice. It is known...
from experience that the most wanted chrysocolla is formed in copper mines, the following in silver mines. The Rio-Tinto mines in southwestern Spain are usually considered the cradle of biohydrometallurgy. These mines have been exploited since pre-Roman periods due to their copper, gold, and silver values. However, with respect to commercial bioleaching operations on an industrial scale, biohydrometallurgical techniques had been introduced to the Tharsis mine in Spain 10 years earlier. As a consequence of the ban of open-air ore roasting and its resulting atmospheric sulfur emissions in 1878 in Portugal, hydrometallurgical metal extraction has been taken into consideration in other countries more intensely. In addition to the ban, cost savings were another incentive for development: Heap leaching techniques were assumed to reduce transportation costs, allowing employment of locomotives and wagons for other services. From 1900 on, no open air roasting of low-grade ore was conducted at the Rio-Tinto mines. The researchers conducted on microbial leaching indicate an increasing rate of recovery and solubility of metals in direct, indirect, Thiosulfate and Polysulfide mechanism, due to microorganism activity. The initial work on uranium bioleaching in the early 1950s was taken to prevent solubilization, but it soon became apparent that this process could be applied for a commercial scale to extract uranium for low-grade ores. During 1952 and 1953, the plant at Urgeirica started a uranium heap leaching process on a commercial scale. This is an early turning point in the microbiological leaching of uranium ores.

Harrison et al. (1966) reported the role of the iron oxidizing Acidithiobacillus ferrooxidans in leaching of uranium. The uranium ore was stacked in heaps, similar to dump leaching of low-grade copper ore, and leached using an acidic ferric sulfate solution at the Elliot Lake Mine, Ontario, Canada. The presence of the bacteria in the heaps was discovered and their role in maintaining oxidizing conditions by conversion of ferrous to ferric iron for extraction of the uranium was defined. Guay’s (1976) investigations showed that the effectiveness and efficiency of microorganism’s influence, such as Thiobacillus, requires the presence of certain amount of iron. He also conducted some research on mixing level, aeration, and oxidation rate of iron that may affect uranium’s microbial leaching. He employed a specific type of microbes such as Thiobacillus in his study. Amongst the various parameters affecting the sufficiency of microbial leaching; he just focused upon the aforementioned parameters. Brierley (1980), one of the distinguished researchers in microbial leaching, has done a case study on uranium ore (coffinite and uraninite) in Gerantez mine in Canada. His investigation’s outcomes demonstrated a promising and positive effect of Thiobacillus ferrooxidans in climbing recovery rate of uranium. Cerda’s studies (1991) on Pitchblende ore in green-grayish shiest samples, collected from Spain’s mines, revealed a close relationship of pyrite and Chalcopyrite in reduction of acid consumption in microbial leaching, in comparison with regular leaching. In continuous surveys carried out over on microbial leaching by different researchers. Gonzalez (1993) utilized column leaching, seepage and shaking table to study the effect of pyrite amount in uranium’s microbial leaching. His investigation’s results showed an augmenting trend of uranium recovery while there is an optimum amount of pyrite. Beyond the optimum level of pyrite, not only the pyrite presence is not beneficiary, but also it may introduce complicity in leaching process. He has also done some research on pH optimization, temperature, and stirred time. In a case study conducted by Junior (1993) on uranium ore in Figopira in Brazil, potentiality of microbial leaching in uranium recovery enhancement has been emphasized. Schipper’s (1995) study on two mines in Germany indicated the identification of microbe variety in...
waste materials. The waste materials (low grade black Schist) consisted of 0.05% of uranium and 0.5 to 7% of carbonate. Sampling showed the microorganisms aerobic and anaerobic were present till 1.5 to 2 meter of surface depth and more than 99% of Thiobacillus ferrooxidans were present within this depth. In this study, there was no investigation on microbial leaching capability and identification of microbes was just concerned. Munoz (1995), dedicated researcher in uranium’s microbial leaching in Spain, has presented his research’s results in many published papers in Elsevier. Bioleaching process, mineralogy of uranium ore, bearing rock type, level of toxic material, and leaching variables are among the factors which have been probed by him. He has worked on pitchblende ore with 0.097% uranium content. Leduc, L.G. (1997) studied ten different isolated of Thiobacillus ferrooxidans with regard to their degree of resistance to the metals copper, nickel, uranium, and thorium. The miscellaneous isolates had different susceptibilities to the tested metals, and moreover none of the metals had a stimulus effect. Uranium and thorium were 20 to 40 times more toxic to ferrous iron oxidation than either copper or nickel. Mathur A.K. (2000) investigated the application of ferric ion as an oxidant and in combination with other anions such as ferric sulfate or chloride as a leachant is well accepted for recovery of metals, particularly from ores of copper, cobalt, nickel, zinc and uranium. Biogenically generated ferric sulfate that has been in vogue for many dump and heap leaching operations, to recover uranium and copper values. Hefnawy (2001) used fungi for Aloga uranium ore bioleaching in Egypt. The amount of uranium solubilized by A. terreus and P. spinulosum was increased by intensifying ore concentrations on the growth media, reaching its maximum at 4% (w/v). Whereas, the highest percentage of uranium released by both fungi was obtained at 1% (w/v), in this concentration the released uranium being 75% and 81.5% respectively for ore and 72.8% and 77.6% respectively for the second ore. The best leaching occurs when the final pH shifts toward acidity. The biosorption of released uranium by fungal Mycelium was also increased by augmenting ore concentrations on the growth media. Kalinowski and Oskarsson (2002) represented common ligand producing bacterial species (Pseudomonas fluorescens, Shewanella putrefactions and Pseudomonas stutzeri) were incubated in a chemically defined medium supplemented U-ore that had been exposed to natural weathering conditions for 30 years having a content of 0.0013% U by weight. For comparison, non-leached uranium ore (0.61% U by weight) from the same area were incubated by P. fluorescens and S. putrefaciens. P. fluorescens is the only species that thrives and manages to mobilize measurable amounts of uranium from the two ores. Despite the extensive increase in pH from 4.7 to 9.3 P. fluorescens supplemented with ore manages to mobilize 0.001-0.005% of the total amount of U from both ores. The release of U was interpreted to be attributed to the production of pyoverdine chelators, which is a typical ligand produced by fluorescent pseudomonades, as U could not be detected in either sterile controls or in experiments with the two other bacteria. In Sumera Saeed (2002) investigation the bioleaching behavior of rock phosphate (Jordan imported) was studied using different strains of Aspergillus Niger. X-ray diffraction analysis revealed the presence of fluorapatite [Ca2(PO4)3F] as the main source of phosphorus. Average content of phosphorus in testing ore was 33.6% scanning electron microscope showed the presence of significant amount of phosphorus. Decrease in pH was observed due to organic acids produced by Aspergillus niger strains during growth on liquid media containing glucose. Akcil (2004) represented an investigation of the potential bioleaching developments in Turkey [27]. Bene Ditto (2005) in his study identified sulfur reduction bacteria in Brazil uranium mine water. This is basically
problem in Brazil nuclear industrial. Choi Moon-sung (2005), schist with 0.01 % U₃O₈ content in S. Korea under study. He could reach 0.8 uranium recovery with use Acidibacillus ferrooxidans. Jong un lee (2005), the effects of several conditional factors on efficiency of U bioleaching via using an iron-oxidizer, Acidithiobacillus ferrooxidans, from U-bearing black shale (349 ppm of U) were investigated. In the case inoculated with the cells, lower pH, higher redox potential and higher amount of aqueous Fe³⁺ than those of non-inoculated reactor observed until 200h. Such development of condition, which was facilitated by microbial activity, can enhance the rate and extent of U leaching from the solid substrate.

3. Bioleaching mechanisms

There are two major mechanisms of bacterial leaching. One involves the ferric-ferrous cycle (indirect or non-contact mechanism), while the other involves physical contact of the organism with the insoluble sulphide (direct or contact mechanism) and is independent of indirect mechanism. Originally, a model with two types of mechanisms which are involved in microbial mobilization of metals has been proposed:

3.1 Direct mechanism

Microorganisms can oxidize metal sulfides by a direct mechanism obtaining electrons directly from reduced minerals. Cells have to be attached to the mineral surface and a close contact is needed. Bioleaching of metal sulfides (MS) can be achieved in direct and indirect modes of bacterial metabolism. Figure 1 (a) shows a scheme of the reaction mechanism for the bio-oxidation of sulphide minerals-direct mechanism. The direct mechanism is given by:

\[ \text{MS} + 2\text{O}_2 \rightarrow \text{MSO}_4 \]  

Where M is a bivalent heavy metal.

The following equations describe the “direct” mechanism for the oxidation of pyrite:

\[ 2\text{FeS}_2 + 7\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{FeSO}_4 + 2\text{H}_2\text{SO}_4 \]  

3.2 Indirect mechanism

The oxidation of reduced metals through “indirect” mechanism is mediated by ferric iron (Fe³⁺) originating from the microbial oxidation of ferrous iron (Fe²⁺) compounds present in minerals. Ferric iron is an oxidizing agent and can oxidize, e.g., metal sulfides and is chemically reduced to ferrous iron which, in turn, can be oxidized microbial again. In this case, iron has a role as electron carrier. It was proposed that no direct physical contact is needed for oxidation of iron. Figure 1 (b) shows a scheme of the reaction mechanism for the bio-oxidation of sulphide minerals-indirect mechanism.

\[ 4\text{FeSO}_4 + \text{O}_2 + 2\text{H}_2\text{SO}_4 \rightarrow 2\text{Fe}_2(\text{SO}_4)_3 + 2\text{H}_2\text{O} \]  

\[ \text{FeS}_2 + \text{Fe}_2(\text{SO}_4)_3 \rightarrow 3\text{FeSO}_4 + 2\text{S} \]  

\[ 2\text{S} + 3\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{H}_2\text{SO}_4 \]  

The indirect mechanisms can be demonstrated, i.e., for uranium leaching as follows:

\[ \text{UO}_2 + \text{Fe}_2(\text{SO}_4)_3 \rightarrow \text{UO}_2\text{SO}_4 + 2\text{Fe}_2\text{SO}_4 \]
However, the model of “direct” and “indirect” metal leaching is still under discussion. Recently, this model has been revised and replaced by another one which is not dependent upon differentiation between a “direct” and an “indirect” leaching mechanisms.

Fig. 1. The reaction mechanism for the bio-oxidation of sulfide minerals (a) Direct mechanism - , (b) Indirect mechanism

### 3.3 Thiosulfate mechanism

The mineral dissolution reaction is not identical to all metal sulfides and the oxidation of different metal sulfides proceeds via different intermediates. This also has been recently reviewed. Briefly, a thiosulfate mechanism has been proposed for oxidation of acid insoluble metal sulfides such as pyrite (FeS$_2$) and molybdenite (MoS$_2$), and a polysulfide mechanism for acid soluble metal sulfides such as sphalerite (ZnS), chalcopyrite (CuFeS$_2$) or galena (PbS). In the thiosulfate mechanism, solubilization is through ferric iron attack on the acid-insoluble metal sulfides with thiosulfate being the main intermediate and sulfate the main end-product. Using pyrite as an example of a mineral, the reactions may be represented as [35, 36]:

$$FeS_2+6Fe^{3+}+3H_2O\rightarrow S_2O_3^{2-}+7Fe^{2+}+6H^+ \quad (7)$$

$$S_2O_3^{2-}+8Fe^{3+}+5H_2O\rightarrow 2SO_4^{2-}+8Fe^{2+}+10H^+ \quad (8)$$

### 3.4 Polysulfide mechanism

Polysulfide and elemental sulfur are the main intermediates in the “polysulfide mechanism” during oxidation of galena, sphalerite, chalcopyrite, hauerite, orpiment and realgar. The presence of iron (III) at the beginning of mineral degradation is an important prerequisite. In the case of the polysulfide mechanism, solubilization of the acid-soluble metal sulfide is through a combined attack by ferric iron and protons, with elemental sulfur as the main intermediate. This elemental sulfur is relatively stable but may be oxidized to sulfate by sulfur-oxidizing microbes such as Acidithiobacillus thiooxidans or Acidithiobacillus caldus according to reaction 11:

$$MS+Fe^{3+}+H^+\rightarrow M^{2+}+0.5H_2S_n+Fe^{2+} \quad (n>= 2) \quad (9)$$

$$0.5H_2S_n+Fe^{3+}\rightarrow 0.125S_8+Fe^{2+}+H^+ \quad (10)$$
The ferrous iron produced in reactions (9), (10) may be reoxidized to ferric iron by iron-oxidizing microorganisms such as Acidithiobacillus ferrooxidans or bacteria of the genera Leptospiillum or Sulfobacillus.

\[ 2\text{Fe}^{2+}+0.5\text{O}_2+2\text{H}^+\rightarrow 2\text{Fe}^{3+}+\text{H}_2\text{O} \quad (12) \]

The role of the microorganisms in the solubilization of metal sulfides is, therefore, to provide sulfuric acid (reaction 11) for a proton attack and to keep the iron in the oxidized ferric state (reaction 12) for an oxidative attack on the mineral.

### 4. Influenced factors on uranium microbial leaching

#### 4.1 Diversity of microbial culture

In general, types of microorganisms found in heap leaching processes are similar to those found in stirred tank processes, however, the proportions of the microbes may vary depending on the mineral and the conditions under which the heaps or tanks are operate. In processes that operate from ambient temperatures to about 40°C, the most important microorganisms are considered to be a consortium of Gram-negative bacteria. These are the iron- and sulfur-oxidizing Acidithiobacillus ferrooxidans, the sulfur-oxidizing Acidithiobacillus thiooxidans and Acidithiobacillus caldus, and the iron-oxidizing leptospiilli, Leptospiillum ferrooxidans and Leptospiillum ferrphilum. Consortia of acidophilic thiobacilli and leptospiilli are believed to be superior to pure cultures for the biological oxidation of sulfide minerals. Microbial diversity is extensive in acid mine waters, including uranium mine leaching solutions. While moderately acidophilic thiobacilli is numerous, thermo acidophilic archaea, resembling *Sufolobus* and *Acidianus*, has been isolated from uranium-mine waste heaps.

#### 4.2 Mineralization of uranium

Another important factor of bioleaching is the mineralization of the uranium. Table 1, 2 show the results of different uranium ores which are subjected to bioleaching. According to this, oxides, phosphates, sulfates and carbonates are solubilized relatively convenient, while dissolution of silicates is difficult or even impossible.

| Uranium oxide | Valence | Natural form | Solution ability |
|---------------|---------|--------------|-----------------|
| UO$_2$        | IV      | Uraninite    | Insoluble       |
| U$_2$O$_5$    | IV      |              | Little soluble  |
| U$_3$O$_8$    | IV,VI   | Pitchblende   | Little soluble  |
| UO$_3$        | VI      | Carnotite    | Soluble         |

Table 1. The type of uranium oxides in nature [40]
Table 2. Uranium mineralization and bioleaching.

| Uranium ores       | Chemical composition                                      | Degree of bioleaching |
|--------------------|----------------------------------------------------------|-----------------------|
| **Uraninite**      | UO₂                                                      | +                     |
| **Gummite**        | UO₃·nH₂O                                                 | +                     |
| **Becquerelite**   | CaU₆O₁₉·11H₂O                                            | +                     |
| **Brammerite**     | (U,Ca,Ce)(Ti,Fe)O₆                                        | +                     |
| **Davidite**       | (Fe₅Ce₅)₂(Ti,Fe,V,Cr)₃O₁₂                                 | +                     |
| **Coffinite**      | U(SiO₄)₅·x(OH)₄x                                        | -                     |
| **Uranophane**     | Ca₃(UO₂)₂Si₃O₇·6H₂O                                     | +·-                   |
| **Sklodowskite**   | Mg(UO₂)₂Si₂O₇·6H₂O                                      | +·-                   |
| **Autunite**       | Ca(UO₂)₂(PO₄)₂·12H₂O                                    | +                     |
| **Torbernite**     | Cu(UO₂)₂(PO₄)₂·8H₂O                                     | +                     |
| **Uramphtite**     | NH₄·UO₂·P₂O₇·3H₂O                                       | +                     |
| **Zeunerite**      | Cu₂(UO₂)₂(AsO₄)₂·12H₂O                                  | +                     |
| **Carnotite**      | K₂(UO₂)₂(VO₄)₂·3H₂O                                     | +·-                   |
| **Tyuyamunite**    | Ca(UO₂)₂(VO₄)₂·8H₂O                                     | +·-                   |
| **Zippeite**       | (UO₂)₂(PO₄)₂·(OH)₂·4H₂O                                 | +                     |
| **Uranopilite**    | (UO₂)₂SO₄·(OH)₁₀·12H₂O                                  | +                     |
| **Johannite**      | Cu(UO₂)₂(PO₄)₂·(OH)₂·12H₂O                              | +                     |
| **Schroeckingerite** | NaCa₃UO₃SO₄(CO₃)₃F·10H₂O                              | +                     |

**Urano-oranic Compounds**

+: easy, -: hard, + -: variable

### 4.3 Country rock type

The bioleaching process also depends upon nature of the country rock. If this is alkaline, it is probable that precipitates would be formed which would lead to causing problem to natural percolation of the leaching. This issue lowers the uranium yield because some pockets of ore are not attacked. On the other hand, when the country rock is acidic, acid consumption by the rock will be low.

### 4.4 Nutrient element

The quantity of nutrient element content in the country rock is an important aspect for microbial leaching. The solid medium should supply at least adequate minerals to microorganism growth of not being stopped. The presence of organic compounds (yeast extract) inhibited pyrite oxidation from *Thiobacillus ferrooxidans*. Certain metals presented in bioleaching environments can inhibit microbial growth; therefore, these metals cause leaching efficiencies to reduce. Based on dry weight, nitrogen is the most important following element after carbon for the synthesis of new cell mass. In commercial operations, inexpensive fertilizer grade ammonium sulfate is typically added to biooxidation tanks or bioleaching heaps to ensure that sufficient nitrogen is available.

### 4.5 Toxic material and resistance to metals

Additions of copper, nickel, uranium, or thorium adversely influenced iron (II) oxidation by *Acidithiobacillus ferrooxidans* with uranium and thorium showing higher toxicities than
copper and nickel. Silver, mercury, ruthenium, and molybdenum reduced the rate of
growth of Sulfolobus grown on a copper concentrate. Resistance to metal ions is a function
of those thiobacilli tested to date. Acidithiobacillus ferrooxidans is resistant to a variety of
metal ions such as chromium, copper, zinc, nickel, thorium, uranium and mercury. The
resistance of Acidithiobacillus ferrooxidans to mercury is ferrous iron dependent.

4.6 Temperature
Temperature is among the most important parameters influencing the diversity of the
microbial communities. Use of thermophiles was found to improve metal sulfide
biooxidation in at least two ways. First, reaction rates increased with increasing
temperature. Second, elevated temperature increased the extent of metal extraction from
certain minerals. Bioleaching processes are carried within range of temperatures from
ambient to a demonstration plant that has been operated at 80°C. Temperature ranges of 2-
35°C and 4-21°C, respectively, were observed. Moderately thermophiles iron-oxidizing and
sulfur-oxidizing bacteria were initially cultured from mining environments and hot springs.
However, psychrophiles have not been isolated from cold tailing effluents where they
would be expected. The temperature used for bioleaching in most of studies is 35°C.
Although Acidithiobacillus ferrooxidans was reported to grow most rapidly at 30°C, it
oxidised iron faster at 35°C. This case has crucial implications for industrial bioleaching
since the oxidation of sulphides is exothermic, and hence cooling may be essential to
maintain a satisfactory industrial process. As it is expected the types of presented iron and
sulfur-oxidizing microbes differentiation depending upon temperature range. The types of
microbes found in processes operating from ambient to 40°C tend to be similar irrespective
of mineral, as are those within the temperature ranges 45–55°C and 75–80°C. As described
below, there are two broad categories of biologically-assisted mineral degrading processes.
An ore or concentrate is either placed in a heap or dump where it is irrigated or a finely
milled mineral suspension is placed in a stirred tank where it is vigorously aerated. In
general, mineral solubilization processes are exothermic and when tanks are used, cooling is
required to keep the processes that function at 40°C at their optimum temperature. At
higher temperatures the chemistry of mineral solubilization is much faster and in the case of
minerals such as chalcopyrite, temperatures of 75–80°C are required for copper extraction to
take place at an economically viable rate. Table 3 shows a Classification of
chemolithotrophic bacteria in terms of their optimum temperature ranges.

| Bacterial class          | Optimum temp. range /°C |
|--------------------------|--------------------------|
| Cryophiles               | < 20                     |
| Mesophiles               | 20-40                    |
| Moderate thermophiles    | 40-55                    |
| Extreme thermophiles     | > 55                     |

Table 3. Classification of chemolithotrophic bacteria in terms of their optimum temperature ranges.

4.7 Pyrite content
Pyrite plays a key role in many biooxidation operations. Its oxidation produces acidity, heat
and dissolved iron. Pyrites vary greatly in their chemical and biological reactivity. It also is
associated with many ores, including zinc, copper, uranium, gold and silver. Pyrite is formed in a reducing environment with a continuous supply of sulphates and iron in the presence of easily decomposable organic matter. In general, microbiological leaching processes of uranium have been applied to the ores that contain accessory Fe-sulfides. The pyrite content of the ore is important and that is why this type of attack has not been widely utilized in extraction of uranium from its ores since the technology is limited to minerals with highly sulfide content. The ores from eastern Canada are especially susceptible to this kind of process since pyrites are associated with uranium. On the other hand the uranium ores along with low pyrite content are less suitable for microbial leaching. In this case, the suitable quantity of pyrite has to be added in medium.

4.8 Hydrogen ion concentration (pH)
Microorganisms that biooxidize sulfide minerals at low pH are resistant to acidic conditions and most heavy metals in process solutions. Typically, microbial cultures are pre-grown or adapted to a particular ore feed in the laboratory or pilot plant. From an industrial perspective it is essential that biomining microorganisms are able to grow at low pH and tolerate high concentrations of acid. Two important reasons for this are to enable iron cycling and to permit reverse electron transportaion to take place. The optimum pH for Acidithiobacillus ferrooxidans is between 2-3, but when the substrate is in large part pyritic, the pH can reach extremely low values, (less than 1). This is because of the availability of abundant sulphur and the precipitation of ferric hydroxide when the solution reaches saturation. Acidithiobacillus caldus is a single mixotrophic species which can utilise sulphur or tetrathionate and yeast extract or glucose. Blais et al. (1993) have demonstrated that less acidophilic bacteria in sludge such as Thermithiobacillus tepidarius, T. aquaesulis, T. denitrificans, T. thioparus and other species formerly placed in Thiobacillus, may initiate the acidification to the point where the acidophilic species can take over. Acidophilic bacteria decreased the pH of a sulphur-containing synthetic salts medium to the level of 1.4-1.6 during 10 days. Table 4 shows pH range for Acidithiobacillus culture.

| Microorganism                | Optimum pH | pH Range |
|------------------------------|------------|----------|
| Acidithiobacillus albertensis| 3.5-4      | 2-4.5    |
| Acidithiobacillus ferrooxidans| 2.0-2.5   | 1.3-4.5  |
| Acidithiobacillus thiooxidans| 2.0-3.0   | 0.5-5.5  |
| Acidithiobacillus caldus     | 2.0-2.5    | 1.0-3.5  |

Table 4. pH range for Acidithiobacillus culture

Mine spoils which were alkaline in nature (pH=9), with low sulphur content and a highly concentration of chlorides tended to be free of Acidithiobacillus ferrooxidans. The limiting pH value for growth of Acidithiobacillus ferrooxidans in rock material and drainage was found to be about 7.2.
4.9 Aerobic/anaerobic growth
Aeration of the solution or slurry is important in all bacterial oxidation processes using obligately acidophilic bacteria cultures. If the oxygen concentration falls to low levels, less than 0.5 to 1.0 mg L\(^{-1}\) for processes carried out in stirred vessels, the culture will normally revert in to its lag phase and the bacterial process stop. A lack of carbon dioxide restricts the culture growth and could limit the rate and amount of reaction of the sulphide mineral. Acidithiobacillus species are strict aerobes with the exception of Acidithiobacillus ferrooxidans, which is a facultative aerobe. In the absence of oxygen, Acidithiobacillus ferrooxidans is able to grow on reduced inorganic sulphur compounds using ferric iron as an alternative electron acceptor. The volume of air that must be supplied is based on the sulfide oxidation required. For example, at Wiluna in Western Australia, about 8 t air was supplied per tonne of concentrate, at typical oxygen utilization efficiencies of 25%. Aeration of bioheaps can accelerate biooxidation reactions, reducing leach cycle time. Air may be delivered via a network of pipes installed in a gravel layer at the base of heaps.

5. Microbial leaching and environmental
Environmental pollution caused by mineral processing is a serious problem; however, microorganisms play crucial roles in bioremediation of toxic metals and radionuclides, and thus are very much involved in resolving such problems. In this technology, microorganisms or their constituents such as enzymes are used to degrade or transform the soil. Much progress has been made with regard to understanding processes involving metal–microbe interactions and new applications of such processes to the detoxification of metal and radionuclide contamination have been developed. Such processes also provide an opportunity to reduce levels of sulphur dioxide polluting the environment and air (Lloyd, 2001, 2002, Morin, 2006). The Gram-negative eubacterium Acidithiobacillus ferrooxidans is important for industry and ecology because firstly, it is capable of solubilizing metals from ores, such as copper, uranium, and cobalt, and decomposing recalcitrant gold-containing ores. Secondly, this bacterium is able to remove heavy metals from contaminated industrial effluents or soils and desulfurize fossil fuels to avoid corrosion and atmospheric acid depositions (Corinne Appia-ayme, 1999). Chemical approaches are available for metal remediation, but are often expensive to apply and lack the specificity required to treat target metals against a background of competing ions. In addition, such approaches are not applicable to cost-effective remediation of large-scale subsurface contamination \textit{in situ}. Biological approaches, on the other hand, offer the potential for the highly selective removal of toxic metals coupled with considerable operational flexibility; they can be used \textit{in situ} in a range of bioreactor configurations (Lloyd, 2001).

6. Discussion and conclusions
The performed investigation into microbial leaching signifies enhancing of metals recovery particularly gold, uranium, copper and zinc, due to microorganism activity in comparison with traditional leaching method. In parallel with the recovery augmenting, utilization of microbial methods as compared with ordinary method like roasting is more environmentally friendly and its cost will be less. Hence, commercial applications in South Africa, Australia, South America, Spain, India and china are increasing in the last two decades. In most of executed operations within laboratory and commercially scale,
Autotrophic bacteria's have been utilized which belong to bacillus species. Nowadays, investigation on new and other microorganism, mixed culture and fungi's is underway in order to probe their function and viability. The appropriately microbe type selection is too important in microbial leaching operations because the rate of being successful is mainly dependent upon environmental conditions, characteristics of mineralogy, country rock and the requisite technique in a way that there might be different results due to using a microbe type for two similar minerals along with divergent environmental conditions. On the other hand, microbial leaching methods include miscellaneous techniques involving; in-situ, dumps, heap, vat and stirred which making use of each of them is dependent on the metal grade, time and capacity. In order to secure the stable supply of raw materials for the industrial needs, it is necessary to develop noble recovery technology of valuable metals from refractory and low-grade mineral ore deposits, intermediate metallurgical products, and waste. Then, it is essential to find methods to treat the ores economically and environmentally to recovery valuable metal. The researches did on Microbial leaching indicating an increase in rate of metal recovery and solubility through direct, indirect, thiosulfate and polysulfide mechanism, due to microorganism activity. Generally, microbial leaching is able to process low grade and marginal ores, mining and industrial waste which couldn’t be processed by other methods including gravity, electrical, magnitude and physiochemical methods. Hence this capability causes mineral ores tonnage to increase. Another advantage of microbial leaching is to use of microorganism and metal metabolically and potentially in order to decrease water and land pollution and control environmental damages. Microorganism could help to selective solution of metals through penetrating into the molecular structure of the materials, breaking existing bond and forming free ions or new compounds.

Regarding nature of uranium mineralization which are generally low grade in most cases (160 cases in the crust), and considering this fact that conventional processing methods won’t be feasible for this low metal content, researches conducted to microbial leaching of uranium ores such as oxides and phosphate like Pitchblende, Uraninite, Cafinite, Gomitite, Uranium shale.

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