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REVIEW

Microbial Leaching of Uranium Ore

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The rapid growth demand for power follows that nuclear power industry has led to progressive exhaustion of high grade reserves of uranium ore and new extraction techniques should be developed to use along with low grade ore. On the other hand, the uranium recovery from low grade and refractory ores with conventional techniques of processing is very expensive and tough because these methods require high level of energy and capital costs. Therefore, making use of low cost and influential method is too significant for this issue. One proposed method is microbial leaching. Microbial leaching is regarded as one of the most promising and certainly the most revolutionary solution to these problems in comparison with pyrometallurgy or chemical metallurgy. Microbial leaching is the extraction of metals from their ores using microorganisms. Microbial technology offers an economic alternative to the mining industry. This paper intends to render a general overview of the microbial leaching research activities all over the world.

Key Words: Uranium, Microbial, Leaching, Nuclear, Pyrometallurgy, Refractory, Processing.

INTRODUCTION

This paper is a review of the microbiological leaching of uranium ores. Microbiological leaching has been use as an alterative approach to conventional hydrometallurgical methods of uranium's extraction. In the microbiological leaching process, iron-oxidizing bacteria oxidize pyretic 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 in reduced state and involves tetravalent form and a redox reaction is involved the oxidation of uranium to the hexavalent form[1]. 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

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of previously existed mining techniques can be resulted in metal recovery by the sources that have not been of economical interest. 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[2,3]. 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'[2]. However, there are some slight differences in definition[4]: 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[5]. 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[6-8].

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 desired 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[1]. 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[1]. From 1900 on, no open air roasting of low-grade ore was conducted at the Rio-Tinto mines. The researches 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[2].

Harrison *et al.*[9] 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 *et al.*[10] investigations showed the effectiveness and efficiency of microorganism's influence, such as *Thiobacillus*, requires the presence of certain amount of iron. They also conducted some research on mixing level, aiersion and oxidation rate of iron that may effect uranium's microbial leaching. Amongst the various parameters affecting the sufficiency of microbial leaching; they just focused upon the aforementioned parameters[11]. Brierley[4], 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[4]. Cerda *et al.*[12] researches 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 *et al.*[13] utilized column leaching, seepage and shaking table to study the effect of pyrite amount in microbial leaching of uranium. The 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. They also done some research on pH optimization, temperature and stirred time[13,14]. In a case study conducted by Junior[15] on uranium ore in Figopira in Brazil, potentiality of microbial leaching in uranium recovery enhancement has been emphasized. Schipper *et al.*[16] studied 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[16,17]. In this study, there was no investigation on microbial leaching capability and identification of microbes was just concerned. Munoz *et al.*[18-20] dedicated researcher in uranium's microbial leaching in Spain, presented research's results in many published papers. 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 them[18-20]. They worked on pitchblende ore with 0.097 % uranium content. Bosecker[21] describes metal extraction and metal detoxification through employing microorganisms activity. Leduc *et al.*[22] studied 10 different isolated of *Thiobacillus ferrooxidans* with regard to their degree of resistance to 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[22]. Mathur *et al.*[23] 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 *et al.*[24] 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[24]. Kalinowski and Oskarsson[25] 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 uranium was interpreted to be attributed to the production of pyoverdine chelators, which is a typical ligand produced by fluorescent pseudomonades, as uranium could not be detected in either sterile controls or in experiments with the two other bacteria[25]. In Saeed *et al.*[26] reported the bioleaching behaviour of rock phosphate (imported from Jordan) was studied using different strains of *Aspergillus niger*. X-ray diffraction analysis revealed the presence of fluorapatite [$Ca_2(PO_4)_3F$] 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[26]. Akcil[27] investigated the potential bioleaching developments in Turkey. Beneditto *et al.*[28] in their study identified sulfur reduction bacteria in Brazil uranium mine water. This is basically problem in Brazil nuclear industries[28]. Choi *et al.*[29], schist with 0.01 % U_3O_8 content in south Korea under study. They could reach 0.8 uranium recovery with application of *Acidbacillus ferrooxidans*[29]. Lee *et al.*[30] mreported the effects of several conditional factors on efficiency of uranium bioleaching *via* using an iron-oxidizer, *Acidithiobacillus ferrooxidans*, from uranium-bearing black shale (349 ppm of uranium) were investigated. In the case inoculated with the cells, lower pH, higher redox potential and higher amount of aqueous Fe^{3+} than those of non-inoculated reactor observed until 200 h. Such development of condition, which was facilitated by microbial activity, can enhance the rate and extent of uranium leaching from the solid substrate.

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[5,31]. Originally, a model with two types of mechanisms which are involved in microbial mobilization of metals has been proposed:

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[2]. Bioleaching of metal sulfides (MS) can be achieved in direct and indirect modes of bacterial metabolism. Fig. 1(a) shows a scheme of the reaction mechanism for the bio-oxidation of sulphide minerals-direct mechanism. The direct mechanism is given by Torma[3]:

$$MS + 2O_2 \rightarrow MSO_4 \tag{1}$$

where M is a bivalent heavy metal.

The following equations describe the "direct" mechanism for the oxidation of pyrite[2,32]:

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2FeSO_4 + 2H_2SO_4 \tag{2}$$

Indirect mechanism: The oxidation of reduced metals through "indirect" mechanism is mediated by ferric iron (Fe^{3+}) originating from the microbial oxidation of ferrous ion (Fe^{2+}) compounds present in minerals. Ferric ion is an oxidizing agent and can oxidize, *e.g.*, metal sulfides and is chemically reduced to ferrous ions 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[2]. Fig. 1(b) shows a scheme of the reaction mechanism for the bio-oxidation of sulphide minerals-indirect mechanism.

$$4FeSO_4 + O_2 + 2H_2SO_4 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O \tag{3}$$

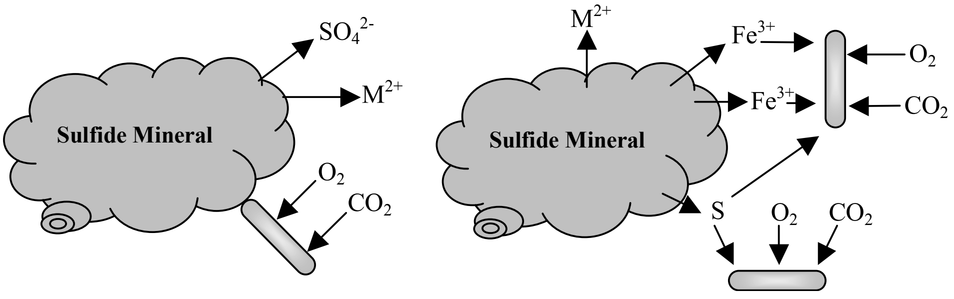
$$FeS_2 + Fe_2(SO_4)_3 \rightarrow 3FeSO_4 + 2S \tag{4}$$

$$2S + 3O_2 + 2H_2O \rightarrow 2H_2SO_4 \tag{5}$$

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

$$UO_2 + Fe_2(SO_4)_3 \rightarrow UO_2SO_4 + 2Fe_2SO_4 \tag{6}$$

However, the model of ‘direct’ and ‘indirect’ metal leaching is still under discussion[33]. 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[34].



(a) (b)

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

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 has also 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 thiosufate 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]:

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

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

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[37]. 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:

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

$$0.5H_2S_n + Fe^{3+} \rightarrow 0.125S_8 + Fe^{2+} + H^+ \quad (10)$$

$$0.125S_8 + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+ \quad (11)$$

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 *Leptospirillum* or *Sulfobacillus*.

$$2Fe^{2+} + 0.5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad (12)$$

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[35,36].

Influenced factors on uranium microbial leaching

Pyrite content: Pyrite plays a key role in many biooxidation operations. Its oxidation produces acidity, heat and dissolved iron. Pyrites vary in their chemical and biological reactivity[38]. It is also 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[39]. In general, microbiological leaching processes of uranium have been applied to the ores that contain accessory Fe-sulfides[1]. 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.

Mineralization of uranium: Another important factor of bioleaching is the mineralization of the uranium. Tables 1 and 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[18].

TABLE-1
TYPES OF URANIUM OXIDES IN NATURE [Ref. 40]

Uranium oxide	Valence	Natural form	Solution ability
UO_2	IV	Uraninite	Insoluble
U_2O_5	V	–	Less soluble
U_3O_8	IV, VI	Pitchblende	Less soluble
UO_3	VI	Carnotite	Soluble

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[18,36].

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*

TABLE-2
URANIUM MINERALIZATION AND BIOLEACHING [Ref. 18]

Uranium ores	Chemical composition	Degree of bioleaching
Uraninite	UO_2	+
Gummite	$UO_3 \cdot nH_2O$	+
Becquerelite	$CaU_6O_{19} \cdot 11H_2O$	+
Brannerite	$(U,Ca,Ce)(Ti,Fe)O_6$	+
Davidite	$(Fe,Ce,U)_2(Ti,Fe,V,Cr)_5O_{12}$	+
Coffinite	$U(SiO_4)_1 \cdot x(OH)_{4x}$	–
Uranophane	$Ca(UO_2)_2Si_2O_7 \cdot 6H_2O$	±
Sklodowskite	$Mg(UO_2)_2Si_2O_7 \cdot 6H_2O$	±
Autunite	$Ca(UO_2)_2(PO_4)_2 \cdot 12H_2O$	+
Torbernite	$Cu(UO_2)_2(PO_4)_2 \cdot 8H_2O$	+
Uramphite	$NH_4\text{-}UO_2\text{-}PO_4 \cdot 3H_2O$	+
Zeunerite	$Cu(UO_2)_2(AsO_4)_2 \cdot 12H_2O$	+
Carnotite	$K_2(UO_2)_2(VO_4)_2 \cdot 3H_2O$	±
Tyuyamunite	$Ca(UO_2)_2(VO_4)_2 \cdot 8H_2O$	±
Zippeite	$(UO_2)_2(SO_4)(OH)_2 \cdot 4H_2O$	+
Uranopilite	$(UO_2)_6SO_4(OH)_{10} \cdot 2H_2O$	+
Johannite	$Cu(UO_2)_2(SO_4)_2(OH)_2 \cdot H_2O$	+
Schroeckingerite	$NaCa_3UO_2SO_4(CO_3)_3F \cdot 10H_2O$	+
Urano-oranic Compounds		+

+ = easy, – = hard, ± = variable.

ferrooxidans. Certain metals presented in bioleaching environments can inhibit microbial growth, therefore, these metals cause reduced leaching efficiencies[2,18]. Based on dry weight, nitrogen is the most important after carbon for the synthesis of new cell mass.

In commercial operations, inexpensive fertilizer grade ammonium sulfate is added to biooxidation tanks or bioleaching heaps to ensure that sufficient nitrogen is available[35].

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[22]. Silver, mercury, ruthenium and molybdenum reduced the rate of growth of Sulfolobus grown on a copper concentrate[40]. 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[41], copper, zinc, nickel, thorium, uranium and mercury[22,42,43]. The resistance of *Acidithiobacillus ferrooxidans* to mercury is ferrous ion dependent[44].

Temperature: Temperature is the most important parameters influencing the diversity of the microbial communities[45]. Use of thermophiles was found to improve metal sulfide biooxidation in atleast two ways. First, reaction rates increased with increasing temperature. Second, elevated temperature increased the extent of metal extraction from certain minerals[46,47]. Bioleaching processes are carried within range of temperatures from ambient to a demonstration plant that has been operated at 80 °C[48]. Temperature ranges of 2-35 ºC and 4-21 ºC, respectively, were observed. Moderately thermopiles iron-oxidizing and sulfur-oxidizing bacteria were initially cultured from mining environments and hot springs[49,50]. However, psychrophiles have not been isolated from cold tailing effluents where they would be expected[51]. The temperature used for bioleaching in most of studies is 35 ºC. Although *Acidithiobacillus ferrooxidans* was reported to grow most rapidly at 30 ºC, it oxidised iron faster at 35 ºC[52]. 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[18,19]. 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.

TABLE-3
CLASSIFICATION OF CHEMOLITHOTROPHIC BACTERIA IN TERMS OF THEIR OPTIMUM TEMPERATURE RANGES

Bacterial class	Optimum temperature range (ºC)
Cryophiles	< 20
Mesophiles	20-40
Moderate thermophiles	40-55
Extreme thermophiles	> 55

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 leptospirilli, *Leptospirillum ferrooxidans* and *Leptospirillum ferriphilum*[35]. Consortia of acidophilic thiobacilli and leptospirili 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[51,53]. While moderately acidophilic thiobacili is numerous, thermo acidophilic archaea, resembling *Sufolobus* and *Acidianus*, has been isolated from uranium-mine waste heaps[53].

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[54]. 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[20]. The optimum pH for *Acidithiobacillus ferrooxidans* is between 2-3, but when the substrate is in large part pyretic, 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[18]. *Acidithiobacillus caldus* is a single mixotrophic species which can utilize sulphur or tetrathionate and yeast extract or glucose[55]. Blais *et al.*[56] 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.

TABLE-4
pH RANGE FOR *Acidithiobacillus* CULTURE [Ref. 57]

Microorganism	Optimum pH	pH range
Acidithiobacillus albertensis	3.5-4.0	2.0-4.5
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

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*[58]. The limiting pH value for growth of *Acidithiobacillus ferrooxidans* in rock material and drainage was found to be about 7.2[59].

Aerobic/Anaerobic growth: Aeration of the solution or slurry is important in all bacterial oxidation processes using 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[32].

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[60]. 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[61].

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.

Conclusion

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 recover 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 physio-chemical 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 and uranium shale.

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