

## 低品位 우라늄의 미생물 침출법†

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### Review on bioleaching of uranium from low-grade ore†

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#### 요 약

본 총설은 광석으로부터 우라늄의 미생물 침출시 사용하는 *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* 그리고 *Leptospirillum ferrooxidans* 등에 역할과 침출반응에 관하여 기술하였다. 미생물에 의한 우라늄의 침출반응은 박테리아가 우라늄 광석과 직접 반응하기 보다는 박테리아가  $U^{4+}$ 를 산화시키는데 필요한  $Fe^{3+}$ 를 공급하고,  $Fe^{3+}$ 가 우라늄 광석과 반응하는 간접반응기구(indirect mechanism)에 의하여 일어난다. 건식제련법과 같은 전통적인 금속회수 공정에 비하여 환경친화적이고 경제적인 장점 때문에 저품위 광물자원으로부터 유가금속을 회수하는데 미생물 제련법이 널리 활용되고 있다. 현재 우라늄은 heap, dump 그리고 in situ를 이용한 미생물 침출법으로 회수되고 있다. Bioheap의 공기 투입량, 교반반응용기의 디자인 및 조업 개선 분야에서 기술개발이 지속적으로 이루어졌으며 최근에는 미생물 침출반응에 투입된 박테리아의 특성 개선 및 균주수를 제어하기 위한 molecular biology 분야에서 활발한 연구가 진행되고 있다.

주제어 : 우라늄, 미생물 침출, 박테리아, 곰팡이

#### Abstract

This review describes the involvement of different microorganisms for the recovery of uranium from the ore. Mainly *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* are found to be the most widely used bacteria in the bioleaching process of uranium. The bioleaching of uranium generally follows indirect mechanism in which bacteria provide the ferric iron required to oxidize  $U^{4+}$ . Commercial applications of bioleaching have been incorporated for extracting valuable metals, due to its favorable process economics and reduced environmental problems compared to conventional metal recovery processes such as smelting. At present the uranium is recovered through main bioleaching techniques employed by heap, dump and in situ leaching. Process development has included recognition of the importance of aeration of bioheaps, and improvements in stirred tank reactor design and operation. Concurrently, knowledge of the key microorganisms involved in these processes has advanced, aided by advances in molecular biology to characterize microbial populations.

**Key words :** Uranium, Bioleaching, Bacteria, Fungi

#### 1. Introduction

The increase in human population has required the utilization of available energy resources to meet the energy requirement. Combustion of coal, oil and other fossil fuel resulted in global warming due to emission

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of CO<sub>2</sub> which provoked to utilize the nuclear energy resources. Despite of various applications like high-density penetrators in the military sector and counterweights for aircraft control surfaces as ballast for missile re-entry vehicles, and as a shielding material, photographic chemicals etc., the demand of uranium has increased due to its utility in nuclear power generation. The increasing use of uranium in nuclear power generation has gradually led to exhaustion of high grade uranium reserves and the use of low grade ores.<sup>1)</sup> New uranium mines have been explored worldwide to meet the demand, but not all mines contained high grade uranium ore. Modern techniques, which have been successfully used to extract valuable metals like copper, gold, are being implemented to recover uranium from high grade as well as from low grade uranium ore to meet the high demand. The current situation of the extractive metallurgy industry dealing with decreasing ore-grades and increasing mining and grinding costs has forced us to consider new low-cost methods that make metal extraction economically feasible. Therefore, in order to cater to the demand, modern techniques are being developed for recovery of copper, uranium and other metals on a commercial scale from its low grade ore using microorganisms.<sup>2,3)</sup> The recent surge of interest in biological leaching of uranium is motivated by the fact that they are relatively inexpensive, involves low energy consumption and is environmentally safe.<sup>4)</sup> At present, uranium ore leaching is an example of successful commercial application of bio-hydrometallurgy.<sup>5)</sup> The process can be further enhanced by employing the native strains from a uranium mine habitat.

Bioleaching is the extraction of specific metals from their ores through the use of bacteria. Metal recovery from sulfide minerals is based on the activity of chemolithotrophic bacteria, mainly *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*, which convert insoluble metal sulfides into soluble metal sulfates. Non-sulfide ores and minerals can be treated by heterotrophic bacteria and by fungi. In these cases, metal extraction is due to the production of organic acids and chelating and complexing compounds excreted into the environment. At present, bioleaching is used essentially for the recovery of copper, uranium and gold, and the main techniques employed are heap, dump and in situ leaching. Different types of bioleaching (in situ and exsitu) are practised for the recovery of different metals.

## 2. Availability of Uranium Ore

According to World Nuclear Association, about 63 percent of the world's production of uranium from mines is from Kazakhstan, Canada and Australia. Since 1993, after a decade of falling mine production, output of uranium has generally risen and now meets 76% of demand for power generation. The world's known uranium resources increased 15% in two years to 2007, due to increased mineral exploration. Uranium is ubiquitous on the Earth, approximately as common as tin or zinc, and it is a constituent of most rocks. The major minerals of uranium are: uraninite, carnotite, pitchblende, coffinite, tobernite, autunite, and tyuyamunite. Table 1. shows different grade of uranium.

Table 1. Classification of uranium ore.

Very high-grade ore (Canada) - 20% U	200,000 ppm U
High-grade ore - 2% U,	20,000 ppm U
Low-grade ore - 0.1% U,	1,000 ppm U
Very low-grade ore* (Namibia) - 0.01% U	100 ppm U
Granite	4-5 ppm U
Sedimentary rock	2 ppm U
Earth's continental crust (av)	2.8 ppm U
Seawater	0.003 ppm U

### 3. Recovery of Uranium by different conventional Process

Since the past four decades most of the leaching processes are followed by agitated acid or alkaline atmospheric leaching of ground ore, with the alkaline option used for ores in which the acid consumption is quite high. New trends which began to appear included pressure leaching, which has been adopted for a few uranium operations treating more refractory ores, especially in the presence of sulphides such as pyrite which can be oxidized to form the acid and ferric iron required for the process. Heap leaching, in-situ leaching, bio-leaching and by-product uranium recovery from phosphoric acid, copper leach solutions and rare earth processing operations. These trends are likely to continue as new projects are developed in the future, depending, of course, on the type and grade of the deposits, and the uranium price.

#### 3.1. Pressure leaching processes

Acid pressure leaching has been adopted for a few uranium operations treating more refractory ores, especially in the presence of sulphides such as pyrite which can be oxidized to form the acid and ferric iron required for the process.

Pressure leaching is incorporated in the new SXR Uranium One Dominion project in South Africa which started in February 2007.<sup>6)</sup> Pressure alkaline leaching was introduced to treat more refractory high acid consuming ores and was successfully used at a number of operations. Although there are no current operations, it will likely be considered in the future for similar ores. For example it was piloted by Western Mining for the Yeelinrie Project near Kalgoorlie in Western Australia,<sup>7)</sup> which is on hold because of the state government's policy, which forbids uranium mining.

#### 3.2. Heap leaching processes

The extensive heap leaching experience gained in copper and gold since the last uranium boom including strong acid cure, heap building techniques and operating strategies. In the seventies a variation of heap leaching consisting of an innovative in-ground vat leaching operation was established in Naturita, New Mexico, USA.<sup>8)</sup> The vat loading/unloading

system, which was developed for this operation went on to be widely used in copper ore heap leaching, in particular for on/off pad type systems. Apart from cost considerations, this had the advantage that the vats could be eventually used for the disposal and burial of leach residue.

#### 3.3. In-situ leaching process

In-situ leaching for uranium was developed in the nineteen sixties and seventies. The relatively low capital investment and absence of surface mining activities are likely to continue to be attractive in the future for processing the mineral ores, especially for smaller and/or lower grade deposits. However, it can only be applied to deposits with adequate permeability and favourable configuration such as relatively shallow roll front type sandstone deposits in confined aquifers, located in a safe distance from ground water used for human or animal consumption. There will be situations where it is feasible to utilize acid systems which generally exhibit faster leaching kinetics. For example, the only commercial ISL project in Australia, Heathgate's Beverley operation, and the upcoming Honeymoon Project have both adopted acid leaching, which is environmentally acceptable as the ground water but unsuitable for humans and animals.<sup>9)</sup>

### 4. Application of microorganism for Uranium leaching

Bio-leaching has been applied commercially for pyritic heap leaching operations and for in-place leaching of low grade underground mine stopes broken by blasting and to old mine stopes. Since the last uranium boom, there have been major advances in the application of both heap bio-leaching of ROM or crushed ore and agitated tank bioleaching of concentrates, such as the use of aeration pipes, addition of nutrients, development of more efficient agitators and the development of new ultrafine grinding equipment. This is likely to lead to a greater use of bio-leaching in the future uranium projects involving sulphidic ores. Heap or in-place systems will be more suitable for lower grade ores, while tank bio-leaching will be more applicable to uranium bearing sulphidic concentrates. Commercial application of bioleaching of uranium from

low-grade ores has been practised since the 1960s.<sup>5)</sup> Best known are the in situ leaching operations in the underground uranium mines in the Elliot Lake district of Canada including the Stanrock, Milliken and Denison mines. At that time, the annual production of uranium from the Stanrock Mine was about 50 000 kg  $U_3O_8$  whereas 60 000 kg  $U_3O_8$  was produced in the Milliken Mine after improvement of the leaching conditions. At the beginning of the 1980s, a distinct drop in uranium production occurred. In 1984, Denison Mines started new activities and in 1988-90 flood leaching stopes were in various stages of operation or in preparation for flood leaching and 347 tons of uranium with a value of over US\$ 25 million were produced from the leaching operation.<sup>5,10)</sup> With the present reduction in world demand for uranium the prices are at a low level and Denison Mines have stopped production. Harrison *et al.*, 1966 reported the role of the iron oxidizing *Acidithiobacillus ferrooxidans* in leaching of uranium.<sup>11)</sup> 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. A unique commercial application for extraction of uranium from underground low-grade ore was demonstrated at the Denison Mine, Ontario, Canada.<sup>5)</sup> A system for intermittent flooding of blasted ore in an underground stope, sealed with a concrete wall, demonstrated the utility for a bio-hydrometallurgical process for uranium extraction. This process also considered the important requirements of the microorganisms for optimal activity. Nutrients and aeration were provided to promote bacterial growth. However, the economics of uranium have precluded further use and advancement in development of microbial processes for extraction of uranium.

## 5. Mechanism of bioleaching

Microbial processes facilitating mineral bio-oxidation and bioleaching are defined in terms of contact, non-contact mechanism and the cooperative mechanism. In the contact mechanism (Fig. 1a) the bacterial cells attach with the aid of extracellular polymeric substances

(EPS) layer to the mineral surfaces, resulting in dissolution of the sulphide minerals at the interface by an electrochemical process. In the non-contact mechanism (Fig. 1b) the ferric iron, produced through bio-oxidation of ferrous iron comes in contact with the mineral surfaces, oxidises the sulphide mineral and releases ferrous iron back into the cycle. While, in the cooperative mechanism (Fig. 1c) planktonic iron and sulphur oxidisers oxidises colloidal sulphur, other sulphur intermediates and ferrous iron in the leaching solution, releasing protons and ferric iron which is further used in non-contact leaching.<sup>12)</sup>

Uraninite, the main uranium ore, has the ideal chemical composition of  $UO_2$ , although in nature, a material of this stoichiometry does not exist because uranium appears partially oxidized in the form of  $U^{6+}$ . The extension of this oxidation varies between 17 and 60%.<sup>13)</sup> In conventional chemical leaching of uranium ores, particularly those with quartz gangue, acid solutions of ferric ion are used. Uranium (VI) is soluble under these conditions, whereas uranium (IV) needs previous oxidation using  $Fe^{3+}$  as the main oxidant.<sup>14)</sup> Dutrizac and MacDonald in 1974 have suggested the use of high ferric iron concentrations to promote the uranium leaching.<sup>15)</sup> However, this technique can only be applied if  $Fe^{2+}$  produced during leaching can be reoxidized to  $Fe^{3+}$  and recycled in the process. This could be performed continuously by bacteria which catalyse the oxidation of  $Fe^{2+}$  to  $Fe^{3+}$ . The acidophilic iron and/ or sulfur-oxidising bacteria, are extensively employed for the oxidation of pyritic and sulfide minerals. During the process, bacteria derive energy for their growth and other metabolic functions from the oxidation of inorganic materials such as sulfide/pyrite minerals.<sup>16-20)</sup> The bioleaching of uranium generally follows indirect mechanism in which bacteria don't attack the uranium ore directly<sup>21-24)</sup> but produce ferric sulfate by oxidizing the small amount of pyrite or other available sulfide minerals present in the ore. Bacteria provide the ferric iron required to oxidize  $U^{4+}$ .

There are five major chemical reactions to be considered in the process of indirect bioleaching of uranium.<sup>25)</sup> Pyrite, in the presence of water and available oxygen, is slowly oxidized to ferrous sulphate and sulphuric acid; *Acidithiobacillus ferrooxidans* acts as a catalyst to increase the reaction rate.

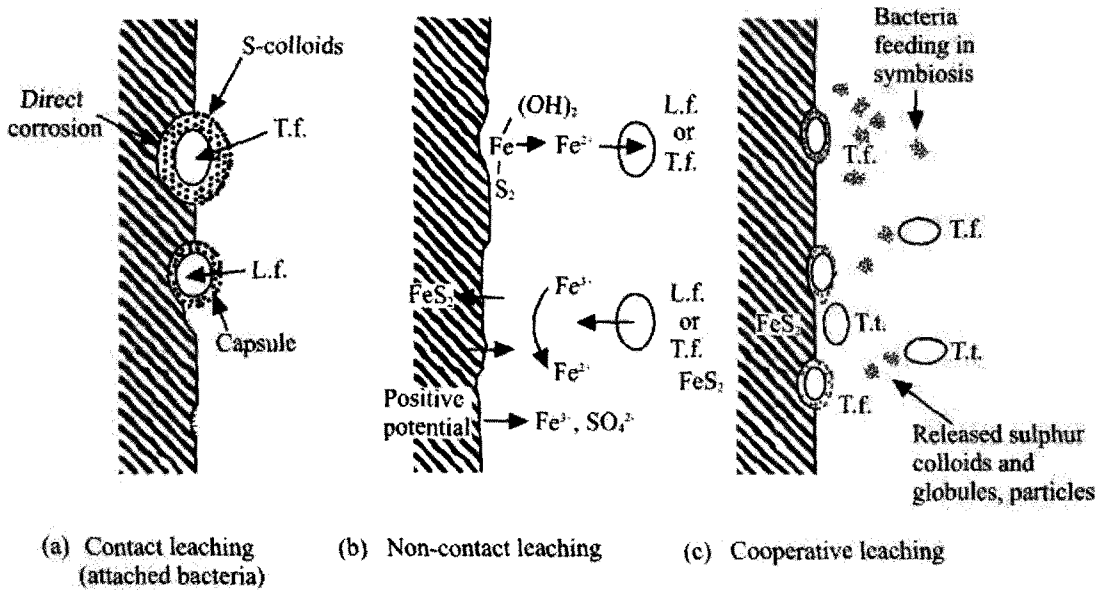
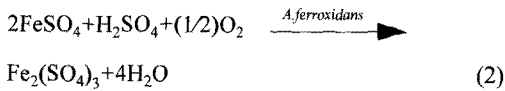


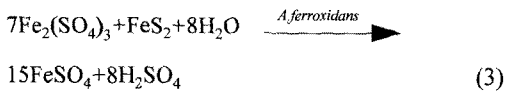
Fig. 1. Patterns of direct and indirect interaction of the bacteria with pyrite (a) contact leaching; (b) non-contact leaching; (c) cooperative leaching. (Figure reprinted from reference 10)



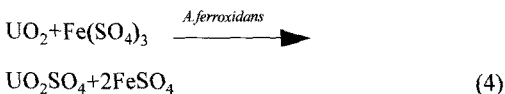
The ferrous sulphate is oxidized to ferric sulphate by the *Acidithiobacillus ferrooxidans* as follows:



Some of the ferric sulphate reacts with additional pyrite forming more sulphuric acid and ferrous sulphate, according to the following reaction:



In the final step, the remaining  $\text{Fe}^{3+}$  oxidizes the non-soluble tetravalent uranium to water soluble hexavalent uranium.<sup>26)</sup>



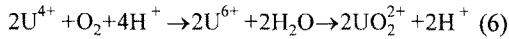
and the reverse reaction occurs in the liquid phase:



The role of *Acidithiobacillus ferrooxidans* in the microbiological leaching of uranium is in the regeneration of the oxidant (ferric sulphate) and in producing the dissolving agent (sulphuric acid). It also physically attacks pyrite exposing more radioactive minerals.

Besides indirect leaching there is some evidence that bacteria might attack the uranium oxides directly since oxidation is more rapid in the presence of *Acidithiobacilli* than ferric iron alone.<sup>27)</sup> *A. ferrooxidans* can oxidize directly from  $\text{U}^{4+}$  to  $\text{U}^{6+}$  by enzymatic action of metal-oxidising bacteria.<sup>28-30)</sup> Aerobic oxidation of  $\text{Fe}^{2+}$  and  $\text{S}^0$  provides energy, which indicates the aerobic oxidation of  $\text{U}^{4+}$  and provides potential energy to the organism such as *A. ferrooxidans*.<sup>30)</sup> In the laboratory, strains of *Acidithiobacillus* can be adapted to tolerate higher  $\text{U}^{6+}$  concentrations, thus more accurately modeling the organisms found within leach piles.<sup>31)</sup> Carbon fixation and oxygen uptake by the adapted cultures of *A. ferrooxidans* and *A. acidophilus*, indicate these organism's couple metabolic processes associated with the oxidation

of  $U^{4+}$  compounds.<sup>28-30)</sup> These observations raise the possibility that uranium solubilisation during acidic bioleaching processes results from both direct and indirect microbial oxidation.<sup>10,32,33)</sup>



## 6. Uranium recovery by different microorganism

The mineral sulphide oxidising microorganisms are acidophilic bacteria as their optimal growth varies between pH 2-4. They are autotrophic in nature as they use inorganic carbon ( $CO_2$ ) as carbon source. They are strictly chemolithotrophic, i.e., derive energy for growth from oxidation of reduced sulphur compounds, metal sulphides and some species also derive energy through oxidation of ferrous iron while some species also can derive energy by oxidation of hydrogen. The mesophiles actively involved in biooxidation and bioleaching are *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Acidithiobacillus caldus*,<sup>34)</sup> *Leptospirillum ferrooxidans*, *Leptospirillum ferrodiazotrophum*, *Leptospirillum thermoferrooxidans* and *Leptospirillum ferriphilum*, the moderately thermophiles are *Acidimicrobium ferrooxidans*, *Acidithiobacillus caldus* and *Sulphobacillus thermosulphooxidans*, while the thermophiles are *Sulfolobus metallicus*, *Sulphobacillus* sp. and *Metallosphaera sedula*. Most *Acidithiobacilli* are chemolithoautotrophic species, which use atmospheric carbon dioxide as their carbon source and the energy derives from the oxidation of sulfur compounds, including sulfides, elemental sulfur and thiosulfate, the final oxidation product being sulfate.<sup>35,36)</sup> Principally the bacteria of the genus *Acidithiobacillus* and *Leptospirillum* can grow in highly acidic environments with high heavy metal content are, and mostly take part in the transformation of uranium to a soluble form.

### 6.1. *Acidithiobacillus ferrooxidans*

*Acidithiobacillus ferrooxidans* is a gram negative bacteria containing lipopolysaccharide outside the peptidoglycan layer, rod shaped, acidophilic by nature and obligately chemolithotrophic for nutrition. They grow with ferrous iron as the sole energy substrate.<sup>3)</sup> The

optimum pH for growth is 2.5 and temperature ranges from 30-35°C. The G+C content of the DNA is 58-59 mol%.<sup>37)</sup> This bacterium was isolated from acid coal mine drainage.<sup>38)</sup> Morphologically the cells are identical to *A. thiooxidans*, but they differ from the latter by the much slower rate of the oxidation of elemental sulfur. *A. ferrooxidans* differs from all other *Acidithiobacilli* by the fact that besides deriving energy from the oxidation of reduced sulfur compounds ferrous iron can be used as an electron donor. In the absence of oxygen *A. ferrooxidans* is still able to grow on reduced inorganic sulfur compounds using ferric iron as an alternative electron acceptor.<sup>39)</sup> Uranium oxidation proceeds more rapidly in the presence of *A. ferrooxidans* than in the presence of ferric ions alone.<sup>11),17),40)</sup> Metal toxicity and nutrient ion effects are assessed for pure culture studies of *Acidithiobacillus ferrooxidans* and contrasted with dump and heap leaching applications.<sup>41)</sup>

### 6.2. *Acidithiobacillus thiooxidans*

*Acidithiobacillus thiooxidans* is an extremely acidophilic chemoautotroph and plays an important role in biohydrometallurgical processes. They derive energy solely through oxidation of reduced sulphur compounds, and cannot oxidise iron or pyrite but has been able to grow on sulphur from pyrite in co-culture with *Leptospirillum ferrooxidans*, which is an iron oxidizing bacteria. They are gram negative rod shaped bacteria acidophilic by nature and obligately chemolithotrophic for nutrition. The optimal pH for growth is 2-3 and the temperature range for optimal growth is between 28-30°C. The G+C content of the DNA is 52 mol%. *A. thiooxidans*, isolated in 1922 by Waksman and Joffe is well known for its rapid oxidation of elemental sulfur.<sup>42)</sup> Other partially reduced sulfur compounds are also utilized and sulfuric acid is generated, decreasing the pH in the medium to 1.5 to 1 and even lower. The intensive sulfuric acid production leads to a rapid decomposition of rocks so that acid-soluble metal compounds can pass into solution as sulfates.

### 6.3. *Acidithiobacillus plumbophilus*

*Acidithiobacillus plumbophilus* was isolated from an uranium mine in Germany and was named so because

of its ability to grow on the expense of lead compounds like galena (PbS) as sole source of energy, which may have been formed as decay products from uranium.<sup>43)</sup> The cell grows at pH values 4.0-4.65 and at about 9-41°C (optimum around 27°C). The bacteria obtain energy for growth from oxidation of galena (PbS), hydrogen sulfide (H<sub>2</sub>S) and molecular hydrogen (H<sub>2</sub>); Anglesite (PbSO<sub>2</sub>) is formed from oxidation of galena. No growth was observed on elemental sulfur, thiosulfate, tetrathionate, ferrous sulfate, synthetic and natural metal sulfides and uranite. No oxidation of Fe<sup>2+</sup> to Fe<sup>3+</sup> was detected neither when FeSO<sub>4</sub> was the sole energy substrate nor in combination with PbS. Growth was neither stimulated nor inhibited by the addition of organic substrates

#### 6.4. *Leptospirillum*

They are gram negative, aerobic and motile by means of a single polar flagellum. Cells are vibrioid to spirilla shaped and sometimes they can form into cocci or pseudococci shapes too. These bacterial cells measure 0.2-0.6 by 0.9-3.5 μm. Acidophilic and have optimum growth at a pH of 1.3-4.0. Some of the species of *Leptospirillum* are either mesophilic or moderately thermophilic and the maximum temperature ranges up to 55°C. They are chemoautotrophic and grow on iron substrate by iron oxidation, but cannot oxidise sulphur or thiosulphate. The G+C content of the DNA is 50-57 mol%.<sup>44)</sup> The different types of species of *Leptospirillum* involved in metal sulphide oxidation are *Leptospirillum ferrooxidans*, *Leptospirillum ferrodiazotrophum*, *Leptospirillum thermoferrooxidans* and *Leptospirillum ferriphilum*.<sup>45)</sup> *Leptospirillum ferrooxidans* is another acidophilic obligately chemolithotrophic ferrous iron oxidizing bacterium, which was first isolated by Markosyan from mine waters in Armenia.<sup>45)</sup> This microorganism tolerates lower pH values and higher concentrations of uranium, molybdenum and silver than *A. ferrooxidans*, but it is more sensitive to copper and unable to oxidize sulfur or sulfur compounds.<sup>46,47)</sup> Therefore, by itself, *L. ferrooxidans* cannot attack mineral sulfides. This can only be done together with *A. ferrooxidans* or *A. thiooxidans*.

#### 6.5. *Sulfobacillus*

The moderately thermophilic microorganisms ca-

pable of growing at temperatures of 45-50°C which are easily reached in the centre of heaps,<sup>48)</sup> has been detected. Since 1977, many of such organisms have been detected, among which are the genus *Sulfobacillus*.

#### 6.6. *Sulfolobus*

The extremely thermophilic bacteria *Sulfolobus* is capable of oxidizing sulphur, both autotrophically and heterotrophically, and it grows at 60-80°C (temperatures reached in some industrial operations).<sup>49)</sup>

#### 6.7. Cyanobacteria

Uranium (VI) leaching, as uranyl ions, from ores with a poor content in util minerals, using some algae as: *Porphyridium cruentum*, *Spirulina platensis* and *Nostoc linkia*. These microorganisms have facilitated the leaching of the U<sup>6+</sup> as UO<sub>2</sub><sup>2+</sup> ions. The microbial leaching degree was comprised between 40-90%, depending on the used alga and experimental conditions.<sup>50)</sup>

#### 6.8. Fungi

Fungi have the potential to solubilize metals and metalloids from insoluble compounds such as ores metal phosphates, sulfides and oxides.<sup>2)</sup> Very few studies have been carried out with heterotrophy microorganisms.<sup>51)</sup> Fungal and other microbial derivatives from heterotrophs can be effective at neutral pH for the extraction of uranium from calcareous type of ore. Bioleaching of metals by fungal species had been reported to be mediated by organic acid formation by the fungi which chelate the metal ions as well as lowers the media pH.<sup>51-53)</sup> Fungi belonging to genus *Aspergillus* and *Penicillium* have received much attention in this regard.<sup>54, 55)</sup> It has been reported by<sup>51)</sup> that isolates from uranium ore like *Aspergillus terreus* and *Penicillium spinulosum* gave highest leaching of uranium obtained at 1%(w/v), at this concentration the released uranium being ~80%. The maximum recovery of 71% uranium was obtained with the strain *Cladosporium oxysporum* and the other two strains belonging to *Aspergillus flavus* and *Curvularia clavata* gave 59% and 50% of metal recovery respectively from the uranium ore of from Jaduguda, Bhatin and Nawapahar uranium mines located in the Jharkhand State, India.<sup>56)</sup> Metal localization was investigated in the lichenized

*Trappelia involuta* growing on a range of uraniferous minerals, including metazeunerite  $[Cu(UO_2)_2(AsO_4)_2 \cdot 8H_2O]$  metatorbernite  $[Cu(UO_2)_2(PO_4)_2 \cdot 8H_2O]$ , autunite  $[Ca(UO_2)_2(PO_4)_2 \cdot 10H_2O]$ , and uranium-enriched iron oxide and hydroxide minerals. The highest uranium, iron, and copper concentrations occurred in the outer

parts of melanized apothecia, indicating that metal biosorption by melanin-like pigments was likely to be responsible for the metal fixation.<sup>57)</sup> The metal solubilisation by fungi may have biotechnological potential for metal recovery from industrial byproducts/ wastes and low grade ores.

Table 2. Microorganisms involved in the bioleaching of metals

Microorganism	Main leaching agent	pH range	Temperature range	References
<b>Heterotrophic Bacteria</b>				
<i>Acetobacter methanolicus</i>	Gluconate	Acidiphilic		58
<i>Acidianus brierleyi</i>	Sulfuric acid	1.0-6.0	45-75	59
<i>Acidophilum cryptum</i>	Organic acids	2.0-6.0	Mesophilic	60
<i>Chromobacterium violaceum</i>	Cyanide			60
<i>Crenothrix</i> sp.	Ferric iron	5.5-6.2	18-24	61
<i>Metallogenium</i> sp.	Ferric iron	3.5-6.8		60
<i>Pseudomonas putida</i>	Citrate, Gluconate	7.0-8.5	4-43	62, 63
<b>Chemolithoautotrophic bacteria</b>				
<i>Leptospirillum ferrooxidans</i>	Ferric iron	1.5-4.0	20-55	45, 60,
<i>Leptospirillum ferrodiazotrophum</i>	Ferric iron			45
<i>Leptospirillum ferriphilum</i>	Ferric iron			45
<i>Leptospirillum thermoferrooxidans</i>	Ferric iron	1.7-1.9	45-50	45, 59
<i>Metallosphaera sedula</i>	Ferric iron, Sulfuric acid	Acidophilic	Extr. thermophilic	60
<i>Thermothrix thiopara</i>	Sulfuric acid	Neutral	60-75	64
<i>Thiobacillus acidophilus</i>	Sulfuric acid	1.5-6.0	25-30	59
<i>Thiobacillus ferrooxidans</i>	Sulfuric acid	1.2-6.0	5-40	59
<i>Thiobacillus plumbophilus</i>		4.0-4.65	9-41	53
<i>Thiobacillus thiooxidans</i>	Sulfuric acid	05-6.0	10-40	52
<i>Thiobacillus thioparus</i>	Sulfuric acid	4.5-10.0	11-25	60
<b>Mixotrophic bacteria</b>				
<i>Sulfurococcus</i> sp.	Ferric iron, Sulfuric acid	Acidophilic	Extr. thermophilic	59
<i>Thiobacillus delicatus</i>	Sulfuric acid	5.0-7.0	25-30	61
<i>Thiobacillus kabobis</i>	Sulfuric acid	1.8-6.0	28	61
<i>Thiobacillus organoparus</i>	Sulfuric acid	1.5-5.0	27-30	61
<b>Chemolithoautotrophic Archaea</b>				
<i>Sulfolobus thermosulfidooxidans</i> sub. <i>thermotolerans</i>	Ferric iron, Sulfuric acid	Extr. acidophilic	37-42	59
<i>Sulfolobus thermosulfidooxidans</i> sub. <i>asporogenes</i>	Ferric iron, Sulfuric acid	Extr. acidophilic	37-42	59
<i>Sulfolobus acidocaldarius</i>	Ferric iron, Sulfuric acid	2.0-7.0	55-85	45, 60
<i>Sulfolobus ambivalens</i>	Ferric iron, Sulfuric acid		Extr. thermophilic	59, 61
<i>Sulfolobus solfataricus</i>	Ferric iron, Sulfuric acid		Extr. thermophilic	59, 61
<i>Sulfolobus thermosulfidooxidans</i>	Ferric iron, Sulfuric acid	1.9-3.0	20-60	59, 61
<i>Sulfolobus brierleyi</i>	Ferric iron, Sulfuric acid		Extr. thermophilic	64
<i>Sulfolobus yellowstonii</i>	Ferric iron, Sulfuric acid		Extr. thermophilic	59



Table 2. Continue-1

Microorganism	Main leaching agent	pH range	Temperature range	References
<b>Cyanobacteria</b>				
<i>Porphyridium cruentum</i>	Alkaline medium	7.5	5-35	50
<i>Spirulina platensis</i>	Alkaline medium		25-30	50
<i>Nostoc linkia</i>	Alkaline medium	7-8.5		50
<b>Fungi</b>				
<i>Actinomucor</i> sp.	Oxalate, Malate, Pyruvate, Oxalacetate			65
<i>Alternaria</i> sp.	Oxalate, Malate, Pyruvate, Oxalacetate			65
<i>Aspergillus clavatus</i>	Aspartate			65
<i>Aspergillus ficuum</i>	Oxalate			66
<i>Aspergillus niger</i>	Oxalate, Citrate, Gluconate, Lactate			67, 68
<i>Aspergillus ochraceus</i>	Citrate, Glutamate			65
<i>Candida</i> sp.				65
<i>Fusarium</i> sp.	Oxalate, Malate, Pyruvate, Oxalacetate			65
<i>Mucor</i> sp.	Fumate, Gluconate			65
<i>Paecilomyces variotii</i>	Malate			65
<i>Penicillium notatum</i>	Oxalate	5.0	15-30	61, 65
<i>Penicillium ochrochloron</i>	Oxalate			65
<i>Penicillium oxalicum</i>	Oxalate			66
<i>Rhizopus</i> sp.	Lactate, Fumarate, Gluconate	5.0	24-27	61, 65
<i>Trapelia involuta</i>			Extr. thermophilic	57

## 6.9 Other microorganisms

The involvement of *Pseudomonas fluorescense*, *P. putida*, *Achromobacter*, *Bacillus licheniformis*, *B. cereus*, *B. luteus*, *B. polymyxa*, *B. megaterium* have been reported in leaching processes.<sup>69)</sup>

## 7. Extraction by different Bioleaching methods

### 7.1. Bacfox Process

The bioleaching of uranium was first mooted at the Uranium Corporation of India at Jaduguda through the help of Bhaba Atomic Research Centre at Atomic Minerals Division in India. The microorganisms were isolated from the stopes and mine waters of the uranium mines and a process similar to BACFOX was designed to leach uranium.<sup>70)</sup> Air agitation leaching tests were conducted in rubber lined pachuca columns of 0.035 m capacity. 5 kg of Jaduguda feed sample (250 µm) was mixed with 5 liters of biogenically generated ferric

sulfate solution using Bacfox process agitated at 50% solids at room temperature and for 12 hours.

### 7.2. Shake flask bioleaching

A bacterial leaching program was carried out in order to evaluate the potential of applying this process to leach uranium from the ore of Figueira-PR, Brazil. The experiments were carried out in shake flasks, column percolation (laboratory and semi-pilot scale) and in heap leaching. In shake flasks and in column percolation experiments at laboratory scale, bacterial activity on the ore was confirmed. Approximately 60% of uranium was leached, against around 30% in sterilized controls. Column percolation experiments at semi-pilot scale and heap leaching (850 tons of ore) showed uranium extractions of approximately 50%. In both experiments, a complementary sulfuric acid attack, after the bacterial leaching phase, was necessary to reach this level of uranium extraction.<sup>71)</sup> In orbital shaking

experiment, the successful extraction of uranium using different purecultures of *A. ferrooxidans*, *A. thiooxidans* and *L. ferrooxidans* as well as a natural culture from FE mine on uranium ore from the same mine with no additional bacterial nutrients or energy sources was demonstrated.<sup>72)</sup> In shake flask bioleaching studies, the isolated strains of *A. ferrooxidans* and *A. thiooxidans* solubilized 91% and 88%  $U_3O_8$ , respectively, from low-grade sandstone uranium ore (0.023%  $U_3O_8$ ) in 30 days of incubation.<sup>73)</sup>

### 7.3. Column Bioleaching

A uranium ore from the F6 mine (Ciudad Rodrigo, Spain) was leached in the presence of bacteria using large columns (24 cm diameter and 275 cm height). The most important results were: (i) the ore contains sufficient pyrite in order for uranium bioleaching to take place under optimum conditions; (ii) shaker experiments showed that temperature, pH and type of inoculum are the most important variables in bioleaching; (iii) two different ores were attacked in the columns: altered and non-altered. In the first case, the extraction rate of uranium was higher. However, in both cases the final efficiency was very similar (95%).<sup>74)</sup> The percolation leaching of various uranium ore from the FE mine (Saelices el Chico, Spain) was carried out in semi-pilot PVC column experiment in the presence of micro-organisms. The larger-scale experiments established the importance of the inoculation and aeration in these systems. The application of two kinetic models showed the decisive role played by the micro-organisms, particularly those of the species *Acidithiobacillus ferrooxidans*, in ore disintegration and uranium dissolution.<sup>75)</sup> PVC column leaching studies revealed that when sandstone ore was amended with elemental sulfur and mine water at a pH-value of 3.5, a uranium recovery of 66%  $U_3O_8$  was achieved during the 50 days of leaching experiments with indigenous microbial populations of acidophilic *Acidithiobacilli*. However when mine water, with a pH of 7.4, was used the uranium solubilisation was found to be up to 48%  $U_3O_8$  under similar conditions. The addition of ammonium sulfate (3.0g/l) in mine water of an adjusted pH-value 3.50, was found to increase the microbial populations concomitantly enhancing the uranium leaching to 90%  $U_3O_8$  from column filled with

ore amended sulfur slag. Similarly, maximum uranium recovery of 84.08%  $U_3O_8$  was obtained from PVC column leaching studies on mill tailings residue during 100-days of leaching time.<sup>73)</sup>

### 7.4. Stirred tank Bioreactor

Bioreactor leaching studies showed a uranium recovery of 87.3%  $U_3O_8$  from low-grade sandstone uranium ore by a mixed culture of *A. ferrooxidans* and *A. thiooxidans* during 20-days of incubation.<sup>73)</sup> The acidophilic bacteria, which are screened from this uranium mine and acid mine water, are identified as *A. ferrooxidans*, *A. thiooxidans* and *L. ferrooxidans*. These bacteria are domesticated by UV mutagenesis and low pH, as well as some toxic ions such as high F and the high toxic metal ions in culture. They can tolerate high fluoride and high toxic metal ions and can grow fast in low pH value ranging from 1.0 to 2.0. For the magnetic stirring reactor the rate of uranium leaching can reach 91.1%, while, the leaching rate for the gas stirring reactor is lower at 85.6%.<sup>76)</sup> A generalized flow sheet of extraction of  $U_3O_8$  by bioleaching process is given in Fig. 2.

### 7.5 Heap bioleaching

Uranium recovery of 4.9%  $U_3O_8$  was obtained (calculated on the basis of heap effluents) from low-grade sandstone ore by microbial heap process for 150 days. But on the basis of chemical data of leached residues (core samples taken at depths of 00-100cm), an average uranium recovery of 31.64%  $U_3O_8$  was leached out during heap operation. During microbial heap leaching process on sandstone uranium ore, an off-white fluffy solid material (sludge) was observed, emerging along with heap effluents, which contained a significant amount of uranium (0.0517% to 0.6283%  $U_3O_8$ ). The formation of off-white sludge and precipitation of uranium in these sludge samples might be due to the presence of high calcium content (150 mg/L) in subsoil water, which is being used for inocula preparation for microbial heap leaching process.<sup>72)</sup>

## 8. Large scale uranium recovery by bioleaching process

A pilot plant unit was designed by Livesey-Goldblatt known as the BACFOX process is used for rapid

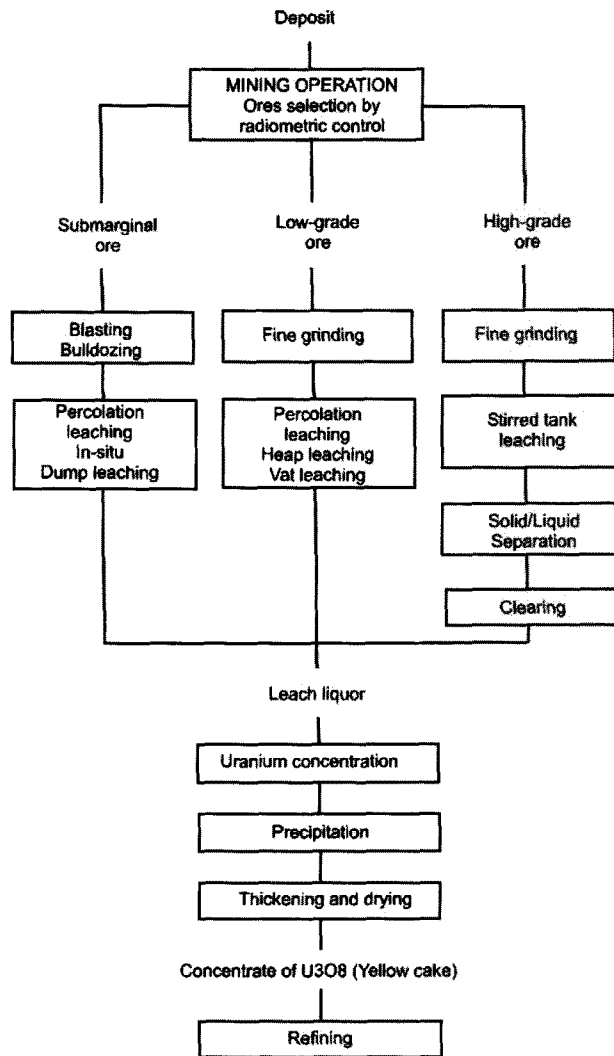


Fig. 2. Flow chart of extraction of  $U_3O_8$  by bioleaching process (Figure reprinted from reference 62).

oxidation of acidified ferrous sulphate solution which is passed over a film of immobilized *A. ferrooxidans*.<sup>77)</sup> This can achieve oxidation rates of 7.5 g ferrous to ferric iron/m<sup>2</sup> bacterial surface/ hour. From low grade uranium ore in the Elliott Lake mining district of Northern Ontario, Canada, commercial uranium leaching was practiced in percolation columns to yield 85% uranium recovery in 20 weeks.<sup>78)</sup> In a bench-scale counter-current system, 90% uranium was recovered within 31 hours retention time. An FBR process (ferric leach, bacterial re-

generation) was developed at Warren Spring Laboratory, Stevenage, UK, using a pilot plant with five columns for continuous operation. From 0.12%  $U_3O_8$ , 95% recovery was obtained in 10 days, which was cheaper than conventional acid slurry leaching.<sup>79)</sup> If uranium leaching was carried out in fermentors, 100%  $U_3O_8$  recovery in 5 days was possible.<sup>17)</sup> It was difficult to carry out uranium ore leaching in actual mine sites, but at Stanrock Uranium Mines near Elliot Lake, Canada, the estimated uranium recovery from mine water in 1963

was 48,000 kg.<sup>80)</sup> At nearby Milliken mines in 1964, 58,000 kg of uranium was recovered. In 1977, At Agnew Lake mine in Canada, stope leaching started for uranium ores, but the results of this operation are not yet available.<sup>81)</sup> Several microorganisms have been found in leach liquors as well as ore-heaps of uranium at Mecsek Ore Mining Enterprises at Pecs, Hungary and their importance was studied in the laboratory.<sup>82,83)</sup>

## 9. Future prospects of Uranium Bioleaching

The future of bio-mining is challenging, as it offers advantages of operational simplicity, low capital and low operating cost and shorter construction times that no other alternative process can provide. In addition, minimum environmental impact and the use of this technology in the mining industry are set to increase.

Once commercial scale high-temperature processes have been designed, the variety of minerals that will become acquiescent to bio-mining will increase. Although the viability of microbes that flourish at temperatures 55°C is not yet well-proven commercially, it appears that one can isolate iron- or/and sulfur-oxidizing organisms at whatever temperature is required, up to at least 80°C. Therefore, while suitable microbes for mineral biodegradation at a given temperature are not yet known, they can probably be found.

The uranium market has been in the doldrums for most of the past 25 years, but in the past few years the price of uranium has risen dramatically. A number of new uranium exploration projects are being undertaken, and a few have recently been brought into production. This renewed activity in the uranium market has sparked fresh interest in uranium extraction technologies. Some of the older wisdom, developed during the previous uranium boom, is being revived. In 1985 Wadden and Gallant described experiments on in situ uranium heap leaching using intermittent flooding and forced aeration.<sup>84)</sup> For uranium ores bearing uraninite together with either naturally-occurring or admixed pyrite, bioleaching of the material would generate acid, ferric iron and heat in the leach from the oxidation of the sulfide, rather than having to add fresh reagents. This could be considered in a heap bioleaching process,<sup>62)</sup> or a tank bioleaching process.<sup>85)</sup> The advances that have occurred in bioleaching during the period of the suppressed

uranium market have made new technologies available that have not been applied to large scale uranium extraction before, which can now be considered for new uranium ventures.

Despite of the several advantages when comparing to the conventional extraction process of the Uranium ore, still there are some areas need to be focused in terms of economical and environmental prospective. Technology should be developed to reduce the slow rate of bacterial leaching process, which is the main cause for less profit as well as a significant delay in cash flow for industries. Toxic chemicals like sulfuric acid and H<sup>+</sup> ions are produced in the process can leak into the ground and surface water turning it acidic, causing environmental damage. Heavy ions such as iron, zinc, and arsenic leak during acid mine drainage. For these reasons, a setup of bioleaching must be carefully planned, since the process can lead to a biosafety failure.

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