Bioleaching of low grade uranium ore containing pyrite using *A. ferrooxidans* and *A. thiooxidans*

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Abstract A process of uranium extraction from ore containing 3.1 % pyrite by bacterial leaching was investigated in shaken flasks during 90 days. The highest uranium recovery amounting to 85.1 % was obtained using binary mixture of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* that was exceeding results obtained by traditional acid leaching technique up to 27 %. High uranium recovery was founded to be due to the high degree of pyrite dissolution that can be readily achieved by bacterial leaching (up to 98.0 %).

Keywords Uranium leaching · Bacterial leaching · *Acidithiobacillus ferrooxidans* · *Acidithiobacillus thiooxidans*

Introduction

Acid leaching is known to be predominant process for uranium recovery from ores, usually with sulfuric acid because its relatively low cost. Uranium that mostly presented in ores in tetravalent state must be oxidized to hexavalent state U(VI) before it can be dissolved. In acid leaching, the uranium oxidation reaction requires the presence of ferric ion, regardless of the reagent used as an oxidant, since that appears that the ferric ion actually oxidizes the uranium while the oxidant reagent oxidizes ferrous ion to ferric ion [1].

So the dissolution of tetravalent uranium in sulfuric acid leaching system follows equation [2]:

$$UO_2 + Fe_2(SO_4)_3 = UO_2SO_4 + 2\ FeSO_4 \tag{1}$$

Various oxidants such as MnO_2, $NaClO_3$, H_2O_2 are widely employed as a ferrous ion oxidant in uranium leaching processing [3].

But these oxidants are very costly or cause very serious environmental pollution than sulfuric acid because of release of heavy metals to the environment.

One more alternative to these oxidants is the use bacterial leaching of uranium. The bioleaching of uranium ores arose from the need to develop economically viable processes in treatment of low grade ores. Of the total costs of the process of yellow cake production, 75 % is due to the first steps required to obtain the pregnant solution [3]. The importance of having a cheap leaching process can therefore be understood.

The capacity of the micro-organisms which take part in these transformations, principally bacteria of the genus *Acidithiobacillus*, to grow in highly acidic environment with high heavy metal content makes this technique a very interesting alternative to conventional processes. The only main drawback from a commercial point of view is the long contact time needed for the uranium solubilization; if environmental conditions are suitable, the process is autosufficient. In addition, savings of up to 50 % of the operation costs of a conventional plant can be achieved [4].

The principle of the method lies in leaching the uranium ore with ferric sulfate produced from the pyrite of the ore by bacterial action and which, in turn, can be regenerated by the bacteria. In other words, during the process in the micro-organisms do not attack the uranium ore directly but

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create the chemical conditions necessary for its dissolution. Zajic [5], however, suggests that the bacteria might attack the uranium oxides directly since oxidation is more rapid in the presence of *Acidithiobacilli* than of ferric iron alone. Recent calorimetric and respirometric research [6] supports this hypothesis, although the rate of such reaction is 30 times less than that of the microbial oxidation of the iron.

The literature [7–9] agrees that it is basically the indirect process which acts in this type of system since, during heap or dump leaching, the direct microbial leaching of the uranium ores is not significant, as a consequence of the large quantities of ferric iron generated from pyrite. That is to say, the bacterial leaching of uranium ores is two steps process: the bacterial leaching of pyrite and the leaching of uranium ore with the resulting metabolic products of the pyrite biological attack.

So it is clear that this leaching technique can be readily accomplished in heap leaching of uranium ores that contains large amounts of pyrite.

Ores of Russian deposit have large amounts of pyrite (up to 10 % wt). That is why in this work an attempt was made to investigate process of uranium bacterial leaching from typical samples of ores.

Experimental

Materials

Sulfuric acid and microbial tests were performed on samples of uranium-bearing candy clay from typical deposits (Russia). The chemical composition of the sand (wt%): 0.052 U, 80.1 SiO_2, 10.2 Al_2O_3, 3.1 FeS_2, 0.00 FeO, 0.05 Fe_2O_3, 1.11 K_2O, 0.21 CaO, 0.07 MgO, 0.17 Na_2O, 0.003 MnO, and 0.025 P_2O_5. The all uranium content in ore was caused by occurrence of coffinite that was founded by means of X-ray diffraction spectroscopy and by making of scanning electron microscopy (SEM) images. In all tests samples of sand were used that had not been grinded.

In each bioleaching experiment 1,000 ml of 9K medium according to Mackintosh [10], without iron(II) sulfate were adjusted to pH 1.9 with sulfuric acid and introduced into the flask with ore that was previously inoculated.

For inoculum a pyrite oxidizing cultures of *Acidithiobacillus ferrooxidans* (strain R1) and *Acidithiobacillus thiooxidans* (strain R20) originated from mine water, were used.

For inoculation these cultures, enriched from mining waters were grown either together or separately for 14 days in shaken flasks (140 rpm, 25 °C) on the medium supplemented with 2 % (w/v) pyrite concentrate (16.5 % pyritical sulfur) from a metallurgical plant.

Equipment and procedure

All reagents used were obtained at analytical grade from commercial sources. For bioleaching experiments, 2,000 ml conical opened flasks containing 200 g of uranium-bearing ore were utilized.

In each bioleaching run a 100 ml culture fluid was used for inoculation of uranium ore just before leaching experiment began. The vitality of cells was checked previously by a cell count determination by the most probable number technique using medium with iron (II) sulfate [11].

During all work four runs were performed: (1) sulfuric acid leaching with typical leaching solution taken from real uranium leaching plant, (2) leaching with 9K medium solution containing *A. ferrooxidans* culture, (3) leaching with 9K medium solution containing *A. thiooxidans* culture, (4) leaching with 9K medium solution containing a mixture of *A. ferrooxidans* and *A. thiooxidans* cultures.

The first run was carried out using 1,000 ml of typical uranium mining solution after its treatment with H_2O_2 that contains (g/l): 0.002 U(VI), 1.02 Fe(III), 0.00 Fe(II), 0.45 Al(III), 0.27 g/l Ca(II), 0.22 Mg(II), and 6.5 H_2SO_4, with pH 1.9. This solution may take place on the real leaching Russian plant.

The pH of solutions during all runs was loosed to change freely. Evaporation losses during runs were routinely compensated with demonized water. In all runs all flasks were shaken (140 rpm) at 25 ± 0.5 °C in special orbital shaker for 90 days.

The temperature inside the flasks was controlled by means of a Honeywell type R7086A controller.

Temperature, pH, and Eh (redox potential) values were measured during all tests. pH and Eh were measured with a glass electrode and smooth platinum electrode, respectively, its data being recorded automatically by a computer in the automatic regime.

During leaching experiments 1 ml samples of solution were taken at intervals for chemical analysis. Both total and ferrous iron were measured by photocolorimetry, using orthophenanthroline as the colorimetric reagent [12]. Sulfuric acid was determined by titration with 0.1 M solution of NaOH. Uranium concentration in samples was determined using a spectrometer ICP-MS.

The mineralogical analyses were carried out with a conventional Phillips X-ray powder diffractometer and scanning electron microscope JEOL JSM-6340F.

Results

The time course of leaching processes is characterized in Figs. 1, 2, 3, 4, 5, 6 by uranium recovery from ore; pH,
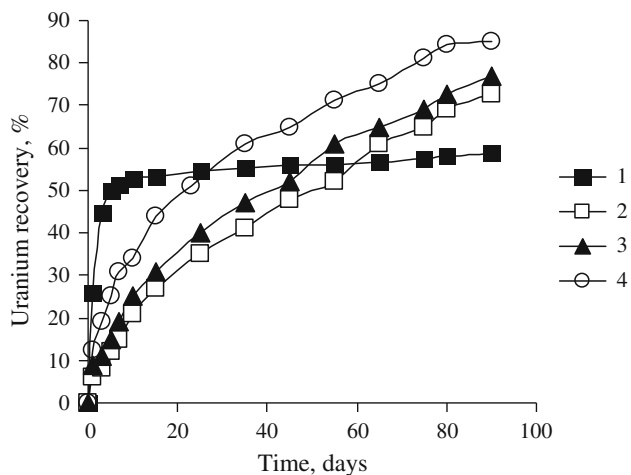
Fig. 1 Evolution of uranium extraction in the following leaching tests: with typical leach solution from the plant (*1*), with *A. thiooxidans* (*2*), with *A. ferrooxidans* (*3*), with a mixture of *A. ferrooxidans* and *A. thiooxidans* (*4*)
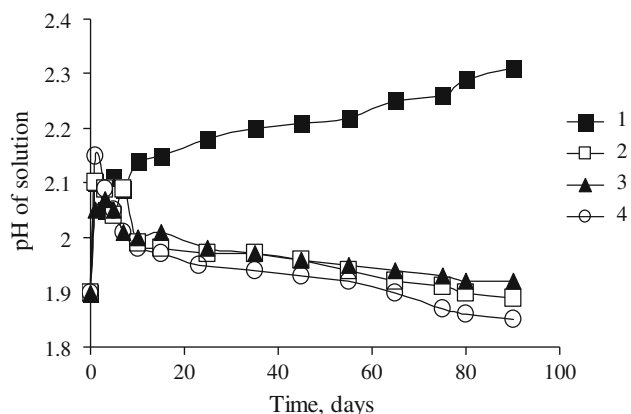

Fig. 2 Evolution of pH of solutions in the following leaching tests: with typical leach solution from the plant (*1*), with *A. thiooxidans* (*2*), with *A. ferrooxidans* (*3*), with a mixture of *A. ferrooxidans* and *A. thiooxidans* (*4*)
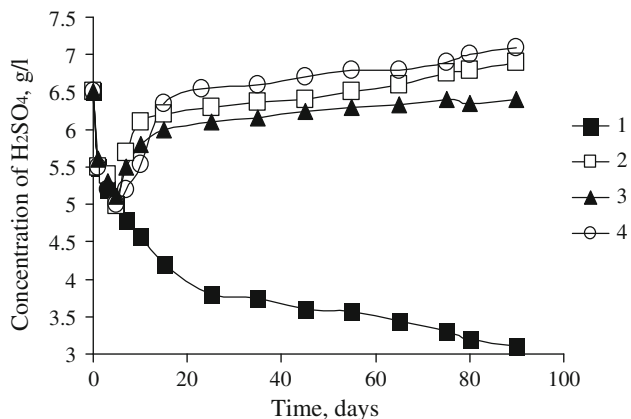

Fig. 3 Evolution of H_2SO_4 concentration in solution in the following leaching tests: with typical leach solution from the plant (*1*), with *A. thiooxidans* (*2*), with *A. ferrooxidans* (*3*), with a mixture of *A. ferrooxidans* and *A. thiooxidans* (*4*)
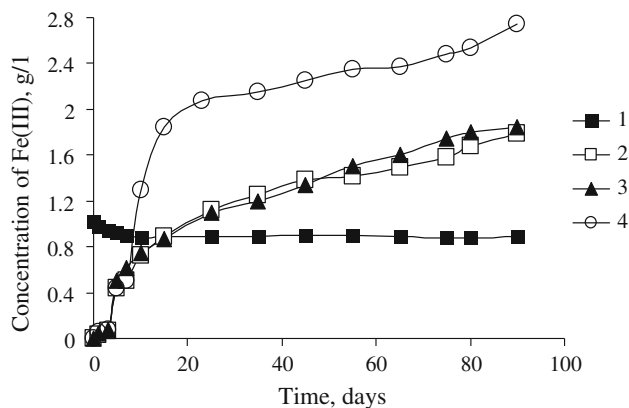

Fig. 4 Evolution of Fe(III) concentration in solution in the following leaching tests: with typical leach solution from the plant (*1*), with *A. thiooxidans* (*2*), with *A. ferrooxidans* (*3*), with a mixture of *A. ferrooxidans* and *A. thiooxidans* (*4*)
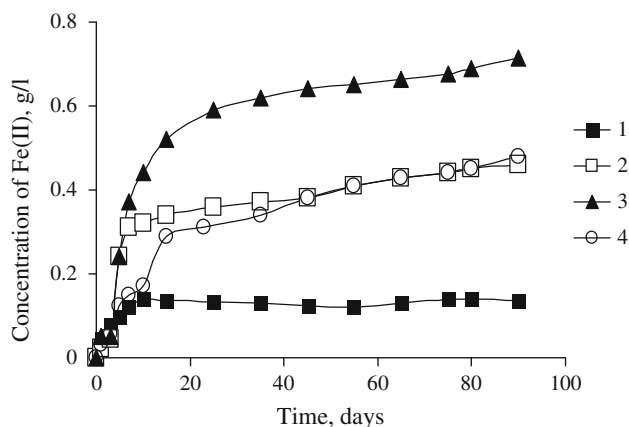

Fig. 5 Evolution of Fe(II) concentration in solution in the following leaching tests: with typical leach solution from the plant (*1*), with *A. thiooxidans* (*2*), with *A. ferrooxidans* (*3*), with a mixture of *A. ferrooxidans* and *A. thiooxidans* (*4*)
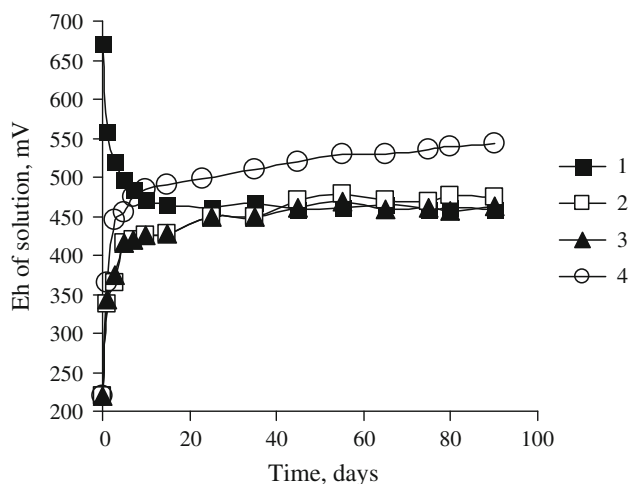

Fig. 6 Evolution of Eh of solution in the following leaching tests: with typical leach solution from the plant (*1*), with *A. thiooxidans* (*2*), with *A. ferrooxidans* (*3*), with a mixture of *A. ferrooxidans* and *A. thiooxidans* (*4*)

redox potential and the concentrations of ferric and ferrous iron in solution.

At the beginning of experiments the uranium extraction was better with uninoculated system since there was no induction period. This is due to the fact that the liquid from the industrial plant contained ferric ions which would favor tetravalent uranium solubilization.

During induction period in tests with inoculation bacterial attack was almost non-existent. However, this period ended in very short time (of about 10 days). After a lapse of this time the bacterial activity was raised sharply, and the system responded by dissolving substantial quantities of pyrite and uranium.

But as can be seen from the Fig. 1 great differences in uranium extraction degrees were observed between the different experiments at the end of experiments: the best results (85.1 % recovery) were obtained with system inoculated with a mixture of *A. ferrooxidans* and *A. thiooxidans*, followed by system with *A. thiooxidans* (76.8 %) and the system with *A. ferrooxidans* (72.5 %). The worst results of uranium recovery (58.1 %) were obtained with an application of leaching solution from uranium plant that was not inoculated at all.

The differences between system with leaching solution from plant and systems with inoculums represent up to 27 % of final recovery of uranium from ore.

From Figs. 2, 3 one can see that the inoculum application resulted in increase of acid concentration in solution up to 2.5–3 g/l, the total amount of acid generated in 90 days being very slightly influenced by the nature of inoculum. The maximal value of acid generation was founded to be in the test with the mixture of cultures, amounting to 3.2 g/l.

So it is clear that acid generation was due to the process of pyrite degradation, its amount corresponding to the increase in degree of pyrite dissolution. In the lapse of 90 days of leaching with the presence of inoculums generation of additional acid detected due to pyrite degradation was comparable to acid consumption in experiment without inoculation. So it is clear that bioleaching application can be resulted in acid feeding reduction up to 100 % compared with traditional technique that is very appropriate because of economics consideration.

As regards to the other parameters of the process, the high quantity of iron in solution (Figs. 4, 5), principally as Fe(III), is of note as this confirms the importance of the indirect mechanism in uranium solubilization during bioleaching. This led to high potential values of solution (Fig. 6), which were sufficient to transform U(IV) into U(VI). That is why there were differences between inoculated and uninoculated systems, the former reaching more oxidizing conditions.

Discussion

As can be seen from Fig. 1 the maximal degree of uranium dissolution using investigated leach solution was unusually low, not exceeding a value of 58.1 %. Its dissolution kinetic showed two phases. The first phase, represented by the line from 0 to 50 %, was characterized by a high dissolution rate as a consequence of a strongly oxidizing conditions existing during the process. The second phase was represented by the slightly sloping straight line extending from 50 % dissolution degree. The quantity of uranium solubilized diminished sharply, suggestive of the process being controlled at this stage by the surface exposed to attack, since the concentrations of Fe(III) and acid during experiment were much higher than 0.5 g/l necessary for tetravalent uranium leaching to be effective [2].

A comparison of bioleaching tests results showed that extraction of uranium was strongly favored by a mixture of cultures.

Contrary to the uninoculated test in experiments with inoculation the kinetics of the process after the induction period are higher at all intervals of experiment. In the case of inoculation the kinetic of the process represents an absence of the second straight line with the slight slope indicating that the process was not being controlled by the surface exposed to attack by reagents.

These findings can be accounted for by the fact that because of large amounts of pyrite in ore particles of coffinite in ore are enclosed into pyrite particles thus preventing the lixivant from contacting with uranium (IV) from coffinite.

In order to assure whether this statement was right or not the sample of uranium-bearing sand was subjected to SEM.

Investigations with SEM showed that about half of coffinite particles in ore having the diameter of 10–200 μm are encapsulated in pyrite particles that had linear dimensions of about 100–1,000 μm. It can be proved by the typical SEM image presented in Fig. 7.

This fact wholly corresponds to the findings derived from Fig. 1 as to the uranium dissolution kinetic and final degree of uranium recovery in solution obtained from industrial plant.

From Fig. 7 it is clear that the presence of large particles of pyrite in ore with conjunction of very small coffinite particles prevented coffinite surface from being exposed to the lixivant attack.

One can deduce from this that high uranium recovery can only be achieved by ore grinding or chemical pyrite dissolution. In order to prove that high pyrite degradation must be strictly followed by high uranium recovery the samples of ore after tests ended were subjected to X-ray and chemical analysis.
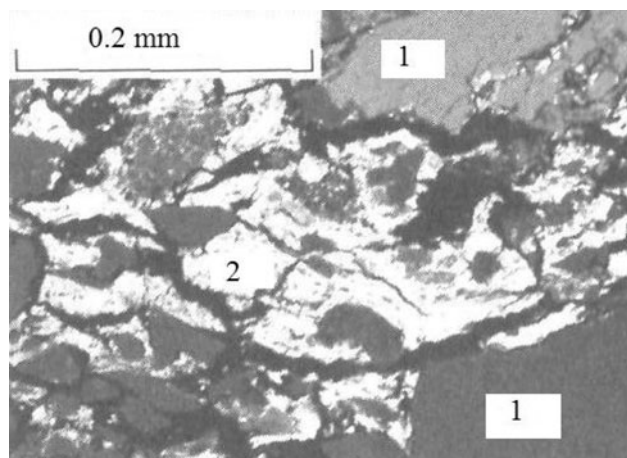
Fig. 7 SEM image of the polished thin section of ore sample. *Gray phase* (*1*) is a pyrite; *white phase* (*2*) is a coffinite

From leach residue investigation it was founded that in the test with typical industrial solution pyrite removal degree was not exceeding 0.2 %, this value being comparable with an error of analysis. In the test with *A. thiooxidans* pyrite was removed in 90 days to a value of 67.5 % while the test with *A ferrooxidans* resulted in removal of 77.9 % of pyrite. At the test with mixed cultures pyrite removal sharply increased amounting to 98.0 %.

It was founded by X-ray diffraction patterns that Fe(III) presented in ore as a magnetite was absolutely insoluble in all leach tests.

Thus, experiments without bacteria were founded to result in very negligible pyrite dissolution that corresponded to the worst results of uranium recovery obtained.

The best result in pyrite dissolution (98.0 %) corresponded to the system inoculated by binary mixture of cultures that showed highest uranium recovery (up to 85.1 %).

The good results obtained in experiments with shaken flasks led us to conclude that percolation leaching process for this ore is viable.

Our conclusion is approved by the findings made by previous researchers [3, 9] who stated that bioleaching had been successfully used in the extraction of uranium from ores when uranium minerals were associated with a high pyrite content.

Ores from Elliot Lake (Canada) containing of about 1–2 % pyrite showed uranium recovery of 80–90 % if processed by bioleaching technology [13].

It was founded that Indian and Portuguese uranium ores that has a lack of pyrite mineralization itself can be processed well by bacterial leaching with an addition of no less than 5 kg of pyrite per ton of ore [14, 15].
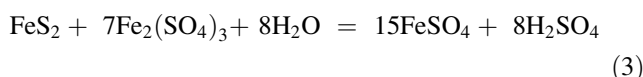
On the other hand, the uranium ores from New Mexico, the Rocky Mountains, and southern Texas (USA), with their low pyrite content (less than 0.05 %), are not suitable

for bacterial leaching showing the uranium recovery of no more than 20–30 % [15].

As can be seen from the Figs. 1, 2, 3, 4, 5, 6 in our test the best results were obtained with mixture of cultures.

Such results can be explained by the following considerations.

In the literature [2, 16, 17] biological pyrite oxidation was described by the following competing reactions that proceed in the presence of bacteria as a catalyst:

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

$$FeS_2 + 7Fe_2(SO_4)_3 + 8H_2O = 15FeSO_4 + 8H_2SO_4 \tag{3}$$

$$FeS_2 + Fe_2(SO_4) = 3FeSO_4 + S^0 \tag{4}$$

Ferrous iron liberated according to Eqs. (2–4) is further oxidized by oxygen in the presence of bacteria as catalyst [2, 9, 15] to ferric iron:

$$4FeSO_4 + O_2 + 2H_2SO_4 = 2Fe_2(SO_4)_3 + 2H_2O \tag{5}$$
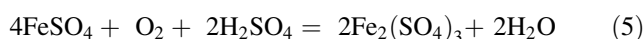
Ferric sulfate, produced by Eq. (5), in its turn, reacts with uranium(IV) according to the Eq. (1), thus enhancing the uranium dissolution rate.

One can see that the process described by Eq. (3) accounts for the additional acid generation detected in bioleaching tests.

Because of kinetic data it became obvious that a sulfur coating according to Eq. (4) can appear on the surface of pyrite preventing it from further dissolution.

The formation of elemental sulfur during pyrite biooxidation was quantified by previous researchers [18].

In pure culture of *A. thiooxidans* generally less than 1 % of the oxidized pyrite sulfur moiety was elemental sulfur, indicating nearly complete oxidation to sulfate. In a pure culture of *A. ferrooxidans* about 10 % elemental sulfur was formed in pyrite oxidation [18].

At the same time pyrite dissolution was founded to proceed more rapidly if *A. ferrooxidans* was used [18].

Some researches stated that *A. thiooxidans* are not able to oxidize iron(II) at all and did not oxidize pyrite. This finding contradicts to the results of Lizama and Suzuki [19] who concluded from oxygen consumption measurements that *A. thiooxidans* are able to oxidize pyrite though with very low rate.

Obviously, *A. thiooxidans* can readily oxidize intermediary sulfur that is formed during biooxidation being not fast themselves in pyrite attacking at the same time.

So it can be proposed that in the case of mixed cultures of *A. ferrooxidans* and *A. thiooxidans* pyrite would be nearly completely dissolved because of the capacity of these cultures to oxidize both pyrite and intermediary sulfur.

Results of our experiments showed that in the test with a mixed culture of *A. ferrooxidans* and *A. thiooxidans* pyrite

and uranium dissolution rates were significantly enhanced in comparison to the test with pure cultures of *A. ferrooxidans* and *A. thiooxidans* just as had been predicted from the considerations mentioned above. It is clear that based on this mechanism a complete dissolution of pyrite can readily be achieved at practice.

The worst results of pyrite and uranium dissolution degree during biooxidation (67.5 and 72.6 %, respectively) just as can be expected were obtained with a culture of *A. thiooxidans*.

Conclusion

The bioleaching of a low-grade uranium ore in shaker using natural, pure, and mixed cultures of *A. ferrooxidans* and *A. thiooxidans* has been studied.

It was founded that the uranium recovery from ore of off-balance sheet type containing 3.1 % pyrite is very effective using *A. ferrooxidans* or a mixture of *A. ferrooxidans* and *A. thiooxidans*. The best results were obtained with the mixture of *A. thiooxidans* and *A. ferrooxidans* (up to 85.1 % of uranium recovery), followed by system with *A. ferrooxidans* (76.8 % of uranium was extracted) and *A. thiooxidans* (of about 72.5 %). The worst results evaluated to 58.1 % of uranium recovery were obtained using traditional technique of leaching with ferric sulfate acidic solution.

An application of bioleaching technology to uranium leaching results in an additional acid generation as a consequence of microbial activity. That, in its turn, would lead to a decrease of acid consumption at uranium industrial plants.

It was proved, that the uranium recovery from ore by bioleaching can be up to 15–27 % higher compared to traditional way of leaching, working with ores that contained fine grained uranium minerals enclosed in pyrite matrix.

Thus, bioleaching technique can be recommended for processing of ores with high pyrite concentrations by means of heap leaching.

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