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Microorganisms in the processes of underground leaching

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Abstract: Microbial diversity in pumping-out solutions of uranium by underground leaching was studied. It was established that pumping-out solutions in pH 3.0 and higher intensifies and stimulates development of mixotrophic thionic bacteria. Supplying oxygen by pumping air into wells leads to intensification of uranium carryout, which is accompanied by considerable increase in growth of almost all studied groups of microorganisms, especially those related to thionic neutrophils and *Pseudomonas* genus bacteria. The increased activity of these groups of microorganism may lead to higher concentration of uranium in solution.

Keywords: microorganisms, underground leaching, uranium, thionic, pH.

Introduction

Last decades were characterized by accelerated development of hydrometallurgical methods for processing mineral raw materials. The development of new methods is stipulated by necessity to process ore with lower content of useful components [1,2]. One of the most prospective directions are microbiological methods of processing such poor ores in order to extract the inside metals, and are based on the application of either microorganisms or their metabolites [3].

Uranium is a radioactive silver-gray metal which today with its slow radioactive decay provides the main source of heat and concentrated energy inside the Earth. It is mostly used to fuel commercial nuclear power plants. It is known that uranium deposits are explored differently nowadays. Uranium deposits of hydrogenic origin are characterized by diversity of minerals composition, by mining, technical and hydrogeological conditions. All these factors make considerably difficult exploitation of the uranium deposits by traditional mining methods. That is why the most developed method is well-drilled underground extraction based on chemical leaching.

Uranium leaching in extreme acidic mode is used in non-calcareous ores. The main advantage of this scheme is high kinetics of the leaching process. But, negative factors are prevailing in this case such huge wastes of the acid, chemical colmatation and environment pollution. An alternative to this scheme is the application of bacterial leaching, which are environment friendly technologies. Besides, they are capable to increase the ratio of uranium extraction as well.

In this present work, we studied microorganisms in both conditions of acidic and reagent-free leaching. Development of microorganisms in pumping-out solutions of underground leaching of uranium was analyzed and considerable microbial diversity was reported. Intensification of activity of all studied groups of microorganisms will promote the increase in concentration of uranium in solution, thus enhancing productivity of the process. [4-8].

It is known that in nature the active carryout of uranium from ore takes place in conditions of pyrite oxidation, which is one of the components of these ores, where thionic bacteria such as *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* play an active role [7,9,10]. The use of different microorganisms for bioleaching is observed not only in acid industrial solutions of uranium, but also in leaching of uranium ore with soda solutions. The presence of active heterotrophic and autotrophic microflora developing at pH 9.0-10.0 in these solutions was reported earlier [11,12]. Isolated strains of thionic bacteria were identified as different species belonging to genus *Acidithiobacillus*. These bacteria, developing at neutral and alkali levels of pH, are capable of stimulating uranium leaching. The addition of phosphorus and nitrogen to the leaching medium stimulated further the process of bacterial oxidation of uranium. The process of biological leaching of uranium with the use of heterotrophic microorganisms may proceed according to more complex schemes which, possibly, involves organic acids and enzymatic activity of microorganisms compared to metal oxidation by autotrophic microorganisms which conversely, takes place mainly according to the simple scheme linked to

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chemical oxidation of uranium with trivalent iron [13,14].

It is known that underground leaching is conducted with the use of acid, so called acidic leaching. We can also distinguish other types of leaching like bicarbonate and reagent-free leaching. Currently, the study of different microbiota that may be involved in different types of leaching represents considerable interest due to existing possibility of application of microorganisms in underground leaching of various metals [15].

In these regards, a study was conducted to identify microorganisms present in pumping-out solutions of different types of uranium leaching.

Materials and Methods

Industrial uranium underground leaching was conducted by the Navoyi mining and smelting enterprise on the deposit Ketmenchi (Uzbekistan). Pumping-out solutions from wells after bioleaching was object of this study (Figure 1). Temperature of solutions was 25°C, the ore was poor in sulfide.

Figure 1. Site for collection of the pumping-out solution

Leaching was conducted with and without air supply to pumping-in solution. Air was supplied to well by industrial pumps (1-2 l/min). Analysis of indigenous microflora was conducted on following groups of microorganisms: thiosulphate oxidizing bacteria, autotrophic and heterotrophic denitrifying bacteria, mixotrophic thionic bacteria by cultivation of samples on elective nutrient media. Determination of microorganisms'development in pumping-out solutions from the acidic underground leaching was conducted as well. pH level of pumping-out solutions was determined on-site with use of portable pH-meter (Mettler-Toledo).

Analysis of indigenous microflora was conducted on the following groups of microorganisms: acidophilic iron- and sulphur-oxidizing microorganisms, thiosulphate oxidizing bacteria, autotrophic and heterotrophic denitrifying bacteria, mixotrophic thionic microorganisms.

Classical microbiological methods, based on the study of morphological, cultural, physiological and biochemical properties of microorganisms, including Gram staining, were applied for identification of isolated microorganisms. The determination of species was done according to Bergey [16].

Results and Discussion

The study conducted on pumping-out solutions of reagent-free leaching revealed that intensification of uranium yield is observed when supplying air (oxygen) in pumping-in wells, which is accompanied by considerable increase in growth of microorganisms belonging to the group of thionic neutrophils and *Pseudomonas* genus (Table 1).

Table 1. Microorganisms of pumping-out solutions of reagent-free leaching

Sample	Thionic neutrophils		Thionic mixotrophs	Oligonitrophilic bacteria	Denitrifying bacteria	Bacteria growing on beef extract agar
	Thionic denitrifying	Thiosulphate oxidizing				
Pumping-out solution with air supply (well №1)	2.5×10^5	2.5×10^3	2.5×10^4	7.5×10^4	6.0×10^4	**8.3×10^3**
Pumping-out solution with air supply (well №2)	6.0×10^5	6.0×10^3	2.5×10^4	6.4×10^4	2.5×10^5	**9.7×10^4**
Pumping-out solution with air supply (well №3)	6.0×10^5	6.0×10^3	6.0×10^3	9.1×10^4	2.5×10^4	**8.8×10^4**
Pumping-out solution without air supply (well №1-a)	2.5×10^2	2.5×10^2	2.5×10^2	3.2×10^3	2.5×10^2	**6.5×10^2**
Pumping-out solution without air supply (well №2-a)	2.5×10^4	2.5×10^2	2.5×10^2	7.6×10^2	6.0×10^2	**2.0×10^2**
Pumping-out solution without air supply (well №3-a)	**6.0×10^2**	**6.0×10^2**	**-**	**5.5×10^2**	**6.0×10^2**	**7.5×10^2**

It is known that many microorganisms produce short-chain organic acids and element-specific ligands, which can change pH and promote synthesis of chelates leading to increased leaching of many elements contained in ores in diminishing quantities. Uranium extraction, apparently, is due to production of pyoverdine chelates, which are typical ligands produced by fluorescents of *Pseudomonas*. Intensification of activity of these groups of microorganisms may promote an increased concentration of metal in solution.

We also studied the development of microorganisms in pumping-out solutions of acidic underground leaching (Figure 2).

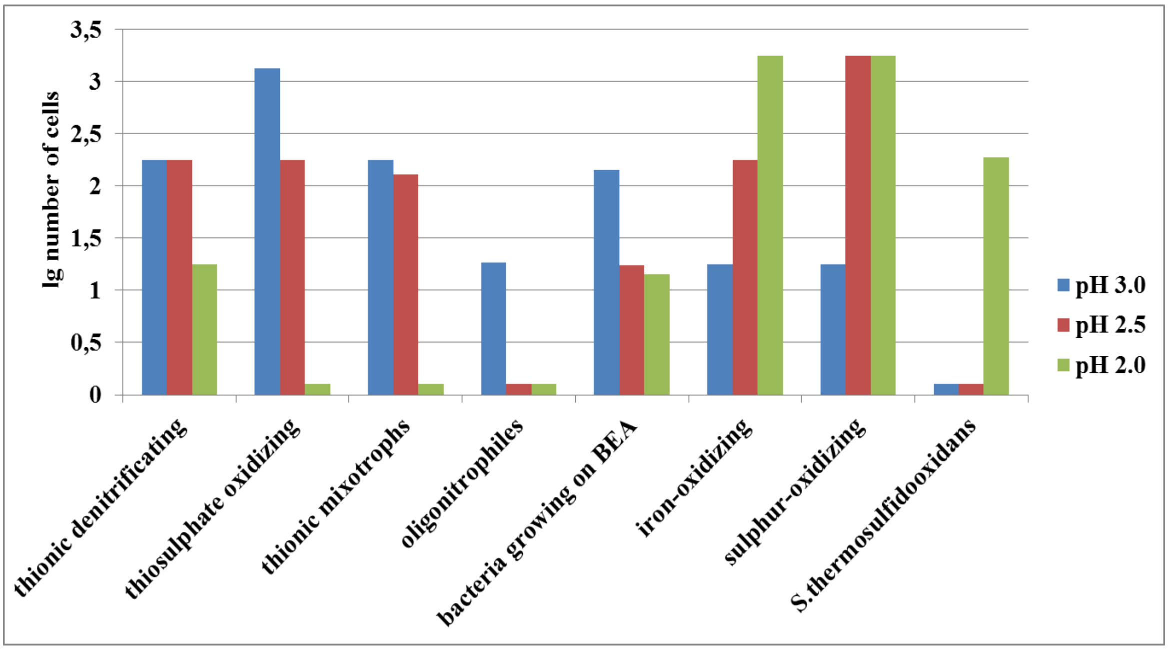
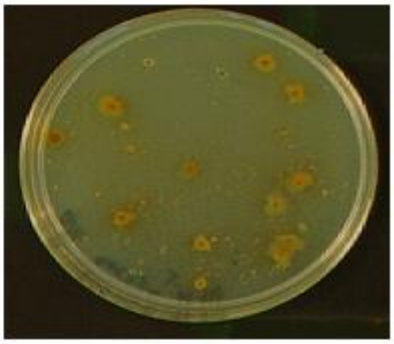
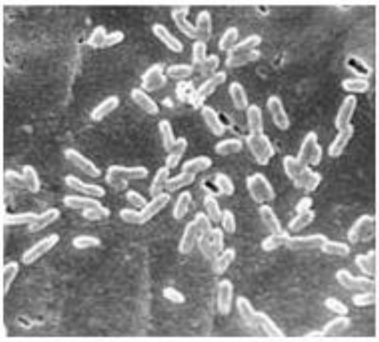


Figure 2. Microorganisms of pumping-out solutions of acidic leaching with different pH levels

Bacteria identified as *Acidithiobacillus ferroxidans,* which oxidize almost all known sulphide minerals, were isolated from the majority of samples (Figure 3).





a) b)

Figure 3. Colonies of *A.ferrooxidans* on medium 9K (a), cells of *A.ferrooxidans* (20x5,000) (b).

By raising the pH from 2.5 to 3.0 in pumping-out solutions, the number of microorganisms belonging to oligo-nitrophiles increased and bacteria close to genus *Pseudomonas* were isolated (Figure 4).

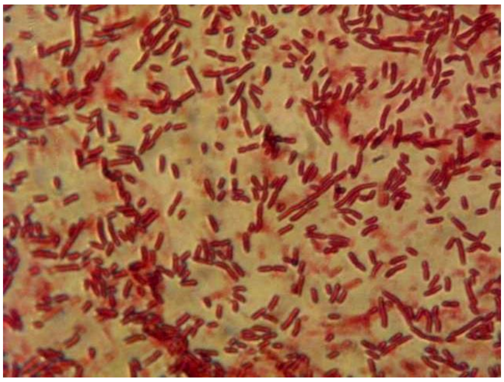


Figure 4. Gram stained cells of *Pseudomonas sp*. (10x100).

At pH 3.0 of pumping-out solutions the different types of thionic bacteria prevailed (Figure 2). But, change of acidity of pumping-out solutions stimulates also development of mixotrophic thionic bacteria and heterotrophic microorganisms.

Analysis of microbiota of pumping-out solutions revealed the presence of sulphur-oxidizing bacteria *A.thiooxidans.* The presented data reveals that with decrease of pH level of pumping-out solutions the quantitative content of *A. ferrooxidans*, which are isolated on medium 9K, considerably grows. The use of Manning medium resulted in the isolation of microorganisms identified as *Sulfobacillus* sp., which are capable to grow on medium with minimum addition of organic substrate (Figure 5).

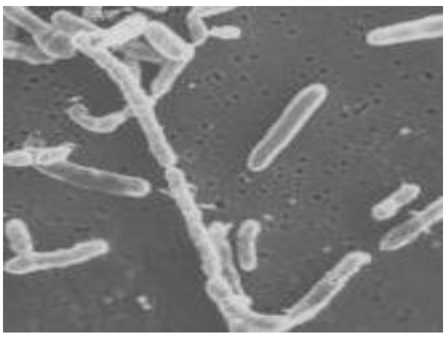


Figure 5. Cells of *Sulfobacillus* sp.(10x10,000).

The decrease in pH level of pumping-out solutions increases number of thionic acidophiles *A.ferrooxidans* and *A.thiooxidans* (Figure 2).

It is necessary to note that in tailing solutions microscopic fungi belonging to genera *Aspergillus, Fusarim* and *Penicillium* were isolated (Figure 6).

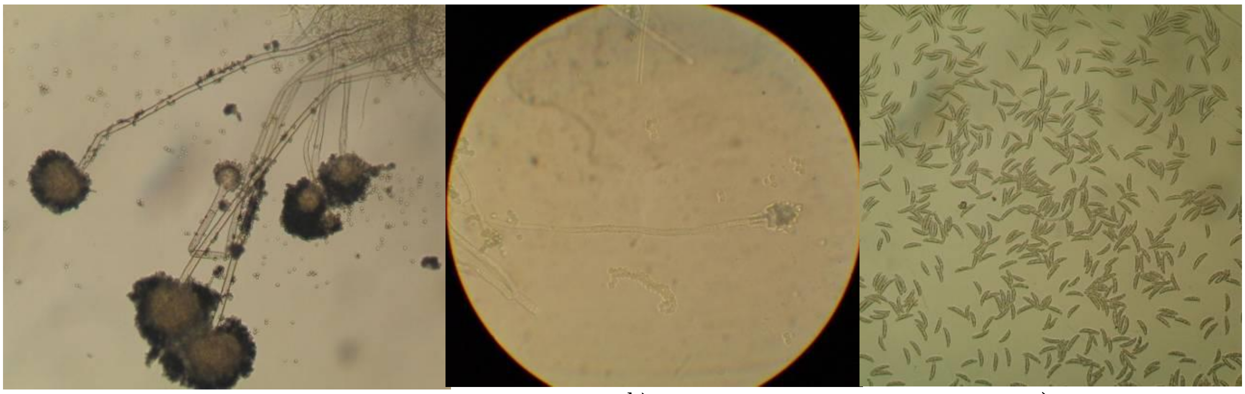

a) b) c)

Figure.6. *Aspergillus flavus* (a), *Penicillium sp*. (b), *Fusarium sp.* – conidia (c); (10x100)

Conclusion

The analysis of microorganisms developing in pumping-out solutions of underground leaching revealed certain microbial diversity. For example in acidic leaching, oligonitrophilic microorganisms and *A.thiooxidans* were isolated in small quantities also, thionic acidophilic iron- and sulpher-oxidizing microorganisms, related to *A.ferrooxidans*, were often isolated.

The presence of heterotrophs even in such extreme conditions of high pressure was observed, but extensive resistance of bacteria to high pressure was reported by a number of researchers and sporogenous species were able to stand the highest pressure and their spores germinated even after 20,000 atmospheres [9, 11].

We showed that air supply to the pumping-in well positively affects diversity of the microorganisms compared to microbiota present in solutions without access to air (oxygen).

The increased activity and biodiversity of these groups of microorganism may lead to higher concentration of uranium extracted in solution and may be used to extract other metals present in small quantities from ores.

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