

MECHANISMS OF BIOLOGICAL OXIDATION OF URANIUM IN NATURAL ECOSYSTEMS

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ABSTRACT. The utilization of mesophilic and moderately thermophilic chemolithotrophic bacteria, possessing ferroxidizing activity and producing ferric ions at acidic pH of the medium, is currently applied under industrial scales for extraction of uranium from mineral raw materials (mainly ores and concentrates), as well as for cleaning soils polluted with this element. The studies carried out in some of the developed in the past and, at the present, recultivated to a different stage uranium deposits in Bulgaria revealed that in these biotopes uranium is oxidized and passes in soluble form also by other microorganisms as the extreme thermophilic archaea, heterotrophs producing hydrogen peroxide, nitrifying and denitrifying bacteria producing uranium oxidising nitrogen oxides (NO_2 , NO , N_2O) as a result of the processes of nitrification and denitrification. The possibilities for biological removal of uranium from polluted waters and soils were tested.

Keywords: uranium, microorganisms, biological oxidation

МЕХАНИЗМИ НА БИОЛОГИЧНО ОКИСЛЕНИЕ НА УРАН В ПРИРОДНИ ЕКОСИСТЕМИ

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РЕЗЮМЕ. Използването на мезофилни и умерено термофилни хемолитотрофни бактерии, притежаващи ферооксидазна активност и генериращи ферри йони при кисело рН на средата, се прилага понастоящем в промишлени мащаби за извличане на уран от минерални суровини (главно руди и концентрати), както и за пречистване на почви, замърсени с този елемент. Изследванията, проведени в някои от разработваните в миналото и понастоящем рекултивирани в различна степен уранови находища в България показаха, че в тези биотопи урана се окислява и преминава в разтворена форма и от други микроорганизми, като екстремни термофилни археи, хетеротрофи, продуциращи водороден перекис, нитрифициращи и денитрифициращи бактерии окисляващи урана чрез азотни окиси (NO_2 , NO , N_2O) в резултат на процесите нитрификация и денитрификация. Тестирани са възможностите за биологично отстраняване на урана от води и почви, замърсени с този елемент.

Ключови думи: уран, микроорганизми, биологично окисление

Introduction

Different microorganisms are able to leach uranium from the relevant minerals and ores. The industrial-scale bioleaching of uranium which is currently applied in several countries is based on the activity of some acidophilic chemolithotrophic microorganisms (bacteria and archaea) which are natural inhabitants of the uranium deposits. These microorganisms are able not only to leach the easily soluble hexavalent uranium present in some oxide minerals such as carnotite and pitchblende but can oxidize the insoluble tetravalent uranium (e.g. in uraninite) to the soluble hexavalent form. This oxidation is carried out by different bacteria and archaea able to oxidize the ferrous iron to the ferric state and/or the sulphur (present as S^{2-} in the sulphide minerals) to sulphuric acid or the relevant sulphates (Karavaiko et al., 1988; Schippers et al., 1995; Tuovinen and Bhatti, 2002).

The industrial bioleaching of uranium is applied by different systems: as underground in situ leaching by injecting solutions of sulphuric acid and, in some cases, ferric ions usually obtained as a result of the preliminary bacterial oxidation of ferrous ions, via boreholes to the uranium bearing ore layers;

as heap leaching using heaps with the shape of a truncated pyramid and consisted of ore crushed usually to minus 1 – 3 cm particle size, aerated by air supplied to the ore through several perforated pipes and irrigated by solutions containing sulphuric acid and ferric ions. The heaps are inhabited by acidophilic chemolithotrophic bacteria and, apart from the sulphuric acid and ferric ions, some nutrients (mainly sources of nitrogen and phosphorous) are added to the ore;

as reactor bioleaching in agitated tanks containing suspensions of finely crushed ore or even concentrate, under conditions optimum for the growth and activity of the relevant microorganisms.

The uranium deposit Curilo located in a short distance from Sofia, for a long period of time was a site of intensive mining activities including both open-pit and underground mining techniques as well as in situ leaching of uranium. The main uranium-bearing minerals in the ore were nasturane, torbernite, metatorbernite, pitchblende, metaautunite and baselite. The ore was rich in pyrite and, apart from uranium, contained some non-ferrous metals such as copper, zinc and lead, present mainly in the form of sulphide minerals. Quartz and feldspars were the main minerals in the host rock. Clay minerals and some iron hydroxides were also present.

The mining operations in the deposit were ended in 1990 but since that time the deposit was for several years a source for generation of acid mine drainage waters. These waters had low pH (usually in the range of about 2 – 3) and contained radionuclides (mainly uranium and radium), heavy metals (mainly iron, copper, zinc and manganese), arsenic and sulphates in concentrations usually much higher than the relevant permissible levels for waters intended for use in agriculture and/or industry. A portion of these waters consisted of groundwaters arisen after the closure of the underground mine and coming from the underground mining works. The other portion consisted of drainage waters arisen after rainfall from the fractured ore body and from the dumps consisted of rich-in-pyrite mining wastes and low-grade ores. The generation of these acid drainage waters was connected with the growth and activity of the indigenous chemolithotrophic bacteria which are able to oxidize the pyrite and other sulphide minerals present in the ore body (Suzuki and Suko, 2006; Nicolova et al., 2010). These microorganisms are of a great scientific and practical interest since are largely used in commercial-scale operations for extracting uranium and different non-ferrous metals from ores, concentrates and mineral wastes, as well as for pretreatment of different sulphide-bearing substrates (mainly concentrates) for exposing the precious metals and different rare earth elements finely disseminated in these substrates accessible to subsequent leaching. On the other side, the spontaneous and without an efficient control growth of such microorganisms can be the reason for heavy contaminations of the soil and water ecosystems located near the dumps of toxic mineral wastes. The data present in this paper contain some results from studies on the character of contamination of some of the ecosystems of this type before their effective remediation.

Materials and Methods

The sample of mining wastes used in this study was taken from the dumps located in the Curilo deposit before their recultivation. The sample contained 0.1 % uranium, mainly in the form of uraninite, nasturane and pitchblende. Apart from uranium, the sample contained some non-ferrous metals (copper, zinc, lead) and arsenic present as the relevant sulphide minerals. However, the pyrite was the main ore mineral in these dumps. The mineral composition of the dumps made them after rainfall an intensive source of acid drainage waters heavily contaminated by radionuclides, heavy metals, arsenic and sulphates in concentrations much higher than the relevant permissible levels for waters intended for use in the agriculture and/or industry (Table 1). The contamination was connected with the activity of some microorganisms, mainly chemolithotrophic bacteria, which were inhabitants of the rich-in-sulphides and still non-recultivated sites in the deposit.

On the basis of the data on the rates of oxidation of Fe^{2+} and S^0 some of the microbial strains isolated in this study, related to different taxonomic species and characterized by different levels of their oxidative ability, were used in experiments for bioleaching of the uranium ore sample mentioned above. The bioleaching was carried out by the shake-flask technique using Erlenmeyer flasks of 250 ml containing 90 ml of 9K nutrient medium, 10 ml microbial inoculum taken from the late-log-phase during the cultivation of the relevant strains on different

substrates (Fe^{2+} , S^0 or ore) and 20 g of the ore milled to minus 100 microns. The cultivation of the microorganisms and the bioleaching experiments were carried out at different temperatures (usually at 30 – 35 °C for the mesophilic bacteria, 50 – 55 °C for the moderate thermophilic bacteria and 70 °C for the extreme thermophilic archaea).

Bioleaching experiments were performed also in percolation columns each of them containing 30 kg of ore crushed to minus 2.0 cm. The leach solutions consisted of diluted sulphuric acid with and without dissolved iron ions (as Fe^{2+} and/or Fe^{3+}) in different concentrations. The columns were inoculated by mixed microbial cultures able to grow at the different temperatures mentioned above.

Table 1.

Data about the chemical composition of the acid drainage waters generated in the dumps of mining wastes

Parameters	Content	Permissible levels
Temperature, °C	(+0.1) - (+29.7)	–
pH	1.81 – 4.40	6 – 9
Total content of dissolved substances, mg/l	510 – 3740	1500
Insoluble substances, mg/l	21 – 212	100
Dissolved organic carbon, mg/l	0.3 – 6.2	20
Sulphates, mg/l	332 – 1918	400
Uranium, mg/l	0.21 – 5.90	0.6
Radium, mg/l	0.05 – 0.62	0.15
Copper, mg/l	0.28 – 17.2	0.5
Zinc, mg/l	0.80 – 28.0	10
Cadmium, mg/l	0.01 – 0.14	0.02
Lead, mg/l	0.08 – 0.91	0.2
Nickel, mg/l	0.32 – 8.24	0.5
Cobalt, mg/l	0.21 – 7.12	0.5
Iron, mg/l	64 – 1928	5
Manganese, mg/l	0.71 – 68	0.8
Arsenic, mg/l	0.01 – 0.71	0.2

The parameters measures of the leach and pregnant solutions included pH, Eh, dissolved oxygen, chemical composition, total dissolved solids, temperature and concentrations of the different microorganisms. Elemental analysis of the solid samples was performed by digestion and measurements of the ion concentrations in solution by atomic absorption spectrometry and inductively coupled plasma spectrometry. Mineralogical analysis was carried out by X-ray diffraction techniques.

The isolation, identification and enumeration of microorganisms were carried out by the classical physiological and biochemical tests (Karavaiko et al., 1988) and by the molecular PCR methods (Escobar et al., 2008; Sanz and Köchling, 2007).

Results and Discussions

The microbiological analyses of the dumps located in the Curilo deposit revealed that a large number of different microorganisms were present in this quite specific ecosystem (Table 2). The acidophilic chemolithotrophic bacteria able to

oxidize the sulphide minerals (mainly pyrite), the ferrous iron, elemental sulphur and tetravalent uranium were the prevalent microorganisms in the dumps and in the acid drainage waters generated after rainfall as a result of the microbial oxidation of these inorganic substrates to sulphuric acid, soluble ferric and sulphate ions and hexavalent uranium. The mesophilic *Acidithiobacillus ferrooxidans* was the main representative of these bacteria and its number in some ore and water samples was higher than 10^8 cells/g (or cells/ml). *Leptospirillum ferrooxidans* was also present in large numbers in the dumps but usually in lower concentrations the *At. ferrooxidans*. This was probably due to the fact that *L. ferrooxidans* is able to oxidize only ferrous iron but not any form of the sulphur. However, mixed cultures of *L. ferrooxidans* with *Acidithiobacillus thiooxidans*, which possess only sulphur-oxidizing ability, were quite efficient during the oxidation of pyrite and the leaching of the mineral wastes from the dumps.

Table 2.

Microflora of the acid drainage waters generated in the dumps of mining wastes

Microorganisms	Cells/ml
Mesophilic microorganisms	
Fe ²⁺ -oxidizing chemolithotrophs (at pH 2)	$10^3 - 10^8$
S ⁰ -oxidizing chemolithotrophs (at pH 2)	$10^3 - 10^7$
S ⁰ -oxidizing chemolithotrophs (at pH 7)	$10^3 - 10^7$
Aerobic heterotrophic bacteria	$10^2 - 10^6$
Nitrifying bacteria	$0 - 10^3$
Fungi	$0 - 10^3$
Anaerobic heterotrophic bacteria	$0 - 10^3$
Denitrifying bacteria	$0 - 10^1$
Sulphate-reducing bacteria	$0 - 10^3$
Fe ³⁺ -reducing bacteria	$0 - 10^2$
Methanogenic bacteria	$0 - 10^1$
Moderate thermophilic microorganisms	
Fe ²⁺ -oxidizing chemolithotrophs (at pH 2)	$0 - 10^6$
S ⁰ -oxidizing chemolithotrophs (at pH 2)	$0 - 10^6$
Aerobic heterotrophic bacteria	$0 - 10^3$
Nitrifying bacteria	$0 - 10^2$
Anaerobic heterotrophic bacteria	$0 - 10^2$
Sulphate-reducing bacteria	$0 - 10^2$
Thermophilic archaea	
Fe ²⁺ -oxidizing archaea	$0 - 10^2$
S ⁰ -oxidizing archaea	$0 - 10^2$
Aerobic heterotrophic archaea	$0 - 10^2$

In some rich-in-pyrite parts of the dump the internal temperature usually was higher than 40 – 42 °C and sometimes reached about 50 °C. Some moderate thermophilic bacteria, mainly of the species *Sulfobacillus thermosulphidooxidans*, *Acidithiobacillus caldus*, *Acidimicrobium ferrooxidans* and *Leptospirillum ferrophilum* were isolated from these parts and usually were prevalent microorganisms in the biotopes. *S. thermosulphidooxidans* was able to oxidize Fe²⁺ and S⁰ and even as pure cultures oxidized efficiently by tetravalent uranium. However, the highest oxidation rates towards the uranium and pyrite were achieved by mixed cultures of *S. thermosulphidooxidans* with *At. caldus*.

Some extreme thermophilic archaea also found and isolated from some rich-in-pyrite parts of the dumps. Most of these archaea were related to the species *Sulfobacillus metallicus* and *Metallosphaera hakonensis* and were able to oxidize the Fe²⁺, S⁰, pyrite and U(IV) at temperatures higher than 70 °C. Archaea related to *Ferroplasma acidiphilium* were found only sporadically.

Three cultures of nitrifying bacteria, two cultures of denitrifying bacteria and two cultures of hydrogen peroxide producing bacteria were also isolated from the soils and drainage waters polluted or in contacts with the mining wastes in the deposit.

The cultures consisted of nitrifying bacteria oxidized relatively small portions of the ferrous ions present in the leach solutions, i.e. about 0.8 – 1.4 g/l from the initial content of 9 g/l or 4.5 g/l added as ferrous sulphate by solutions containing also other components of the nutrient medium of Gilty. The oxidation was carried out by means of the oxygen contained in the nitrogen oxides, mainly in NO₂ and NO₃, generated during the process of nitrification. The ferric ions obtained in this way oxidized a portion of the tetravalent uranium in the ore to the soluble hexavalent state. The extraction of uranium from the ore by this treatment was low (less than 15 % for 30 days of leaching). It must be noted that the chemical leaching of the ore by means of the sterile nutrient medium of Gilty without the nitrifying bacteria resulted in uranium extraction of about 8 – 10 % for the same period of time. These extractions were due to the molecular oxygen present in the well-aerated system for cultivation of the nitrifying bacteria (the content of O₂ dissolved in the leach solution was usually higher than 5 mg/l).

The nitrogen oxides mentioned above were generated also during the process of denitrification in which the nitrates were reduced to molecular nitrogen (N₂) through the consecutive generation of intermediate gaseous nitrogen oxide products. Several bacterial species were involved in the denitrification process and some of the intermediate products reacted with sunlight and ozone producing nitric acid, a component of acid rain. The effects of the nitrogen oxides generated during the denitrification on the oxidation of uranium, ferrous ions and sulphidic and elemental sulphur was negligible and the extraction of uranium from the ore by this mechanism was low (about 5 – 7 % within 30 days of leaching).

Data about the maximum oxidation rates towards the most essential energy sources for these microorganisms, i.e. the Fe²⁺ and S⁰, as well as data about their ability to leach uranium from the ore used in this study, are shown in Tables 3 and 4. The data revealed that the efficiency of uranium leaching depended on the rates of Fe²⁺ and S⁰ oxidations by the relevant microbial strains. In general, the highest oxidation rates during these experiments were achieved by some strains of the extreme thermophilic archaea related to the species *Sulfolobus metallicus*. At the same time, it was clear that the individual distinctions between the strains related to one and the same taxonomic species were quite great, e.g. some mesophilic strains were able to oxidize Fe²⁺ and/or S⁰ at rates higher than these of some of the moderate thermophilic or even of some of the extreme thermophilic strains.

Table 3.

Maximum rates of oxidation of Fe^{2+} and S^0 by different strains of chemolithotrophic microorganisms at the relevant optimum temperatures

Microorganisms	Number of strains	$^{\circ}\text{C}$	Maximum oxidation rate, mg/l.h	
			Fe^{2+}	S^0
<i>Acidithiobacillus ferrooxidans</i>	17	35	125 – 271	42 – 95
<i>Leptospirillum ferrooxidans</i>	12	35	114 – 235	–
<i>Sulfobacillus thermosulfidooxidans</i>	12	50	159 – 284	68 – 132
<i>Sulfolobus metallicus</i>	10	70	177 – 295	82 – 145
<i>Metalosphaera hakonensis</i>	6	70	155 – 280	62 – 114

Table 4.

Bioleaching of uranium ore by the shake-flask technique at different temperatures by different strains of chemolithotrophic bacteria and archaea

Microorganisms	Number of strains	$^{\circ}\text{C}$	Maximum oxidation rate, % for 30 days
<i>Acidithiobacillus ferrooxidans</i>	8	35	41 – 75
<i>Leptospirillum ferrooxidans</i>	6	35	35 – 68
<i>Sulfobacillus thermosulfidooxidans</i>	6	50	59 – 84
<i>Sulfolobus metallicus</i>	8	70	64 – 86
<i>Metalosphaera hakonensis</i>	4	70	44 – 77

The oxidation rates achieved by some mixed cultures of chemolithotrophic microorganisms towards the Fe^{2+} and S^0 , as well as the extractions of uranium from the mining wastes used in this study, were slightly higher than the relative data obtained by the same strains when they were used as pure cultures (Table 5). However, this symbiotic effect was not present by some other mixed cultures.

Table 5.

Microbial oxidation of Fe^{2+} and S^0 and bioleaching of uranium ore by means of different mixed cultures at different temperatures

Type of the mixed culture	Number of culture tested	$^{\circ}\text{C}$	Rates of Fe^{2+} and S^0 oxidation, mg/l/h, and U extraction, % for 30 days		
			Fe^{2+}	S^0	U
Mixed cultures of mesophiles	8	35	250 - 280	82 - 93	59 - 79
Mixed cultures of moderate thermophiles	6	50	64 - 293	86 - 140	68 - 88
Mixed cultures of extreme thermophiles	6	70	271 - 299	91 - 147	71 - 95

It was found that the considerable distinctions between the strains related to one and the same taxonomic species were connected with the activity of the different enzymes participating in the oxidation of the energy sources, i.e. of U^{4+} , Fe^{2+} and S^0 , and in the CO_2 fixation. The strains differ also with

respect to the optimum conditions for their growth and activity (such as pH, type and concentrations of some nutrients such as sources of nitrogen and phosphorus) and with respect to their stability to different toxic elements. It must be noted that most of the strains tested in this study were able to oxidize U^{4+} directly, i.e. in the absence of dissolved iron, peroxides and/or the nitrogen oxides present during the processes of nitrification and/or denitrification. It must be noted that the sample of uranium-bearing mineral wastes used in this study contained some iron (mainly in pyrite but a small portion in oxide minerals) and a portion of it dissolved during the leaching. In the leach solutions acidified by means of diluted sulphuric acid the dissolved uranium is present as uranyl sulphate, i.e. as the uranyl cation (UO_2^{2+}). In some cases it is possible this uranium to inhibit the microbial oxidation of Fe^{2+} ions. It is also known that even the tetravalent uranium can inhibit the microbial oxidation of Fe^{2+} . For that reasons, the well-known BACFOX (BACterial Film OXidation) process, which is largely applied under industrial-scale conditions, is based on the microbial oxidation of Fe^{2+} in the absence of uranium, i.e. after the removal of dissolved uranium from the pregnant leach solutions.

The microbial transformations of uranium, including its oxidation to soluble forms, were applied in large-scale operations for extractions of this element from mineral raw materials as well as for remediation of contaminated waters and soils located within or near uranium deposits (Groudev et al., 2008; 2010).

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The article has been recommended for publication by department "Engineering geocology".