ACTA MICROBIOLOGICA BULGARICA

Review

Biohydrometallurgy in Bulgaria - Achievements and Perspectives

Stoyan Groudev

University of Mining and Geology "Saint Ivan Rilski", Sofia, Bulgaria

Abstract

The past and current research and industrial activities in the field of biohydrometallurgy in Bulgaria cover a large number of problems: microflora of different mineral deposits (such as of ores of non-ferrous metals, uranium and gold; non-metallic mineral raw materials such as coal, oil, kaolins, quartz sands, etc.); physiology, biochemistry and genetics of the microorganisms participating in the transformations of the above-mentioned mineral substrates; bioleaching of copper and other non-ferrous metals and uranium by means of in situ, dump, heap and reactor techniques; pretreatment of gold and silver-bearing sulphide concentrates and ores by means of chemolithotrophic bacteria to expose these precious metals; combined microbial and chemical leaching of precious metals from oxide ores; microbial removal of iron from quartz sands and kaolins, of sulphur from coal, of silicon from low-grade bauxites, of phosphorus from iron ores; improvement of the ceramic properties of kaolins; microbial enhanced oil recovery; electricity production from organic wastes by means of microbial fuel cells.

Several of the above-mentioned activities are connected with the solution of essential environmental problems: prevention of the generation and/or treatment of toxic waters polluted by heavy metals, radionuclides, arsenic, oil, organochemicals, etc. by means of active and/or passive systems, reactive zones, rock filters, etc.; treatment of soils polluted with the above-mentioned pollutants by different reactors, heap and in situ techniques; bioremediation of post-mining landscapes and wastes.

Keywords: biohydrometallurgy, bioleaching, bioremediation, chemolithotrophic bacteria, mineral biotechnology

Резюме

Научните и промишлени дейнсоти в областта на биохидрометалургията в миналото и понастоящем в България обхващат голям брой проблеми: микрофлора на различни минерални находища (на руди на цветни метали, уран и злато; неметални минерални суровини като въглища, нефт, каолини, кварцови пясъщи и т.н.); физиология биохимия и генетика на микроорганизм,и участващи в трансформациите на посочените минерални субстрати; биологично излугване на мед и други цветни метали и уран посредством техники, приложенени *in situ*, в насипища и реактори; предварително третиране на златни и сребърни сулфидни концентрати и руди чрез хемолитотрофни бактерии за разкриване на тези благородни метали от минералните структури; комбинирано микробно и химично излугване на благородни метали от окисни руди; микробно отстраняване на желязо от кварцови пясъци и каолини, на сяра от въглища, на силиций от нискокачествени боксити, на фосфор от железни руди; подобряване на керамичните свойства на каолини; микробно повишаване на добива на нефт; получаване на електричество от органични отпадъци чрез микробни горивни клетки.

Редица от горепосочените дейности са свързани с решаването на съществени екологични проблеми: предотвратяване на генерирането и/или третиране на токсични води, замърсени от тежки метали, радионуклиди, арсен, нефт, органохимикали и т.н., посредством активни и/или пасивни системи, действащи in situ зони, скални филтри и т.н.; третиране на почви, замърсени с горепосочените замърсители, чрез техники, приложени в реактори, конструирани насипища и in situ; биоремедиация на ландшафти и отпадъци след рудодобив.

Introduction

The first studies in the field of biohydrometallurgy in Bulgaria were started in 1967 by a joint group of investigators and students from the University of Mining and Geology "Saint Ivan Rilski" - Sofia and the University of Sofia "Saint Kliment Ohridski". In the course of time, engineers from the then-existing institute NIPRORUDA and from some industrial enterprises were involved in these activities.

The realization of efficient large-scale biotechnologies for processing different mineral raw materials and recovery of the relevant valuable components required preliminary studies on the chemical and mineral composition and structure of the raw materials, the composition of the indigenous microflora of the relevant deposits, as well as on the real participation and potential of this microflora in the processes of mineral transformations and leaching of different components. Initially, special attention was paid to the oxidation of sulphide minerals, ferrous iron and elemental sulphur by different acidophilic chemolithotrophic bacteria, the mechanisms and the optimum conditions for these processes, which were essential for the leaching of copper, other non-ferrous metals and uranium from the relevant mineral raw materials (Gaidarjiev *et al.*, 1975; Genchev *et al.*, 1978; Groudev *et al.*, 1978; Karavaiko and Groudev, 1985; Karavaiko *et al.*, 1988). The data from these studies made possible the efficient application of the relevant processes under real commercial-scale conditions.

Until now, there were five commercial-scale operations for dump bioleaching of copper from low-grade ores and mining wastes. One of them, located near to the Vlaikov Vrah copper mine, for a long period of time (1972 - 2003) was the largest operation of this type in Europe. More than forty million tons of ore with an average copper content of about 0.15 % were leached by indigenous populations of acidophilic chemolithotrophic bacteria, mainly of the species Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans and Leptospirillum ferrooxidans. The growth and activity of these bacteria were stimulated by suitable changes in the levels of some essential environmental factors such as humidity, pH and content of air and nutrients (mainly of ammonium and phosphate ions) in the ore dumps. Initially, copper was recovered from the pregnant leach solutions by cementation with metallic iron (Fe0) but later solvent extraction followed by electrolysis was used for this purpose (Groudev and Genchev, 1976; Groudev *et al.*, 1978; Groudev and Groudeva, 1993; Groudeva *et al.*, 2009; 2011) (Tables 1 and 2, and Figures 1 - 5).

Table 1. Data about the recirculating solutions at the Vlaikov Vrah copper dump leaching operation

Year	pH	Cu, g/l	Fe^{2+}, g/l	Fe^{3+}, g/l
	Solution to dump			
1968 - 1971	Rainfall			
1972 - 1975	3.0 - 4.3	0.05 - 0.24	2.4 - 3.9	0.05 - 0.2
1976 - 1985	3.1 - 4.5	0.04 - 0.12	2.6 - 4.2	0.07 - 0.3
1986 - 1990	3.4 - 4.6	0.01 - 0.04	2.3 - 4.1	0.10 - 0.3
	Solution from dump			
1968 - 1971	2.1 - 4.4	0.3 - 0.9	0.03 - 0.2	1.6 - 2.3
1972 - 1975	1.9 - 3.0	1.0 - 3.2	0.01 - 0.2	1.7 - 3.0
1976 - 1985	2.0 - 3.4	0.5 - 1.5	0.01 - 0.3	1.8 - 3.2
1986 - 1990	2.6 - 3.5	0.2 - 0.4	0.01 - 0.3	1.4 - 2.8

Table 2. Microorganisms in the dump effluents in industrial copper dump leaching operations in Bulgaria

Microorganisms, cells/ml	Bioleaching operations			
	Vlaikov Vrah	Tzar Asen	Assarel	Medet
Thiobacillus ferrooxidans	10^2-10^8	10^1-10^6	10^2-10^7	10^2-10^6
Thiobacillus thiooxidans	10-10^5	1-10^3	1-10^4	1-10^4
Leptospirillum ferrooxidans	10^1-10^5	10^1-10^4	1-10^3	1-10^2
Bacteria capable of oxidizing S^o at neutral pH (*T. thioparus, T. neapolitanus, T. denitrificans*)	0-10^3	10^2	0-10^3	0-10^3
Moderately thermophilic chemolithotrophic bacteria (*Thiobacillus caldus, Sulfobacillus thermosulphidooxidans*)	0-10^3	0	0-10^1	0-10^1
Extremely thermophilic chemolithotrophic archaea (*Sulfolobus, Acidianus*)	0-10^2	0	0	0
Saprophytic bacteria (*Acidiphilium, Pseudomonas, Bacillus, Aerobacter, Caulobacter*, etc.)	1-10^3	10^1-10^3	1-10^4	10^1-10^3
Fungi and yeasts (*Cladosporium, Penicillium, Trichosporon, Rhodotorula*, etc.)	0-10^3	1-10^3	0-10^3	1-10^3
Algae	0-10^2	0-10^1	0-10^1	0-10^1
Protozoa	0-10^2	0-10^1	0-10^1	0-10^1

Note: The genus *Thiobacillus* for the acidophilic bacteria was later transformed in the genus *Acidthiobacillus*.

Fig. 1. A general view of the operation for copper dump bioleaching at Vlaikov Vrah mine

Fig. 2 - 3. A general view of the operation for copper dump bioleaching at Vlaikov Vrah mine

Fig. 4 - 5. The bioleaching of dumps of low-grade copper ores near Radovish, Macedonia

Three commercial-scale operations for in situ underground bioleaching of uranium existed until 1991 (Fig. 6 and 7). The largest of these operations was put into action in 1984 near the town of Simitli. Leach solutions containing sulphuric acid, ferric ions and chemolithotrophic Fe^{2+}-oxidizing bacteria (*Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*) were grown in aerated bioreactors by the well-known BACFOX (BACterial Film OXidation) process, in which biofilms of the above-mentioned bacteria formed on inert plastic surfaces together with a matrix of jarosites oxidized ferrous ions to the ferric state in diluted sulphuric acid (at pH of about 1.7 - 1.9). The leach solutions prepared in this way were injected through a large number of boreholes to a depth greater than 100 meters below the earth surface to reach the uranium-bearing ore layers. The sulphuric acid dissolved the hexavalent uranium present in the ore to the soluble uranyl sulphate, and bacteria and ferric ions oxidized the tetravalent uranium in the ore to the hexavalent state, which dissolved in the sulphuric acid. The pregnant leach solutions containing the dissolved uranium, the ferrous ions generated by the reduction of the ferric ions at the oxidation of uranium, as well as the viable bacteria, were recovered by a set of production boreholes and directed to the surface for uranium extraction by ion exchange resins. After the removal of uranium, the leach solutions were regenerated in the BACFOX reactors and were recycled to the uranium ores. The uranium retained by the ion exchange resins was solubilized and then recovered as a primary uranium-bearing concentrate (the so called "yellow cake").

Several pilot-scale operations for bacterial pretreatment of gold-bearing sulphide ores to liberate the gold (and silver) encapsulated in the sulphide matrix were constructed and tested by the heap leaching technique using mixed cultures of acidophilic chemolithotrophic bacteria (Figure 8). The amount of ores tested in the relevant heaps largely varied (from 8 to about 4600 tons). The treatment was carried out until the sulphide oxidation reached the extent at which almost the whole quantity of gold was exposed and liberated from the sulphides. The ores pretreated in this way were washed by water to remove the residual acidity and then were leached by means of thiosulphate at a slightly alkaline pH (usually within the range of 8 - 9) to solubilize gold and silver. A real commercial-scale operation of this type was constructed and efficiently used at the Elshitza mine in the period 1993-96. Some oxide ores containing mainly well exposed free gold were subjected directly to leaching by thiosulphate (Groudev, 1987; 1999; Groudev *et al.*, 1999; Spasova *et al.*, 2015).


Fig. 6 - 7. *In situ* and heap bioleaching of uranium ores in Curilo deposit

It must be noted, however, that much higher extractions of gold (in some cases even about 95%) were achieved by microbial pretreatment and subsequent leaching (by thiosulphate or cyanide) of concentrates obtained by dressing (usually by flotation) of the relevant gold-bearing ores. The microbial pretreatment was carried out by continuous leaching in systems consisting usually of three or four aerated and stirred bioreactors with acidified leach solutions containing nutrients, chemolithotropic microorganisms and concentrate at initial pulp densities of 10 - 20 %. Different microbial cultures at different temperatures were tested to establish the

Fig. 8. Pilot-scale operation for microbial pretreatment and leaching of gold-bearing sulphide ores

optimum pretreatment conditions: mixed mesophilic cultures, containing mainly *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*, at 30 - 37 °C; mixed cultures of moderately thermophilic bacteria, mainly of *Sulfobacillus spp.* and *Acidithiobacillus caldus* at 45 - 55 °C; mixed cultures of extremely thermophilic archaea, containing mainly species of the genera *Sulfolobus*, *Acidianus* and *Metallosphaera*, at 68 - 80 °C (Spasova *et al*., 2015) (Tables 3 and 4).

It is possible to say that samples from practically all Bulgarian gold deposits (more than 10 in numbers) were tested by the above-mentioned methods for gold (and silver) extraction.

Pilot-scale operations were also constructed and efficiently used for removal of iron impurities from quartz sands, kaolins and clays by leaching in stirred reactors with culture solutions containing oxalic and citric acids produced by *Aspergillus niger* grown on a nutrient medium with sucrose as a source of carbon and energy; for production of high-quality kaolins by treatment with “silicate” bacteria of the species *Bacillus mucilaginosus* and *Bacillus circulans*, (Table 5) and the operation for microbial enhanced oil recovery in Tulenovo, located near the Black Sea coast (Groudev *et al*., 1985, 1989; Groudev, 1987, 1999; Karavaiko *et al*., 1988).

Several activities in the field of biohydro-metallurgy were connected with relevant environmental problems: prevention of the generation of toxic acid drainage; treatment of waters polluted by heavy metals, radionuclides, arsenic, oil, organochemicals, etc. by means of active and/or passive systems (Table 6 and Figures 9 and 10); treatment of soils polluted with the above-mentioned pollutants by different reactor, heap and in situ techniques (Tables 7 - 9); bioremediation of post-mining landscapes (Georgiev *et al*., 2013; 2014; Groudev *et al*., 2005, 2010; Nicolova *et al*., 2014) and recovery of valuable components from technological wastes (Spasova *et al*., 2007).

The largest operations of this type were constructed and used in the uranium deposit Kurilo located within a relatively short distance of Sofia (Table 8). Uranium was recovered for a long period of time from this deposit and after the end of the recovery acid drainage waters containing radionuclides, heavy metals (copper, zinc, cadmium, lead, nickel, cobalt, iron, manganese), arsenic and sulphates were generated in concentrations usually much higher than the relevant permissible levels for waters intended for use in agriculture and/or industry. Different passive systems such as natural and constructed wetlands (Fig. 9), alkalizing limestone drains, permeable reactive multibarriers (Table 6 and Fig. 10), reactive in situ zones, rock filters, were used separately or in different combinations to treat the polluted waters. The most efficient in this respect was a multicomponent system consisting of a permeable multibarrier (with an alkalizing lime-

Table 3. Effect of the prior bacterial oxidation of sulphides on the extraction of precious metals during the subsequent leaching

Contenet of sulphidic sulphur, g	Degree of sulphide oxidation, %	Extraction of precious metals, %	
		Au	Ag
42.8	0	23.0	19.4
38.5	10	41.0	32.5
33.8	21	62.2	50.3
25.3	41	81.1	70.7
20.1	53	90.5	79.0
16.3	62	93.2	83.3
12.4	71	94.1	85.1

Table 4. Data about the continuous - flow microbial leaching of a gold - bearing pyrite concentrate

Parameters	Values
Pulp density, %	10
Residence time, h	768
Dilution rate, h^{-1}	0.00595
Sulphidic sulphur oxidation, %	62
Sulphidic sulphur oxidation rate, g/l	0.1577
Sulphate ions generated during the leaching, g/l	79.5
Sulphate generation rate, mg/l.h	473.21
Iron content in the effluent, g/l	23.90
Iron extraction rate, mg/l.h	142.26
Solid residue after leaching, g	62.8
Weight losses, %	37.2

Table 5. Effect of the way of microbial treatment on the ceramic properties of kaolin

Parameters	Before treatment	After treatment	
		By contact in humid medium	By leaching with stirring
Al_2O_3, %	32.7	32.3	25.0
SiO_2, %	51.2	50.7	40.1
Fe_2O_3, %	1.16	0.82	0.78
TiO_2, %	0.24	0.23	0.15
CaO, %	0.82	0.77	0.24
Loss on drying, %	0.35	0.91	1.25
Bending strength, MPa	1.4	3.5	2.1
Plasticity, %	32.3	36.9	33.6
Drying shrinkage, %	3.5	4.2	3.9
Firing shrinkage at 960 °C, %	4.2	4.6	3.9
Water saturation capacity at 960 °C, %	28.0	30.2	27.5
Formation water, %	32.1	34.1	32.5
Whiteness on drying, %	76.5	82.0	82.8
Size fractions, % :			
>60 µm	0.02	0.02	0.02
10-60 µm	1.25	1.14	1.07
5-10 µm	14.1	11.32	14.01
1-5 µm	33.95	31.12	33.7
<1 µm	50.68	56.4	51.2

stone drain and an anoxic section for microbial sulphate reduction, biosorption and additional chemical neutralization) and a constructed wetland. Efficient removal of pollutants was achieved by means of this system during a period of about ten years and during the different climatic seasons, even during the cold winter months at water and ambient temperatures close to 0 °C (Groudev *et al*., 2008).

A very efficient operation for cleaning acid-leached cinnamonic forest soil heavily contaminated with radionuclides (mainly uranium and radium) and non-ferrous metals (mainly copper, zinc and cadmium) was constructed and tested in situ under real field conditions using the activity of the indigenous soil microflora. This activity was enhanced by suitable changes of some essential environmental factors such as pH and water, oxygen and nutrient contents of the soil (Figure 11). The treatment was connected with solubilization and removal of contaminants from the top soil layer (horizon A) due to the joint action of the soil microorganisms (mainly of the acidophilic chemolithotrophic bacteria) and leach solutions (diluted sulphuric acid) used to irrigate the soil. The dissolved contaminants were transferred through the drainage soil effluents to the deeply located soil subhorizon B_2 where they precipitated as the relevant insoluble forms (uranium as uraninite, and the non-ferrous metals as the relevant sulphides) under the action of the sulphate-reducing bacteria inhabiting that soil subhorizon. The effluents from the subhorizon B_2 containing much lower concentrations of inorganic contaminants, but enriched in dissolved biodegradable organics, were subjected to additional treatment in a constructed wetland for removing these residual contaminants. However, it was demonstrated that an efficient treatment of the effluents from the subhorizon B_2 could be achieved by a microbial fuel cell in which the water clean-up was connected with electricity generation (Groudev *et al*., 2010; 2014) (Table 10).

Some other essential problems in the field of

Table 6. Data about the acid mine drainages in the uranium deposit Curilo before and after their treatment by means of the permeable reactive barrier

Parameters	Acid mine drainage	Multibarrier effluents	Permissible levels
Temperature, °C	(+1.2) - (+25.1)	(+1.4) - (+27.5)	-
pH	2.42 - 4.25	6.22 - 7.83	6 - 9
Eh,mV	(+290) - (+597)	(-140) - (-280)	-
Dissolved O_2, mg/l	1.7 - 6.0	0.2 - 0.4	2
TDS, mg/l	930 - 2972	545 - 1827	1500
Solids, mg/l	41 - 159	32 - 104	100
DOC, mg/l	0.5 - 2.1	51 - 159	20
SO_4^{2-}, mg/l	532 - 2057	275 - 1225	400
U, mg/l	0.10 - 2.75	< 0.05	0.6
Ra, Bq/l	0.05 - 0.5	< 0.03	0.15
Cu, mg/l	0.79 - 5.04	< 0.2	0.5
Zn, mg/l	0.59 - 59.8	< 0.2	10
Cd, mg/l	< 0.01 - 0.1	< 0.004	0.02
Pb, mg/l	0.08 - 0.55	< 0.02	0.2
Ni, mg/l	0.17 - 1.49	< 0.03 - 0.1	0.5
Co, mg/l	0.12 - 1.22	< 0.03 - 0.1	0.5
Fe, mg/l	37 - 671	0.5 - 9.5	5
Mn, mg/l	2.8 - 79.4	0.5 - 5.2	0.8
As, mg/l	0.15 - 0.59	< 0.01	0.2

Table 7. Bioremediation in situ of contaminated soils (horizon A) in uranium deposits in Bulgaria

Parameters	U	Ra	Cu	Zn	Pb	Cd
	Contents of contaminants, ppm					
Vromos Bay deposit						
Before treatment pH - 7.5	41	280	611	251	268	7.3
After treatment pH - 7.3	7.1	55	242	99	109	1.7
Permissible levels (pH>7.0)	10	60	280	370	80	3.0
Kurilo deposit						
Before treatment pH - 4.40	68	510	190	215	122	4.5
After treatment pH - 3.21	8.0	65	35	48	55	0.4
Permissible levels (pH<4.3)	10	65	20	30	60	0.5

Fig. 9. Treatment of acid mine drainage by constructed wetland in the uranium deposit Kurilo

Fig. 10. Treatment of acid mine drainage by means of permeable reactive multibarrier in the Kurilo deposit

biohydrometallurgy which are currently developed in Bulgaria can be also mentioned:

•Test systems of the "push-pull" and "push-through" types for studying the possibilities for efficient *in situ* underground bacterial leaching of non-ferrous metals and uranium from ores were developed. Such systems were prepared for application in the deposit located near Radovish, Macedonia, in connection with the possible future copper bioleaching operation, which will be the largest operation of this type in Europe. These test systems can be also applied in other ore deposits intended for underground in situ bioleaching.

•Characterization of the processes of dump and heap bioleaching of copper in the commercial-scale operation near Radovish, Macedonia, the largest operation of its type in Europe, was carried out with recommendations for stimulation of the *in situ* bacterial activity and improvement of the processing of the copper-bearing pregnant solutions.

•Systems connecting the bioremediation of polluted waters and soils with electricity or hydrogen generation were developed on the basis of specific microbial fuel (or electrolytic) cells. Elec-

Table 8. Toxicity of the soils in uranium deposits before and after the treatment and remediation

Test - organisms	Toxicity		
	Before treatment (pH 7.52)		After treatment and remediation (pH 7.81)
Soil in the Vromos Bay area			
Bacillus cereus	40		NOEC at 100
Pseudomas putida	40		100
Lactuca sativa	50		NOEC at 100
Trifolium repens	50		NOEC at 100
Avena sativa	30		90
Lumbricus terrestris	30		90
Soil in the Kurilo deposit			
	pH 4.4	pH 3.2	pH 4.4 (by lime)
Bacillus cereus	40	40	80
Pseudomas putida	30	40	100
Lactuca sativa	40	40	NOEC at 100
Trifolium repens	40	50	NOEC at 100
Avena sativa	30	40	90
Lumbricus terrestris	20	10	60

Notes: The toxicity was expressed as the lowest observed effect concentration (LOEC) at different contents (in wt. %) of contaminated soil in a mixture with clean soil at the relevant type: NOEC - Not observed effect concentration.

Table 9. Bioremediation in situ of soil in the Tulenovo oil deposit polluted by oil and toxic heavy metals

Pollutants	Content in the soil		Pollutant removal, %
	Before treatment	After treatment	
Hydrocarbons, g/kg dry soil			
Parafins	3.8	0.01	99.7
Naphthens	10.6	0.08	99.3
Aromatics + polars	3.6	1.61	55.3
Total hydrocarbons 18.0	18.0	1.7	90.6
Heavy metals and arsenic, mg/kg dry soil			
Lead	185	64.0	65.4
Cadmium	5.1	2.3	54.9
Copper	262	140.0	46.6
Zinc	212	95.0	55.2
Arsenic	32	18.0	43.7

Table 10. Data about the electricity generation from wastewater by means of microbial fuel cell (MFC)

Parameters	Values
Influents in the MFC	
Chemical oxygen demand, mg/l.day	2500 - 3000
pH	6.8 - 7.1
Eh	(-210) - (-230)
Temperature, °C	32
COD used in the MFC, mg/l.day	2300 - 2700
% from the initial	90 - 92
Current density, mA/cm^2	0.08 - 0.18
Voltage of the open circuit, mV	86 - 170
External resistance	20 - 80
Power, mW/m^2	770 - 980

Fig. 11. *In situ* treatment of soil polluted with heavy metals

trochemically active bacteria able to transfer electrons directly to the anode electrode were selected and used in some of these systems (Groudev *et al.*, 2010; 2014).

•Experimental leaching of certain precious and rare elements (such as the platinum group of metals, gold, silver, rhenium) from different ores, mineral processing wastes, and pyrometallurgy, by combined chemical and biological treatments using different heterotrophic and chemolithotrophic microorganisms.

References

Gaidarjiev, S. S., S. N. Groudev, F. N. Genchev (1975). Direct mechanism of bacterial oxidation of sulphide minerals. In: Proceedings of the IX International Mineral Processing Congress, Cagliari, Italy, April 1975, pp. 945-958.

Genchev, F. N., S. N. Groudev, S. S. Gaidarjiev, G. K. Gushterov, Z. P. Todorov (1978). Microbial mutualism in the leaching of a sulphide ore, in: Proceeding of the Fourth Congress of the Bulgarian Microbiologists, Sofia, 21-24 November 1977, Sofia, 1978, III, pp. 241-244.

Georgiev, P. S., I. I. Spasova, M. V. Nicolova, S. N. Groudev (2013). In situ treatment of polluted soil with power generation. In: Proceedings of the 8th Symposium "Recycling Technologies and Sustainable Development", Borsko Jezero, Serbia, 3 - 5 July 2013, M. Z. Trumic and G. Bogdabovic, eds., pp. 253-258.

Georgiev, P. S., S. N. Groudev, I. I. Spasova, M. V. Nicolova (2014). Ecotoxicological characteristic of a soil polluted by radioactive elements and heavy metals before and after its bioremediations. *J. Geochem. Explor.* **142**: 122-129.

Groudev, S. N., F. N. Genchev (1976). Monitoring of the microbial population in the operation for bacterial leaching in Vlaikov Vrah. In: Proceedings of the conference “Problems of the Present - Day Methods for Management in the Mining Industry”, Sofia, pp. 111-120.

Groudev, S. N., F. N. Genchev, S. S. Gaidarjiev (1978). Observations on the microflora in an industrial copper dump leaching operation. In: Metallurgical Phenomena, L. E. Murr, A. E. Torma and J. A. Brierley, eds., Academic Press, New York, 1978, pp. 253-274.

Groudev, S. N., V. I. Groudeva, F. N. Genchev, D. I. Mochev, E. Petrov (1985). Biological removal of iron from quartz sands, kaolins and clay, in: Proceedings of the XVth International Mineral Processing Congress, Cannes, June 1985, vol. II, Flotation, Hydrometallurgy. pp. 378-387.

Groudev, S. N. (1987). Use of heterotrophic microorganisms in mineral biotechnology, *Acta Biotechnol.* **7**: 299-306.

Groudev, S. N., V. I. Groudeva, S .G. Buchvarov, T. Datskova (1989). Improvement of the ceramic properties of kaolins by means of microbial treatment. In: Proceedings of the Second World Congress on Non-metallic Minerals, Beijing, China, 17-21 October 1989, vol. III, pp. 722-726.

Groudev, S. N., V. I. Groudeva (1993). Microbial communities in four industrial copper dump leaching operations in Bulgaria. *FEMS Microbiol. Rev.* **11**: 261-268.

Groudev, S. N. (1999). Biobeneficiation of mineral raw materials. *Metall. Process.* 1999, **16 (4)**:19-28.

Groudev, S. N., I. I. Spasova, I. M. Ivanov (1999). A chemical and biological heap leaching of an oxide gold - bearing ore. *Physicochem. Probl. Miner. Process*. **33**: 55-61.

Groudev, S. N., M. V. Nicolova, I. I. Spasova, V. I. Groudeva, P. S. Georgiev, L., Diels (2005). Bioremediation acid drainage by means of a passive treatment system. In: Proceedings of the 16th International Biohydrometallurgy Symposium, Cape Town, South Africa, 2005, S. T. L. Harrison, D. E. Rawlings and J. Petersen, eds., pp. 473-478.

Groudev, S. N., P. S.Georgiev, I. I. Spasova, M. V. Nicolova (2008). Bioremediation of acid mine drainage in a uranium deposit, *Hydrometallurgy*. **94**: 93-99.

Groudev, S. N., I. I. Spasova, M. V. Nicolova. P. S. Georgiev (2010). *In situ* bioremediation of contaminated soils in uranium deposits, *Hydrometallurgy*, **104**: 518-523.

Groudev, S. N., P. S.Georgiev, I. I. Spasova, M. V. Nicolova (2014). Decreasing the contamination and toxicity of heavily contaminated soil by *in situ* bioremediation. *J. Geotech. Explor.* **144**: 374-379.

Groudeva, V. I., K. Krumova, S. N. Groudev (2007). Bioleaching of rich-in-carbonates copper ore at alkaline pH. *Adv. Mat. Res.* **20-21:** 358-361.

Groudeva, V. I., D. V. Vasilev, G. I. Karavaiko, S. N. Groudev (2009). Changes in an ore dump during a 40- year period of commercial - scale and spontaneous natural bioleaching. *Adv. Mat. Res.* **71-73**: 373-376.

Groudeva, V. I., D. V. Vasilev, G. I. Karavaiko, S. N. Groudev (2011). Inoculation of chemolithtrophic bacteria into heaps consisting of a freshly excavated copper mixed ore, in: Biohydrometallurgy: Biotech Key to Unlock Mineral Resources Value, Proceedings 19th International Biohydrometallurgy Symposium, Changsha, China, 18 - 22 September 2011, G. Qui, T. Jiang, W. Qin, X. Liu, Y. Yang and H. Wang eds., **1:** 533-539, Central South University Press, Changsha, China.

Groudeva, V. I., M. V. Iliev, R.V. Ilieva, S. N. Groudev (2013). Bioleaching of a copper sulphide ore at different temperatures. *Adv. Mat. Res.* **825**: 258-261.

Karavaiko, G. I., S.N., Groudev (1985). Biogeotechnology of Metals. Centre for International Projects GKNT, Moscow.

Karavaiko, G. I., G. Rossi, A. D Agate, S. N. Groudev, Z. A. Avakyan (1998). in: Biogeotechnology of Metals. Mannual, Centre of International Projects GKNT, Moskow.

Nicolova, M. V., I. I. Spasova, P. S. Georgiev, S. N. Groudev (2011). Treatment of activated sludge for utilization in agriculture and for recovery of non-ferrous metals. In: Proceedings of the International Symposium “The Environment and Industry”, Bucharest, Romania, vol. I, pp. 139-147.

Nicolova, M. V., I. I. Spasova, P. S. Georgiev, S. N. Groudev (2014). Bioremediation of polluted waters in a uranium deposit by means of a passive system, Annual of the University of Mining and Geology, Sofia, 2014, vol. 57, part II, pp. 133-136

Spasova, I. I., M. V. Nicolova, P. S. Georgiev, S. N. Groudev (2007). Biological and chemical leaching of metals from electronic scrap. In: Proceedings of the XII Balkan Mineral Processing Congress, Delphi, Greece, 10 - 14 June 2007, G. N. Anastassiakis, ed., pp. 571-575.

Spasova, I. I., M. V. Nicolova, P. S. Georgiev, S. N. Groudev (2015). Comparative variants of microbial pretreatment of a gold - bearing sulphide concentrate under different growth and technological conditions. In: Proceedings of the XVI Balkan Mineral Processing Congress, Belgrade, Serbia, June 17 - 19, 2015, vol. II, pp. 787-790.