Application of *Acidithiobacillus ferrooxidans* VKM B-3655 for bioleaching silicate ore

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Abstract. Acid metal bioleaching is common and classical for nickel recovery from the sulfide refractory ores: various microorganisms can oxidize sulfides as energetic substrates. Silicate nickel ores are widespread in the world but their bioleaching is more problematic because silicates cannot serve as energetic substrates. Meanwhile iron in the silicate nickel ores presents a significant part and can be used by some acidophilic autotrophic microorganisms for the ore destruction. In model experiments, we studied application of acidophilic autotrophic sulfur- / iron-oxidizing bacteria *Acidithiobacillus ferrooxidans* VKM B-3655 for the nickel recovery from the nickel-bearing silicate ore with high content of iron. The strain was selected by its ability of iron oxidation and resistance to arsenic which also presented in the ore. We also evaluated possibility to stimulate the bioleaching with formate as additional energetic substrates or with persulfate for increasing the medium redox. It was shown that low concentrations of sodium formate (0.3%) and persulfate (0.1%) stimulated growth of *A. ferrooxidans* while higher persulfate concentration (1.0%) stimulated the ore bioleaching.

1 Introduction

Nickel mining is of great importance for the global industry as a main source of this valuable metal. In this regard [1], promising mineral raw materials include not only easily oxidized sulfide ores, but also difficult to develop silicate ones. It is necessary to mention that the low-grade ore have a Ni content only of between 0.6-1.2%.

Various groups of microorganisms have been tested for their ability to leach silicate ores. Commonly, chemolithoautotrophic bacteria are the most convenient for industry, since they do not require any introduction of additional substrates as sources of energy and carbon. However, in sulfide ores, sulfides can serve as an energy source, while silicates are not using energy sources in lateritic ores. In this regard, the main efforts in the bioleaching of nickel from silicate ores have been focused on heterotrophic organisms (including fungi) which form leaching organic acids [2-4].

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As a result of such microbiological studies, a strong understanding has been established in world practice that heterotrophic microorganisms forming organic metabolic acids are preferable for nickel leaching from silicate ores [5, 6].

Some published experiments about leaching of silicate ore with autotrophic iron-oxidizing bacteria *Acidithiobacillus ferrooxidans* were accompanied with some doubts on perspectives of this leaching [7]. At the same time, the high content of iron, including ferrous iron, in lateritic ores suggests that such a process could be possible for certain types of silicate mineral raw materials.

This paper describes attempts to use autotrophic iron-oxidizing bacterium *A. ferrooxidans* to leach nickel from lateritic ore. Since the ore contained arsenic, an arsenic-resistant strain VKM B-3655 was used. In the course of the work, additional additives were used to increase both intensity of bacterial growth and leaching activity of bacteria.

2 Materials and methods

2.1 Silicate ore

Data on the composition of silicate ore used in this work are presented in Table 1. Main sulfide mineral of the concentrate was silicate. The ore samples and information on its composition were provided by Resources and Technologies Ltd. and supplemented by Test-Pushchino LLC, Russia. Mechanical composition of the samples was presented with a fine (<1 mm) fraction.

As a classical laterite ore, the mineral material contained high concentration of iron and aluminum oxides and about 1% of nickel.

Table 1. Chemical components of the silicate iron-nickel ore, mg/kg.

Components	Content, mg/kg
Fe total	402278
Si	196000
Al	28227
Cr	11832
Ni	9659
Mg	2243
Mn	1107
S total	<1000
S sulfate	<1000
S sulfides	<1000
Ca	873
Co	666
K	525
Zn	353
Na	301
P	124
As	107
Cu	63
Cd	31
Ba	30

Pb	24
Sr	8
O	No data
Cl	No data
TOTAL	654451

2.2 Microorganisms and culturing medium

The used strain was *A. ferrooxidans* VKM B-3655 - acidophilic chemolithoautotrophic bacteria initially isolated from the gold-bearing pyrite-arsenopyrite concentrate obtained from the ore of the Bakyrchik deposit (Republic of Kazakhstan) [8, 9]. The strain was selected by its following properties: i) autotrophy as ability to grow without organic substrates, ii) ability to oxidize iron as energetic substrate, and iii) resistance to arsenic that presented in the ore composition [10]. The strain was cultivated in the Silverman-Lundgren 9K medium [11] containing the following components (g/L): $(NH_4)_2SO_4$ - 3, KCl - 0.1, K_2HPO_4 - 0.5, $MgSO_4 \times 7H_2O$ – 0.5, $Ca(NO_3)_2$ - 0.01, $FeSO_4 \times 7H_2O$ - 44.22, 10N H_2SO_4 - 4.5 mL/L. The used reagents were produced by Sigma-Aldrich/Merck (Germany) and Reakhim (Russia).

2.3 Cultivation with additives and bioleaching experiments

Effects format and sodium persulfate were studied using 9K medium supplemented with different concentrations of these additives on bacterial activity. Experiments were based on comparison of bacterial growth in the 9K medium or ore bioleaching with and without (blank) additives. The inoculum was 10 mL of the starting culture, 106-107 cells/mL.
Growth of the culture was evaluated by optical density (OD) measured with Spekol 221 spectrophotometer (Carl Zeiss Industrielle Messtechnik GmbH, Germany) at the wavelength 600 nm. Data present the average of three replicated experiments; variations were within 5%.

Bioleaching experiments with the iron-containing silicate ore were performed using 9K medium without ferrous sulfate. A sample of a fine fraction (<1 mm) of a solid mineral raw material and a leaching solution were placed in a 750 mL shake flask. Each flask contained 200 mL of the 9K medium as the leach solution and 20 g of the concentrate, i.e., the solid and liquid phases were in ratio 1:10. All experiments were carried out in triplicate under aerobic conditions at 28°C using the IBPM shaker (IBPM RAS, Russia) at 180 rpm. Thus, the experiments modeled stirred tank / vat leaching at a constant temperature.

2.4 Chemical analysis

Content of leached iron in the solution was determined by spectrophotometry according to GOST 13195-73. The method is based on the formation of a complex compound during the interaction of iron ions with potassium ferricyanide in an acidic medium. The optical density (OD) was measured at a wavelength of 540 nm using Schimadzu UV-1800 spectrophotometer (Schimadzu, Japan). Iron concentration in the solution was determined by the constructed calibration curve.

Nickel concentration in the solution was measured with spectrophotometer Schimadzu UV- 1800 (Japan) using specific color reaction of nickel with dimethylgloxime. It has already been shown earlier [12] that this analysis has a high specificity and a modest sensitivity at very low concentrations (on human skin etc). To prevent any iron interfering, the nickel analyses were carried with an excess of dry dimethylglyoxime and ammonia until alkaline pH. The value of optical density (OD) was measured at a wavelength of 460 nm.

Concentrations of nickel were calculated according to the constructed calibration graph, the paired linear regression showed coefficient of determination R2 equal 0.9933, which indicates its reliability.

2.5 Statistics

The experiments and analyzes were performed in triplicate. All data were statistically checked using ANOVA / Excel Microsoft. The results presented in the article showed a high level of reliability (p> 0.99).

3 Results and discussion

3.1 Bioleaching iron and nickel from silicate ore by *A. ferrooxidans*

Chemical composition of the treated ore was known only by its elemental analysis, thus it was unknown which shares of metals could be extracted with the hydrometallurgy. We compared chemical leaching Fe and Ni with the sterile acid medium 9K and inoculated medium (Table 2).

It was obvious that the intensity of bioleaching was higher than that of chemical alone. Nickel extraction with bacteria increased up to 20% and iron extraction up to 36%. At the same time, taking into account the initial content of nickel in the ore, it was necessary to take measures to stimulate the process.

Table 2. Leaching and bioleaching the silicate ore presented as Fe- Ni- concentrations in the solution (28°C, 180 rpm).

Time, days	**Sterile blank leaching**			***A. ferrooxidans* bioleaching**			**Bioleaching / leaching, %**	
	pH	**Ni, mg/L**	**Fe, mg/L**	**pH**	**Ni, mg/L**	**Fe, mg/L**	**Ni**	**Fe**
1	2.1	15.0±0.3	1355±10	2.1	16.7±0.4	1855±9	111.3	136.9
3	2.1	15.5±0.5	1470±9	2.1	16.8±0.5	1868±8	108.4	127.1
6	2.0	15.7±0.2	1500±12	2.0	16.9±0.5	1900±9	107.6	126.7
14	2.0	16.6±0.1	1650±12	1.9	20.0±0.1	2075±11	120.5	125.8

3.2 Stimulation of the *A. ferrooxidans* growth and bioleaching with format

Main approach was based on the known data about stimulation of *Thiobacillus ferrooxidans* (later transferred to *A. ferrooxidans*) with formate as an energy substrate [13]. According to Pronk. Format was preferred for use in chemostatic cultivation at a final concentration of 100 μM format to avoid the toxic effect of organics on the culture. In the periodic cultivation of the strain, however, there is an accumulation of formate and the suppression of the activity of the culture as a result.

Our experiments confirmed the growth stimulation for *A. ferrooxidans* VKM B-3655 in the 9K medium with optimum concentration equal to 0.3% format [10]. Example is presented in the Table 3: the biomass density reached the highest value on the 4th day without format and on the 2nd day with the additive.

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Table 3. Formate effect on dynamics of the *A. ferrooxidans* VKM B-3655 growth as the biomass density in the medium 9K (28°C, 180 rpm).

Format concentration, %	**Optical density of the medium inoculated with *A. ferrooxidans***			
	Time, days			
	1	**2**	**3**	**4**
0.0 (blank)	0.06±0.01	0.10±0.01	0.24±0.03	0.29±0.01
0.3	0.12±0.02	0.32±0.03	0.32±0.03	0.32±0.03

Bacterial growth and bacterial participation in ore leaching are two different processes despite of the fact that they both are based on bacterial activity. Bacterial growth is provided on increasing biomass by consumption of carbon and energy sources while bacterial leaching can go on by immobilized cells using energy sources. By this reason, we studied effects of the formate supplementation on the bacterial Ni- and Fe-extraction from the silicate ore into the solution (Table 4). At the formate concentration 0.3%, the nickel bioleaching increased up to 40% and iron bioleaching up to 23%.

Table 4. Formate effect on the Ni- and Fe-bioleaching from silicate ore by *A. ferrooxidans* VKM B-3655 (28°C, 180 rpm). The leaching data are presented as Fe and Ni concentrations in the solution.

Time, days	**Format, %**						**Bioleaching with / without formate, %**	
	0.0 (blank)			**0.3**				
	pH	**Ni, mg/L**	**Fe, mg/L**	**pH**	**Ni, mg/L**	**Fe, mg/L**	**Ni**	**Fe**
1	2.1	16.7±0.3	1855±12	2.1	17.7±0.1	1995±5	106.0	107.5
3	2.1	16.8±0.2	1860±11	2.1	19.5±0.2	2275±10	116.1	122.3
6	2.0	16.9±0.2	1900±13	2.0	19.5±0.1	2345±12	115.4	123.4
9	1.9	17.6±0.4	2050±8	2.0	19.5±0.1	2316±12	110.8	113.0
15	1.9	20.0±0.5	2075±11	2.0	28.0±0.1	2320±10	140.0	111.8

3.3 Stimulation of the *A. ferrooxidans* growth and bioleaching with sodium persulfate

The intensity of chemical reactions, including the interaction of acids with metal compounds of ores, depends not only of pH but also of the solution redox. It was shown that during chalcopyrite leaching by Acidiphilium, redox increased from approximately 600 mV up to about 900 mV [14]. These data are in a good accordance with suggestion to regulate the bioleaching process with redox of the medium [15]. In this regard, leaching of metals from mineral raw materials using acids could be increased with an additional oxidizing agent. Our former investigations showed that persulfate salts effected both bioleaching [10] and chemical leaching [16]. According our experiments with *A. ferrooxidans* VKM B-3655 in the 9K medium, 0.1 % persulfate stimulated growth of the strain with time but the higher concentrations suppressed the growth [10]. An example of the experiments is shown in the Table 5.

Table 5. Persulfate effect on dynamics of the *A. ferrooxidans* VKM B-3655growth as the biomass density in the medium 9K (28°C, 180 rpm).

Persulfate concentration, %	**Optical density of the medium inoculated with A. ferrooxidans**			
	Time, days			
	1	**2**	**3**	**4**
0.0 (blank)	0.10±0.01	0.08±0.01	0.22±0.01	0.28±0.01
0.1	0.07±0.01	0.08±0.01	0.22±0.01	0.41±0.01

0.2	0.07±0.01	0.07±0.01	0.18±0.01	0.25±0.01
0.3	0.08±0.01	0.08±0.00	0.08±0.01	0.12±0.01

Should be considered that the discovered effect of persulfate on bacterial growth in the culture medium is not identical to its effect on possible bioleaching in presence of ore. Interaction of the oxidizer with the mineral compounds can reduce its own toxic effect and increase an availability of the ore for bacteria. For example, peroxides can oxidize arsenic compounds to arsenate which reacts with iron forming insoluble compound. We studied persulfate effects on the Ni- and Fe-bioleaching from the silicate arsenic-bearing ore inoculated with *A. ferrooxidans*; the results are shown in Table 6.

Table 6. Persulfate effect on the Ni- and Fe-bioleaching from silicate ore by A. ferrooxidans VKM B-3655 (28°C, 180 rpm). The leaching data are presented as Ni and Fe concentrations in the solution.

Time, day	**Persulfate, %**											
	0.0 (blank)			**0.3**			**0.5**			**1.0**		
	pH	**Ni, mg/ L**	**Fe, mg/ L**	**pH**	**Ni, mg/ L**	**Fe, mg/ L**	**pH**	**Ni, mg/ L**	**Fe, mg/ L**	**pH**	**Ni, mg/ L**	**Fe, mg/ L**
5	2.2	17.7	2140	2.0	17.7	2694	2.0	18.6	2974	2.0	18.6	2694
10	2.1	17.7	2274	1.9	22.3	2274	1.9	22.4	2834	1.8	23.2	2835
15	2.1	18.0	2300	1.9	24.0	2300	1.8	25.0	2905	1.8	25.0	3000

The Ni- and Fe-leaching from the arsenic-containing silicate ore was higher with addition of persulfate than without it (Tables 6 and Figure 1). The positive effect was also discovered at concentrations which suppressed A. ferrooxidans in culturing medium. This may indicate that persulfate reacted and was partly neutralized with some components of the ore (arsenic, ferrous iron, etc.). In its turn, it could provide a better substrate availability for the leaching bacteria by degrading the ore.

Bioleaching of valuable metals from mineral raw materials is inherently a combination of chemical and biological processes, where microorganisms and their products are not only catalysts for the metal oxidation of metals but are also mutually related to the ore structure, pH, and redox. Thus, the use of any bioleaching microorganisms is advisable with the right approach to the process which include application of selected additives for optimal yield of target components.

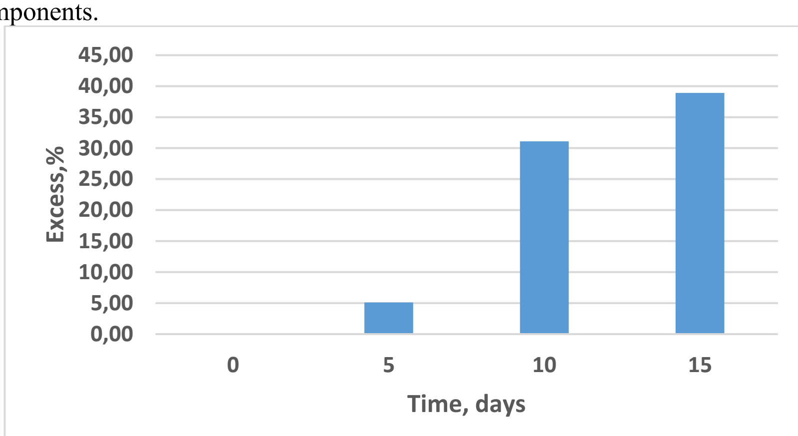

Fig. 1. Excess of Ni bioleaching with 1% persulfate over bioleaching without persulfate from silicate ore by A. ferrooxidans VKM B-3655, %.

4 Conclusions

The paper presents experimental study on the Ni- and Fe-bioleaching by *A. ferrooxidans* VKM B-3655 from silicate iron-nickel ore containing arsenic. The strain was chosen in accordance with its ability to obtain energy from the iron oxidation and its resistance to arsenic. The experiments confirmed possibility of nickel bioleaching from ores of this type.

Due to the low yield of nickel, we also studied use of stimulating supplements, namely: formate as an energy source and persulfate as an additional oxidizing agent. Addition of 0.3% formate increased the bacterial growth rate and increased nickel bioleaching to 40% in two weeks. Addition of 0.1% persulfate accelerated the growth and bioleaching process. Higher concentrations of persulfate inhibited bacterial growth in pure culture but increased the chemo-biological leaching of nickel to 38% in two weeks. The positive effect of 1% persulfate on the Ni leaching is discussed as possible favorable treatment of toxic compounds of the ore.

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