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EXPERIMENTAL ARTICLES

Effect of the Aeration Mode and Yeast Extract on the Oxidation of High-Pyrrhotite Sulfide Ore Flotation Concentrate and on the Composition of the Acidophilic Chemolithotrophic Microbial Community

P. V. Moshchanetskiy[a], T. A. Pivovarova[a], A. V. Belyi[b], A. G. Bulaev[a], V. S. Melamud[a], and T. F. Kondrat'eva[a, 1]

[a] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*

[b] *Polyus Co., Krasnoyarsk, Russia*

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Abstract—Optimal aeration conditions were determined and the effect of yeast extract on biooxidation of high-pyrrhotite sulfide ore flotation concentrate in the course of continuous cultivation of an acidophilic chemolithotrophic microbial community was studied in a line of four sequential laboratory reactors; the aeration rate was 3 L/(L min), yeast extract concentration was 0.02%. The gold recovery level was 96.45% at 2.23% elemental sulfur content in the solid residue. The dominant strains identified in the community responsible for biooxidation were *Acidithiobacillus caldus* OL13-1, *At. caldus* OL13-3 = *At. caldus* OL12-3, and an *'Acidiferrobacter'* strain. Strains *Sulfobacillus thermosulfidooxidans* OL13-2 = *S. thermosulfidooxidans* OL12-1 and *Ferroplasma acidiphilum* OL13-4 = *F. acidiphilum* OL12-4 were isolated in pure culture and identified.

Keywords: sulfide ore flotation concentrate, pyrrhotite, acidophilic chemolithotrophic microbial community, aeration mode, yeast extract, degree of gold recovery

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In most countries (United States, Canada, China, Australia, South Africa) gold is recovered mainly from primary deposits of gold-bearing sulfide ores. Russia is exceptional in this respect, since 75% of gold is recovered from gravel deposits and only 25% from ore deposits. However, 75% of predicted resources and 40–60% of explored resources are concentrated in ore deposits [1].

Biotechnology for processing the ores and concentrates containing noble metals is based on the oxidation of sulfide minerals by acidophilic chemolithotrophic microorganisms (ACM) at low pH (1.7–2.2) within a broad temperature range (28 to 80°C). Biooxidation is carried out to either the complete deterioration of the crystal lattice of sulfide minerals—resulting in the release of gold and silver—or to the degree of oxidation sufficient for unimpeded contact between the metals and the solvent (sodium or potassium cyanides). In the course of this process, sulfide sulfur is oxidized to elemental sulfur and then, through intermediate stages, to sulfate ion. Ferrous iron is oxidized to ferric one. This technology is acceptable for industrial exploitation of large deposits of refractory gold–arsenic sulfide ores, which are impossible or economically inefficient to process by other methods, such as oxidizing roasting or autoclave leaching. These methods, moreover, result in environmental contamination with toxic arsenic and sulfur compounds.

The only gold-recovering factory in Russia (Polyus, Krasnoyarsk krai) using the BIONORD© agitation biotechnology applies it for gold recovery from the concentrates of pyrrhotite–arsenopyrite antimony ore of the Olympiadinskoe deposit [1].

Under acidic conditions pyrrhotite (FeS) is dissolved with proton consumption, resulting in pH increase, which is undesirable for ACM. Considerable amounts of ferrous ions are released into the medium, which, while acting as an energy substrate for microorganisms, decreases the redox potential, thus impeding sulfide oxidation. Sulfide sulfur of pyrrhotite is converted to elemental sulfur via hydrogen sulfide and polysulfides [2]. Pyrrhotite oxidation and microbial oxidation of ferrous iron are described by the following equations:

$$FeS + H_2SO_4 + 0.5O_2 \rightarrow FeSO_4 + S_0 + H_2O, \quad (1)$$

$$4FeSO_4 + O_2 + 2H_2SO_4 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O, \quad (2)$$

$$FeS + 2.25O_2 + 0.5H_2SO_4 \rightarrow 0.5Fe_2(SO_4)_3 + 0.5H_2O, \quad (3)$$

[1] Corresponding author; e-mail: kondr@inmi.ru

$$FeS + Fe_2(SO_4)_3 \rightarrow 3FeSO_4 + S_0. \quad (4)$$

High pyrrhotite content in the oxidized substrate may result in accumulation of excessive elemental sulfur, which is often not oxidized completely by microorganisms [3]. Moreover, precipitation of elemental sulfur at the surface of sulfide minerals prevents their further oxidation [4]. Sulfur reacts with cyanide producing thiocyanate; this reaction consumes an expensive reagent [5]. Incomplete oxidation of sulfide minerals and elemental sulfur also results in high NaCN expenditure for sorption cyanidation and in a loss of gold recovered from solid residues. Thus, the presence of high amounts of easily oxidized pyrrhotite in a mineral concentrate decreases the efficiency of sulfide ore processing.

Selection of a microbial community with a high rate of S_0 oxidation and optimization of the physicochemical parameters of the process will result in an increased rate of biooxidation of pyrrhotite-rich sulfide ore flotation concentrate.

We have previously investigated the oxidation of pyrrhotite-rich sulfide ore flotation concentrate by communities of acidophilic chemolithotrophic microorganisms at different temperatures (35, 40, and 45°C) [6]. The physicochemical parameters of the pulp liquid phase and the results of analysis of the solid residues after biooxidation and cyanidation indicated that the microbial community formed at 40°C carried out the process at a higher rate. Gold recovery at 35, 40, and 45°C was 89.34, 94.59, and 83.25%, respectively. The elemental sulfur content in the solid biooxidation residues of the fourth reactor in the line was low at all temperatures: 2.01, 2.24, and 2.54% at 45, 40, and 35°C, respectively.

At 40°C, the number of microorganisms in the pulp liquid phase was the highest (6.01×10^9 cells/mL). Although the changes in temperature had little effect on microbial species diversity (apart from the absence of *Leptospirillum ferriphilum* at 35°C), the ratio of the species differed considerably. A relatively higher abundance of *Sulfobacillus thermosulfidooxidans* was observed at 40°C.

Investigation of the species and strain diversity of acidophilic chemolithotrophic microbial communities—which play a key role in the oxidation of sulfide minerals in biogeotechnological processes—is, apart from its theoretical interest, important for increasing the rate and efficiency of precious metal recovery from sulfide ores and concentrates.

The goal of the present work was to investigate the effect of the aeration mode and of yeast extract on the rate of biooxidation of pyrrhotite-rich flotation concentrate and on the species composition of the microbial community under continuous cultivation conditions in a line of four sequentially connected reactors.

MATERIALS AND METHODS

The subject of study was pyrite–arsenopyrite flotation concentrate of gold–arsenic sulfide ore with high pyrrhotite content (30–35%) containing the following (%): Fe_{total}, 32.4; Fe_{sulf}, 25.76; As_{total}, 8.45; As_{sulf}, 6.69; S_{total}, 23.5; S_{sulf}, 22.6; S_0, 0.7; Sb_{total}, 2.67; and Ca_{total}, 3.28. Apart from pyrrhotite, sulfide minerals were represented by arsenopyrite, 9–11%; pyrite, 6–8%; and antimonite, 2–3%. Magnetite content was 8–9%. Nonmetallic minerals were quartz, 15–20%; mica, over 10%; and calcite, 6–8%. Gold content was 61 g/t.

A community of acidophilic chemolithotrophic microorganisms with high rate of S_0 oxidation—which was selected in the course of optimization of bacterio–chemical oxidation of flotation concentrate in continuous culture in a line of four laboratory reactors at 40°C [6]—was used as inoculum. Microbial strains identified in the community by molecular genetic techniques were *Acidithiobacillus caldus* OL12-3, *Ferroplasma acidiphilum* OL12-4, *Sulfobacillus thermosulfidooxidans* OL12-1, and *Leptospirillum ferriphilum* OL12-2.

Cultivation conditions in laboratory reactors. Continuous cultivation of the microbial community was carried out in a line of four sequential laboratory reactors (4 L, with 2 L of the pulp at 1 : 5 ratio of the solid and liquid phase) at 40°C, agitation rate (turbine stirrer) 450 rpm, and varying aeration modes. The salt base of the 9K medium [7] without iron salt was used as the liquid phase, with pH in the first reactor adjusted to 2.0. The temperature was maintained with a U-shaped heat exchanger connected to an Elmi TW2.02 thermostat (Latvia). In order to maintain the precise pulp temperature at increased or decreased air flows, two thermostats were used for pairwise heating of reactors 1, 2 and 3, 4 during changes in the aeration mode.

Mass exchange (1600 mL of the pulp) was carried out daily. The total pulp volume was replaced every five days.

Analytical techniques. The values of pH and Eh were determined using a pH-150MA millivoltmeter (Belarus). Eh was expressed relative to the normal hydrogen electrode.

The aeration rate was measured with PM-2.5 GUZ flow rotameter (Russia).

The concentrations of Fe^{2+} and Fe^{3+} ions in the pulp liquid phase were determined by chelatometric titration [8].

The number of microbial cells was determined by direct count under a CX-41 phase contrast microscope (Olympus, Japan) and by inoculating selective media with tenfold terminal dilutions and subsequent incubation at different temperatures. For molecular genetic identification of the components of the community, pure cultures were obtained by repeated transfers of the last growth-positive dilution of the culture

liquid into selective media and subsequent incubation at specified temperatures [9].

The content of sulfide elements in the products of processing of flotation concentrate (solid residue) was determined by the fluorescent X-ray radiometric method after washing off the solid phase for 24 h with 5% HCl at 30°C [10].

Gold content in the solid residue was determined by assay analysis. The degree of gold recovery was determined by sorption cyanidation of the solid residue after biooxidation. Cyanidation was carried out under the following conditions: pulp density 25%, pH 10.2–10.5, 1.0 g/L NaCN, 5% AM-2B anion-exchange resin, cyanidation time 48 h. Gold adsorption to the sorbent was 99–100%.

Investigation of microbial species composition. DNA was extracted from microbial biomass according to [11]. The preparation contained 30–50 μg DNA/mL. RNA was present in trace amounts (below 1% according to electrophoretic analysis).

The universal primer system [12] was used for polymerase chain reaction, cloning of the 16S rRNA gene PCR fragments, and sequencing of the clonal inserts of bacterial origin. The original primer system [13] was used for the PCR and subsequent sequencing of the archaeal component.

In both cases, the amplification mixture (50 μL) contained the following: 1 × BioTaq DNA polymerase buffer (17 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl, 2 mM Mg Cl_2, pH 8.8); 12.5 nmol of each dNTP; 50 ng template DNA; 5 pmol of the relevant primers; and 3 U BioTaq DNA polymerase (Dialat, Russia).

The PCR profile on a Gradient MasterCycler (Eppendorf, Germany) was as follows: first cycle, 94°C for 9 min, 55°C for 1 min, 72°C for 2 min; 30 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min; final cycle of 72°C for 7 min.

PCR products were analyzed by electrophoresis in 2% low-melt agarose gel at 6 V/cm. The PCR products were isolated from agarose and purified using the Wizard PCR Preps reagent kit (Promega, United States) according to the manufacturer's recommendations. Amplification products were cloned using the pGEM-T System reagent kit (Promega, United States) according to the manufacturer's recommendations.

Sequencing of the PCR fragments encoding the 16S rRNA genes was carried out according to Sanger et al. [14] using the BigDye Terminator v. 3.1 reagent kit (Applied Biosystems, United States) on an ABI PRIZM 3730 automatic sequencer (Applied Biosystems, United States) according to enclosed instructions. Both external and internal primers were used for the sequencing; the reading was carried out in two directions.

Primary analysis of the similarity of the 16S rRNA gene sequences was carried out using the BLAST software package (http://www.Blast.ncbi.nlm.nih.gov.blast.cgi). The sequences were aligned with those of the closely related bacterial and archaeal species using CLUSTALW [15]. The sequences were edited using BioEdit (http://jwbrown.mbio.ncsu.edu/BioEdit/bioedit.html).

RESULTS AND DISCUSSION

The effect of aeration on the physicochemical parameters of the pulp liquid phase and microbial species composition was studied in the course of oxidation of pyrrhotite-rich sulfide ore flotation concentrate.

Under industrial conditions, equal amounts of air are supplied to each of sequentially connected reactors [1], with 2–3 L/(L min) being the optimal air flow. In each reactor, microbial oxidation of different, predominantly energy substrates is carried out: sulfide minerals in the first one; sulfide minerals, ferrous iron, and elemental sulfur in the second one; and elemental sulfur with the products of its oxidation in the third and fourth reactors. We therefore expected intensification of biooxidation at increased aeration rates in the individual reactors. The effect of aeration increase to 4 L/(L min) or decrease to 1 L/(L min) in reactors 1–2 and 3–4 compared to uniform aeration of 3 L/(L min) in all reactors was investigated.

Physicochemical parameters of the pulp liquid phase during biooxidation of sulfide ore flotation concentrate under uniform aeration at 3 L/(L min) in all reactors. The data presented in Table 1 show that at uniform aeration of 3 L/(L min) in all reactors, pH gradually decreased from the initial 2.0 to 1.50 in reactor 4; Eh increased from 597 mV in reactor 1 to 696 mV in reactor 4; and total iron concentration increased from 14.6 to 50.0 g/L. The physicochemical parameters of the liquid phase indicated active oxidation of sulfide minerals in flotation concentrate, accompanied by leaching of sulfur and ferrous iron. Oxidation of elemental sulfur commenced in reactor 1, resulting in pH decrease from 2.0 to 1.6. Oxidation of ferrous iron occurred only in reactor 4, with its concentration decreasing from 23.5 to 7.5 g/L. The share of ferric iron increased from 7.53 in reactor 1 to 85.0 in reactor 4. Low pH favored high concentrations of ferric iron, the main oxidant in the process, and this promoted the oxidation of sulfide minerals.

The microbial community of the liquid phase of the pulp was found to contain rod-shaped bacteria of varying size, spirals, and vibrios, as well as archaea. They were tentatively designated as rods (small, medium, and large), leptospirilla, and archaea. Our earlier works reported varying ratios of rods of different sizes in response to the variations in the mode of biooxidation of a substrate in different reactors [6]. Isolation and identification of pure cultures revealed that small rods (0.4 × 1.0 μm) belonged to *Acidithiobacillus caldus*, medium rods (0.3 × 2.0 μm) were *At. ferrooxidans, At. thiooxidans*, and *Acidiferrobacter thiooxidans*, while

Table 1. Average values of the physicochemical parameters of the pulp liquid phase during the oxidation of pyrrhotite-rich flotation concentrate

Experimental conditions	Reactor no.	pH	Eh, mV	Fe^{3+}, g/L	Fe^{2+}, g/L	Fe_{tot}, g/L	Fe^{3+}, %
Aeration 3 L/(L min) in reactors 1–4 (mode 1)	1	1.60	597	1.1	13.5	14.6	7.53
	2	1.59	629	6.2	21.0	27.2	22.79
	3	1.60	649	18.3	23.5	41.8	43.78
	4	1.50	696	42.5	7.5	50.0	85.0
Aeration 1 L/(L min) in reactors 1 and 2 and 4 L/(L min) in reactors 3 and 4 (mode 2)	1	1.64	593	1.0	11.1	12.1	8.26
	2	1.72	634	7.9	19.8	27.7	28.52
	3	1.71	653	18.0	18.0	36.0	50.0
	4	1.53	681	33.7	12.1	45.8	73.58
Aeration 4 L/(L min) in reactors 1 and 2 and 1 L/(L min) in reactors 3 and 4 (mode 3)	1	1.84	581	0.5	8.3	8.8	5.68
	2	1.69	617	2.3	12.8	15.1	15.23
	3	1.60	636	8.0	19.2	27.2	29.41
	4	1.65	650	15.1	18.4	33.5	45.07
Addition of yeast extract (mode 1)	1	1.68	624	4.9	18.2	23.1	21.1
	2	1.48	650	18.9	21.6	40.5	46.7
	3	1.52	697	35.2	10.9	46.1	76.2
	4	1.31	761	44.3	1.0	45.3	97.8

large rods (0.6–0.8 × 1.0–3.0 μm) were represented by *Sulfobacillus* species. Small, medium, and large rods predominated at uniform aeration in all four reactors. Microbial numbers increased from reactor 1 to reactor 3 and decreased in reactor 4 (Table 2). Large rods tentatively identified as *Sulfobacillus* species were present in small numbers. Microscopy revealed some morphologically conspicuous cells of *Leptospirillum* and *Ferroplasma*. Their numbers did not exceed 10^5 cells/mL.

Physicochemical parameters of the pulp liquid phase during biooxidation of sulfide ore flotation concentrate under aeration at 1 L/(L min) in reactors 1 and 2 and at 4 L/(L min) in reactors 3 and 4. Aeration decreased in reactors 1 and 2 to 1 L/(L min) and increased to 4 L/(L min) in reactors 3 and 4, resulting in decreased biooxidation rates (Table 1). It was evident mainly as a decrease in total iron (45.8 g/L) and a decreased rate of ferrous iron oxidation in reactor 4 (Fe^{2+} concentrations in reactors 3 and 4 were 18.0 and 12.1 g/L, respectively). The share of ferric iron in reactor 4 was 73.58%. Decreased aeration in the first two reactors had no considerable effect on the rate of sulfur oxidation: pH in reactor 1 decreased from 2.0 to 1.64 and pH in reactor 4 decreased to 1.53.

A decrease in the rate of biooxidation of the substrate may be explained by insufficient concentrations of dissolved oxygen in reactors 1 and 2, where the oxidation of sulfide minerals, elemental sulfur, and ferrous iron begins. This limitation probably affected the process as a whole, so that enhanced aeration in reactors 3 and 4 did not help to reach the biooxidation parameters observed in the line with uniform aeration at 3 L/(L min).

Under decreased aeration in the first two reactors, the number of rods of different size decreased compared to mode 1 (Table 2). Increased aeration in reactors 3 and 4 resulted in a slight increase in the number of large rods (tentatively *Sulfobacillus* species) and the onset of ferrous iron oxidation. Under these conditions, the number of large rods in reactor 4 was 17.4% higher than under mode 1 of aeration. Sulfobacilli have been found to exhibit high rates of ferric iron oxidation under intense aeration [16].

Physicochemical parameters of the pulp liquid phase during biooxidation of sulfide ore flotation concentrate under aeration at 4 L/(L min) in reactors 1 and 2 and at 1 L/(L min) in reactors 3 and 4. Increase of aeration to 4 L/(L min) in reactors 1 and 2 and its decrease to 1 L/(L min) in reactors 3 and 4 resulted in considerable inhibition of biooxidation. It could be seen from a decrease in total iron concentration (33.5 g/L) and from weak oxidation of ferrous iron in reactor 4 (Fe^{2+} concentrations in reactors 3 and 4 were 19.2 and 18.4 g/L, respectively). Saturation of the pulp with oxygen at the early stages of the process, when ferric iron concentration was low, probably did not result in increased rates of sulfide mineral oxidation. The decrease in aeration in reactors 3 and 4 to 1 L/(L min) resulted in a considerably decreased rate of ferrous iron oxidation. This aeration mode had no significant effect on the oxidation of reduced sulfur compounds: pH in reactors 1 and 4 was 1.84 and 1.65, respectively.

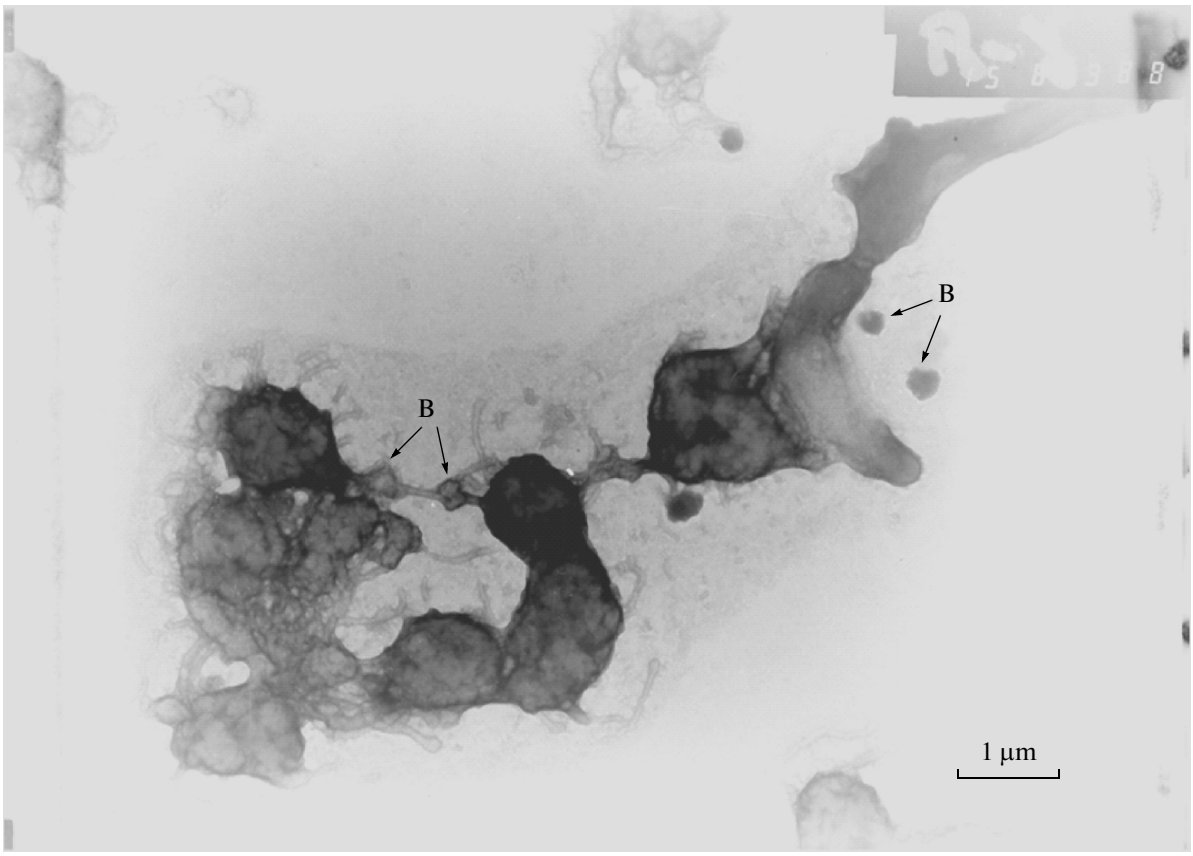
Compared to mode 1, at aeration mode 3, decreased numbers of medium and small rods were found in reactor 1. A considerable increase in abundance of large rods (tentatively *Sulfobacillus* species) was also detected, which confirmed our previous findings concerning the positive effect of enhanced aeration on this microorganism [16]. In reactor 2 at high aeration, the numbers of all microbial forms increased compared to reactor 1. Decreased aeration in reactors 3 and 4 affected the numbers of *Sulfobacillus*, especially in reactor 4, thus resulting in decreased rates of sulfur and iron oxidation.

Physicochemical parameters of the pulp liquid phase during biooxidation of sulfide ore flotation concentrate at mode 1 of aeration in the presence of yeast extract. Addition of yeast extract (YE) to the pulp under mode 1 of aeration resulted in pH decrease to 1.68 in reactor 1 and to 1.31 in reactor 4 (Table 1). Eh increased from 624 to 761 mV, and concentrations of ferric iron increased from 4.9 to 44.3 g/L. Active oxidation of ferrous iron occurred in reactor 3, resulting in its concentration decreasing from 21.6 to 1.0 g/L. The share of ferric iron in the total iron content in reactor 4 was 97.8%.

The lowest pH in reactor 4 (1.31) was achieved in the presence of yeast extract at mode 1 aeration, indicating the highest rate of oxidation of reduced sulfur compounds leached from the sulfide minerals of the concentrate. Eh in the mode 1 aeration without yeast extract (control) was lower by 65 mV, ferric iron concentration was lower by 1.8 g/L, and the rate of ferrous iron oxidation was lower.

Introduction of yeast extract into the pulp during biooxidation of pyrrhotite-rich sulfide ore flotation concentrate at aeration mode 1 resulted in the numbers of large rod-shaped cells (genus *Sulfobacillus*) increasing compared to the control, while the numbers of medium and small rods (probably autotrophic bacteria) decreased (Table 2). The number of archaea in reactor 1 exceeded 10^7 cells/mL, while in reactor 2 it increased sevenfold due to bud separation (figure).

Analysis of the solid residue of biooxidation of pyrrhotite-rich sulfide ore flotation concentrate at different aeration modes. The results presented in Table 3 show that the best levels of gold recovery from solid biooxidation residues were obtained in mode 1, when the aeration conditions were the same in all reactors, i.e., 3 L/(L min). From the residue obtained at mode 1, the gold recovery level was 93.84%. Introduction of yeast extract under aeration mode 1 enhanced biooxidation of the sulfide minerals of pyrrhotite-rich flotation concentrate, thus confirming the physicochemical parameters of the liquid phase (Table 1). Gold recovery level was 96.45%. In two other modes of aeration, gold recovery was lower due to insufficient aer-



Cell morphology of the strain *Ferroplasma acidiphilum* OL12-4. B indicates buds.

ation in the first or last pair of the reactors. Mode 3 resulted in the lowest gold recovery, with 8.51 g/t gold remaining in the residue after cyanidation and 12.74% gold recovery. This resulted from insufficient oxygen inflow to reactors 3 and 4 for the maintenance of high microbial growth rates and for the oxidation of flotation concentrate, as well as of ferrous iron and elemental sulfur leached from sulfide minerals in the first two reactors. In the biogeotechnologies for gold recovery from flotation concentrates of gold-bearing refractory sulfide ores, oxidation of ferrous iron and elemental sulfur is most active in the end reactors of a line due to high abundance of both microorganisms and their growth substrates [1]. Insufficient pulp saturation with oxygen inhibits these processes. Our results indicate the desirability of uniform pulp aeration in all reactors of the line. Increased aeration in some reactors accompanied by decreased aeration in others does not result in more intense oxidation and may considerably impair the process.

The content of elemental sulfur in biooxidation products from four reactors was 2.02 to 2.61% at all aeration modes. Low elemental sulfur content in the residue from reactor 3 was probably an indication of insufficient sulfide oxidation (the reaction releasing this product), rather than of its active microbiological oxidation. This was confirmed by lower values of gold recovery from the solid oxidation residue.

Cyanide consumption for gold recovery varied from 43.4 to 54.5 kg per 1 t residue. However, this high cyanide consumption was 1.3–1.7 times lower than the amount required for sorption cyanidation of solid residues obtained by biooxidation of a similar concentrate at the Polyus factory. The average cyanide expenditure for gold recovery from sulfide ore flotation concentrates with high pyrrhotite content varies from 70 to 100 kg/t. According to [17], cyanidation of the biooxidation residue of the Olympiadinskoe processing enterprise required 89 kg/t cyanide at 86% gold recovery. A decrease of cyanide consumption by 30% under industrial conditions will decrease the operation costs and the net cost of gold production.

The positive effect of YE addition has been reported previously [18] for two-stage oxidation of refractory gold-bearing sulfide ore concentrate. After 240 h of biooxidation, the degrees of biooxidation for pyrite and arsenopyrite were 73.5 and 95.1%, respectively, while gold recovery was 77.9%. Under autotrophic conditions, pyrite and arsenopyrite oxidation was 60.2 and 92.0%, respectively, with 65.7% gold recovery. *S. thermosulfidooxidans* and *A. caldus* were the dominant microorganisms of the community

Table 2. Numbers of rod-shaped microorganisms of different size and of archaea in the community oxidizing pyrrhotite-rich sulfide ore flotation concentrate, cells × 10^9/mL

Experimental conditions	Reactor no.	Rods of different size and archaea			
		1	2	3	4
Aeration 3 L/(L min) in reactors 1–4 (mode 1)	Small	4.0	6.5	8.3	6.2
	Medium	2.8	6.8	7.2	4.7
	Large	0.034	0.043	0.05	0.046
Aeration 1 L/(L min) in reactors 1 and 2 and 4 L/(L min) in reactors 3 and 4 (mode 2)	Small	1.1	1.3	1.3	1.9
	Medium	0.87	1.3	1.4	1.8
	Large	0.03	0.035	0.052	0.054
Aeration 4 L/(L min) in reactors 1 and 2 and 1 L/(L min) in reactors 3 and 4 (mode 3)	Small	1.2	1.5	1.7	1.8
	Medium	1.0	1.6	2.0	2.0
	Large	0.31	0.34	0.28	0.017
Addition of yeast extract (mode 1)	Small	2.0	2.4	2.7	3.5
	Medium	1.6	2.0	2.8	2.4
	Large	0.052	0.092	0.11	0.10
	Archaea	0.05	0.35	0.49	0.53

Table 3. Gold content in the products of cyanidation of the solid residue from four reactors at different aeration modes for biooxidation of flotation concentrate by the microbial community

Experimental conditions	Solid residue, %	Gold content, g/t		Gold recovery, %			Elemental sulfur, %	Cyanide consumption, kg/t
		initial residue	cyanidation tales	on the resin at cyanidation	to cyanidati on tails	of the initial flotation concentrate		
Aeration 3 L/(L min) in reactors 1–4 (mode 1)	68.3	65.7	5.5	91.63	8.37	93.84	2.43	51.4
Aeration 1 L/(L min) in reactors 1 and 2 and 4 L/(L min) in reactors 3 and 4 (mode 2)	73.5	63.16	5.76	90.88	9.12	93.06	2.61	54.5
Aeration 4 L/(L min) in reactors 1 and 2 and 1 L/(L min) in reactors 3 and 4 (mode 3)	80.2	66.81	8.51	87.26	12.74	88.81	2.02	43.4
Addition of yeast extract (mode 1)	67.5	65.40	3.21	95.09	4.91	96.45	2.23	49.5

under both autotrophic and mixotrophic conditions. The archaeon *Acidiplasma* sp. MBA-1 was present as a minor component.

Identification of the dominant bacteria of the community. Two bacterial strains were isolated by tenfold terminal dilutions from the pulp of reactor 4 under optimal aeration conditions (at mode 1 of aeration in the presence of yeast extract): the dominant strain OL13-1 and strain OL13-2.

Analysis of the 16S rRNA gene sequence of strain OL13-1 revealed that it was phylogenetically most closely related to *At. caldus* SM-1 (NR_102970.1). Homology between the sequences of strains OL13-1 and SM-1 (NR_102970.1) was 99%. According to modern concepts [19], this level of the 16S rRNA gene similarity makes it possible to identify the isolate as an *A. caldus* strain. Homology between strain OL13-1 and *At. caldus* OL12-3 (KF356025.1) [6], which was isolated from flotation concentrate oxidized at 40°C, was 95%. Thus, two *At. caldus* strains, OL12-3 and OL13-1 were present in the reactor pulp.

Analysis of the 16S rRNA gene sequence of strain OL13-2 revealed that among described bacteria it was phylogenetically most closely related to *S. thermosulfidooxidans* OL12-1 (KF356023.1). Homology between the sequences of strains OL13-2 and OL12-1 was 100%. This similarity level of the 16S rRNA gene sequences makes it possible to identify the isolate as a *S. thermosulfidooxidans* strain.

From the pulp of reactor 4 in which flotation concentrate was oxidized in the presence of yeast extract, a thermotolerant bacterial strain OL13-3 and an archaeal strain OL13-4 were isolated by tenfold terminal dilutions.

Analysis of the 16S rRNA gene sequence of strain OL13-3, small rods which were predominant in the community, revealed that it was phylogenetically most closely related to *At. caldus* Ol12-3 (KF356025.1). Homology between the 16S rRNA gene sequences of the strains OL13-3 and OL12-3 was 100%. Homology between the sequences of strain OL13-3 and of the type strain *At. caldus* DSM 8584 (Z29975) was 99.7%. According to modern concepts [19], this level of the 16S rRNA gene similarity makes it possible to identify the isolate as an *A. caldus* strain.

Analysis of the 16S rRNA gene sequence of the archaeal strain OL13-4 revealed that it was phylogenetically most closely related to the species *F. acidiphilum*. Homology between the 16S rRNA gene sequences of the isolate and of the strain *F. acidiphilum* OL12-4 (KF356026.1) was 99.9%. Homology between the 16S rRNA gene sequences of strain OL13-4 and of the *F. acidiphilum* type strain (AJ224936) was 99.1%. This similarity level between the 16S rRNA gene sequences makes it possible to identify the archaeal isolate as a *F. acidiphilum* strain.

Medium-sized rods, which were predominant in microbial communities during biooxidation of the pyrrhotite-rich flotation concentrate under all aeration modes, were not obtained in pure culture. Under the optimal temperature (38°C), enrichment cultures containing mostly these microorganisms oxidized ferrous iron and elemental sulfur, not requiring yeast extract for growth. Analysis of the clone library obtained from microbial biomass of the reactor pulp in the course of oxidation of the same concentrate [20] revealed that the sequences of 12 clones out of 51 exhibited 99.5% homology to the 16S rRNA gene of *Acidiferrobacter thiooxidans* DSM 2392T, an acidophilic thermotolerant autotrophic bacterium oxidizing iron, sulfur, and pyrite. The bacterium present in the community oxidizing the pyrrhotite-rich flotation concentrate probably belonged to the same species.

Thus, optimal aeration conditions were determined for continuous cultivation of the ACM community in a line of four sequentially connected laboratory reactors and the effect of yeast extract on biooxidation of pyrrhotite-rich sulfide ore flotation concentrate was studied. The optimal conditions were aeration at 3 L/(L min) and yeast extract concentration 0.02%. The strains *Acidithiobacillus caldus* OL13-1 = *At. caldus* SM-1 (NR_102970.1), *At. caldus* OL13-3 = *At. caldus* OL12-3 (KF356025.1) and probably *'Acidiferrobacter thiooxidans'* were identified as the dominant components of the community. The strains *S. thermosulfidooxidans* OL13-2 = *S. thermosulfidooxidans* OL12-1 (KF356023.1) and *F. acidiphilum* OL13-4 = *F. acidiphilum* OL12-4 (KF356026.1) were isolated as pure cultures and identified.

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