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EXPERIMENTAL ARTICLES

Optimization of Bioleaching and Oxidation of Gold-Bearing Pyrite–Arsnopyrite Ore Concentrate in Batch Mode

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Abstract—Biooxidation of refractory gold-bearing pyrite–arsenopyrite flotation concentrate was optimized and the abundance of predominant groups in the community of thermophilic acidophilic chemolithotrophic microorganisms at various stages of bioleaching was determined. The optimal parameters for growth and leaching/oxidation of the mineral components of the concentrate were pH 1.4–1.8; 47.5°C; and the following salt concentrations in the liquid phase (g/L): $K_2HPO_4 \cdot 3H_2O$ – 0.53, $(NH_4)_2SO_4$, 1.6 and $MgSO_4 \cdot 7H_2O$, 2.5 (or $(NH_4)_2SO_4$, 1.23; ammophos, 0.41; KOH, 0.1) with 0.03% yeast extract. The optimal conditions resulted in high growth rate, high levels of iron and arsenic leaching, of Fe^{2+} and S^{2-}/S^0 oxidation, and predominance of *Acidithiobacillus caldus*, *Sulfobacillus* spp., and *Ferroplasma* spp. in the community.

Keywords: biooxidation, gold-bearing flotation concentrate of sulfide ores, thermoacidophilic microbial community, community composition, optimization of biooxidation

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High gold recovery from sulfide ore concentrates in biogeotechnological processes requires optimal conditions for microbial growth and oxidation of the energy substrates during the biooxidation (BO) stage. Elemental sulfur of the pyrite–arsenopyrite gold-bearing ores impedes the efficiency of the process by decreasing gold recovery from solid biooxidation residue and increasing cyanide expenditure. Microbial communities involved in the oxidation of sulfide concentrates at moderate temperatures and low pH include thermotolerant and moderately thermophilic bacteria and archaea [1–3]. Optimal values for the temperature, pH, medium composition, aeration, and pulp density are the most important conditions for their growth and oxidation of the energy substrates. Research in this field should consider the exothermic nature of oxidation of sulfide ores and concentrates and determine the conditions which, while having no negative effect on the microorganisms, provide for intensified biooxidation.

We have previously obtained a thermophilic community of acidophilic chemolithotrophic microorganisms (ACM) [4]. It included the active strains of two communities: an aboriginal one, which was isolated from the pulp liquid phase during biooxidation of refractory pyrite–arsenopyrite gold-bearing flotation concentrate at 47°C, and the experimental consortium adapted to this concentrate. Chemolithotrophic thermoacidophilic bacteria and archaea were the dominant organisms in the ACM community developed in the course of the biotechnological process. These were bacteria *Acidithiobacillus caldus* PCG-1 and *Sulfobacillus thermotolerans* PCG-2, archaea of the family *Ferroplasmaceae* (*Ferroplasma acidiphilum* PCG-4 and the archaeal strain PCG-5), as well as the heterotrophic bacterium *Alicyclobacillus tolerans* PCG-3 [4].

The goal of the present work was to optimize conditions for biooxidation of refractory pyrite–arsenopyrite gold-bearing flotation concentrate by the ACM thermophilic microbial community in batch mode and to characterize the composition of this community depending on biooxidation conditions.

MATERIALS AND METHODS

Subjects of research. Refractory pyrite–arsenopyrite gold-bearing flotation concentrate and the thermophilic community of acidophilic chemolithotrophic microorganisms [4] were the subjects of investigation.

The concentrate consisted of silica-alumina mass with inclusions of sulfide minerals. Particle size was –0.074 mm. Pyrite (35%) and arsenopyrite (8%) were the major sulfide minerals; small amounts of stibnite (0.56%) were present. Silicate and carbonate minerals constituted 49 and 5.5%, respectively. According to chemical analysis, flotation concentrate contained 20.9% total iron, 19.2% sulfide iron, 3.63% arsenic (total and sulfide one), 20.44% total sulfur, 19.87% sulfide sulfur, 0.08% elemental sulfur, 0.56% total antimony, 0.28% sulfide antimony, 1.42% calcium, 1.58% magnesium, and 0.4% C_{org}. The analysis was carried out using a 3100 atomic absorption spectrom-

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eter (Perkin Elmer, United States) with plasma atomization.

Optimization of biooxidation parameters. For optimization of BO conditions, the thermophilic ACM community was grown for up to 10 days in batch mode in round-bottomed 250-mL flasks with 100 mL of iron-free 9K medium [5] containing sulfide concentrate on a Unimax 1010 shaker with Incubator 1000 (Heidolph, Germany) at 47°C and 190 rpm. The 9K medium contained the following (g/L): $(NH_4)_2SO_4$, 3.0; KCl, 0.1; $K_2HPO_4 \cdot 3H_2O$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; $Ca(NO_3)_2 \cdot 4H_20$, 0.01, and yeast extract (YE), 0.02%; pH 2.0. Pulp density was 80 to 100 g/L (S : L = (0.8–1.0) : 10).

The optimization parameters were pH, cultivation temperature, mineral composition of the medium, and C_{org} content. The values of pH from 0.9 to 1.8 with a 0.1 step were maintained throughout the cultivation by addition of concentrated H_2SO_4 or 10% $NaHCO_3$, and pH variations throughout a day were summarized. Effect of temperature of the rates of growth and sulfide concentrate BO was studied by incubation at temperatures from 40 to 50°C with a step of 2.5°C. Effect of organic compounds on the activity of the ACM thermophilic community was determined by adding yeast extract (0.001, 0.01, 0.02, or 0.03%) to the pulp. The following preparations were used to determine the optimal macroelement source: ammophos (source of nitrogen, potassium, and phosphorus), $(NH_4)_2SO_4$ (nitrogen source), K_2HPO_4 (phosphorus and potassium source), $MgSO_4$ (magnesium source), and KOH (potassium source). Ammophos (Malakhovo Ltd., Moscow, Russia) State Standard 18918-85 contained (by mass) 11–13% nitrogen and 49–51% utilizable phosphates. The first variant of experimental medium (I) contained the following (g/L): $K_2HPO_4 \cdot 3H_2O$, 0.53; $(NH_4)_2SO_4$, 1.6; and $MgSO_4 \cdot 7H_2O$, 2.5. The second variant (II) was full 9K medium (without iron). The third variant (III) contained the following (g/L): $(NH_4)_2SO_4$, 1.23; ammophos, 0.1; and KOH, 0.1. The fourth variant (IV) differed from variant (III) in fourfold increased ammophos concentration. The fifth variant (V) contained the following (g/L): $(NH_4)_2SO_4$, 2.0; $MgSO_4 \cdot 7H_2O$, 0.5; KOH, 0.1; and ammophos, 0.1. The sixth variant (VI) contained (g/L): $(NH_4)_2SO_4$, 0.5; $K_2HPO_4 \cdot 3H_2O$, 0.3; KCl, 0.1; and $MgSO_4 \cdot 7H_2O$, 0.23. All variants, except for (III), contained yeast extract.

Analytical techniques. The analyzed parameters of the pulp liquid phase included the following: pH and Eh (relative to the normal hydrogen electrode) determined with a pH-150M (Belarus); concentrations of ferric and ferrous iron determined by chelatometric titration with Trilon B [6]; total arsenic determined by iodometric titration with iron ions bound with $TiCl_3$ [7]. Dissolved organic carbon (C_{org}) concentrations at the beginning and end of the experiment were determined by the bichromate method and expressed in COD units—glucose equivalents (gl. eq., g/L) [8]. Quantitative assessment of microbial cells was carried out by direct counts and by terminal tenfold dilutions in selective media: 9K medium with $Fe_2SO_4 \cdot 7H_2O$ or with S^0 (10 g/L each) with or without YE. Microscopy was carried out using a Mikmed-2 phase contrast microscope (LOMO, Russia).

All experiments were carried out in three repeats. For statistical treatment, Student's criterion at 5% significance level was used [9].

RESULTS AND DISCUSSION

Optimization of the Biooxidation Stage

Experimental results on determination of the optimal values of pH, temperature, YE concentration, and mineral composition of the pulp liquid phase for biooxidation are presented in Tables 1–4 and Figs. 1–3. The inoculum (10% vol/vol) used in these experiments was grown at initial pH 2.0 at 47°C in 9K medium with the sulfide ore flotation concentrate.

Effect of pH. The ACM community developed at the S : L = 1 : 10 in the pulp. The highest rate of cell division occurred on the third day after inoculation. The highest values of specific growth rate (μ_{max} 0.12 h^{-1}) were observed at pH 1.6, as were the maximum cell numbers (3.25×10^9/mL) . At pH 1.5, the growth rate was somewhat lower ($\mu_{max} = 0.11\ h^{-1}$). At pH values of 1.3 and 1.8, specific growth rate and cell yield were 0.1 h^{-1} and 2.50×10^9/mL, respectively; the values for pH 1.4 were 0.09 h^{-1} and 2.25×10^9/mL. At all pH values, rod-shaped bacterial cells predominated in the ACM community; they decreased in size under less favorable conditions. Cultivation of the ACM community at pH 0.9 provided interesting results. Although cell numbers were lower in this case, the community developed steadily, with μ_{max} close to 0.1 h^{-1}. These extremely acidophilic conditions probably resulted in lysis of some of the cells and synchronized growth of the others, thus causing a prolonged lag phase (Fig. 1a). The values of pH above 1.2 were favorable for growth, with the maximum at pH 1.6. Terminal tenfold dilutions of the pulp liquid phase at low pH revealed predominance of lithotrophic, sulfur-oxidizing gram-negative bacteria *Acidithiobacillus* spp. and iron-oxidizing archaea of the family *Ferroplasmaceae* [4]. Heterotrophic *Alicyclobacillus* spp. and mixotrophic *Sulfobacillus* spp. were present in small numbers.

Analysis of the dynamics of iron leaching and oxidation revealed the absence of ferrous iron (Fe^{2+}), i.e. its complete oxidation by the end of growth at all pH values. Fe^{3+} concentration in the liquid phase was highest (17.5 g/L) at pH 1.4 (Fig. 1d). At pH 1.5 and 1.6, Fe^{3+} were similar (16.4 g/L). Fe^{3+} at pH 1.1 and 1.2 were slightly lower. The highest rate of iron oxidation (4.2 g/L per day) was observed at pH 1.6. At pH

Table 1. Total changes in Σ(ΔpH↑↓) during batch cultivation of the ACM community under stabilized pH values at 47°C

pH	Cultivation time, days									Σ↑ΔpH/↓ΔpH
	1	2	3	4	5	6	7	8	9	
0.9	↑0.06	↓0.03	↑0.13	↓0.02	↓0.02↓↑	↑0.03↓↑	↑0.11	↑0.06	0↓↑	↑**0.5**/↓0.17
1.0	↑0.15	↓0.02	↑0.10	0↑↓	↑0.07	↑0.02↓↑	↑0.07	↑0.04	↓0.04↓↑	↑**0.52**/↓0.13
1.1	↑0.06	↓0.06	↑0.02	↓0.08	↑0.01↓↑	↑0.02↓↑	↑0.06	↑0.01↓↑	↓0.09	↑0.28/↓0.34
1.2	↑0.06	↓0.08	↓0.01	↓0.08	↓0.08	↓0.04↓↑	↑0.06	↑0.01↓↑	↓0.09	↑0.22/↓0.47
1.3	↑0.05	↓0.05	↓0.06	↓0.14	↓0.09	↑0.02↓↑	↑0.03	↓0.04	↓0.08	↑0.10/↓0.46
1.4	↑0.04	↓0.08	↓0.06	↓0.14	↓0.11	↓0.04↓↑	↓0.03	↓0.05	↓0.14	↑0.07/↓0.69
1.5	↑0.02	↓0.08	↓0.14	↓0.15	↓0.13	↓0.08	↓0.05	↓0.11	↓0.13	↑0.02/↓0.87
1.6	↑0.03	↓0.10	↓0.12	↓0.16	↓0.09	↓0.12	↓0.08	↓0.07	↓0.15	↑0.03/↓**0.89**
1.7	↑0.01	↓0.13	↓0.03↓↑	↓0.19	↓0.09↓↑	↓0.10	↓0.05↓↑	↓0.07	↓0.14	↑0.10/↓0.84
1.8	↑0.02	↓0.12	↓0.11	↓0.21	↓0.17	↓0.13	↓0.08	↓0.11	↓0.15	↑0.02/↓**1.08**

Total daily increase or decrease in pH of the pulp liquid phase is denoted by ↑ and ↓, respectively. Fluctuations of pH during the day are denoted by ↓↑. Σ↑ΔpH/↓ΔpH denotes the overall change in the stabilized pH value in a given experimental variant.

1.4, 1.1, and 1.5, the maximal rates were lower: 3.4, 3.3, and ~3 g/L per day, respectively.

All changes in pH associated with substrate oxidation and growth processes were registered according to the accepted procedure [10]. During the day, pH could both decrease (↓ΔpH) and increase (↑ΔpH). Titration returned the pH value to the desired level. All fluctuations from the set pH value during each day of the process summarized in Table 1 were used to confirm the oxidation of reduced sulfur compounds (RSC).

For the community grown at the lowest pH 0.9–1.0, the total acidification (↓ΔpH) of the medium was 0.13–0.17 pH units, while total alkalinization (↑ΔpH) was 0.50–0.52 pH units. Increased pH probably resulted from the lysis of some cells (microscopic data) or from reactions of iron oxidation, decarboxylation, etc. Acidification of the medium increased at higher initial pH. Thus, at pH maintained at 1.8, total acidification and alkalinization of the pulp liquid phase during growth and substrate oxidation were 1.08 and 0.02 pH units, respectively, which indicated predominance of the processes of sulfur oxidation.

It was therefore concluded that both energy substrates (iron and sulfur) were oxidized simultaneously by the microbial community. Thus, when RSC oxidation, accompanied by accumulation of sulfate ions, prevailed, sulfur oxidizers (acidithiobacilli or acidithiobacilli and sulfobacilli) predominated in the community. Plating and inoculation of the pulp liquid phase on selective media and the results of the tests on phenotypic similarity to archaea and bacteria [4] revealed that intense iron leaching and oxidation coincided with active growth of sulfobacilli and archaea involved in these processes. Acidification of the medium coincided with rapid growth of acidithiobacilli and/or sulfobacilli.

Effect of temperature. Figure 2 and Table 2 present the results of optimization of BO of sulfide flotation concentrate by the second parameter, temperature.

At 40°C, the highest cell yield (25.3×10^8/mL) was achieved on the fourth day of growth, μ_{max} was 0.086 h^{-1} (Fig. 2a). Microscopic observations and analysis of the inoculation of the pulp liquid phase on media revealed predominance of two groups of bacteria, *Acidithiobacillus* spp. (dominant) and *Sulfobacillus* spp.; archaea were also present.

At 42.5 and 45°C, specific growth rates (μ_{max} 0.089 h^{-1}) and cell yields (25.0×10^8/mL) indicated almost identical development during the first four days (Fig. 2a). At 42.5°C, *Acidithiobacillus* spp. cells prevailed in the ACM community; the *Sulfobacillus* spp. group, archaea, and small rod-shaped cells comprised 30, 10, and 2–3%, respectively. On the fifth day, cell yield at 42.5°C peaked (35.1×10^8 cells/mL) due to enhanced iron oxidation (Fig. 2). The structure of the ACM community changed due to a 15% increase of the total numbers of sulfobacilli and archaeal cells. At 45°C, acidithiobacilli and sulfobacilli each comprised 40%, while small cells, including archaea, ~20%.

When the ACM community developed at 47.5°C, biomass increased during four days of the experiment; the highest cell yield was 27.5×10^8/mL, and μ_{max} was 0.094 h^{-1}. The dominant groups, *Sulfobacillus* spp. and *Acidithiobacillus* spp., still constituted most of the community (up to 70–80%). After the third day at 47.5°C, as well as at 50°C, larger (0.8–1.0 × 2.5–3.0 μm) cells of heterotrophic bacteria *Alicyclobacillus* spp. became noticeable.

At 50°C, the highest growth rate of the ACM community (μ_{max} = 0.126 h^{-1}) was recorded on the second day of growth. Cell numbers peaked (12.3×10^8 cells/mL) on the third day and remained at the same level throughout the stationary phase. The num-

Table 2. Changes in pH (ΔpH) in the pulp liquid phase during 7 days of the ACM community growth under pH maintained at 1.6 and different cultivation temperatures

T, °C	Cultivation time, days							Σ↑ΔpH/↓ΔpH
	1	2	3	4	5	6	7	
40	↑0.02	↓0.13	↓0.08↓↑	↑0.11↓↑	↓0.09	↓0.09	↓0.08	↑**0.32**/↓0.66
42.5	↓0.09	↓0.27	↓0.18	↓0.09↓↑	↑0.15	↓0.12	↓0.05	↑0.24/↓0.89
45	↓0.08	↓0.12	↓0.10↓↑	↓0.07↓↑	↓0.14	↓0.11	↓0.05	↑0.17/↓0.89
47.5	↓0.10	↓0.30	↓0.21	↓0.17	↓0.16	↓0.12	↓0.16	↑0.0/↓1.22
50	↓0.02	↓0.18	↓0.17↓↑	↓0.21	↓0.24	↓0.17	↓0.19	↑0.17/↓**1.35**

Notation is the same as in Table 1.

ber of sulfobacilli cells in this variant was 10–15% higher than cell numbers of other organisms.

These results show that cell yields within the temperature range from 40 to 47.5°C varied slightly (from 2.5 to 3.5 × 10^9 cells/mL pulp liquid phase); this value was 2–3 times lower at 50°C. Some of the cells in the community responded to temperature increase to 50°C by lysing. As a result, larger cells of mixotrophic or organotrophic bacilli, which utilized organic compounds released by the lytic processes into the pulp liquid phase, constituted a significant portion of the community. Isolation of these bacteria in pure culture made it possible to identify them as heterotrophic *Alicyclobacillus tolerans* [4] with facultative mixotrophic metabolism [11].

Calculation of the total values of pH increase or decrease (Σ↑↓ΔpH) relative to its preset value at a given temperature made it possible to assess the reaction of the ACM community to temperature changes (Table 2). At 40°C the values for the processes resulting in alkalinization and acidification of the pulp liquid phase (Σ↑ΔpH and Σ↓ΔpH) were 0.32 and 0.66 pH units, respectively. The balance was as follows at 42.5 and 45°C: Σ↑ΔpH = 0.24 and Σ↓ΔpH = 0.89 U; Σ↑ΔpH = 0.17 and Σ↓ΔpH = 0.89 U, respectively. At 47.5°C Σ↓ΔpH = 1.22 U, and the rate of RSC oxidation exceeded that of iron oxidation (no pH increase was observed). The ΔpH balance at 50°C was: Σ↑ΔpH = 0.17 and Σ↓ΔpH = 1.35 U. Thus, acidification of the pulp liquid phase was most pronounced at 47.5 and 50°C, indicating increased rates of RSC oxidation at these temperatures.

The maximal rates of iron oxidation at 40, 42.5, 45, 47.5, and 50°C were 1.54, 2.04, 1.86, 2.8, and 2.73 g/L per day (Fig. 2b). Thus, the highest rates were revealed in the communities growing at 47.5 and 50°C. At these temperatures, the concentrations of leached (Fe^{2+}) and oxidized iron (Fe^{3+}), i.e. Fe_{tot}, were higher than at other cultivation temperatures, reaching 12.95 and 12.88 g/L, respectively, on the seventh day. At 42.5°C, total iron concentration in the pulp liquid phase (9.73 g/L) was higher than at 40°C (7.14 g/L) and 45°C (8.61 g/L); cell numbers there also higher at this temperature. It may be suggested that cultivation at 42.5°C stimulates the processes of iron leaching and oxidation by microorganisms, specifically, by archaea and sulfobacilli, which exhibit higher iron-oxidizing activity at this temperature than at 40 and 45°C. The numbers of cells of various morphotypes determined by terminal dilutions of the pulp liquid phase into the medium with ferrous iron were comparable with the results of direct cell count. For the ACM community cultivated at 42.5°C, the number of the dominant group of iron-oxidizing microorganisms (archaea and sulfobacilli) was 19.2 × 10^8/mL. Changes in the numbers of components of microbial communities in the course of oxidation of pyrite–arsenopyrite ore concentrates at temperature variations have been reported previously [1, 12].

The concentration of total arsenic in the pulp liquid phase increased with temperature from 0.96 to 1.24 g/L. Eh remained high (852–860 mV) throughout the temperature interval tested.

These results shed light upon trophic relationships between the components of the microbial community and make it possible to use external factors, such as temperature, to regulate the oxidation of an excessive energy substrate.

Content of organic matter. Our next task was the investigation of the effect of organic matter on bioleaching of sulfide concentrates by enhancing the activity of iron- and sulfur-oxidizing microorganisms. The community was known to contain mixotrophic organisms, with their growth rate depending on the presence of C_{org} in the medium [13]. Yeast extract (0.001 to 0.03%) was therefore added to the pulp (density = 8 g/100 mL). YE is a stimulator of microbial growth and a convenient model to determine the attitude of microorganisms, including ACM, to C_{org} in the medium and their ability to switch from lithoautotrophic to mixo- or organoheterotrophic metabolism. YE contains vitamins, easily utilizable amino acids, nucleotides, carbohydrates, and microelements. Moreover, it is a good antioxidant [14–16].

As was stated above, the sulfide concentrate used in our work contained C_{org}. Concentration of soluble organic carbon in the liquid phase of the medium was 0.40 (control without YE) and 0.41, 0.52, 0.64, and

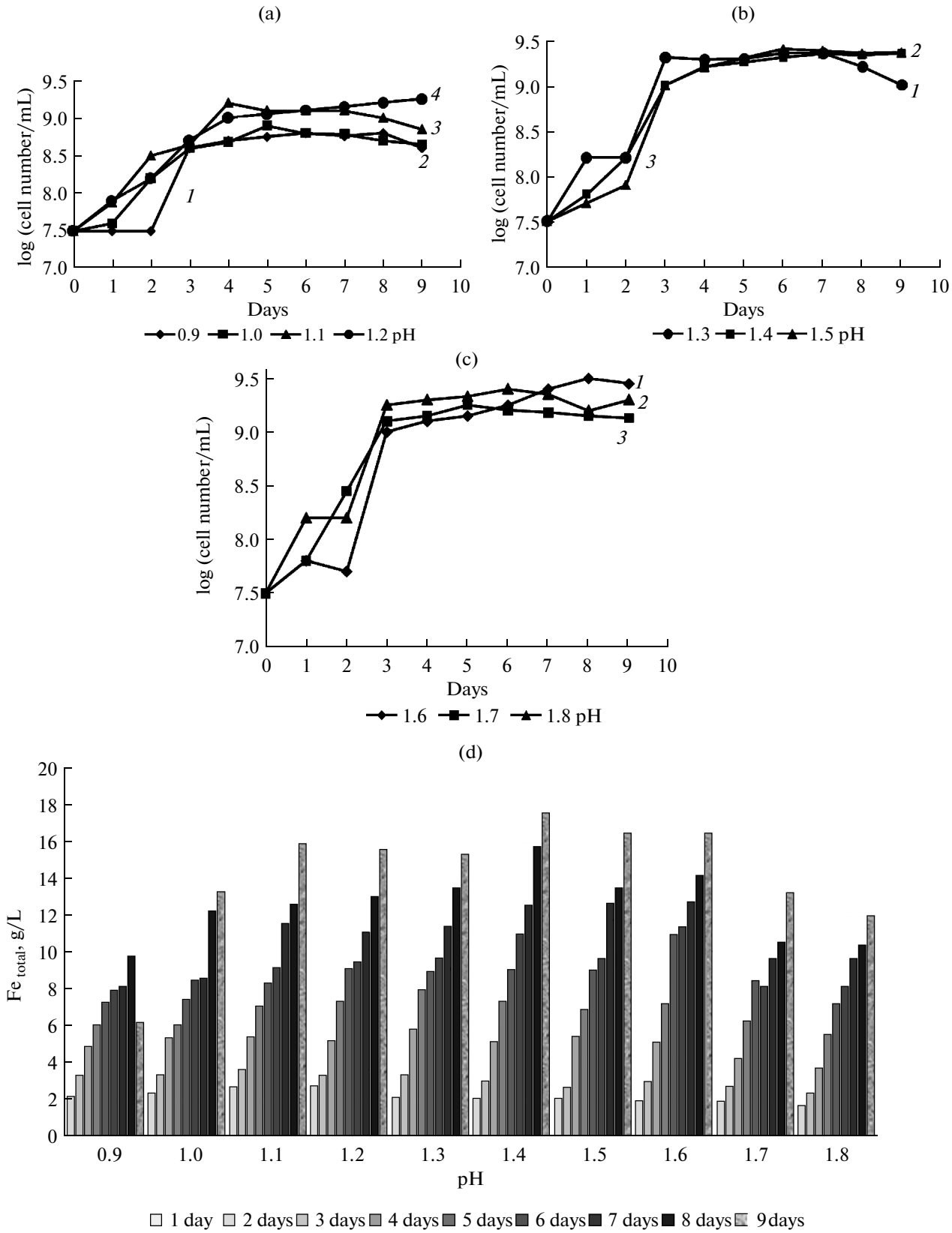

Fig. 1. Cell numbers and iron concentration during cultivation of the ACM community in the medium with sulfide flotation concentrate at different pH values. Growth dynamics of the ACM community at pH 0.9 (*1*), 1.0 (*2*), 1.1 (*3*), and 1.2 (*4*) (a); growth dynamics of the ACM community at pH 1.3 (*1*), 1.4 (*2*), and 1.5 (*3*) (b); growth dynamics of the ACM community at pH 1.6 (*1*), 1.7 (*2*), and 1.8 (*3*) (c); and concentration of total iron (Fe^{3+} + Fe^{2+}) in the pulp liquid phase during growth of the community (d). Pulp density was 100 g/L.

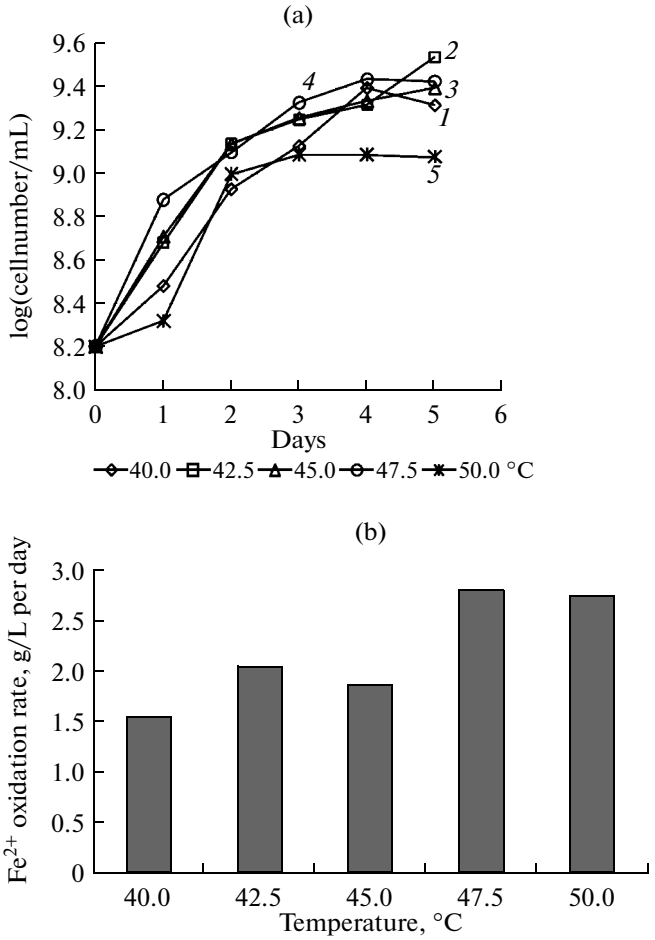

Fig. 2. Growth dynamics of the ACM community and iron oxidation rates at different temperatures. Growth dynamics (a) at 40 (*1*), 42.5 (*2*), 45 (*3*), 47.5 (*4*), and 50°C (*5*). Maximum rate of Fe^{2+} oxidation by the ACM community (b). Pulp density was 90 g/L.

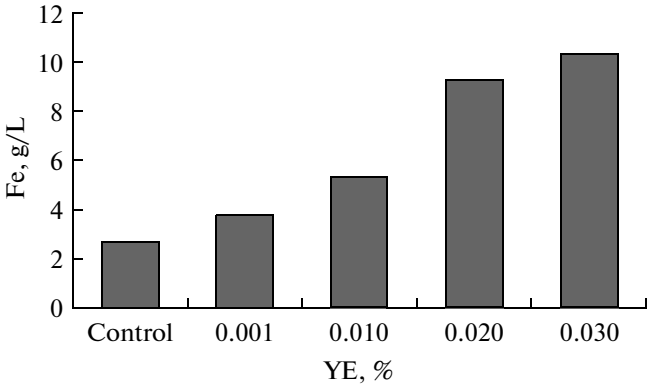

Fig. 3. Total iron concentration during growth of the ACM community in media with different YE concentrations at pH 1.6 and 47.5°C. Pulp density was 80 g/L. Cultivation time was 5 days.

0.76 g/L in the experiments with 0.001, 0.01, 0.02, or 0.03% YE, respectively (Table 3).

After five days of growth (pH 1.6, 47.5°C), content of dissolved C_{org}. in the control and in the variant with 0.001% increased to 0.51 and 0.49 g/L, respectively. In other variants C_{org} concentrations decreased to 0.50–0.53 g/L, indicating consumption of easily utilizable YE. Subtraction of the value for the control (0.40 g/L) from the experimental carbon values showed a comparable increase (by 0.08 to 0.13 g/L) in C_{org} concentrations in all experimental variants. It may therefore be concluded that under mixotrophic conditions the pulp liquid phase contained, apart from unused organic matter of the sulfide concentrate, other organic matter, and the concentration of the latter increased, similar to the control. C_{org} increase in all variants was probably due to the release of organic exometabolites into the liquid phase, including the compounds responsible for cell resistance to heavy metals (As, Sb, Fe), similar to [17]. Similar to our experiment with pyrite–arsenopyrite concentrate of a different composition [10], organic matter of the sulfide ore concentrate also was not utilized by the organisms of the ACM community.

In the control (without YE), the highest number of microbial cells was 5.0×10^8/mL. The cells of gram-negative acidithiobacilli capable of chemolithoautotrophic growth predominated in the ACM community. Abundance of mixotrophic archaea and sulfobacilli, which prefer to use simultaneously both mineral and organic compounds as electron donors and energy sources [13], was lower (20% of the total cell number).

Addition of YE to the medium and its increasing concentrations resulted in increased ACM cell numbers and higher rates of iron/arsenic leaching and oxidation of sulfide sulfur and iron. Cell yield after five days of growth was 7.5 to 15.0×10^8 cells/mL. Iron concentration increased (Fig. 3) from 1.96 Fe^{3+}/0.70 Fe^{2+} or Σ2.66 g/L (in the control) to Σ10.36 g/L in the experiment with 0.03% YE; Eh of the pulp increased from 675 to 840 mV; arsenic concentration increased from 0.6 to 1.24 g/L; and the RSC oxidation (as a decrease in Σ↓ΔpH) increased from 0.4 to 0.91 pH units. In the absence of YE, leached iron was oxidized slowly, with considerable amounts of ferrous iron remaining in the liquid phase of the pulp.

Thus, the highest effect was obtained at the highest of the tested YE concentrations (0.03%).

Mineral elements in the medium. Balanced composition of the major mineral elements in the medium is also an important factor affecting the growth and properties of microorganisms [18]. These elements are mainly nitrogen, phosphorus, potassium, and magnesium. The required microelements were present in the flotation concentrate under study. Adjustment of the source and concentrations of the macroelements was therefore our goal. For all six media listed in Materials and Methods, initial pH was 1.6, growth temperature was 47.5°C, pulp density was 9 g/100 mL, and YE

Table 3. Concentration of dissolved organic matter C_{org} in the liquid phase of 8% pulp after 5 days of cultivation of the ACM community at pH maintained at 1.6 and 47.5°C

Experimental variant, YE, %	Initial concentration of dissolved C_{org}, g/L	Final concentration of dissolved C_{org}, g/L
Control (without YE)	0.40	0.51
0.001	0.41	0.49
0.01	0.52	0.50
0.02	0.64	0.52
0.03	0.76	0.53

content was 0.03%. Variant 3 did not contain YE. The results of this stage of our study are listed in Table 4.

The highest cell numbers of the ACM community achieved in variants I and IV were similar, from 27.5×10^8 to 30.4×10^8 cells/mL. These maxima corresponded with the highest values of total iron concentration ($Fe^{2+} + Fe^{3+}$) in the pulp liquid phase, 17.64 and 17.22 g/L. Results of RSC oxidation were also good. In variants V and II (the control), only cell numbers (26.2×10^8 and 22.4×10^8/mL) and iron concentration in variant II (16.10 g/L) were close to the above values. Variants III and VI exhibited lower results. Investigation of growth kinetics of the ACM communities revealed the maximal growth rates for variants I–VI were 0.087, 0.077, 0.029, 0.063, 0.092, and 0.050 h^{-1}, respectively. The lowest values of specific growth rate, sell yield (4.8×10^8/mL), and iron concentration (6.06 g/L) were found in variant III, which did not contain YE. Investigation of the dynamics of iron and sulfur oxidation revealed that the most active growth of the cells coincided with the highest rates of oxidation of the substrates, RSC, and iron (on the second day of growth). In all experimental variants, however, iron was oxidized throughout the experiment. The highest rate of iron oxidation (3.8 g/L per day) was observed in variant I; it was somewhat lower (3.2 g/L per day) in variant II (the control 9K medium); in variant IV it was 3 g/L per day, confirming the high leaching and oxidative capacity of the iron-oxidizing organisms in the community.

Comparative analysis of capacity of the ACM community for S^{2-}/S^0 oxidation revealed the highest $\Sigma\downarrow\Delta$pH value (0.90 U) in variant IV (Table 4). In the control variant II, the total pH decrease in the pulp was 0.84 U, which also indicated complete RSC oxidation to sulfate. In the pulp of variant I, SO_4^{2-} accumulation was somewhat lower, and $\Sigma\downarrow\Delta$pH was 0.80 U. Variants VI, V, and III with $\downarrow\Delta$pH values of 0.70, 0.67, and 0.45 pH units, respectively, exhibited less pronounced oxidation of the sulfur component of the concentrate. In variant III, Eh (730 mV) was also the lowest, compared to much higher values in other variants (870–890 mV). Arsenic concentration was 0.74 g/L in variant III and 1.12–1.36 g/L in other variants.

Chemolithotrophic bacteria *Acidithiobacillus* spp., *Sulfobacillus* spp. and archaea of the family *Ferroplasmaceae*, which predominated in the communities of the best variants, constituted 45–50, 35–40, and 10–20% of the cells, respectively. In variants III and VI the ACM community consisted mainly of smaller cells with their size and shape changed to those of cocci and ultramicrobacteria. After several transfers on selective media, these cells recovered their initial size. In variants III and VI, archaea and cystlike dormant forms developed. The cells of *Acidithiobacillus* spp. and *Sul-*

Table 4. Cell numbers and physicochemical parameters of the pulp liquid phase in the course of growth of the ACM community in the media with different qualitative and quantitative ratios of the major elements at 47.5°C and pH 1.6

Experimental variant no.	Cell number, 10^8/mL	Fe^{3+}/ Fe^{2+} concentrations, g/L	$\Sigma\downarrow\Delta$pH
I	25.4	17.5/0.14	0.80
II	22.4	15.96/0.14	0.84
III	5.02	5.74/0.28	0.45
IV	30.41	16.94/0.28	0.90
V	26.24	11.76/0.14	0.67
VI	12.18	11.06/1.2	0.70

Experimental media contained the following (g/L): variant I, $K_2HPO_4 \cdot 3H_2O$, 0.53; $(NH_4)_2SO_4$, 1.6; $MgSO_4 \cdot 7H_2O$, 2.5; variant II (control, 9K medium), $(NH_4)_2SO_4$, 3.0; KCl, 0.1; $K_2HPO_4 \cdot 3H_2O$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; $Ca(NO_3)_2 \cdot 4H_2O$, 0.01; variant III, $(NH_4)_2SO_4$, 1.23; ammophos, 0.1; KOH, 0.1; variant IV, $(NH_4)_2SO_4$, 1.23; ammophos, 0.41; KOH, 0.1; variant V, $(NH_4)_2SO_4$, 2.0; $MgSO_4 \cdot 7H_2O$, 0.5; KOH, 0.1; ammophos, 0.1; variant VI, $(NH_4)_2SO_4$, 0.5; $K_2HPO_4 \cdot 3H_2O$, 0.3; KCl, 0.1; and $MgSO_4 \cdot 7H_2O$, 0.23. Variant III did not contain YE. $\Sigma\downarrow\Delta$pH is a total pH decrease in the course of cultivation.

fobacillus spp. with characteristic morphology constituted only 20–25% of the population.

As a result of investigation on optimization of the quantitative composition of the most important mineral elements for cultivation of the thermophilic ACM community, variant I was recommended (with nitrogen and magnesium concentrations half of those in the control medium). It may be substituted by variant IV (medium with ammophos and high phosphorus content) or by variant II (control – 9K medium without iron).

Microscopy of the cells in the course of biooxidation of flotation concentrate makes it possible to monitor the physiological state of microorganisms. Vegetative cells retaining the characteristic size of the relevant group should predominate in an active microbial community. To a considerable degree, cell morphology is an indicator of activity of the community.

Optimization of the medium composition and conditions of batch cultivation provided for stable and efficient functioning of the ACM community. The microorganisms were able to carry out leaching and oxidation of the sulfide concentrate not only under the optimized conditions, but also at a significant increase/decrease in temperature and pH, which made it possible for the community to survive varying conditions in industrial reactors.

In industry, the biotechnological process of sulfide concentrate oxidation is carried out in a continuous mode. Investigation of the processes of biooxidation of refractory pyrite–arsenopyrite flotation concentrate by multistage continuous cultivation of the ACM community under the optimal conditions determined in the present work is therefore planned.

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