ISSN 0026-2617, Microbiology, 2012, Vol. 81, No. 3, pp. 288–298. © Pleiades Publishing, Ltd., 2012.
Original Russian Text © I.A. Tsaplina, A.E. Zhuravleva, N.V. Grigor'eva, A.V. Belyi, T.A. Pivovarova, A.G. Bulaev, V.S. Melamud, T.F. Kondrat'eva, 2012, published in Mikrobiologiya, 2012, Vol. 81, No. 3, pp. 314–324.

EXPERIMENTAL ARTICLES

Biooxidation of a Gold-Containing Sulfide Concentrate in Relation to Changes in Physical and Chemical Conditions

I. A. Tsaplina[a, 1], A. E. Zhuravleva[a], N. V. Grigor'eva[a], A. V. Belyi[b], T. A. Pivovarova[a], A. G. Bulaev[a], V. S. Melamud[a], and T. F. Kondrat'eva[a]

[a] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia*
[b] *Polyus Closed Joint-stock Company, Krasnoyarsk Region, Russia*

Received September 15, 2011

Abstract—The growth of a microbial community and the oxidation of iron- and sulfur-containing substrates in batch culture during the leaching/oxidation of the flotation concentrate of refractory gold–arsenic sulfide ore were optimized with respect to the following medium parameters: temperature, pH, and requirement in organic substances. It was revealed that the optimum mode is (i) to maintain the pH at 1.6–1.7 and the temperature at 34–35 and 38°C and (ii) to add C_{org} in the form of yeast extract (0.02%). Mutually beneficial or competitive relationships among groups of microorganisms of the community were established, depending on the cultivation conditions.

Keywords: biooxidation of the flotation concentrate of sulfide ore, mode optimization, microbial community, trophic functions.

DOI: 10.1134/S0026261712030149

Optimizing the technological process-related variables is a prerequisite for increasing the rate of biological oxidation of sulfide minerals and the degree of recovery of noble metals. The temperature and pH values and the requirement in organic substrates are the most important factors influencing the growth and energy metabolism of microorganisms, their developmental dynamics, succession of their dominant groups in the microbial community, and efficiency in leaching/oxidizing the mineral components of ore concentrates.

According to the literature, sulfur oxidizers (*Acidithiobacillus caldus, Acidithiobacillus thiooxidans*, and *Sulfobacillus* species) prevail during the initial stages of leaching gold-, copper-, and nickel-containing sulfide ore concentrates at mesophilic (30 and 35°C) and moderately thermophilic conditions (39 and 45°C). During the intermediate and the late stages of the process, sulfur oxidizers are replaced by iron oxidizers. As a rule, *Leptospirillum ferriphilum* is the dominant species [1–4]. Raising the temperature results in decreasing *Acidithiobacillus* numbers and increasing the *Sulfobacillus* share, while retaining the dominance of the leptospirilli. Decreasing the pH to 1.2–1.0 gives an advantage to the microorganisms resistant to high acidity values. These are *L. ferriphilum* and some mixotrophs similar to the *Ferroplasma* archaea, whose growth is activated by the acid-induced lysis of a part of the cells of the microbial community and, accordingly, the release of C_{org} into the medium liquid phase [1]. The presence of heterotrophic cultures in the community, such as *Alicyclobacillus* spp., *Ferroplasma* spp., and *Ferrimicrobium acidiphilum*, increases the degree and rate of pyrite leaching and oxidation [1, 2, 5, 6]. For instance, addition of an organic substrate (0.005–0.05% of yeast extract) significantly influenced the succession of the predominant species in the community and stimulated the leaching and oxidation of chalcopyrite upon raising the temperature to 40–60°C [4] and of pyrite at 30°C [6]. Hence the most efficient community includes Fe^{2+}-, S^{2-}/S^0-oxidizers and heterotrophic microorganisms.

The goal of this work was to elucidate the influence of (i) the pulp temperature and pH values maintained during the biooxidation of ore flotation concentrate and (ii) yeast extract-induced changes in the medium composition on the process-related variables during the growth dynamics of the microbial community.

MATERIALS AND METHODS

Research subject. An enrichment culture of microorganisms was obtained from ore flotation concentrate at 35–37°C. The microbial community used as inoculum was supplemented with bacteria and archaea isolated from pulp samples from the reactor of the gold-recovery plant of the Polyus Closed Joint-stock Company and with the following cultures from the collection of cultures of chemolithotrophic microorganisms

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

of the Institute of Microbiology, Russian Academy of Sciences: *Acidithiobacillus ferrooxidans* OL10-01, *Leptospirillum* sp. OL10-02, *Sulfobacillus thermosulfidooxidans* OL10-03, *Ferroplasma acidiphilum* OL10-04, and *Alicyclobacillus tolerans* OL10-05 [7].

Flotation concentrate composition. The contents of the main elements of the sulfide flotation concentrate of the ore was as follows: Au, 79.0 g/t; Fe_{total}, 25.40%; As_{total}, 7.23%; S_S, 19.80%; and Sb_{total}, 3.69%. Pyrite, arsenopyrite, pyrrhotite, and antimonite were the main sulfide minerals of the flotation concentrate.

Cultivation conditions. To obtain an enrichment microbial culture, flotation concentrate samples were placed in 250-mL flasks with 100 mL of Silverman–Lundgren 9K medium [8] supplemented with 1 g/L elemental sulfur. The medium was adjusted to pH 2.0 with 10 N H_2SO_4. The flasks were placed on a shaker (150 rpm) in a thermostat at 35–37°C. After 10 days, a decrease in medium pH and growth of microorganisms (small and large rods and archaea) were observed.

The inoculum was cultured as follows. The samples of disintegrated ore concentrate (5 g) were placed in 250-mL flasks supplemented with the above medium without additional mineral energy sources and 10% inoculum (vol/vol). The total volume of the pulp was 100 mL. Cultivation was carried out at a temperature of 36°C and the initial pH value of 1.8. The medium composition in the experiments was the same as above. The flasks were incubated in a thermostat at the specified temperature under dynamic conditions, i.e. they were rotated on a shaker (Incubator 1000 Heildolph, Germany, 180 rpm). The microorganisms were grown in batch cultures, and the pulp density in all experiments was 7%.

Studies aimed at determining the pH optimum for the oxidation of the flotation concentrate sulfides were conducted at a temperature of 36°C. The preset experimental pH level (below 1.8) was maintained by acidifying the medium with concentrated H_2SO_4 or alkalifying it with 20% $NaHCO_3$ solution during the day. The differences in the preset pH level between experimental variants were multiples of 0.1 pH unit. The culture grew overnight without pH control, whereupon pH was readjusted to the initial value; the overnight pH change was recorded.

The studies aimed at determining the temperature optimum were conducted at a constant temperature within the 34–38°C range. The temperature differences between experimental variants were multiples of 1°C. The pulp pH was maintained at the optimum level of 1.7 that was determined in the previous studies.

In the studies on the influence of C_{org}, i.e., yeast extract (YE), on sulfide oxidation in the concentrate, the microorganisms of the tested community were cultivated at a temperature of 36°C and an initial pH level of the liquid phase ~2.0. The YE concentration was 0.02%.

In the studies aimed at determining the dominant microbial group within the community under various cultivation conditions, the flotation concentrate was replaced with either S^0 (0.5%) or Fe^{2+} (8 g/L) as the sole energy source in the growth medium. The microbial community was grown at 34 or 38°C at the optimum initial pH value. The inocula were microbial community cells grown at various temperature and pH values, under the conditions that stabilized them on the medium with sulfide concentrate.

Analytical techniques. The pH and Eh values were monitored during the cultivation using a pH-150M device (Belarus). The tri- and bivalent iron contents were determined by trilonometric titration [9]. Total arsenic content in the liquid phase of the pulp was determined by iodometric titration, which was based on binding iron ions with titanium chloride $TiCl_3$ [10]. The sulfite ion content was determined turbidimetrically [11]. The number of microbial cells was determined by direct counts and by the terminal dilution method. The physiological state of the microorganisms was monitored with a Lyumam I1 microscope (LOMO, Russia) equipped with a phase-contrast device. The C_{org} concentration at the beginning and at the end of the experiment was determined by the bichromate method and expressed in COD (chemical oxygen demand) units, i.e. glucose equivalents [12].

The results were statistically processed using the Student's test at a 5% significance level [13].

RESULTS

Effect of pH

The growth of the microbial community. The dynamics of growth of the microbial community at controlled pulp pH values is shown in Fig. 1a. Obviously, pH levels of 1.6–1.8 were the optimum for microbial growth; the maximum was attained at pH 1.8. The cell number was $26.28 \times 10^7 - 28.35 \times 10^7$ cells/mL if the pH value was maintained within the above range. At more acidic pH values, the microbial cell concentration was lower: it was 16.74×10^7 cells/mL at pH 1.4. At the optimum pH values, the microbial community grew almost without a lag phase. At pH 1.7, it developed at a low rate for the first two days of cultivation. Thereupon, the division rate constant of the community population drastically increased to the maximum value of 0.06 h^{-1} and remained constant for 3–4 days of the experiment, resulting in a high biomass yield. If the pH value was maintained at 1.8, the cell division rate constant was somewhat lower (0.05 h^{-1}); however, the cell number at the end of the growth phase was the highest.

During the whole observation period, rounded and mostly small ($0.3–0.5 \times 0.8–1.0$ μm) cells prevailed in the community. They were morphologically similar to

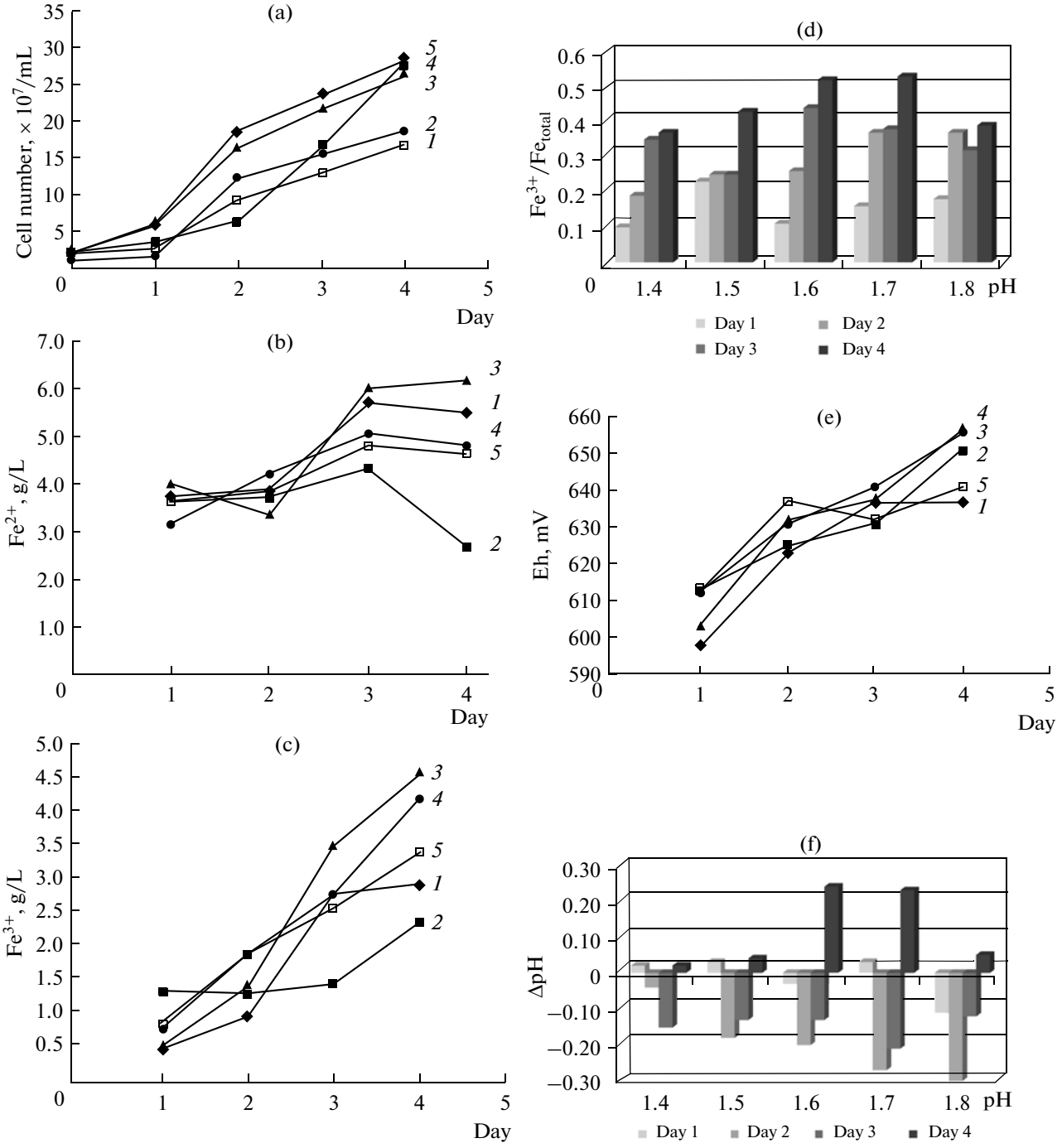

Fig. 1. Growth-related variables of the microbial community and the biological leaching/oxidation activity at controlled pH values of the pulp. The dynamics of growth (a); Fe^{2+} leaching (b); Fe^{2+} oxidation (based on monitoring Fe^{3+} concentrations) (c); changes in the Fe^{3+}/Fe_{total} ratio (d); redox potential (ΔEh) (e); and pH value (ΔpH) during the unregulated period (f). Designations in (a), (b), (c), and (d): *1*, pH 1.4; *2*, pH 1.5; *3*, pH 1.6; *4*, pH 1.7; and *5*, pH 1.8.

the cells of acidithiobacilli and archaea. Medium-size (0.6–0.8 × 1.2–1.5 μm) rods were less numerous. A minimal number of large rod-like forms (0.7–1.0 × 1.5–2.5 μm) occurred in the total population. In morphological terms, the latter two bacterial groups could be affiliated with typical sulfobacilli and alicyclobacilli, respectively. The dominance of the above microbial groups in the tested variants was confirmed by inoculating the community on media with Fe^{2+}, S^0, and YE.

Maintaining the pH values in the tested variants required the addition of 0.4–0.6 mL of H_2SO_4 per 100 mL of the pulp.

Iron leaching and oxidation. Starting from the first days of growth, leachable Fe^{2+} accumulated in the liquid phase of the pulp (Fig. 1b). Its content reached values of 4.0 to 4.8 g/L at pH levels of 1.5 and 1.7, respectively. Maximum concentrations of 6 g/L were attained on day 3 of growth at pH 1.6. In this variant, the maximum iron leaching rate, 3.0 g/(L × day), was recorded. High Fe^{2+} leaching rates also occurred at the lowest tested pH values, particularly at pH 1.4 (1.9 g/(L × day), apparently because extreme acidophilic bacteria and archaea were selected for [1].

Starting from the first days of the experiment, Fe^{2+} oxidation proceeded concomitantly with leaching (Fig. 1c). Subsequently, on day 3 of cultivation, the biomass (Fig. 1a) and the amount of iron leached and oxidized increased at all pH values (Figs. 1b, 1c). The same pattern persisted during the fourth day of cultivation, although the rate of iron leaching decreased. The maximum iron oxidation rates (2.2 and 1.5 g/(L × day)) were achieved in experimental variants, with pH values of 1.6 and 1.7 during the third and the fourth day of population growth, respectively. The Fe^{3+} content in the liquid phase of the pulp was maximum at pH 1.6 (Fig. 1c). The oxidation of leached iron also proceeded in a stable manner at pH 1.8 with an average rate of 0.8 g/(L × day).

In Fig. 1d, the ratio between the ferric iron and the total iron (Fe^{3+} + Fe^{2+}) content during the community's growth is plotted. It is evident that the ratio was maximum at pH 1.6–1.7 after 4 days of growth. During day 5, the same pattern persisted, although iron oxidation slowed down (data not shown).

Dynamics of Eh, pH, and arsenic leaching. On day 1 of growth, while Fe^{2+} ions were accumulating in the liquid phase at all pH values, the Eh level indicated that the quantity of ferrous iron significantly exceeded that of ferric iron (Fig. 1e). After 2–3 days, the redox potential value increased to 637 mV at pH 1.4 and to 641 mV at pH 1.7. The fourth day of growth was characterized by an additional increase in Eh to 656–657 mV while the pH of the medium was stabilized at 1.6 and 1.7, respectively.

Measuring arsenic concentrations revealed that the ΣAs content in the liquid phase of the pulp (1.20–1.28 g/L) agreed with the pattern observed while cultivating the microbial community at pH 1.7–1.8. ΣAs at other pH values fluctuated within the 0.92 ± 0.12 g/L range.

Based on the comprehensive studies on the leaching/oxidation of metal sulfides of the Olympiadinskaya ore flotation concentrate by the microbial community at various pH values, the conclusion was drawn that the optimum cultivation mode is to maintain the pH value at a level of 1.6–1.7.

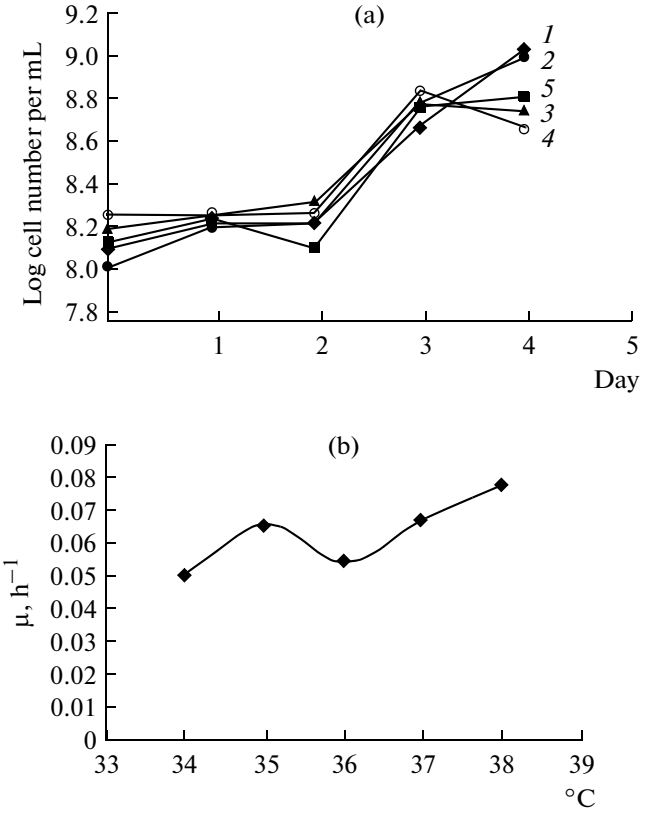

Fig. 2. Growth dynamics (a) and the maximum value of the cell division constant (b) plotted against cultivation temperature. Designations (a): *1*, 34°C; *2*, 35°C; *3*, 36°C; *4*, 37°C; and *5*, 38°C.

The pulp pH fluctuations during the uncontrolled night period were plotted (Fig. 1f). These data made it possible to characterize the trophic requirements of the microbial community. The growth dynamics was characterized by a succession of groups of organisms that played dominant roles in oxidizing the iron or the sulfur of the sulfide concentrate. For instance, on day 1 (at pH 1.6 and especially at pH 1.8) and day 2 (at all tested pH values) we found a drop in pH by 0.05–0.30 units overnight. On day 3 of the experiment (during the night), the pH level decreased by 0.13–0.23 units. Subsequently, after stabilizing the pH value and the daytime growth, the pH value increased overnight. The maximum alkalization (by 0.21–0.23 units) occurred in the variants with pH values of 1.7 and 1.6 on day 4.

Taken together, the data obtained suggested that two processes, iron oxidation and sulfur oxidation, were concomitantly carried out in the pulp. In the course of iron oxidation, an increase in Fe^{3+} concentration was recorded (Figs. 1c and 1d), especially in the variants with pH 1.7 and 1.6 on day 4 of growth. Concomitantly, an increase in pH of the medium was noted (Fig. 1f). Sulfur oxidation to sulfate ions is always accompanied by a pH decrease. This was found

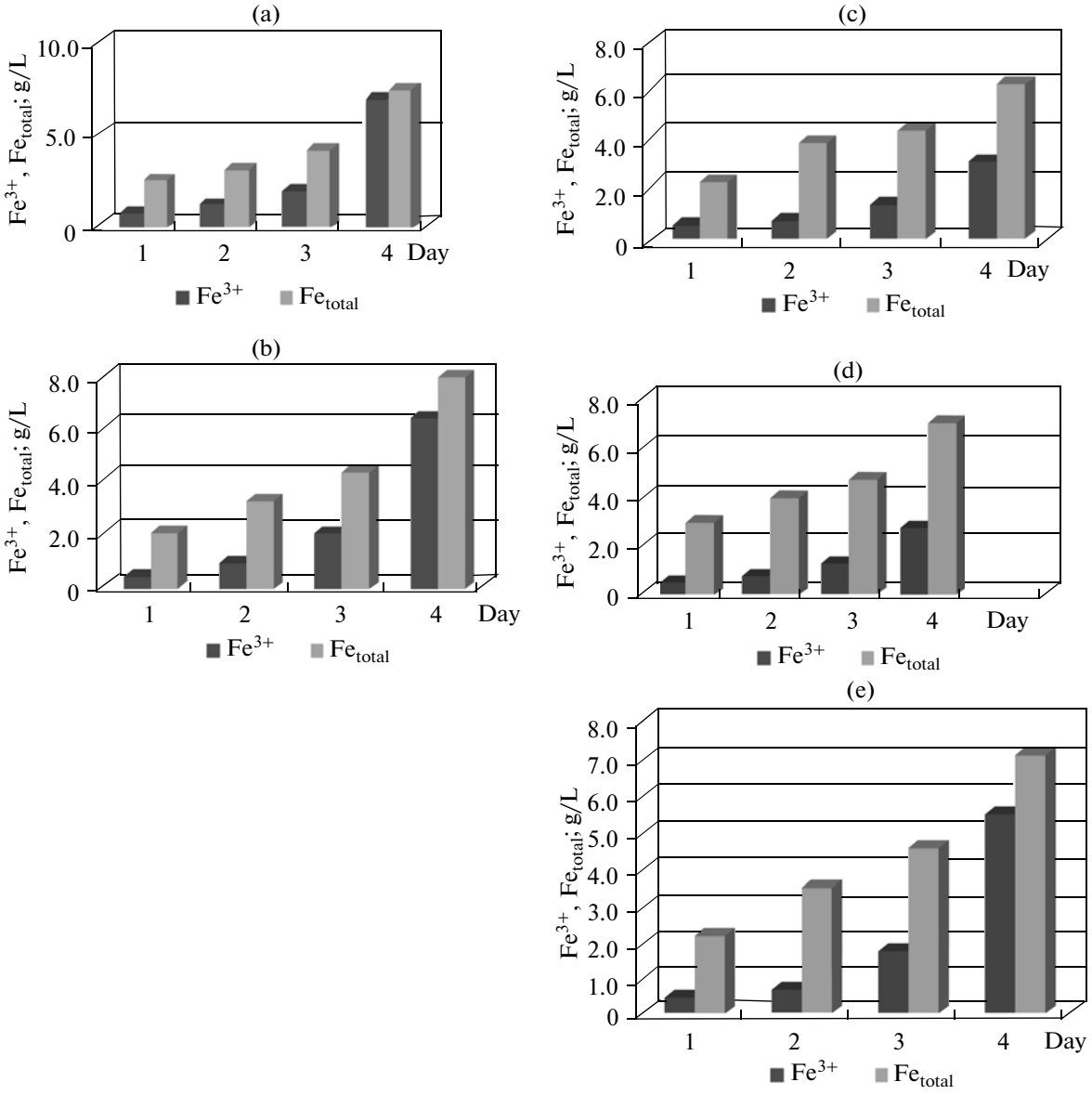

Fig. 3. Changes in the Fe^{3+} and Fe_{total} content in the liquid phase of the pulp during the cultivation of the microbial community at various temperatures: 34°C (a); 35°C (b); 36°C (c); 37°C (d); and 38°C (e).

in our variants, particularly at pH 1.7 and 1.8 during the first two days of the experiment and, in all tested variants, on day 3 (Fig. 1f). A slight increase in pH (starting from the zero point) at a low concentration of Fe^{2+} oxidized overnight could result from the parallel oxidation of reduced inorganic sulfur compounds (RISCs) and Fe^{2+}.

It was established that RISC oxidation was the predominant process during the second–third days of cultivating the microbial population at a temperature of 36°C and all tested pH values (particularly, in the variants with pH 1.7 and 1.8 on day 2 and with pH 1.7 also on day 3). This was due either to the dominance of S oxidizers or to the activation of S substrate oxidation by bacteria capable of oxidizing the sulfur component of the sulfide ore. Temperature was a possible factor that could "tease apart" the organisms similar in terms of substrate specificity and shed light on the substrate requirements of the tested groups of organisms.

Temperature Influence

The 34–38°C temperature range was investigated in this work. The physical, chemical, and microbiological process-related variables are plotted in Figs. 2–4.

Microbial community growth. Fig. 2a, represents the growth dynamics of the community cells population at various temperatures. During days 1 and 2, the microorganisms gradually adapted to the substrate and

the temperature mode. The variant with a temperature of 38°C was exceptional: the cell number dropped on the second day of incubation owing to the lysis of a part of the cells. Apparently, these were the cells of the mesophilic cultures that formed a part of the microbial community and were more responsive to an increase in temperature from 36 to 38°C. At 38°C, sulfobacilli (~80%) clearly prevailed over acidithiobacilli on day 2 of cultivation. They accounted for >90–95% of the total cell population after 4 days of growth.

Changes in the minimum cell generation time at all tested temperatures were monitored (Fig. 2b). Evidently, the division rate constant was maximum (0.08 h^{-1}) at 38°C. This also suggested a succession of dominant groups within the community. The prevalent group comprised the mixotrophic microorganisms with the highest growth rate that were adapted to cultivation at high temperatures and utilized organic substances resulting from the prior lysis of the cells of mesophilic lithoautotrophs.

At 36 and 37°C, the growth period was terminated earlier than at other tested temperatures. Partial cell lysis also occurred on day 4. Cell division persisted only at 34 and 35°C. By the end of day 4, the maximum population cell number in the 34°C and the 35°C variants reached 10.4 × 10^8/mL and 9.01 × 10^8/mL, respectively (Fig. 2a).

Monitoring the morphological alterations in the community population revealed that two microbial groups, *Sulfobacillus* spp. and *Acidithiobacillus* spp., dominated the community on days 2 and 3 of growth. Their ratio was 1 : 1 or sulfobacilli were slightly more numerous (50–60% of the total cell population). After 4 days, the share of sulfobacilli increased to 65–70%, while that of the acidithiobacilli, in contrast, decreased due to a partial lysis of the cells within the population. At 34–36°C, all morphological cell types occurred in the microbial community; at 34–35°C, acidithiobacilli and archaea dominated the community. Control studies in which the population was inoculated confirmed these results.

Iron leaching and oxidation. The concentration of leached iron significantly exceeded that of ferric iron at all tested temperatures during day 1, when the community cell number changed insignificantly (Figs. 3a–3e, 4a). The pattern persisted on day 2 of growth, i.e., the rate of iron leaching from the concentrate was higher than that of iron oxidation. Thereafter, the iron oxidation rate increased. The maximum values were characteristic of the community cultivated at 34, 35, and 38°C; they were 4.7, 4.4, and 3.7 g/(L × day), respectively (Figs. 3a, 3b, and 3e). The Fe^{3+}/Fe_{total} ratio approached unity, which was indicative of a high rate of metabolic reactions and lent additional support to the conclusion that temperatures of 34, 35, and 38°C were the optimum. At temperatures of 36 and 37°C, the iron oxidation rates were considerably lower

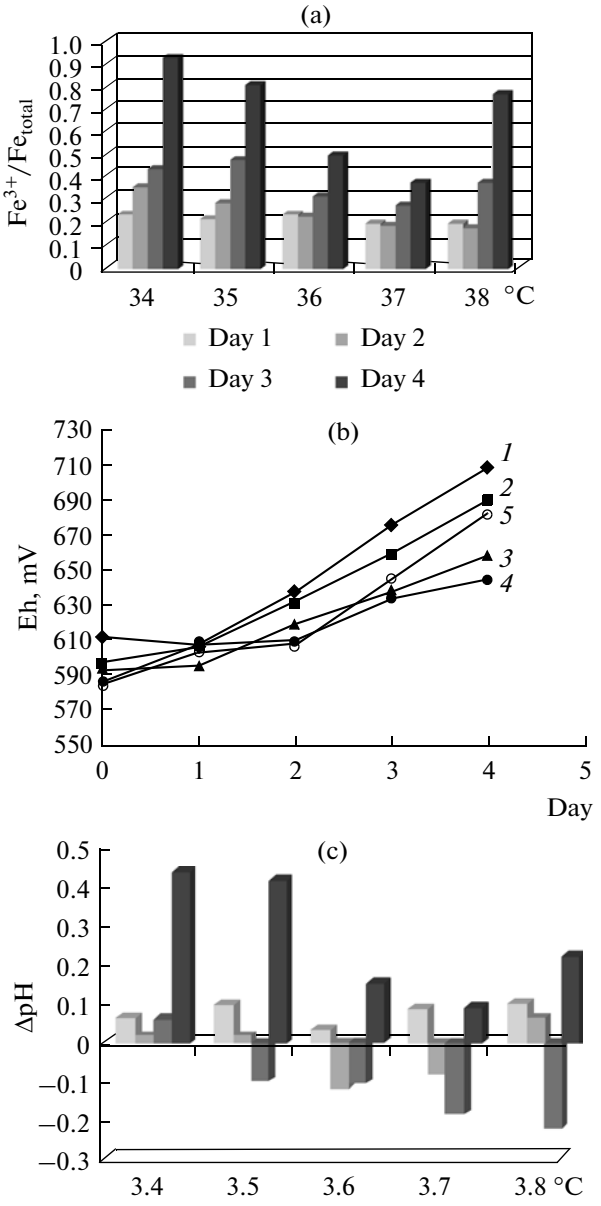

Fig. 4. Changes in the Fe^{3+}/Fe_{total} ratio (a), redox potential (b), and pH (during the uncontrolled period) (c) in the liquid phase of the pulp during the cultivation of the microbial community at various temperatures. Designations (a): *1*, 34°C; *2*, 35°C; *3*, 36°C; *4*, 37°C; and *5*, 38°C.

(Figs. 3c–3d), as the data of the plot (Fig. 4a) demonstrate.

The dynamics of Eh and pH and arsenic leaching. Measuring the Eh values (Fig. 4b) during the growth of the community confirmed the findings that 34–35°C and 38°C were the most favorable temperatures for carrying out the biooxidation of sulfide minerals. The Eh values reached 683–709 mV by the end of the process.

Similar arsenic concentrations were found in the liquid phase of the pulp in all tested variants. The maximum level was 1.44–1.76 g/L if the microbial com-

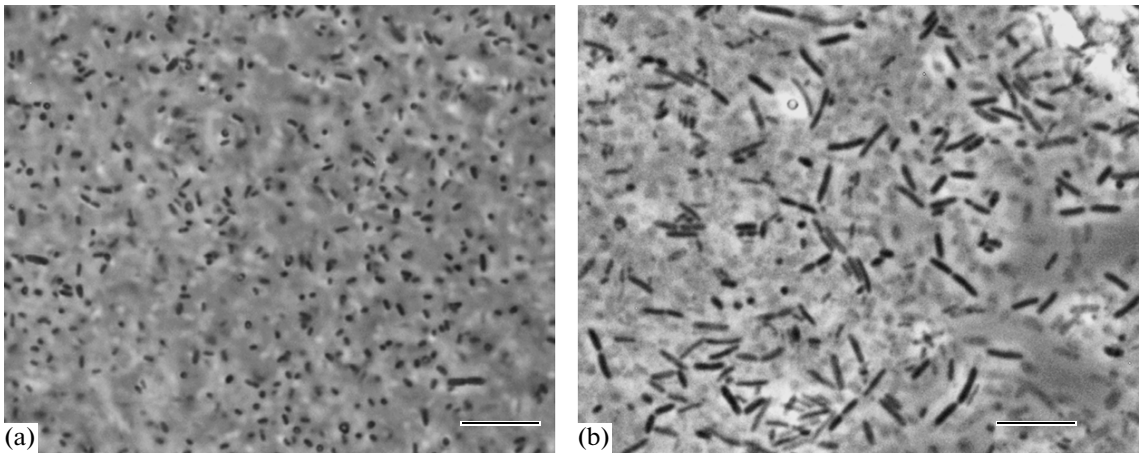

Fig. 5. General view of the cells of the microbial community grown at 34°C (a) and 38°C (b) on ore flotation concentrate. Scale bar, 10 μm.

munity grew at 34–35°C. It was 1.50 g/L at 38°C. Arsenic concentrations were at intermediate levels (1.24–1.36 g/L) within the 36–37°C range.

As was mentioned above, the pH value was maintained at a level of 1.7 during the daytime. On day 1, after an uncontrolled period, iron-oxidizing microorganisms prevailed at all tested temperatures because the ΔpH value was positive, due to iron oxidation and leaching (Fig. 4c). During this period, we could not rule out the concomitant oxidation of S^{2-}/S^0 that was made available for the cells by opening-up the sulfides. During the second day, the process rates changed only insignificantly, except for the 36°C and the 37°C variants, where pH level dropped below zero, similar to the first series of experiments at pH 1.7 (Fig. 1f). On day 3, the overnight pH drop was detected in virtually all variants (except the 34°C variant), which confirmed the conclusion concerning the active sulfur oxidation (in all "pH variants"). The RISC oxidation process was carried out particularly actively at 37 and 38°C. After 4 days, the pH increment (ΔpH) was characterized by the maximum positive values. This is consistent with the results of direct measurement of iron oxidation and provides additional evidence that the microbial community always preferably utilized ferrous iron as the main energy substrate on day 4 of growth. The maximum iron oxidation-related data were recorded at 34, 35, and 38°C at pH 1.7 (Figs. 3a, 3b, and 3e) and at 36°C at pH 1.6, 1.7, and 1.8 (Fig. 1c).

Hence, the organisms with a high cell yield and high iron-oxidizing activity at pH 1.6–1.7 dominated the 34–35°C community. If the community was cultivated at 36–38°C, sulfur oxidation was markedly promoted. These temperatures also correspond to the highest growth rates of the microbial community (0.067–0.080 h^{-1}).

Determining the Dominant Microbial Group within the Community under Various Cultivation Conditions

In our subsequent studies, we tested the suggestion that Fe- and S-oxidizers are involved in replacing the complex energy source in the medium, i.e., the flotation concentrate of sulfide minerals, with a sole energy source such as S^0 or Fe^{2+}.

We confirmed that S^0 oxidation proceeded particularly actively at the above temperatures and at pH 1.7. This was the initial pH value in the studies on the influence of various growth temperatures and the optimum pH value in the first experiment concerning ΔpH. The cell population of the community grew at a higher rate at 38°C; sulfobacilli prevailed in it, while archaea and acidithiobacilli represented minor groups. The conclusion also confirmed that the temperature of 34°C stimulated the growth of mesophiles in the community. At 34°C, acidithiobacilli prevailed at the end of the growth period; they displayed lesser S-oxidizing activity in comparison to sulfobacilli (at 38°C). In Figs. 5a and 5b, the morphology of the microbial community at 34 and 38°C, respectively, is shown. The sulfate ion was the main product of S^0 oxidation as the control studies revealed in which sulfate salts were replaced with chloride salts (38–40 mM) in the incubation medium.

An analogous series of experiments on the medium with Fe^{2+} revealed that the optimum for the manifestation of the iron-oxidizing activity of the microbial community pre-cultivated at various temperature and pH values behaved in a more complicated manner. Apparently, the reason was that a greater number of groups of organisms specializing in iron oxidation (*Acidithiobacillus ferrooxidans, Ferroplasma* spp., and *Sulfobacillus* spp.) were present in the community. Nonetheless, we confirmed that high substrate oxidation rates and Fe^{3+} concentrations (up to 7.2 g/L) at

Table 1. Organic matter concentration in the liquid phase of the pulp

Variant	Initial concentration C_{org}, g/L	Final concentration, C_{org}, g/L
Control	0.16	0.23
Experiment	0.40	0.25

the end of the growth period were attained at 34 and 38°C and pH 1.6.

In addition, a number of experimental variants were revealed that exhibited equal iron- and sulfur-oxidizing activities. This indicated that these microbial groups established competitive relationships if they were used for oxidizing sulfide-containing ores and concentrates. In continuous cultures, the relationships between microorganisms within the community can be mutually beneficial if the pH and temperature modes are optimum for the oxidation of sulfide minerals.

Requirement in Organic Substances

One of the goals of this work was to determine the effect of adding organic matter (C_{org}) to the pulp on the growth rate of the microbial community and on the rate of leaching/oxidation of the sulfide minerals of the flotation concentrate. Yeast extract (YE, 0.02%) was added as an organic carbon source to 7% pulp.

YE is known to stimulate microbial growth. It contains vitamins, peptides, amino acids, nucleotides, organic acids, organic iron-containing complexes, essential microelements, and other substances. This system is a convenient model for testing the requirements of microorganisms with respect to C_{org} in the medium and ability of microbial cells to switch from a lithotrophic to a mixo- or organotrophic nutrition mode.

The sulfobacilli and archaea of the community are mixotrophic organisms whose growth rates vary depending on the presence of small C_{org} amounts in the medium. Upon addition of organic substances, the rates of oxidation of the mineral substrate and growth processes significantly increase [14]. Moreover, heterotrophic organisms are also present in the community.

C_{org} concentration. Table 1 gives the content of soluble organic substance (C_{org}) in the control (without YE) and experimental (with YE) variants without inoculum and after the end of the cultivation of the microbial community. The original medium that included sulfide mineral concentrate as the main energy source for the growth of the organisms contained 0.16 g/L C_{org}. Supplementing the original experimental pulp with an additional amount of C_{org} in the form of 0.02% of YE increased the C_{org} content in the medium 2.5-fold.

Toward the end of the cultivation of the microbial community, i.e., after 4 days, the C_{org} content in the control variant increased 1.4-fold, amounting to 0.23 g/L. This attested either to the autolytic processes in the cell population, or to the accumulation of organic products of metabolism by microbial cells and their release into the liquid phase of the pulp, or to a combination of both factors. In the experimental variant, the total concentration of organic substances decreased 1.6-fold and was 0.25 g/L after 4 days of cultivating the community. It can be concluded that the organisms of the community utilized the C_{org} added [$0.4 - 0.16 = 0.24$ (g/L)]. However, they did not use,

Table 2. Growth dynamics of the microbial community supplemented with 0.02% of yeast extract

Day	Variants	pH	Eh, mV	Fe^{2+}, g/L	Fe^{3+}, g/L	Fe_{total}, g/L	Cell number, 1×10^7/mL
1	Control	1.91	614	3.25	0.99	4.24	5.90
	Experiment	1.89	627	2.52	1.23	3.75	5.95
2	Control	1.65	624	3.90	1.26	5.16	6.10
	Experiment	1.80	633	3.71	2.73	6.44	163.0
3	Control	1.78	648	3.92	2.94	6.86	228.0
	Experiment	1.96	667	3.01	5.18	8.19	260.0
4	Control	1.94	653	4.20	3.92	8.12	233.0
	Experiment	1.85	732	0.21	8.12	8.33	279.0

under indisputably mixotrophic conditions, the organic substances contained in the pulp, the concentration of which increased by approximately the same amount (0.08 ± 0.01 g/L) as in the control variant.

The growth dynamics of the community. Table 2 contains the results of experiments in which additional organic matter was introduced. In the control variant, the cell number only insignificantly changed during two days of growth, whereas after 4 days it increased from 6.1×10^7 to 23.3×10^8/mL. According to the microscopic data, small forms such as, probably, acidithiobacilli, unevenly dividing sulfobacilli, very small rod-shaped bacteria, and archaea, predominated in the control variant. The percentage of large rod-shaped bacteria such as sulfobacilli or some alicyclobacilli did not exceed 10%.

Changes in the pH value of the liquid phase of the pulp testified to the prevalence of oxidation processes. A marked decrease in pH during the first day of growth—by 0.16 units in the control and by 0.11 units in the experimental variant—probably indicated more active oxidation of sulfur compounds, particularly in the former variant where the autotrophic acidithiobacilli were not exposed to the inhibitory influence of YE. On day 4 of the experiment, a pH increase occurred (by approximately 0.3 units, compared to day 2), corresponding to an increase in the rate of iron leaching and oxidation by Fe oxidizers, such as acidithiobacilli, archaea, and sulfobacilli.

In the experimental variant, a pH increase by 0.16 units, i.e. to a greater extent than the preceding pH decrease, was detected on day 3 with YE. This attested to alterations in the structure of the dominant microbial groups of the community. Iron oxidizers could dominate the community again. During day 4, the pulp was acidified once more, by 0.11 units. This suggested that sulfur oxidizers functioned more actively. Microscopy of the liquid phase of the pulp revealed that the growth of the cells of the microbial community in the medium with YE continued until the end of the experiment. The cell number increased from 5.95×10^7/mL to 27.9×10^8/mL. It increased significantly as early as during day 2 of cultivation: from 5.95×10^7 to 16.3×10^8 cells/mL. Morphologically different groups of microorganisms were present, including very small and, apparently, heterotrophic bacteria and the bacteria *Acidithiobacillus* spp., *Ferroplasma* spp., and *Sulfobacillus* spp. (about 20%). During days 3 and 4, the cells of the community divided at a slower rate; the cell yield was $26.0–27.9 \times 10^8$/mL. The community structure did not change. The number of sulfobacilli increased. Motile forms were detected among the smaller bacterial forms. Hence the microbial community responded by approximately twofold acceleration of growth and cell division to the addition of supplementary C_{org} to the medium.

Iron leaching and oxidation. Iron leaching and oxidation also proceeded more actively upon addition of YE to the pulp (Table 2). The maximum total content of all iron forms in the liquid phase in the control and the experimental variant was similar. It was 8.12–8.33 g/L after 3–4 days and 8.1 g/L after 4 days in the experimental and the control variants, respectively. However, by the end of the process, the level of unoxidized iron in the control variant remained significant—4.2 g/L (compared to 0.2 g/L in the experiment). The maximum Fe^{2+} oxidation rate in the control variant (without YE) was 1.7 g/(L × day) on day 3, i.e., it was below that in the organic substance-containing variant where it was as high as 3 g/(L × day) on day 4. By the end of the experiment, the Fe^{3+} content was 8.1 g/L with YE and 3.9 g/L in the control variant. Based on the data on the pH drop in the experimental variant during the same period, the conclusion may be drawn that not only iron (Table 2) but also RISCs were oxidized during the four days. In contrast, active leaching of iron from the concentrate proceeded in the control variant during the four days. The periods of the most active oxidation of RISCs and iron were separated in time and corresponded to days 2 and 3 of the microbial community growth. The control variant required an increase in the incubation time for a more complete oxidation of iron.

Arsenic leaching and the Eh dynamics. Arsenic concentration in the liquid phase of the pulp was 1.5 times higher in the experimental variant (2.2 g/L) than in the control variant (1.4 g/L).

During the growth of the community, the Eh level upon adding C_{org} to the pulp was higher in the experimental variant. At the end of the process, it reached 732 mV if YE was added and 653 mV in the control variant (Table 2).

DISCUSSION

The rise in the temperature maintained in a reactor is conditional on the rates of the exothermic reactions associated with metal sulfide oxidation. The pulp pH dynamics is influenced by the mineralogical composition of the flotation concentrate, i.e., by the content of calcites, pyrrhotite, and other components. The variability of the composition of the flotation concentrate of sulfide ore is the reason for the metabolic alterations in the microbial community. Since mixotrophs requiring small amounts of organic substances play an important role in the communities of acidophilic chemolithotrophic microorganisms, monitoring their content in the liquid phase of the pulp is of special importance. Maintaining the temperature and pH values and organic substance amounts in the reactor that are optimum for the microbial community is necessary for stabilizing and enhancing the process efficiency.

Investigating the influence of pH in the liquid phase of the pulp on the growth dynamics and the oxidative activity of the microbial community and on the dynamics of the physicochemical variables made it

possible to determine the optimum acidity values for the medium. At pH values of 1.6 and 1.7, higher iron oxidation rates were detected, and a decrease in pH was accompanied by the oxidation of S^{2-}/S^0, the more energy-demanding component of sulfide minerals. The dynamics of pH changes during the uncontrolled night period provided material for evaluation of the trophic links within the microbial community that reflect the mutually beneficial and competitive relationships.

Analysis of the dynamics of the growth of the microbial community and iron leaching and oxidation (with the temperature varied within the 34–38°C range) revealed that the optimum temperatures for the process were 34, 35, and 38°C. At 34–35°C and pH 1.6–1.7, the microbial community utilized Fe^{2+} as the main oxidizable substrate. At 37–38°C and pH 1.7–1.8, the processes associated with sulfur oxidation were activated.

The results obtained not only provide insights into the trophic interactions between the components of the microbial community. They may also be used to regulate the oxidation of the mineral energy substrate with external factors that are present in excess. For instance, excess iron in the concentrate enables us to cultivate the community at pH 1.4–1.5 and a temperature of 34°C. Under these conditions, the most active and low pH-tolerant organisms of the community are selected for. The process duration is to some extent increased because sulfur oxidation is slowed down.

We revealed the effects of C_{org} added to the medium (YE) on the process dynamics. The organic substance contained in the original sulfide concentrate was not utilized if the microbial community grew without YE. The reason was that the cells of chemolithoautotrophs lacked the relevant enzyme systems [15, 16]. Upon addition of the easily utilizable YE to the pulp, the microbial community still failed to utilize some of the organic substances of the medium, even though organic compounds could support the growth of the mixotrophic components of the community (sulfobacilli and archaea) [1, 14]. The C_{org} content increased in the control variant, and this provided evidence that the increase was due to the presence of organic exometabolites in the liquid phase. It was revealed that a complex ensemble of metabolites including amino acids, nucleotides, organic acids, and other substances was released into the medium during the growth of the autotrophic bacterium *Thiobacillus* (*Acidithiobacillus*) *ferrooxidans*, which provided for its resistance to molybdenum in the presence of Fe^{3+} [17]. It seems likely that the protection of a microbial community from heavy metal ions accumulating in the liquid phase (As, Sb, etc.) implicates the synthesis and excretion from the cell of metabolites that can stabilize the community and maintain the active viable state of the cells. The feasibility of this scenario is consistent with the fact that a lag phase occurs during the initial stage of community growth under autotrophic conditions (without YE). There is no manifest lag phase in the presence of YE. Mixotrophic organisms are able to use a portion of the organic substances from yeast extract in order to incorporate it in the protective complex. The other part can be utilized in their metabolism, which is more active than that of the autotrophs [16].

It was established during our experiments that the cells of the microbial community undergo marked metabolic changes. The community is switched over to preferentially leaching/oxidizing iron or to oxidizing the RISCs of the flotation concentrate, depending on the physicochemical parameters of the process. The results obtained make it possible to draw guidelines for regulating and optimizing the continuous bioleaching process and oxidation of sulfide concentrate components, taking account of relevant physical and chemical factors and the dominance of specific groups of microorganisms.

ACKNOWLEDGMENTS

The work was supported by the Polyus Closed Joint-stock Company and by the Russian Foundation for Basic Research, project no. 10-04-00589.

REFERENCES

1. Okibe, N. and Johnson, D.B., Biooxidation of Pyrite by Defined Mixed Cultures of Moderately Thermophilic Acidophiles in pH-Controlled Bioreactors: Significance of Microbial Interactions, *Biotech. Bioeng.*, 2004, vol. 87, no. 5, pp. 574–583.
2. d'Hugues, P., Joulian, C., Spolaore, P., Michel, C., Garrido, F., and Morin, D., Continuous Bioleaching of Pyrite Concentrate in Stirred Reactors: Population Dynamics and Exopolysaccharide Production vs. Bioleaching Performance, *Hydrometallurgy*, 2008, vol. 94, pp. 34–41.
3. Hao, C., Wang, L., Dong, H., and Zhang, H., Succession of Acidophilic Bacterial Community during Bio-Oxidation of Refractory Gold-Containing Sulfides, *Geomicrobiology*, 2010, vol. 27, pp. 683–691.
4. Li, Q., Tian, Y., Fu, X., Yin, H., Zhou, Z., Liang, Y., Qiu, G., Liu, J., Liu, H., Liang, Y., Shen, L., Cong, J., and Liu, X., The Community Dynamics of Major Bioleaching Microorganisms during Chalcopyrite Leaching under the Effect of Organics, *Curr. Microbiol.*, 2011. doi: 10.1007/s00284-011-9960-y. vol. 63, pp. 164–172
5. Johnson, D.B., Biodiversity and Ecology of Acidophilic Microorganisms, *FEMS Microbiol. Ecol.*, 1998, vol. 27, pp. 307–317.
6. Bacelar-Nicolau, P. and Johnson, D.B., Leaching of Pyrite by Acidophilic Heterotrophic Iron-Oxidizing Bacteria in Pure and Mixed Cultures, *Appl. Environ. Microbiol.*, 1999, vol. 65, pp. 585–590.
7. Bulaev, A.G., Pivovarova, T.A., Melamud, V.S., Bumazhkin, B.K., Patutina, E.O., Kolganova, T.V., Kuznetsov, B.B., and Kondrat'eva, T.F., Species Composition of the Association of Acidophilic Chem-

olithotrophic Microorganisms Participating in the Oxidation of Gold-Arsenic Ore Concentrate, *Microbiology*, 2011, vol. 80, no. 6, pp. 842–849.

8. Silverman, M.P. and Lüdgren, D.G., Studies on the Chemoautotrophic Iron Bacterium *Ferrobacillus ferrooxidans*. I. An Improved Medium and a Harvesting Procedure for Securing High Cell Yields, *J. Bacteriol.*, 1959, vol. 77, no. 5, pp. 642–647.
9. Reznikov, A.A., Mulikovskaya, E.P., and Sokolov, I.Yu., *Metody analiza prirodnykh vod* (Methods for Analysis of Natural Waters), Moscow: Nedra, 1970, pp. 140–143.
10. Suvorovskaya, I.A., Titov, V.I., Brodskaya, V.M., Vasil'ev, P.I., Lipshchits, B.M., and Elentur, M.P., Arsenic Determination, in *Tekhnicheskii analiz tsvetnoi metallurgii* (TRANSLATION), Moscow: Metallurgizdat, 1957, pp. 182–184.
11. Cypionka, H. and Pfenning, N., Growth Yields of *Desulfotomaculum orientis* with Hydrogen in Chemostat Culture, *Arch. Microbiol.*, 1986, vol. 143, pp. 396–399.
12. Orlov, D.S. and Grishina, L.A., *Praktikum po khimii gumusa. Ucheb. posobie* (Practical Course in Humus Chemistry), Moscow: Mosk. Gos. Univ., 1981.
13. Lakin, G.F., *Biometriya* (Biometrics), Moscow: Vysshaya shkola, 1990.
14. Tsaplina, I.A., Krasil'nikova, E.N., Zakharchuk, L.M., Egorova, M.A., Bogdanova, T.I., and Karavaiko, G.I., Carbon Metabolism in *Sulfobacillus thermosulfidooxidans* subsp. *asporogenes*, Strain 41, *Microbiology*, 2000, vol. 69, no. 3, pp. 271–276.
15. *Biology of the Prokaryotes*, Lengeler, J.W., Drews, G., and Schlegel, H.G., Eds., Blackwell, 1999.
16. Zhuravleva, A.E., Ismailov, A.D., and Tsaplina, I.A., Electron Donors at Oxidative Phosphorylation in Bacteria of the Genus *Sulfobacillus, Microbiology*, 2009, vol. 78, no. 6, pp. 811–814.
17. Pivovarova, T.A., Dzhansugurova, R.S., and Karavaiko, G.I., Role of Exometabolites in *Thiobacillus ferrooxidans* to Molybdenum, *Microbiology*, 1991, vol. 60, no. 4, pp. 609–616.