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EXPERIMENTAL ARTICLES

Oxidation of Gold–Antimony Ores by a Thermoacidophilic Microbial Consortium

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Abstract—Antimony leaching from sulfide ore samples by an experimental consortium of thermoacidophilic microorganisms, including *Sulfobacillus, Leptospirillum,* and *Ferroplasma* strains was studied. The ores differed significantly in the content of the major metal sulfides (%): Sb_S, 0.84 to 29.95; Fe_S, 0.47 to 2.5, and As_S, 0.01 to 0.4. Independent of the Sb_S concentration in the experimental sample, after adaptation to a specific ore and pulp compaction, the microorganisms grew actively and leached/oxidized all gold–antimony ores at 39 ± 1°C. The lower was the content of iron and arsenic sulfides, the higher was antimony leaching. For the first time the investigations conducted with the use of X-ray microanalysis made it possible to conclude that, in a natural high-antimony ore, Sb inhibits growth of only a part of the cell population and that Ca, Fe, and Sb may compete for the binding centers of the cell.

Keywords: gold–antimony sulfide ores, microbial consortium, chemolithotrophs, thermoacidophilic organisms, leaching, inhibition

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Antimonite, or stibnite (Sb_2S_3), the major mineral of antimony ores, contains up to 71.4% Sb. Less common antimony ores contain complex sulfides of antimony, copper, mercury, lead, and iron (e.g., berthierite, jamesonite, etc.), or antimony oxides and oxychlorides (e.g., senarmontite, nadorite). Antimony trisulfide is known in its amorphous and crystalline, more stable modification [1, 2]. According to the oxidation level of antimonite from the Olympiadinskoe deposit, sulfide ore concentrate (usually less than 10%) is the third one, after pyrrhotite and arsenopyrite. Bacterial transformation of antimonite from gold–antimony concentrates results in production of the material suitable for cyanidation. Biohydrometallurgical treatment of high-antimony gold–sulfide concentrates at Olympiadinskoe factory causes certain problems, since antimony has a negative effect on bacterial oxidation of sulfides and on subsequent gold recovery by cyanidation of the residues of bacterial oxidation. Incomplete oxidation of antimony-containing sulfide minerals results in high cyanide consumption and loss of gold. In the course of antimonite oxidation, Sb(III) is oxidized to Sb(V), resulting in formations of poorly soluble antimony oxides and hydroxides [3]. Investigation of antimony behavior in the course of biooxidation of gold–antimony ores with various Sb concentrations is of importance.

Antimony belongs to heavy metalloids, whose small number of molecules penetrating into the cytoplasm act as a signal to change the normal course of metabolic processes. Antimony ions may cause protein denaturation by damaging the internal bonds responsible for the secondary and tertiary structure of the proteins. Antimony therefore belongs to the toxicants of the group of so-called thiol poisons. Their interaction with SH groups of amino acids modifies the properties of proteins. Inhibition of enzymatic systems may impair respiration, protein and RNA synthesis, and the functioning of the cytoplasmic membrane [4]. The rate of Sb(III) absorption by suspensions of bacterial cells not resistant to this metalloid increases with Sb concentrations in the solution, so that its intracellular concentration may approach the minimal inhibitory concentration (10 μM) [5]. Antimony adsorption on the biomass increases with pH rise [5]. Antimony transport inside the cell may, however, not occur at all [4, 6], with its absorption limited to accumulation at the cell surface. The process is more intense for the cells secreting extracellular polysaccharides and for immobilized cells.

Resistance to heavy metals is a prerequisite characteristic for the microorganisms of associations oxidizing sulfide ores in the framework of biohydrometallurgical technologies.

The goal of the present work was to investigate the effect of antimony content in the ores of the Olympiadinskoe deposit on the parameters of their bacterial–chemical oxidation (BO) and on the viability of microorganisms at 39 ± 1°C.

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Table 1. Elemental composition of the ores oxidized by the thermoacidophilic microbial consortium

Component content, %	Ore sample no.					
	1	2	3	4	5	6
Sb_S	29.95	21.85	14.50	6.91	2.89	0.84
Ca	1.72	8.31	2.60	9.20	6.24	7.00
Au, g/t	7.60	2.80	11.50	1.10	10.10	0.80
S_{total}	11.51	7.94	6.70	3.40	2.80	0.60
Fe_S	0.50	0.47	2.50	0.47	2.40	0.60
As_S	0.16	0.06	0.40	0.01	0.25	0.01
C	0.94	2.90	1.34	3.30	2.29	2.60

MATERIALS AND METHODS

Antimony ores. The Olympiadinskoe deposit ores used in the work contained mostly antimonite and small amounts of berthierite. They differed in the quantitative content of antimony and other components. The antimony sulfide ores used are characterized in Table 1.

According to the data presented in Table 1, content of sulfide antimony in the ores varied significantly, from 0.84 to 29.95%. The highest content of antimony sulfide was found in ore samples nos. 1, 2, and 3. Sample no. 2 had also a high Ca content, while two other samples contained considerably less Ca. The highest Ca content (9.2%) was found in sample no. 4, which contained 6.91% of the antimony component. High carbonate content resulted in alkalinization of the liquid phase of the pulp and had a negative effect on microbial viability and activity. The ore samples differed in the content of sulfur (0.6 to 11.51%), iron (0.47 to 2.5%), arsenic (0.01 to 0.4%), and carbon (0.94 to 3.3%). The size of ore particles differed insignificantly.

Preliminary treatment of the ores. Prior to the experiment, the ore of all six samples was pretreated with 10 N H_2SO_4. The ore (1 to 5 g) was placed in a 250-mL Erlenmeyer flask with 100 mL of the Silverman and Lundgren mineral medium 9K containing the following (g/L): $(NH_4)_2SO_4$, 3.0; KCl, 0.1; KH_2PO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; and $Ca(NO_3)_2 \cdot 4H_2O$, 0.01 [7] on a shaker (200 rpm) at 39 ± 1°C. An increase in pH to 4.0–8.0 was detected. To adjust pH of the medium to 1.8–1.9, 10 N sulfuric acid was used.

Composition of the microbial consortium. A chemolithotrophic microbial consortium was formed to carry out the process of bacterial oxidation of antimony sulfide ore at 39°C. It contained sulfur- and iron-oxidizing microorganisms as monocultures of bacteria of various species: *Sulfobacillus sibiricus* strains OFO, SSO, and B1 isolated from sulfide ores of the Olympiadinskoe deposit [8], *S. thermotolerans* Kr1, and *S. sibiricus* N1, isolated from dense pulp of the reactors testing the biohydrometallurgical technology for the oxidation of Olympiadinskaya factory pyrite–arsenopyrite concentrate and Nadezhdinskaya factory concentrate, respectively [9, 10]. The consortium was supplemented with the population of the bacterial community, containing archaea of the genus *Ferroplasma*, as well as bacteria of the genera *Sulfobacillus* and *Leptospirillum*, which was isolated from the pulp of the Olympiadinskaya factory pyrrhotite pyrite–arsenopyrite concentrate. Sulfobacilli predominating in the consortium had high rates of oxidation of sulfide minerals in a broad temperature range (20 to 60°C).

Cultivation. The stable community was obtained by joint cultivation of these organisms in flasks under shaking (200 rpm) at 39 ± 1°C in 9K medium [7] with initial pH 1.8–2.0, containing microelements of the Brierley medium [11] and yeast extract (0.02%). Elemental sulfur (1%) or ferrous iron (10 g/L $FeSO_4 \cdot 7H_2O$) and further antimony sulfide ores of the Olympiadinskoe deposit (1% wt/wt) were used as energy sources and electron donors. The flasks were inoculated with the population of the microbial consortium (10% vol/vol). For inoculation of the medium with antimony ore, 0.3 g/L ferrous iron was added to stimulate the growth of the organisms adapted to sulfide ore (adaptation is described below). Cultivation was carried out in flasks or in a 3-L reactor (pulp volume, 1 L; aeration rate, 3 volumes of air per volume medium per min; agitation rate, 400 rpm). In case of acidification of the liquid phase in the course of bacterial oxidation, 5% $NaHCO_3$ solution was used to adjust pH to the initial level.

Preparation of the consortium for investigation. The microbial consortium was adapted to the oxidation substrate, i.e., to each of the antimony ore samples. Adaptation was carried out first by triple transfers of microorganisms in the media with antimony ores, starting at the 1 : 100 solid-to-liquid ratio and sequentially increasing the pulp density by addition of the ore. Transfer and addition of new portions of ore were carried out after a pH decrease in the liquid phase of the pulp due to the oxidative processes or after pH remaining stable for 24 h. Morphology of the cells within the consortium was considered during assess-

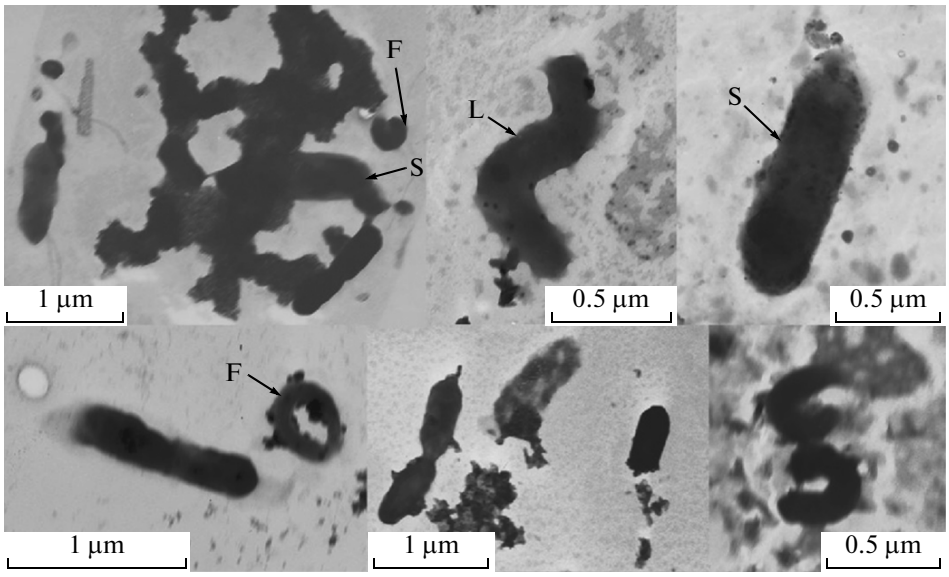

Fig. 1. Microbial consortium on the Olympiadinskoe deposit antimony ore (Sb_S = 29.95%) at 12% pulp density: *Sulfobacillus* spp. (S), *Leptospirillum* spp. (L), and *Ferroplasma* spp. (F).

ment of the adaptation as a criterion of their physiological state. During the adaptation of the consortium to increasing pulp density, BO was carried out in batch mode, using increased mass exchange to maintain the microorganisms in the most viable state. The liquid phase of the pulp (1/3 of the volume) was exchanged to an equal volume of 9K medium. The cells and ore particles were separated by centrifugation and returned to the process.

The cultivation of microorganisms in tenfold serial dilutions was carried out in 9K medium, which was supplemented with yeast extract for enumeration of archaea and sulfobacilli.

Microscopy. Microscopy and direct cell count were carried out under a LUMAM I1 microscope (LOMO, Russia) under phase contrast.

During the experiment, quantitative assessment of the microorganisms belonging to different taxonomic groups was carried out by tenfold serial dilutions in order to compare the values obtained with the results of direct count. The morphological state of microbial cells was assessed under a JEM-100CXII electron microscope (JEOL, Japan) at 80 keV and instrumental magnification 14000 to 40000. Electron microscopy was carried out as described in [12]. Elemental content of microbial cells was determined on a JEM-100CXII electron microscope equipped with an EM-ASID4D scanning attachment and a Green Star X-ray microanalyzer (Russia) with an E5423 detector at 60 keV. Elemental content of the cells was assessed as the heights of the peaks of characteristic lines in induced X-ray radiation. Typical spectra of the cells from the community collected at the phase of active growth in the medium with gold–antimony ore with Sb_S content 29.95% are listed in the Results and Discussion section.

Chemical analytical techniques. Bacterial oxidation was terminated by the end of acidification of the liquid phase or when a significant portion of the cells was found to enter a dormant state. In the liquid phase of the pulp, Eh and pH were measured with a pH-150M pH-meter (Belarus), ferric and ferrous iron concentrations were determined by chelatometric titration with Trilon B [13], sulfate concentration was determined turbidimetrically [14], total arsenic was determined by iodometric titration involving iron ions binding with $TiCl_3$ [15]. Solid residues of bacterial oxidation were removed by filtration and dried. Antimony content was then determined in the Polyus factory laboratory [16].

RESULTS AND DISCUSSION

Development of the Thermoacidophilic Chemolithotrophic Microbial Consortium Oxidizing Antimony Ores and Its Adaptation to Different Pulp Densities at 39 ± 1°C

Formation of the consortium. The procedure described above resulted in development of a stable community of chemolithotrophic thermoacidophilic microorganisms initially with iron and sulfur as the oxidation substrates, and subsequently in media with the samples of gold–antimony ores (1–5%). Microscopy of the liquid phase revealed archaea, leptospirilla, and sulfobacilli (Fig. 1). The cells of sulfobacilli of various sizes predominated in the consortium.

Table 2. Parameters of the liquid phase of the pulp during the adaptation of the microbial consortium to various values of pulp density of antimony sulfide ores with Sb_S content from 14.5 to 29.95%

Ore sample no.	1				2				3				
Stage no.	I	II	III	IV	I	II	III	IV	I	II	III	IV	V
Pulp density, % solid	4.8	7.0	9.5	12	4.8	7.0	9.5	12	4.8	8	9.5	12	14
BO duration, days	3	4	4	2	4	3	4	2	3	4	2	2	2
Cell number, 1×10^8 cells/mL	8.0	4.5	3.5	4.5	8.4	5.3	4.6	4.8	8.0	7.5	9.1	9.9	15.0
pH													
ini.	1.88	1.86	1.73	1.60	1.80	1.88	1.76	1.68	1.83	1.85	1.80	1.80	1.80
fin.	1.63	1.59	1.60	1.50	1.57	1.72	1.50	1.50	1.60	1.65	1.57	1.60	1.71
Eh, mV													
ini.	586	719	722	813	578	721	693	702	642	811	805	775	795
fin.	815	845	845	825	804	804	811	823	812	830	834	831	828
Fe^{3+}_{fin}, g/L	0.25	0.35	0.40	0.46	0.23	0.32	0.45	0.48	1.30	1.60	2.20	2.80	2.42
SO_4^{2-}, g/L	1.9	2.4	3.5	3.8	2.1	3.2	3.2	4.15	1.8	2.5	3.3	3.5	3.9

Sb_S content in samples nos. 1, 2, and 3 was 29.95, 21.85, and 14.50%, respectively.

Table 3. Parameters of the liquid phase of the pulp during the adaptation of the microbial consortium to various values of pulp density of antimony sulfide ores with Sb_S content from 0.84 to 6.91%

Ore sample no.	4				5						6			
Stage no.	I	II	III	IV	I	II	III	IV	V	VI	I	II	III	IV
Pulp density, % solid	4.8	7.0	9.5	12	4.8	8	9.5	12	14	16.7	4.8	7.0	9.5	12
BO duration, days	4	3		2	3	4	2	2	2	7	3	4	3	4
Cell number, 1×10^8 cells/mL	8.8	4.0	4.0	4.5	6.2	8.1	6.2	5.0	4.9	4.3	5.4	3.5	5.5	4.5
pH														
ini.	1.83	1.84	1.71	1.7	1.92	1.82	1.87	1.96	1.83	1.96	1.8	1.86	1.81	1.81
fin.	1.55	1.71	1.60	1.6	1.65	1.7	1.67	1.78	1.70	1.77	1.73	1.71	1.71	1.61
Eh, mV														
ini.	584	715	709	764	636	715	798	723	780	780	627	716	702	731
fin.	840	764	812	822	829	842	840	848	824	863	808	833	837	824
Fe^{3+}_{fin}, g/L	0.55	0.42	0.30	0.28	1.2	1.5	1.95	2.03	2.34	3.28	0.22	0.38	0.45	0.52
SO_4^{2-}, g/L	1.6	2.5	3.4	3.7	1.2	1.6	2.0	2.3	2.5	2.9	0.75	1.1	1.2	1.4

Sb_S content in samples nos. 4, 5, and 6 was 6.91, 2.89, and 0.84%, respectively.

Adaptation of the Microbial Consortium to Different Pulp Densities at 39 ± 1°C

Increasing pulp density. Sequential transfers of the consortium into the media with pulp density from 1 to 5 g/100 mL containing different amounts of Sb_S resulted in adaptation of the organisms to the oxidation of a specific ore sample with a shortened lag phase.

In all variants, the microbial consortium was preserved at pulp density increasing to 12% (after 11–13 days). For ore samples nos. 3 and 5 (Sb_S content 14.5 and 2.89%, respectively), 14 and 16.7% density were achieved 13 and 20 days after the onset of the experiment, respectively. Compared to other ores, these samples had higher levels of Fe_S and As_S (Table 1).

Dynamics of the liquid phase parameters during adaptation of the microbial consortium to different pulp densities. During adaptation of the microorganisms to different pulp densities, BO of sulfide minerals was studied. Dynamic parameters of the liquid phase of the pulp during arsenic ore BO in the course of adaptation are listed in Tables 2 and 3.

At low pulp density, the microbial population developed rapidly, with high levels of cell yield (5.4–8.8 × 10^8 cells/mL) after 3–4 days. At all levels of pulp density, the consortium oxidized all ores actively. After 3–4 days of BO at 4.8% pulp density, pH decreased by 0.15–0.28 units. (by 0.07 units for sample no. 6). This pH decrease resulted mainly from S^{2-}/S^0 oxidation. The concentration of sulfate ion, the terminal product of S^{2-}/S^0 oxidation, was 0.75 to 2.1 g/L of the bacterial solution. Depending on Fe_S content in the sample, ferric iron concentration in the liquid phase of the pulp varied from 0.22–0.25 g/L (ore samples nos. 1, 2, 4, and 6) to 1.2–1.3 g/L (samples nos. 5 and 3). The redox potential value increased to 804–840 mV. Microscopy revealed that half of the cells of the community population were sulfobacilli, while archaea and leptospirilla constituted another half. Most sulfobacilli cells were in a dividing state. Motile cells were numerous. The share of refractory cells, which indicates transition into a dormant state, was small (~7% of the total population in the case of the ore with the highest antimonite content). In the course of oxidation of the ore sample with the lowest Sb_S content (0.84%, sample no. 6), the microbial community encountered limitation by energy sources. The oxidation occurred mainly during the first two days of cultivation, and both refractory cells and low numbers of sulfobacilli spores were observed after 24 h of BO.

By the end of the period of adaptation of the consortium to 7, 9.5, 12, and 14% pulp, high cell yield (7.5, 9.1, 9.9, and 15 × 10^8 cells/mL) was observed for ore no. 3 (Sb_S = 14.5%; Fe_S = 2.5%; As_S = 0.4%). In the variant no. 5 (Sb_S = 2.89%; Fe_S = 0.4%; As_S = 0.25%), the highest cell yield (8.1 × 10^8cells/mL) was achieved at the second stage of BO, while later, at higher pulp densities it decreased two fold. In other experimental variants, a similar decrease in cell numbers by approximately two times (to 3.5–5.5 × 10^8 cells/mL) was observed.

Comparison of the results of direct cell count and quantitative enumeration of various microbial groups by tenfold terminal dilutions revealed the following. Dilutions of the pulp samples, from which viable organisms of three taxonomic groups (sulfobacilli, archaea, and leptospirilla) were retrieved, corresponded to the 10^8 (sometimes to 10^7) dilution; the result was almost identical with that of direct count. Thus, microbial populations within the consortium remained viable in the course of BO of antimony ores. Archaea and sulfobacilli were the predominant cells in the developing community. For example, abundance of these groups in pulp samples (ore samples nos. 1 and 3) was 2–4 × 10^8 cells/mL and 2–3.5 × 10^8 cells/mL, respectively. The number of leptospirilla cells was lower, 8–10 × 10^7 cells/mL. The tendency for modification of the morphological parameters mentioned above persisted, indicating the possible transition of some of the cells into a dormant state at high values of pulp density and antimonite concentrations. All adapted populations retained, however, their leaching and oxidative activities. Eh values were high, exceeding 800 mV, while pH values gradually decreased. While pulp density increased to 12%, sulfate concentrations increased to 3.8, 4.15, 3.3, 3.7, 2.03, and 1.4 g/L for variants nos. 1, 2, 3, 4, 5, and 6, respectively (Tables 2 and 3). The rate of iron oxidation by microbial populations was higher at higher iron content within the ore, which made it possible to increase pulp densities of variants nos. 3 and 5 to 14 and 16.7%, respectively. The concentrations of leached and oxidized iron at the maximal pulp density were 2.8 and 3.3 g/L for samples nos. 3 and 5, respectively, with the respective SO_4^{2-} concentrations 3.9 and 2.9 g/L of the liquid phase.

Arsenic concentrations in the liquid phase were 0.3–0.7 g/L (for ore samples nos. 1, 3, and 5).

Ore Oxidation by the Chemolithotrophic Thermoacidophilic Microbial Consortium Adapted to Dense Pulp

The consortium adapted to dense pulp was subsequently cultured at the same pulp density. The data on BO of antimony sulfide ores for pulp density 12% (samples nos. 1, 2, 4, and 6), 14% (sample no. 3), and 16.7% (sample no. 5) are presented in Tables 4 and 5. Duration of cultivation was 14 days for ore no. 5 and 10 days for other samples.

The specific growth rate of microbial populations depended on the ore composition. The highest growth rate (0.053 h^{-1}) was observed for sample 5 with low Sb_S content (2.89%) and Fe_S content 2.4%. The lowest μ_{max} (0.04 h^{-1}) was revealed in the case of the population growing in the medium with ore no. 6, which was poor in energy sources (S^{2-}/S^0, and Fe_S).

At the pulp densities listed above, the consortium continued intense oxidation of sulfide ores after sequential transfers. Apart from high cell density, this was confirmed by other characteristics of the process: decrease in pH of the liquid phase, high Eh, and oxidation of S^{2-} and Fe_S (Table 5). The rate of sulfur oxidation for ore no. 6 was somewhat lower (0.21 g L^{-1} day^{-1}), while the highest rate (0.71 g L^{-1} day^{-1}) was observed for ore no. 3. The microorganisms growing on ores nos. 1, 2, and 4 had similar kinetic characteristics of S^{2-}/S^0 oxidation: 0.62, 0.59, and 0.57 g L^{-1} day^{-1}, respectively.

Higher average rates of ferrous iron oxidation by the consortia preadapted to dense pulp (0.36 and 0.30 g L^{-1} (day^{-1}) were recorded for ore samples nos. 3 and 5, i.e., for the ores with higher iron content. The rates of iron oxidation in other variants were 0.056–0.078 g L^{-1} day^{-1}.

Similar to the adaptation of the consortium to increasing pulp density, the tendency for induction of

Table 4. Parameters of bacterial oxidation of antimony sulfide ores by adapted consortia of thermoacidophilic microorganisms at 39 ± 1°C

Technological parameters of bacterial oxidation	Values of the parameters for BO of the ore samples; initial Sb_S content in the sample					
	no. 1, 29.95%	no. 2, 21.85%	no. 3, 14.5%	no. 4, 6.91%	no. 5, 2.89%	no. 6, 0.84%
Maximal specific growth rate of the microbial community, h^{-1}	0.045	0.047	0.051	0.047	0.053	0.040
Acidity of the bacterial solution, pH	2.0 → 1.44	2.0 → 1.43	2.0 → 1.40	2.0 → 1.5	2.0 → 1.54	2.0 → 1.60
Redox potential (Eh), mV	589 → 848	580 → 825	640 → 834	583 → 843	635 → 850	625 → 839
Total concentration of sulfate ion, g/L	6.2	5.9	7.1	5.7	4.9	2.1
Total Fe(III) concentration, g/L	0.63	0.60	3.60	0.56	4.11	0.78

Pulp density was 12% for samples nos. 1, 2, 4, and 6; 14% for no. 3, and 16.7% for no. 5. The values for SO_4^{2-} and Fe(III) concentrations allow for mass exchange.

sporogenesis and development of dormant forms (refractory cells), as well as formation of morphologically peculiar cells (swollen, thinned, close to coccoid, or circularly curved leptospirilla cells), persisted during BO. Increased mass exchange resulted in a smaller number of such cells, thus retaining the oxidative activity of the main biomass.

The final results of leaching the samples of antimony ores form the Olympiadinskoe deposit by the thermoacidophilic microbial consortium are presented in Table 6. The values of antimony leaching were obtained at pulp density 12% (ore samples nos. 1, 2, 4, and 6), 14% (sample no. 3), and 16.7% (sample no. 5).

The results presented confirmed that the efficiency of oxidation of the ores differing in elemental composition and antimony content by the thermoacidophilic microbial consortium was different. More complete ore oxidation and antimony leaching were achieved at lower initial antimony content. Thus, the ore containing 2.89% Sb_S (2.4% Fe_S and 0.25% As_S) was leached by 86.2%. Similar levels of leaching (81.3 and 78.2%) were obtained with ore samples with initial sulfide antimony content 6.91 and 0.84%, respectively. The latter ores also contained lower amounts of other sulfides: 0.47 and 0.6%, respectively, Fe_S and the lowest amount of As_S (0.01% each). It may be concluded that the ores with low Sb_S content (0.84–6.91%) were oxidized more completely if an electron donor and energy source were present, apart from antimony. The ores with high initial Sb_S content (21.85 and 29.95%) were leached by 34.5 and 30.9%, respectively. These ores contained Fe_S (0.47 and 0.5%, respectively) and As_S (0.06 and 0.16%). Thus, the lower Fe_S and As_S content in high-antimony ores, the higher the absolute concentration of leached antimony. The intermediate ore sample no. 3 (14.5% Sb_S) had the Fe_S and As_S levels (2.5 and 0.4%) close to those of ore no. 5 (see above), for which the highest antimony leaching was found. Ore no. 3 had five times the initial Sb_S concentration of ore no. 5 and exhibited ~4 times lower antimony leaching. These results indicate the possible existence of a threshold ratio of the concentrations of sulfide antimony (~15%) and iron and arsenic concentrations, with Sb_S leaching/oxidation suppressed above this value.

Thus, depending on antimony content in the original ore, the degree of its leaching at 39 ± 1°C varies significantly, depending on the content of other sulfide minerals containing iron and arsenic.

Assessment of the Effect of Antimony Content in the Liquid Phase on Microbial Cells

Sample no. 1 was used to investigate the effect of antimony leached into the liquid phase of the pulp in

Table 5. Average rates of antimony sulfide ore oxidation by microbial consortia adapted to different pulp densities

Sample no.	Average rate of S^{2-}/S^0 oxidation to SO_4^{2-}, g L^{-1} day^{-1}	Average rate of Fe^{2+} oxidation, g L^{-1} day^{-1}
1	0.62	0.063
2	0.59	0.060
3	0.71	0.360
4	0.57	0.056
5	0.35	0.300
6	0.21	0.078

Pulp density was 12% for samples nos. 1, 2, 4, and 6; 14% for no. 3, and 16.7% for no. 5. The oxidation rates allow for mass exchange.

Table 6. Antimony leaching from the Olympiadinskoe deposit sulfide ores by the thermoacidophilic microbial consortium at 39 ± 1°C

Ore sample no.	Sb_S content in the original ore, %	Solid biooxidation product yield, %	Sb_S content in the residual product, %	Antimony leaching, %
1	29.95	81.54	25.38	30.9
2	21.85	92.31	15.50	34.5
3	14.50	85.29	13.5	20.6
4	6.91	57.69	2.24	81.3
5	2.89	50.00	0.80	86.2
6	0.84	83.08	0.22	78.2

the course of biooxidation of the Olympiadinskoe deposit ores. This ore sample had the highest antimony content (29.95% Sb_S), the lowest content of Ca (1.72%), which is the first element to be absorbed by microbial cells [4, 17], and low content of iron (0.5%), which is absorbed at pH 0.5 [17]. The observations were carried out by phase contrast microscopy and by electron microscopy coupled to X-ray microanalysis.

Light microscopic investigation showed that antimony ore-adapted microorganisms retained their viability. Transition of some of the cells into another physiological state (dormant state, with emergence of refractory forms and spores) was a visible response of the microorganisms to antimony in the liquid phase of the pulp. The share of the dormant forms increased with increasing concentrations of leached antimony. Cell lysis and changed cell morphology were other indications of the inhibitory effect. The majority of the cell population retained, however, the morphological stability. A mucous polysaccharide layer facilitating cell attachment to ore particles was visible on electron micrographs taken at the initial stage of antimony ore BO (Fig. 2). "Encrusted" cells, with small adsorbed particles associated with the mucous matrix covering the cells, occurred (Fig. 3).

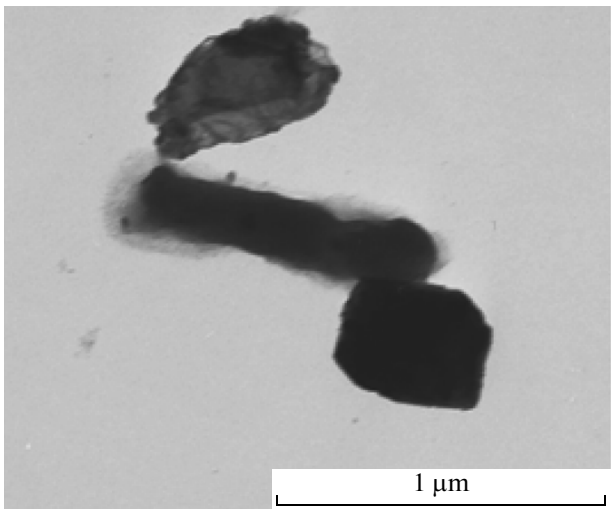

Fig. 2. A cell of *Sulfobacillus* sp. attached to the crystals.

X-ray microanalysis was used to investigate the elemental composition of the cells. The presence and height of the peaks of Sb, Ca, S, and Fe in the spectra of the cells were used to assess the sorption of these elements. The elemental composition of the cells attached to small or large solid sediment particles, of those sorbing the elements on the surface or inside the cells, and of free-living cells from the liquid phase was also analyzed.

Many free-swimming cells were shown to contain no antimony, while some of them contained iron. Attached cells exhibited heterogeneous distribution of metals and sulfur. Some particle-associated cells contained S, Fe, Ca, and probably Sb (Fig. 4a). Other similar cells contained no antimony, which probably remained dissolved after leaching. The cells accumulating Ca, as well as the cells with particles containing S (Fig. 4b), Sb an S (Figs. 4c, 4d), or Sb and Fe (Figs. 4e, 4f) were observed. Some of them looked as cells covered with small particles (Fig. 3). Such cells sorb these elements on their surface or contain them intracellularly due to the presence of the surface structures, a mucous polysaccharide capsule or an S-layer [4].

The spectra of the cells sorbing antimony, elemental sulfur, iron, and calcium, did not contain the peaks of potassium and phosphorus, which was probably an indication of impaired cytoplasmic membrane (CM). In some cells, the sulfur peak was also absent (Figs. 4e, 4f), while antimony and iron were detected, which was also an indication of damaged CM. The metals affecting CM may penetrate inside the cell and accumulate in the intracellular volume, resulting in cell death.

Antimony was not always detected in the dense particles containing no cells. This was an indication of Sb leaching from the ore. The peaks corresponding to other elements of the ore (S, Ca, and Si) were present.

The mechanisms responsible for binding of the metal ions to microbial cell surface include electrostatic attraction, weak van der Waals forces, covalent binding, reductive interaction, extracellular precipita-

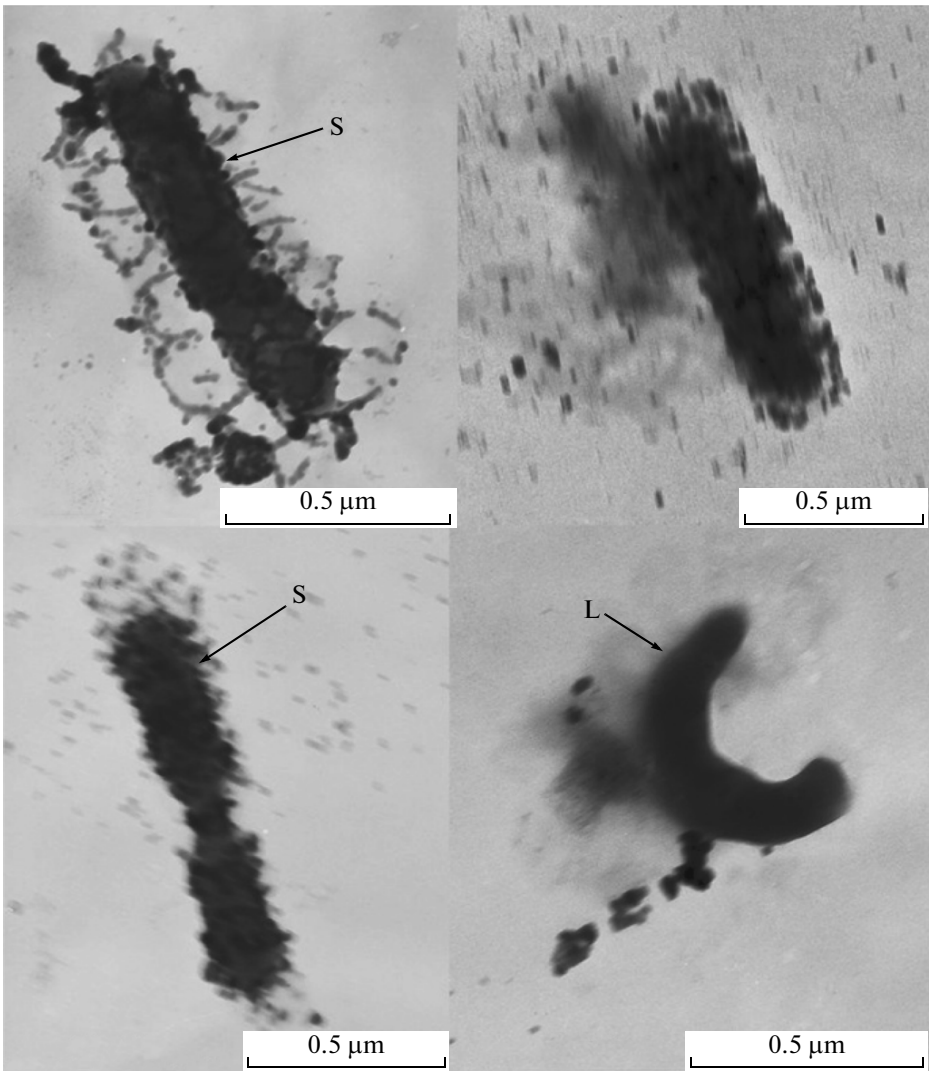

Fig. 3. Cells with solid ore particles attached to their surface structures: *Sulfobacillus* spp. (S) and *Leptospirillum* spp. (L).

tion, or some combinations of these factors [15]. The metals penetrating inside the cell initiate the synthesis of cytoplasmic compounds, resulting in binding with polyphosphates or metalloproteins [17, 18].

Gram-positive bacteria are known to have higher sorption capacity for heavy metal ions adsorbed from aquatic environment than gram-negative ones [19]. In our work, binding of Sb, Ca, and Fe with *Leptospirillum* cells was detected quite rarely.

Some microorganisms are able to adapt to high concentrations of heavy metal ions due to low permeability of their membranes for these ions, transport of the metals out of the cell, and intercellular detoxification [20]. In heterotrophic microorganisms of acidophilic microbial communities carrying out bioleaching, resistance to heavy metals is known to be determined by plasmids [21, 22], while obligate and facultative chemolithoautotrophs have their resistance determinants localized in the chromosome [23, 24].

Our results make it possible to conclude that during the oxidation of gold–antimony ores, the cells of the thermoacidophilic microbial consortium encounter an inhibitory effect of antimony ions, which mostly affects the physiological state of the cells and their transition to dormant forms. Results of X-ray microanalysis support the conclusion that antimony, calcium, iron, and elemental sulfur may act as inhibitors of cellular metabolism in a part of the cell population. Dividing vegetative cells, which are known to have the highest sorption capacity [18], did not contain extracellular or intercellular antimony. Some cells were found to contain calcium or iron. Calcium, which accompanies antimony, the major component of the ore, decreases the sorption capacity of the biomass [4] and prevents antimony sorption. Fe(III), one of the strong inhibitors of biological sorption of other metals from the solution, is already sorbed at pH 0.5–1.5 [17, 25] and may also prevent (completely or par-

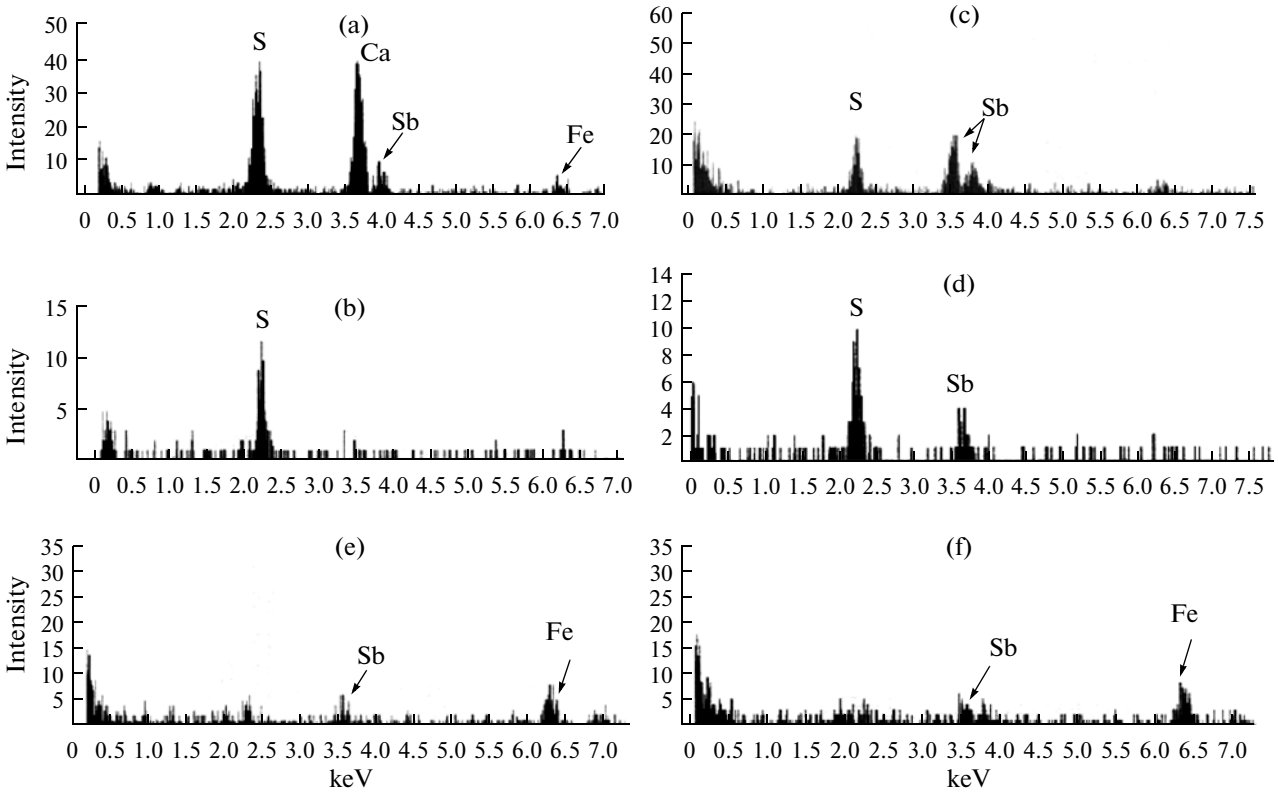

Fig. 4. X-ray spectra of microbial cells from the thermoacidophilic microbial consortium oxidizing antimony sulfide ore (Sb_S content 29.95%) at 39 ± 1°C.

tially) antimony binding to the sorption centers of the cells (Sb sorption occurs at pH 1.6 and higher). The sorption of penta- and trivalent antimony by ferric hydroxides, $Fe(OH)_3$, or iron oxides and oxyhydroxides Fe_2O_3 (hematite) and FeOOH (goethite) was shown, which was over 80% at pH 1–12 and ~95% at pH 2.5–7 [26, 27]. It was shown (Table 4) that during biooxidation of ore sample no. 1 pH varied within the range from 2.0 to 1.5, which probably supplemented iron binding by the cells with binding of the hydrolyzed Fe ions.

The following conclusions were made based on the results of the present work. The microbial consortium was able to grow well at 39 ± 1°C and to oxidize the ores with both high and low antimony content within the range of 0.84–29.95% Sb_S in the ore. The degree of antimony leaching during biooxidation depended on the ratio of the oxidized energy sources in the ore. At high Sb_S content in the ore, high levels of easily oxidized substrates (Fe_S and As_S) resulted in lower activities of antimony leaching, while at lower content of iron and arsenic sulfides in high-antimony ores the absolute concentration of leached antimony was higher. While antimony concentration in the liquid phase was ~20 times higher than the minimal inhibitory concentration for *Escherichia coli* [28], the microorganisms of the consortium were able to adapt to the inhibitory effects of antimony. The inhibition/limitation of the growth processes in the cells of the microbial community was evident from low H^+ accumulation rate in the liquid phase of the pulp, decreased biomass (total number of microorganisms), and altered physiological state of the cells within the population (emergence of refractory cells, spores, or cells with modified morphology). Antimony, calcium, iron, and sulfur were able to compete for the cellular binding centers.

For processing of gold–antimony ores, it should be kept in mind that a low degree of ore oxidation may be achieved at high Sb_S content of 14.5% or higher.

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