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EXPERIMENTAL ARTICLES

Biooxidation of a Double-Refractory Gold-Bearing Sulfide Ore Concentrate

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Abstract—The efficiency of biooxidation for treatment of a double-refractory gold-bearing sulfide ore concentrate from the Bakyrchik deposit (East Kazakhstan) was defined. The experiments were conducted in two different modes, i.e., with the standard liquid medium and the medium imitating the chemical composition of the Bakyrchik deposit groundwater and containing high concentrations of sodium, magnesium, and chloride. The concentrate contained 17.5% of organic carbon, 6% of pyrite, and 13% of arsenopyrite. Gold content was 57.5 g t^{-1}. Direct gold recovery by cyanidation was very low (2.8%). While biooxidation was efficient in both cases (approximately 90% of sulfide sulfur was oxidized), the efficiency of cyanidation was low (39 and 32%, respectively). This fact suggests high efficiency of biooxidation is insufficient for efficient treatment of double-refractory gold-bearing sulfide ore concentrates.

Keywords: refractory gold-bearing sulfide ore concentrate, biooxidation, cyanidation, gold extraction, composition of microbial population, DGGE

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The technology for tank biooxidation of various sulfide ore concentrates using acidophilic microorganisms was developed in the 1980s and has been successfully applied worldwide (van Aswegen P.C. et al., 2007; Gericke et al., 2009; Sovmen et al., 2007; Sovmen et al., 2009). At least 15 industrial-scale facilities for tank biooxidation of sulfide concentrates are presently in operation, and the construction of new ones is planned. While tank biooxidation is used mainly for processing of gold-bearing concentrates, it has been successfully used for cobalt and nickel recovery (Gericke et al., 2009; Morin, d'Hugues P., 2007). Gold-bearing sulfide ore concentrates are refractory, since gold recovery from them by cyanidation is insignificant due to its fine dispersion in sulfide minerals. Biooxidation of sulfide minerals results in deterioration of their crystal lattice, so that gold becomes available for cyanidation (Lodeishchikov, 1999).

Biohydrometallurgical technologies rely on acidophilic microorganisms oxidizing ferrous iron and sulfur compounds. Oxidation of sulfide minerals is exothermic, which results in heating of the pulp in industrial reactors. Industrial biooxidation is therefore carried out at 38–50°C. Under these conditions, moderately thermophilic and thermotolerant microorganisms predominate. The composition of microbial communities may vary, depending on the temperature and mineralogical composition of the oxidized concentrate (Table 1).

In spite of successful industrial application of biogeotechnologies, certain factors may limit their applicability at specific sites, which should be taken into account in the course of engineering the relevant facilities. The content of various cations and anions in the water used in hydrometallurgical processes is among the factors affecting the efficiency of biooxidation. Metal deposits are often located in the areas with limited supply of freshwater, such as Chilean or Australian deserts, and water used for biotechnological operations may contain high concentrations of the ions suppressing the growth and oxidative activity of acidophilic microorganisms (Zammit et al., 2012). Compared to other anions (sulfate and phosphate), chloride was shown to have the most pronounced inhibitory effect and may suppress oxidation of iron, sulfur, and pyrite (Zammit et al., 2012; Shiers et al., 2005; Gahan et al., 2009). For laboratory investigation on adaptation of BIOX™ (the most widespread technology for vat biooxidation) to specific concentrates, BIOMIN (the company holding the relevant patent) uses water from sources planned to be used for industrial application of this technology (biomin.co.za).

The Bakyrchik deposit (East Kazakhstan) is characterized by considerable gold reserves (geoportal-kz.org). Gold in the ore of this deposit is mainly associated with sulfide minerals (pyrite and arsenopyrite).

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Table 1. Species composition of acidophilic chemolithotrophic microbial communities in vat reactors for biooxidation of sulfide ore concentrates

Sulfide minerals present in the concentrate	Temperature, °C	Microbial community	Reference
Pyrite, arsenopyrite, pyrrhotite	38–40	*Acidiferrobacter thiooxidans, Leptospirillum ferriphilum, Sulfobacillus thermosulfidooxidans, Ferroplasma acidiphilum, Acidithiobacillus ferrooxidans, Alicyclobacillus tolerans, Acidiphilium cryptum*	(Bulaev et al., 2011)
Pyrite	42	*Leptospirillum ferriphilum, Acidithiobacillus caldus, Ferroplasma acidiphilum, Sulfobacillus benefaciens*	(Morin, d'Hugues, 2007, Johnson et al., 2008)
Pyrite, chalcopyrite, sphalerite	45	*Acidithiobacillus caldus, Leptospirillum ferriphilum, Sulfobacillus* sp., *Ferroplasma* sp.	(Okibe et al., 2003)
Pyrite, arsenopyrite, chalcopyrite	45	*Acidithiobacillus caldus, Sulfobacillus thermosulfidooxidans, "Sulfobacillus montserratensis"*	(Dopson et al., 2004)
Pyrite, arsenopyrite	40–50	*Sulfobacillus* sp., *Acidithiobacillus caldus, Leptospirillum ferriphilum, Ferroplasma* sp., *Acidiplasma* sp.	(van Hille et al., 2011 and 2013)

It is characterized by high content of organic carbonaceous matter represented by schungite. The content of noble metals in this mineral may be significant and comparable to their content in sulfides (Marchenko, 2012). High content of coaly matter capable of gold sorption is responsible for the highly refractory character of this ore in terms of gold recovery. The ores of the Bakyrchik deposit are therefore double-refractory due to significant amount of organic carbonaceous matter and incorporation of gold into the crystal lattice of sulfide minerals. In spite of the high gold content in the ore and its large reserves, no efficient technology exists for the ores of the Bakyrchik deposit (mining.kz). Exploitation of the Bakyrchik deposit is also prevented by water shortage due to its great distance from large surface water bodies, its unavailability to moist wind, and its location on the open terrain, which results in relatively low atmospheric precipitation, the only source of groundwater replenishment.

Several concentrates of the Bakyrchik deposit ores of different composition were used for development of the tank technology for sulfide ore biooxidation in the 1980s. Application of biooxidation resulted in a considerably higher degree of gold recovery by cyanidation (Table 2) (Karavaiko et al., 2000). Unfortunately, no pilot tests of this technology were carried out. The composition of currently recovered ore may vary considerably, and prolonged trial is required for assessment of its suitability for biohydrometallurgical processing.

Apart from biohydrometallurgical technologies, REDOX process, one of the variants of the autoclave process, was proposed for processing the Bakyrchik deposit ore. It involves leaching of sulfide minerals in the presence of nitrogen oxides at a temperature 180°C or higher (Sobel et al., 1995). Although a high degree of gold recovery by cyanidation was achieved, after pilot trials the technology was not introduced due to its high cost.

The goal of the present work was to determine the efficiency of biooxidation of the Bakyrchik deposit ore in two processing modes (using the standard mineral medium and the medium approximating the composition of the Bakyrchik groundwater with high concentrations of sodium, magnesium, and chloride), as well as to investigate the composition of the microbial community formed at different biooxidation modes.

MATERIALS AND METHODS

Subjects of research. The pyrite–arsenopyrite concentrate of the Bakyrchik deposit ore was the subject of investigation. The mineral and chemical composition of the concentrate is listed in Tables 3 and 4. The particles of the –74 μm fraction constituted 80% (P_{80} =

Table 2. Gold recovery by cyanidation from Bakyrchik ore concentrates and the products of their biooxidation

Year of trial	Gold recovery, %	
	From the concentrate	From the products of concentrate biooxidation
1979	10.0	88.3
1991–1992	12.6	94.0

Table 3. Mineral composition of the concentrate determined by X-ray diffraction analysis

Mineral phase	Formula	Content, mass %
Quartz	SiO_2	18
Partially hydrated potassium mica	$KAl_3Si_3O_{10}(OH)_2$	21
Plagioclase	$(Na,Ca)AlSi_3O_8$	3
Pyrite	FeS_2	6
Ankerite	$Ca(Mg,Fe)(CO_3)_2$	1.5
Rutile	TiO_2	1
Gypsum	$CaSO_4 \cdot 2H_2O$	1
Kaolin	$Al_4(Si_4O_{10})(OH)_8$	3
Arsenopyrite	FeAsS	13
Clinochlore	$(Mg,Fe)_6(Si,Al)_4O_{10}(OH)_6$	1
Siderite	$FeCO_3$	1
X-ray amorpous phase		30.5

74 μm). The total content of sulfide minerals in the concentrate was ~20%.

Direct cyanidation resulted in 2.8% gold recovery, which was an indication of the highly refractory nature of the ore concentrate.

Biooxidation was carried out in two sequentially connected reactors with mechanical agitation (500 rpm) and aeration (4 L/min). In each reactor, pulp volume was 1 L, and pulp density was 200 g concentrate per 1 L of the medium. The incubation time was 240 h. The temperature was maintained at 38–39°C by means of a U-shaped heat exchanger connected to an Elmi TW2.02 thermostat (Latvia), pH was maintained within the1.35–1.55 range by addition of sulfuric acid or calcium carbonate.

The acidophilic enrichment culture obtained in the course of oxidation of flotation tailings of high-pyrite polymetallic ore (Kondrat'eva et al., 2012).

Two media of different composition were used. The first one (medium 1, Table 5) has been repeatedly used in laboratory experiments on vat oxidation of various sulfide concentrates. The second medium (medium 2, Table 5) was composed considering the data on the composition of the Bakyrchik deposit groundwater and had higher concentrations of sodium, magnesium, and chloride (Table 5).

Table 4. Chemical composition of the concentrate

Element	Content, %
ΣFe	11.6
Fe_s	10.2
ΣAs	4.8
As_s	4.4
ΣS	7.4
S_s	6.8
C_{org}	17.5
Au	57.5 g/t

The values of pH and Eh were measured using a pH-150MA pH meter–millivoltmeter (Belarus); Eh was measured relative to the normal hydrogen electrode. The concentrations of Fe_{2+} and Fe_{3+} ions were determined by trilonometric titration (Reznikov et al., 1970); As concentration was measured by iodometric titration (Surovskaya et al., 1957). The content of iron, arsenic, and sulfur was determined by phase analysis (Filippova, 1975).

Gold recovery from biooxidation residues was carried out by cyanidation at 33% pulp density, 0.1% NaCN, resin load 3–5% (wt/wt) of the pulp, and process duration of 24 h.

Composition of the microbial community. The species composition of the inoculum and of the communities developed during the experiment was analyzed by DGGE. The pulp was sampled 35 days after the onset of the experiment. DNA was isolated using the standard procedures (Maniatis et al., 1984). Amplification was carried out in 20 μL of the mixture containing *1* reaction buffer (Evrogen, Russia), 200 μg of each deoxyribonucleotide triphosphate, the relevant amount of each primer, 1 activity U *Taq* polymerase (Evrogen), and 1 μL of template DNA solution (1–10 ng). Amplification was carried out using the primers Univ515F 5'-GTGBCAGCMGCCGCGGTA-3' (universal) and either Bac907R 5'-CCGTCAATTC-MTTTGAGTTT-3' (bacterial) or Arch915R 5'-GTGCTCCCCCGCCAATTCCT-3' (archaeal). The temperature program was adjusted experimentally, based on the standard PCR protocol. Prior to DGGE analysis, in order to add a G + C-rich fragment to the amplicons, reamplification was carried out with the primers containing the 40-nucleotide GC-clamp, 515FGCclamp 5'-CGCCCGCCGCGCCCCGCG-

Table 5. Composition of nutrient media

Compound	Concentration, g/L	
	Medium 1	Medium 2
$K_2HPO_4 \cdot 3H_2O$	0.12	0.12
KCl	0.05	0.08
NaCl	0	0.15
$MgSO_4 \cdot 7H_2O$	0.125	1.0
$(NH_4)_2SO_4$	0.75	0.75

CCCGTCCCGCCGCCCCCGCCCGGTGBCAG-CMGCCGCGGTAAA-3'. Reaction without template DNA was used as one of the negative controls in all experiments. Amplification was carried out using a Tercyc multichannel thermocycler (DNA-Technology, Russia). Amplification products were analyzed by electrophoresis in 1.5% agarose gel. The amplicons were separated according to their melting characteristics in 8% (vol/vol) polyacrylamide gel with the 30 to 70% relative gradient of denaturing agents. Electrophoresis was carried out for 20 h on a TV400-DGGE (SCIE-PLAS, United Kingdom) at 70 V and 60°C. After electrophoresis, the gel was washed with double-distilled water and stained with SYBR Gold (Molecular probes, The Netherlands) for 40 min in the dark. The stained amplification products were visualized under 470-nm illumination, and the bands were excised for subsequent analysis. The bands were transferred to test tubes with 20 μL distilled water and stored overnight in a refrigerator for DNA elution. The eluate (1 μL was used as a template for PCR with the relevant primers. The reamplification products were purified by electrophoresis in 1.5% agarose gel, DNA was excised and purified using the kit for DNA purification from gels and reaction mixtures (Cytokine, Russia). Sequencing was carried out according to Sanger (Sanger et al., 1977). Primary analysis of the similarity of the obtained sequences was carried out using the BLAST server (ncbi.nlm.nih.gov/blast). The MEGA 6 software package (Tamura et al., 2013) was used for aligning the sequences and construction of the phylogenetic tree.

RESULTS AND DISCUSSION

Biooxidation of the concentrate. The average values for the physicochemical parameters of the pulp liquid phase during 35 days of each experiment are presented in Table 6. It can be seen that in experiment 1 (in which medium 1 was used, see Table 5), low concentrations of ferrous iron were present in the first reactor, while no Fe^{2+} was found in the second reactor. This result was confirmed by Eh measurements (802 and 840 mV for the first and second reactor, respectively). In the second experiment, no Fe^{2+} was found in the pulp of both reactors, which agreed with higher Eh (819 and 857 mV, respectively). Thus, it may be concluded that changes in the composition of the medium did not result in decreased rates of iron biooxidation.

Table 6. Physicochemical parameters of the pulp in the course of biooxidation

Parameter	Experiment 1		Experiment 2	
	Reactor 1	Reactor 2	Reactor 1	Reactor 2
pH	1.42	1.37	1.38	1.30
Eh, mV	802	840	819	857
Fe^{3+}, g/L	9.1	12.1	13.0	14.0
Fe^{2+}, g/L	0.02	0	0	0
ΣFe, g/L	9.1	12.1	13.0	14.0
ΣAs, g/L	6.2	5.9	6.6	7.5

Table 7. Composition of biooxidation residues and residiues yields

Element	Experiment 1	Experiment 2
ΣFe, %	4.3	2.9
Fe_s, %	1.77	1.83
ΣAs, %	2.1	1.15
As_s, %	0.2	0.3
ΣS, %	2.5	2.8
S_s, %	1.2	1.28
Solud phase output, %	61.6	60.5

Table 8. Oxidation level of the concentrate and gold recovery by cyanidation

Parameter	Experiment 1	Experiment 2
Oxidation level:		
Fe_s, %	89	89
As_s, %	97	96
S_s, %	89	88
Gold recovery Au, %	39	32

In the second experiment, the total iron concentration in the pulp was even higher than in the first one.

The results on arsenic concentrations in the pulp were similar. The values of pulp pH and consumption of H_2SO_4 and $CaCO_3$ for pH adjustment may be used as indirect indicators of sulfur oxidation. In our experiments, addition of sulfuric acid to the pulp was not necessary, since the pulp pH did not increase. Moreover, calcium carbonate was added to the pulp in order to increase pH. Calcium carbonate consumption per 1 t of the concentrate was similar in two experiments (4.00 and 4.25 kg, respectively). Thus, analysis of the rates of oxidative processes based on the parameters of the pulp liquid phase did not indicate suppressed activity of acidophilic microorganisms resulting from the changed salt composition of the medium.

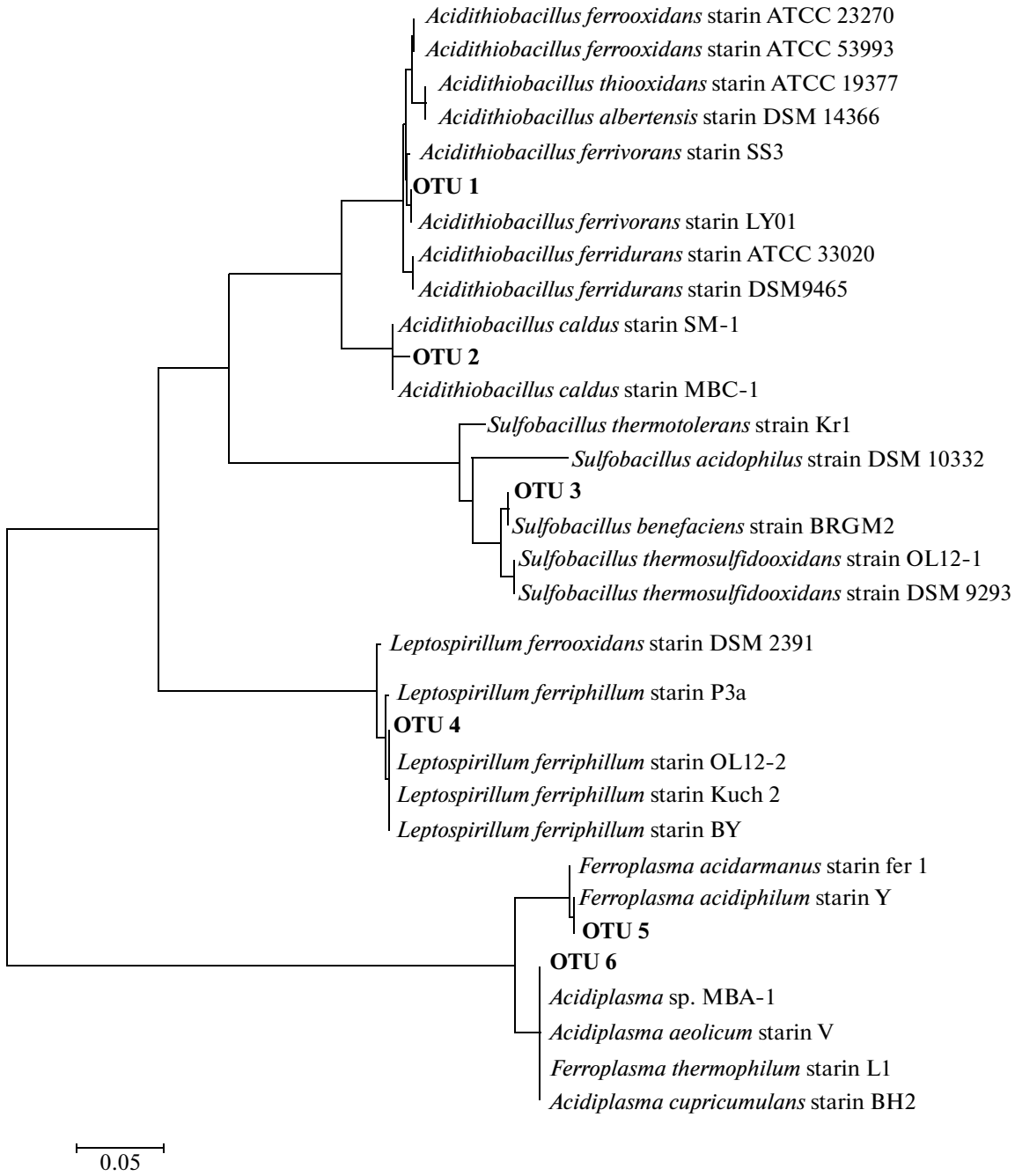
Analysis of the composition of solid biooxidation residues (Table 7) confirmed the conclusions made for the liquid phase of the pulp. The solid phase yield was almost the same in two experiments (61.6 and 60.5%, respectively), and the content of elements in sulfide form (iron, arsenic, and sulfur) in the biooxidation residue was considerably lower than in the original concentrate. The rates of oxidation of the elements were calculated using the data on biooxidation residues composition and solid phase output (Table 8). In both experiments, the oxidation levels for sulfide iron, arsenic, and sulfur were almost the same. The data presented in Table 8 demonstrate relatively high oxidation levels of these elements, indicating a high oxidation level of sulfide minerals in both experiments. The oxidation levels for sulfide minerals were calculated. For the first and second experiment they were approximately 85 and 84% for pyrite and 97 and 96% for arsenopyrite, respectively.

Gold recovery by sorption cyanidation. Almost no gold was recovered from the unoxidized concentrate (2.82 %), indicating its highly refractory nature. While recovery for biooxidation residues was higher by an order of magnitude (Table 8), it was relatively low (39 and 32% in the first and second experiments, respectively) in spite of the high oxidation level of sulfides. Cyanide consumption was high (10 kg/t concentrate). This is another confirmation of high refractivity of the concentrate, which may be explained by high content of organic carbon (17.5%). Preg-robbing, i.e., sorption of gold cyanide complexes on the coaly matter, occurs during cyanidation of such concentrates. As a result, cyanidation efficiency decreases.

Composition of the microbial community formed during biooxidation. The DNA sequences obtained by PCR and separated by DGGE were sequenced in order to establish phylogenetic position of the microorganisms (operational taxonomic units, OTU) in the inoculum and in the microbial communities of the reactor pulp. *Acidithiobacillus ferrivorans, At. caldus, Leptospirillum ferriphilum, Sulfobacillus benefaciens, Ferroplasma acidiphilum*, and *Acidiplasma* sp. were identified in the community (Figure).

The composition of the community developed in the course of ore concentrate oxidation was different from that of the inoculum (Table 9). *S. benefaciens* was not revealed in the inoculum, which indicated low abundance of this species. This organism was, however, detected in the pulp in both experiments. In the course of biooxidation, *At. ferrivorans*, which was present in the inoculum, was partially or completely displaced from the community. In the first and second experiment, the composition of microbial communities in both reactors was identical (Table 9) and included *At. caldus, L. ferriphilum, S. benefaciens, F. acidiphilum*, and *Acidiplasma* sp. Thus, changed composition of the growth medium did not result in significant changes in the composition of the microbial community developing in the course of biooxidation.

Our results show that, although acidophilic microbial communities are able to adapt to various unfavorable environmental factors, such as high concentrations of sodium, magnesium, and chloride ions in the water, the traditional scheme for biooxidation of gold-bearing concentrates is not always applicable.



Phylogenetic tree of the 16S rRNA gene sequences constructed using the neighbor joining algorithm. Bootstrap support is over 70%. The sequences (OTU) obtained in the present work are marked by boldface. The scale of evolutionary distances is shown in the lower left corner.

In this particular case, in spite of the highly efficient biooxidation of sulfide minerals, gold recovery was low due to high content of organic carbon in the concentrate.

For efficient processing of double-refractory concentrates, such as the Bakyrchik deposit ore concentrate, modifications of the traditional scheme of biotechnological treatment are required. Modification of the cyanidation process is one of the possible approaches. Biomin Co. proposed cyanidation at high temperatures (HiTeCC technology), which decreases the sorption capacity of the coaly matter of double-refractory concentrates (biomin.co.za). Modifications of the technological scheme of biooxidation may broaden the range of efficient application of biohydrometallurgical technologies.

Table 9. Species composition of microbial communities

	Inoculum	Experiment 1		Experiment 2	
		reactor 1	reactor 2	reactor 1	reactor 2
Acidithiobacillus ferrivorans	+	–	–	–	–
Acidithiobacillus caldus	+	+	+	+	+
Leptospirillum ferriphilum	+	+	+	+	+
Sulfobacillus benefaciens	–	+	+	+	+
Ferroplasma acidiphilum	+	+	+	+	+
Acidiplasma sp.	+	+	+	+	+

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