Dependence of the Phenotypic Characteristics of *Acidithiobacillus ferrooxidans* on the Physical, Chemical, and Electrophysical Properties of Pyrites

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Abstract—Comparison of *Acidithiobacillus ferrooxidans* strains TFV-1 and TFBk with respect to their capacity to oxidize pyrite 1, with an electron-type (*n*-type) conductivity, or pyrite 2, with hole-type (*p*-type) conductivity, showed that, at a pulp density of 1%, both before and after its adaptation to the pyrites, strain TFBk, isolated from a substrate with a more complex mineral composition, grew faster and oxidized the pyrites of both conductivity types more efficiently than strain TFV-1, which was isolated from a mineralogically simple ore. At a pulp density of 3–5%, the oxidation of pyrite 2 by strain TFV-1 and both of the pyrites by strain TFBk began only after an artificial increase in Eh to 600 mV. If the pulp density was increased gradually, strain TFBk could oxidize the pyrites at its higher values than strain TFV-1, with the rate of pyrite 2 oxidation being higher than that of pyrite 1. During chemical oxidation of both of the pyrites, an increase was observed in the absolute values of the coefficients of thermoelectromotive force (K_{TEMF}); during bacterial–chemical oxidation, the K_{TEMF} of pyrite 1 changed insignificantly, whereas the K_{TEMF} of pyrite 2 decreased.

Key words: Acidithiobacillus ferrooxidans strains, properties of pyrites, adaptation, growth rate, oxidation rate.

Acidithiobacillus ferrooxidans is a gram-negative acidophilic chemolithotrophic mesophilic bacterium deriving its energy from oxidation of inorganic substrates, such as ferrous iron; elemental sulfur and its reduced compounds; and sulfide minerals. In natural ecosystems, the main habitats of this bacterium are sulfide ore deposits, where it is a dominant microorganism under mesophilic conditions.

Pyrite is the most widespread sulfide mineral on Earth. Pyrite with the formula FeS_2 consists of 46.6% Fe and 53.4% S. However, the actual ratio of these elements in pyrites vary from $FeS_{1.78}$ to $FeS_{2.2}$, or from $Fe_{1.1}S_2$ to $Fe_{0.9}S_2$. The physical, chemical, and electrophysical properties of pyrites vary considerably depending on the pyrite origin.

Earlier, Grudev [1] and Karavaiko *et al.* [2] reported that the same strains oxidize different pyrites at different rates. However, this phenomenon has not been studied further.

The aim of the present study was to investigate the effect of the physical, chemical, and electrophysical properties of two types of pyrites on the phenotypic characteristics of two *A. ferrooxidans* strains, TFV-1 and TFBk.

MATERIALS AND METHODS

Bacterial strains and cultivation conditions. Experiments were conducted with two *A. ferrooxidans* strains, TFV-1 and TFBk, stored in the culture collection of the Laboratory of Chemolithotrophic Microorganisms at the Winogradsky Institute of Microbiology (Russian Academy of Sciences). Strain TFBk had been isolated from the pulp of a plant processing a gold-arsenic high-sulfur ore concentrate from the Bakyrchikskoe deposit; this concentrate is mainly composed of pyrite and arsenopyrite and provides high concentrations of iron and arsenic in solutions. Strain TFV-1 had been isolated from the poor copper ore of the Volkovskoe deposit, which is relatively simple in its mineralogical composition. The strains were grown in 500-ml Erlenmeyer flasks containing 200 ml of Silverman–Lundgren medium [3] supplemented with Fe^{2+} or with one of the two pyrite types on a rotary shaker (150 rpm) at $28 \pm 2°C$. Cells of *A. ferrooxidans* from a log-phase cul-

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Table 1. Characteristics of pyrites

Characteristic	Pyrite	
	1	2
Physical properties:		
density, g/cm^3	5.6	5.72
microhardness, kG/mm^2	1360–1780 (1458)	1119–1233 (1162)
	794–824 (802)*	
Surface features	Pyrite grains have uneven steplike chips; some grains have shelllike fractures; inclusions of feldspar are often observed	Higher density of surface imperfections than in pyrite 1; cavities and acute-angled points occur; uniform planes occur very rarely
Chemical composition of natural samples, %		
Fe	40.78–50.08	40.35–44.64
S	49.45–59.08	56.27–59.09
Co	–	0.21–0.62
Ni	0.052–0.26	0.204–0.37
V	0.032–0.16	–
As	–	0.252–0.81
Ag	–	0.164–0.82
Sb	0.084–0.42	–
Electrophysical properties:		
conductivity type	*n* electron-type	*p* hole-type
resistance value, ln$R$ (R, Ohm)	from 5 to 11	from 7.5 to 10
K_{TEMF} value, μV K^{-1}	from –203 to –16	from 110 to 274
Reaction with HNO$_3$ (0.67 N) pH 0.95, 18°C	More soluble than pyrite 2 (contains less sulfur)	Less soluble than pyrite 1 (contains more sulfur)

Note: "–" stands for "absent".
        * Pyrite 1 was heterogeneous with respect to its microhardness, being composed of two generations.

ture grown in a medium with Fe^{2+} were used as the inoculum, which was introduced at a dose of 10% by volume.

Adaptation of the strains to pyrites was performed by successive passages on a Silverman–Lundgren medium containing 1% of a new oxidation substrate instead of ferrous iron. In each passage, cultures were grown until the stationary growth phase. The adaptation process was considered completed when the maximum growth rate and the highest activity of pyrite oxidation were achieved and did not change in further passages.

Experiments on increasing the pulp density were started at a pyrite concentration of 1%. After the culture achieved the stationary growth phase, new portions of pyrite were added, thus increasing the pulp density to 2 and, then, to 3%. When a 3% density was reached in the pulp, the cultures were transferred to a fresh medium with the same pulp density. After the stationary growth phase had been attained, the pulp density continued to be increased up to 7% for pyrite 1 and 10% for pyrite 2.

The experiments used pyrite grains that were less than 180 μm in size and sterilized by flowing steam. The experiments were performed in duplicate. Variants without bacterial inoculation served as the controls. On

completion of the experiment, the pyrites were sedimented by centrifugation at 100 *g* for 1 min, washed three times with distilled water, and dried in air, after which the resistance value and the coefficient of thermoelectromotive force (K_{TEMF}) were determined.

The rate of pyrite oxidation was judged from the accumulation of the oxidation product, i.e., ferric iron. The coefficient of substrate utilization was calculated from the increase in the cell number ($\times 10^{11}$ per l) as related to the amount of Fe^{3+} produced (g/l).

The number of *A. ferrooxidans* cells was determined by a direct count under a LOMO Mikromed 2 light microscope equipped with a phase-contrast device. The Fe^{3+}/Fe^{2+} ratio was determined by complexometric titration with Trilon B [4]. The pH value was measured with a pH150M pH-meter, and the Eh value was determined with an I-130.2M.1 ionometer.

Analysis of pyrite properties. The physical, chemical, and electrophysical properties of the pyrites were analyzed by the conventional methods: density was determined by the microburet method, microhardness was measured with a PMT-3 microhardness gage at a 100-g load and a 10-s exposure, and the chemical com-
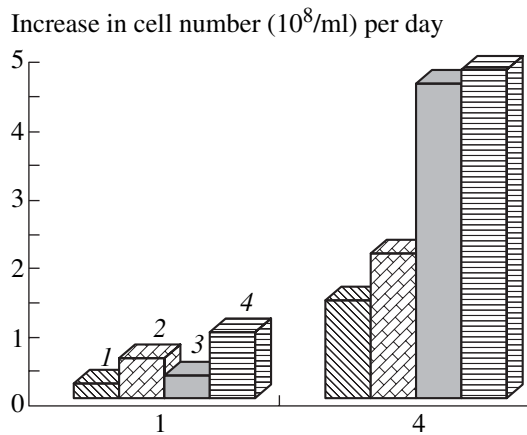
Increase in cell number (10^8/ml) per day

Fig. 1. Growth rates of *A. ferrooxidans* strains (*1, 2*) TFV-1 and (*3, 4*) TFBk on (*1, 3*) pyrite 1 and (*2, 4*) pyrite 2 in the (1) first and (4) fourth passage at a pulp density of 1%.
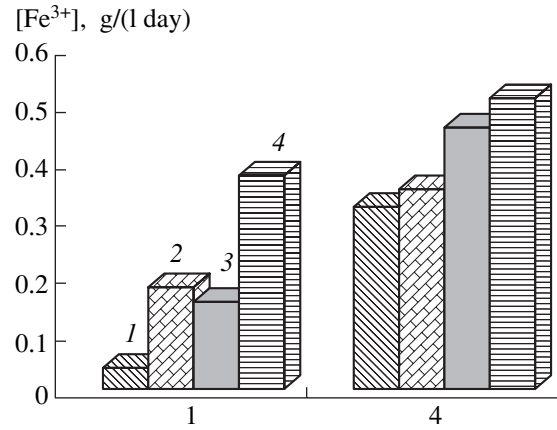
[Fe^{3+}], g/(l day)

Fig. 2. Oxidation rates of (*1, 3*) pyrite 1 and (*2, 4*) pyrite 2 by *A. ferrooxidans* strains (*1, 2*) TFV-1 and (*3, 4*) TFBk in the (1) first and (4) fourth passage at a pulp density of 1%.
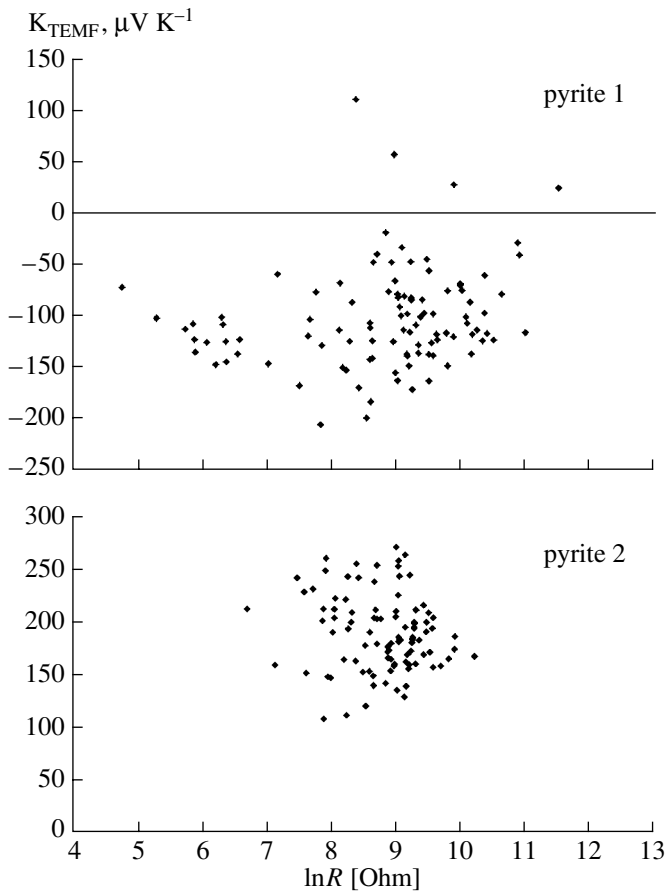
K_{TEMF}, μV K^{-1}

Fig. 3. Relation between the thermoEMF coefficients and resistance logarithm in samples of pyrites 1 and 2.
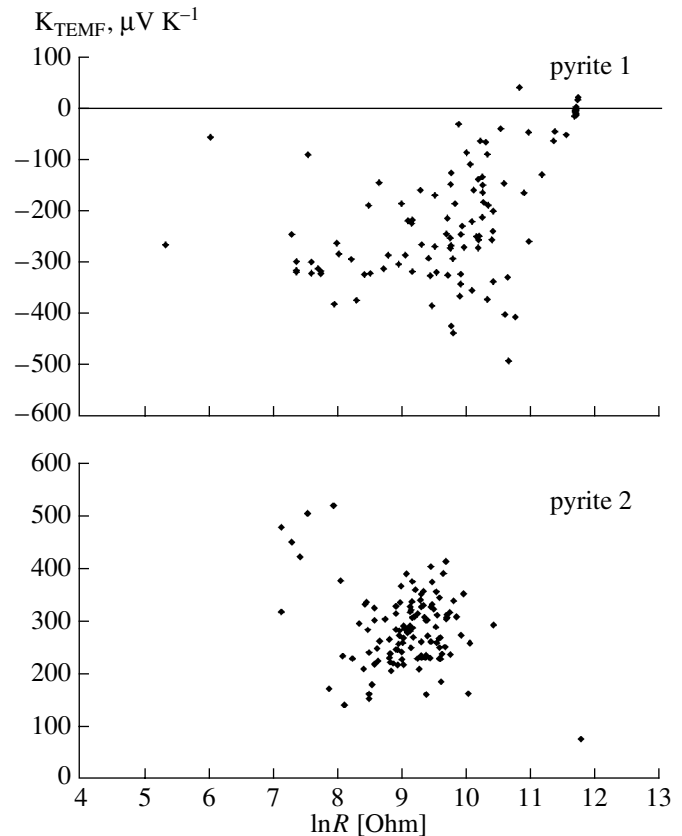
K_{TEMF}, μV K^{-1}

Fig. 4. Relation between the thermoEMF coefficients and resistance logarithm in samples of pyrites 1 and 2 after chemical oxidation.

position was determined with the microprobe analyzer of a JSM-5300 (Japan) scanning electron microscope. The conductivity type, value of resistance, and coefficient of thermoelectromotive force (K_{TEMF}) were determined with a modified device as described in [5]. A representative

sampling of 125 pyrite grains was used for the analyses. Grains of a similar size (100–180 μm) were selected, which allowed us to compare their resistance instead of specific resistance. The mean values of K_{TEMF} and dispersion were calculated using Microsoft Excel.

Table 2. parameters of growth and pyrite oxidation in original and adapted strains *A. ferrooxidans* TFV-1 and TFBk

Strain	Pyrite	Passage	Parameters of growth and pyrite oxidation				
			Specific growth rate (μ_{max}), h^{-1}	Generation time (t_d), h	Yield coefficient	Initial and final values of	
						pH	Eh
TFV-1	1	1	0.01	64.2	0.43	2.05 1.84	639 725
		4	0.052	13.3	4.47	1.88 1.36	673 735
	2	1	0.016	43.3	3.18	2.08 1.82	639 735
		4	0.064	10.83	6.03	1.94 1.41	697 737
TFBk	1	1	0.02	34.7	1.93	2.14 1.65	697 743
		4	0.065	10.66	12.39	1.86 1.32	679 757
	2	1	0.026	26.7	2.84	2.12 1.67	701 734
		4	0.073	9.49.	9.39	1.85 1.30	689 775

RESULTS AND DISCUSSION

Characteristics of pyrites. The properties of the pyrites used in this work are presented in Table 1. The pyrites were of different origin: pyrite 1, possessing

Table 3. Physicochemical characteristics of the pulp at a 5% density in the course of bacterial–chemical oxidation of pyrite 2 by *A. ferrooxidans* strain TFV-1

Days	Growth and oxidation parameters				
	pH	Eh, mV	Fe^{3+}, g/l	Fe^{2+}, g/l	Cells per ml of the pulp
0	1.94	600	0.28	0.35	4.39×10^7
2	1.77	540	0.14	0.91	0
Repeated inoculation to 1×10^7 cells/ml					
10	1.42	560	0.42	3.49	0
pH adjustment to 2.0					
16	1.63	560	0.48	5.3	0
Replacement of the liquid phase with fresh medium and inoculation to 1×10^7 cells/ml					
36	1.33	580	0.84	3.6	0
Addition of Fe^{3+} (4.88 g/l), increase of Eh to 643 mV, pH adjustment to 1.67					
43	1.33	670	7.4	2.4	3.59×10^7
pH adjustment to 1.84					
47	1.4	640	4.6	0.56	2.05×10^8
pH adjustment to 1.47					
54	1.45	710	2.44	0.07	3.56×10^8

n-type conductivity, was obtained at Mount Karpushikha in the Central Urals (Russia), and pyrite 2, which had *p*-type conductivity, was obtained from the Angren coal field (Uzbekistan).

Adaptation of *A. ferrooxidans* strains to pyrites. Earlier, we showed that *A. ferrooxidans* strains TFV-1 and TFBk differ in relation to the activity of oxidation of ferrous iron, elemental sulfur, and various sulfide ore concentrates [6]. We compared the dynamics of their adaptation to the oxidation of pyrites 1 and pyrite 2 at a 1% pulp density. In the first passage, the duration of the lag phase of strain TFV-1 when grown on pyrite 1 and pyrite 2 was 48 and 24 h, respectively, whereas strain TFBk started to grow on both pyrites immediately. Strain TFV-1 grew exponentially on pyrite 1 for 2 days and on pyrite 2 for 3 days, reaching, by the end of this growth phase, a cell density of 7.57×10^7 cells/ml on pyrite 1 and 2.2×10^8 cells/ml on pyrite 2. Strain TFBk grew exponentially for 2 days on both pyrites. In the stationary phase, the activity of pyrite oxidation decreased in strain TFV-1 but did not change in strain TFBk. In the first passage, the growth rate was rather low (Fig. 1, *1*), but it increased after each passage. After four passages, both strains achieved the limit of adaptation. In the fifth passage, both strains exhibited the same growth rate as in the fourth passage. The adapted culture of strain TFBk grew better on both of the pyrites than strain TFV-1 did. Both strains accumulated greater biomass when grown on pyrite 2 (Fig. 1, *4*). In the course of adaptation, the maximum specific growth rates increased in both strains, the generation times decreased accordingly, and acidification of the medium became more pronounced (Table 2).

Over the whole course of adaptation, both strains oxidized pyrite 2 more actively than pyrite 1 (Fig. 2). During adaptation at the pulp density of 1%, the activity of pyrite oxidation increased. Strain TFBk oxidized both pyrites more actively than strain TFV-1 did. The rate of pyrite oxidation was already rather high (close to the maximal one) in the second passage (data not shown). In the course of adaptation, the catabolic reactions of metabolism were activated faster than the biosynthetic ones.

The more efficient adaptation to the oxidation of the two types of pyrite observed in strain TFBk as compared to strain TFV-1 can probably be explained by the prehistory of strain TFBk, which was isolated from a more complex gold–arsenic concentrate. This concentrate favoured the development of a labile system regulating the oxidation of different types of substrates.

Under the continuous industrial processing of ores and concentrates, pyrites, mixed with other sulfide minerals, are oxidized at a pulp density of 20% and at even higher densities. However, in our experiments, a batch culture of *A. ferrooxidans* strain TFV-1 failed to develop and even died in a 5% monomineral pulp, regardless of the type of pyrite. This phenomenon was accompanied by the accumulation of Fe^{2+} in the pulp, presumably due to chemical oxidation, and by a decrease in the redox potential (Table 3). Reinoculation of the pulp did not yield positive results. The replacement of the liquid phase of the pulp with a fresh medium resulted in rapid accumulation of Fe^{2+} at a relatively low Eh value of 580 mV. The bacteria died in the pulp, and, after 16 days, their number, determined by the method of serial dilutions, did not exceed 10^2 cells/ml. It could be suggested that the unfavorable conditions developed in the liquid phase. However, when the liquid phase was separated from the solid material and reinoculated with *A. ferrooxidans* TFV-1, normal growth of the strain and oxidation of Fe^{2+} were observed. Thus, the liquid phase on its own, without the solid phase, did not inhibit growth of the bacteria. An increase in the redox potential (Eh) in the pulp via the addition of trivalent iron in the form of $Fe_2(SO_4)_3$ stimulated oxidation of pyrite 2 by strain TFV-1 at a pulp density of 5%. As can be seen from Table 3, the growth of bacteria and active oxidation of pyrite 2, resulting in the decrease in the bivalent iron concentration, started only after the increase in Eh in the pulp to 643 mV as a result of the addition of 4.88 g/l Fe3+. Under the same conditions, pyrite 1 was not oxidized by strain TFV-1 (data not shown).

Taking the above data into account, further experiments with strain TFBk were conducted in a more diluted pulp. At a pulp density of 2%, strain TFBk grew and oxidized both types of pyrite. However, when the pulp density was increased to 3%, the strain started to grow and oxidize the pyrites of both types only after the addition of Fe^{3+}, which resulted in an increase in the Eh value in the medium to 600–640 mV followed by its

Table 4. Physicochemical characteristics of the pulp at a 3% density in the course of bacterial–chemical oxidation of pyrite 1 by *A. ferrooxidans* strain TFBk

Days	Growth and oxidation parameters				
	pH	Eh, mV	Fe^{3+}, g/l	Fe^{2+}, g/l	Cells per ml of the pulp
0	1.96	560	0.21	0.14	1.3×10^7
10	1.84	570	0.28	1.64	0
Repeated inoculation to 1×10^7 cells/ml					
13	1.65	570	0.56	2.09	0
Addition of Fe^{3+} (2.34 g/l), increase of Eh to 600 mV					
20	1.43	660	5.58	1.35	2.66×10^7
pH adjustment to 1.95					
22	1.39	610	3.91	0.98	2.2×10^8
pH adjustment to 1.98					
23	1.50	690	4.05	0.07	2.83×10^8

Table 5. Physicochemical characteristics of the pulp at a 3% density in the course of bacterial–chemical oxidation of pyrite 2 by *A. ferrooxidans* strain TFBk

Days	Growth and oxidation parameters				
	pH	Eh, mV	Fe^{3+}, g/l	Fe^{2+}, g/l	Cells per ml of the pulp
0	1.98	570	0.28	0.21	1.37×10^7
7	1.93	550	0.14	0.98	0
Repeated inoculation to 1×10^7 cells/ml					
10	1.86	560	0.21	1.19	0
Addition of Fe^{3+} (2.16 g/l), increase of Eh to 640 mV					
17	1.42	720	6.7	0	3.05×10^8
pH adjustment to 1.88					
19	1.44	660	7.54	0.07	8.29×10^8
pH adjustment to 1.95					
21	1.43	770	5.86	0	1.08×10^9

further increase due to bacterial–chemical oxidation of Fe^{2+} to Fe^{3+} (Tables 4, 5).

During chemical oxidation (without bacteria) of both of the pyrite types at a 5% pulp density, bivalent iron accumulated in the pulp and Eh remained in the range 510–560 mV, i.e., approximately in the same range as it was in the above-mentioned experiments with nongrowing bacteria in dense pulps (Tables 3, 6). The oxidation proceeded slowly, as is indicated by the insignificant decrease in pH and negligible change in the Fe^{2+}/Fe^{3+} ratio over the 40-day experiment.

The results obtained allowed us to suggest that the main factor preventing the growth of bacteria and bacterial–chemical oxidation of pyrites in pulps at a density of above 3% was the electrochemical interaction

Table 6. Physicochemical characteristics of the pulp at a 5% density in the course of bacterial–chemical oxidation of pyrites of two types

Days	Pyrite							
	1				2			
	Growth and oxidation parameters							
	pH	Eh, mV	Fe^{3+}, g/l	Fe^{2+}, g/l	pH	Eh, mV	Fe^{3+}, g/l	Fe^{2+}, g/l
0	1.8	560	0.21	0.56	1.9	510	0.07	0.78
5	1.8	540	0.07	1.26	1.9	530	0.07	0.84
10	1.7	550	0.07	1.33	1.8	540	0.07	1.26
21	1.6	560	0.14	2.23	1.7	550	0.14	2.00
40	1.5	560	0.14	3.00	1.6	560	0.14	2.51

between the liquid phase of the pulp and its solid phase. It is evident that, in a batch culture, the similarity of the Eh values of the medium and the values of the electrode potential (EP) of the pyrites precluded the possibility of pyrite utilization as the source of energy for bacterial growth. Similar results have previously been reported [2, 7]. It has been shown [7] that the val-
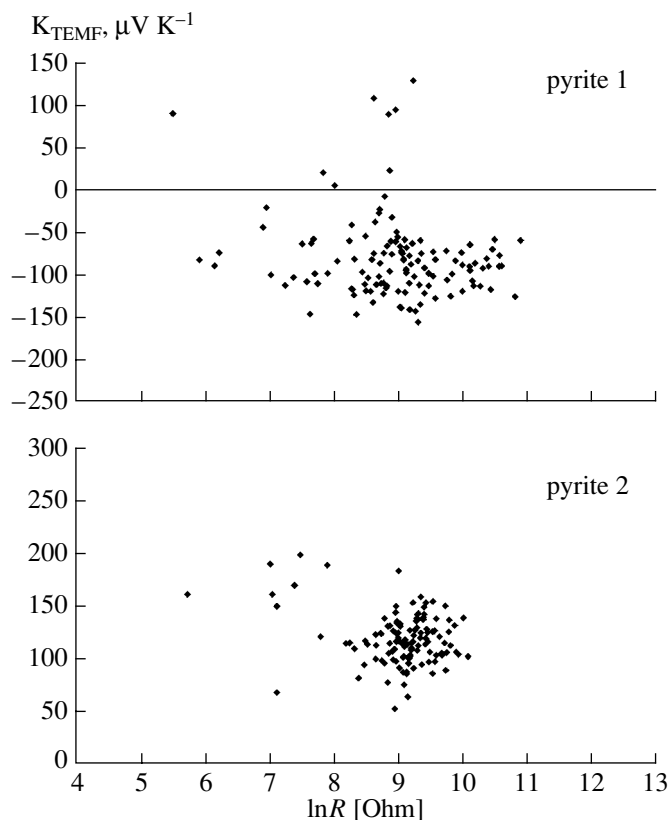

Fig. 5. Relation between the thermoEMF coefficients and resistance logarithm in samples of pyrites 1 and 2 after bacterial–chemical oxidation by *A. ferrooxidans* strain TFBk.

ues of the liquid phase Eh and of the pyrite EP level off in the course of bacterial–chemical oxidation of the mineral, resulting in equilibrium conditions in the Eh–EP system and preventing pyrite oxidation. This equilibrium is reached faster in the case of *n*-pyrite oxidation.

The main technological prerequisite for an efficient bacterial–chemical process is the onset of a nonequilibrium system in the pulp. To some extent, we managed to provide these conditions by adding pyrites to a medium containing bacteria that were in the stationary growth phase, i.e., when a high Eh value had been established in the pulp. Under such conditions, both *A. ferrooxidans* strains demonstrated greater resistance to an increase in the pulp density. In both strains, the activity of pyrite oxidation changed with the increase in the pulp density: in most variants, the activity first increased and then decreased. Strain TFV-1 demonstrated the highest oxidation rate on both pyrites at a pulp density of 4%; on pyrite 2, the rate was considerably higher (0.68 g/(l day)) than on pyrite 1 (0.24 g/(l day)). Over the entire period of increase in the pulp density, strain TFBk was superior to strain TFV-1 with respect to the activity of oxidation of both pyrites. The maximal rate of pyrite 1 oxidation by strain TFBk was observed at a pulp density of 5% (0.99 g/(l day)), and the rate of pyrite 2 oxidation at a pulp density of 6% was 3.33 g/(l day). At a pulp density of 10%, both *A. ferrooxidans* strains oxidized pyrite 2, but no culture growth occurred.

The higher resistance of strain TFBk to increased densities of pulp with monomineral pyrites (compared to strain TFV-1) can be explained by the greater ability of the former strain to oxidize pyrites and to maintain a greater difference between the Eh of the medium and the EP of the pulp.

The results obtained confirm our earlier suggestion that the activity of bacterial growth and pyrite oxidation is mainly determined by the level of Eh of the medium and EP of the minerals and by the difference between their values, which governs the electromotive force in the pulp system. When the above values are close, the oxidation does not proceed. A high pulp density stabilizes the equilibrium electrophysical conditions and indirectly affects the growth and oxidizing activity of the bacteria.

Electrophysical characteristics of the pyrites during their chemical and bacterial–chemical oxidation. The mineral structure (deviations from stoichiometry and the presence of electrically active impurities of rare-earth elements) exerts a pronounced effect on the kinetics of pyrite oxidation during its chemical and bacterial–chemical processing. The above structural parameters can be evaluated by measuring the electrophysical characteristics of the mineral samples, i.e., the conductivity type, the values of resistance, and K_{TEMF}.

As was mentioned above, pyrite 1 had electron-type (*n*-type) conductivity (Fig. 3). The logarithm of resistance (ln*R*) of its particles ranged from 5 to 11, and the

value of K_{TEMF} ranged from –16 to –203 $\mu V\ K^{-1}$ (–100 ± 49 $\mu V\ K^{-1}$). Pyrite 2 had hole-type (*p*-type) conductivity. The ln*R* values of its crystal particles varied in a narrower range, from 7.5 to 10, and the values of K_{TEMF} ranged from 110 to 274 $\mu V\ K^{-1}$ (192 ± 36 $\mu V\ K^{-1}$).

During chemical oxidation of the *n*-type and *p*-type pyrites, the range of K_{TEMF} values extended (Fig. 4). The values of K_{TEMF} ranged from 0 to –400 $\mu V\ K^{-1}$ (–172 ± 148 $\mu V\ K^{-1}$) for the crystal particles of *n*-type pyrite 1 and from 150 to 400 $\mu V\ K^{-1}$ (288 ± 71 $\mu V\ K^{-1}$) for pyrite 2. The patterns of changes of both pyrite types were similar. It is possible that, at low pH, H^+ ions penetrate pyrite crystals and increase their electroconductivity, which results in an increase in the absolute values of K.

During bacterial–chemical oxidation of pyrite 1 by strain *A. ferrooxidans* TFBk, the values of K changed insignificantly, varying in the range of 0 to –150 $\mu V\ K^{-1}$ (–79 ± 50 $\mu V\ K^{-1}$) (Fig. 5). This may indicate that, during the bacterial–chemical oxidation of pyrite 1, iron and sulfur were oxidized in nearly equimolar amounts. After the bacterial–chemical oxidation of pyrite 2 by *A. ferrooxidans* strain TFBk, the K_{TEMF} value shifted considerably, from 110–274 $\mu V\ K^{-1}$ (192 ± 36 $\mu V\ K^{-1}$) in the initial pyrite 2 to 50–200 $\mu V\ K^{-1}$ (119 ± 24 $\mu V\ K^{-1}$) in the treated pyrite (Figs. 3, 5). This finding may indicate that the extraction of sulfur was more active than that of iron. Thus, the bacterial–chemical oxidation caused different changes in the electrochemical properties of the two types of pyrite. However, after the chemical and bacterial–chemical oxidation of both pyrites, the values of ln*R* remained within the same ranges as in the initial unoxidized pyrite samples.

The bacterial–chemical oxidation of pyrites 1 and 2 by strain *A. ferrooxidans* TFV-1 resulted in analogous changes (data not shown).

Thus, we demonstrated the influence of the physical, chemical, and electrophysical properties of pyrite on the growth and oxidation rates in two *A. ferrooxidans* strains of different origin. The Eh value of the liquid phase was shown to play a decisive role in the establishment of a nonequilibrium system with the pyrite EP, which is necessary for initiation of the oxidation process. Changes have been found to occur in the electrophysical characteristics of pyrites during their chemical and bacterial–chemical oxidation.

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