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## Patterns of Pyrite Oxidation by Different Microorganisms

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Received February 19, 2008; in final form, June 26, 2008

**Abstract**—The bacterial-chemical oxidation of natural pyrites with different physical, chemical, and electro-physical characteristics by bacteria *Acidithiobacillus ferrooxidans*, *Sulfobacillus thermotolerans*, and the archaeon *Ferroplasma acidiphilum* were studied. The electrophysical characteristics of three natural pyrites differed in the  $K_{\text{thermoEMF}}$  value (pyrites 3, 4, hole conduction (p-type conductivity); pyrite 5, mixed type conductivity (n-p)) and in the logarithm of electric resistance. Chemical oxidation of pyrites 3 and 5 resulted in no changes of  $K_{\text{thermoEMF}}$ . When pyrite 4 was oxidized chemically, the  $K_{\text{thermoEMF}}$  values remained in the same range as in the initial sample, but the ratio of grains with different  $K_{\text{thermoEMF}}$  values in the sample was changed: the number of grains with a higher  $K_{\text{thermoEMF}}$  value increased. The same changes were also observed in the course of bacterio-chemical oxidation of pyrite 4. Of the three pyrites studied, an increase in the logarithm of resistance was observed only for chemical oxidation of pyrite 4 at 28°C. At higher experimental temperatures, the logarithm of resistance increased accordingly; more active bacterial-chemical oxidation resulted in a more pronounced increase in the logarithm of resistance than chemical oxidation. On bacterial-chemical oxidation of pyrites 3 and 5 by *A. ferrooxidans* and *S. thermotolerans* strains, iron was leached more actively than sulfur. Preferred bacterial-chemical oxidation of certain fractions from the pyrite samples was shown, namely of the pyrite 3 fraction with higher  $K_{\text{thermoEMF}}$  values by the *F. acidiphilum* strain and of a fraction from the pyrite 5 sample with medium  $K_{\text{thermoEMF}}$  values by the *A. ferrooxidans* and *S. thermotolerans* strains. The comparative assessment of bacterial-chemical pyrite oxidation by three types of microorganisms showed the direction of changes in the  $K_{\text{thermoEMF}}$  values to be the same in the case of bacteria *Acidithiobacillus ferrooxidans* and *Sulfobacillus thermotolerans* and different in the case of the archaeon *Ferroplasma acidiphilum*.

**Key words:** *Acidithiobacillus ferrooxidans*, *Sulfobacillus thermotolerans*, *Ferroplasma acidiphilum*, pyrites, electrophysical properties of pyrites.

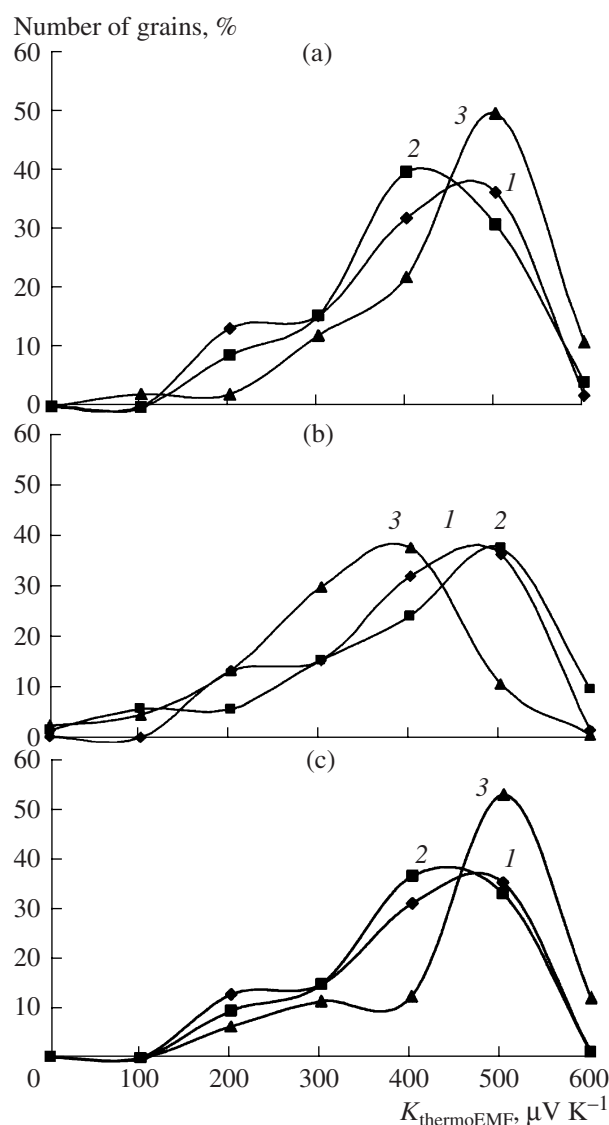
**DOI:** 10.1134/S0026261709020052

The first attempts to study the differences in the character of bacterial-chemical oxidation of pyrites with the hole (n) and electronic (p) types of conductivity by the bacterium *Acidithiobacillus ferrooxidans* were made by Yakhontova and Karavaiko in 1976 [1]. A relationship between the character and the rate of iron leaching from pyrite and the type of pyrite conductivity was shown. Pyrite with the hole type of conductivity was oxidized by *A. ferrooxidans* not only more intensely but also continuously, unlike pyrite with the electronic type of conductivity. It was not until 1994 that Karavaiko et al. resumed their investigations [2]. The oxidation of six pyrite samples by two species of microorganisms, *A. ferrooxidans* and *Sulfobacillus thermosulfidoxidans*, was studied. The differences were demonstrated both in the oxidation of different types of pyrites by the same microorganism and in the oxidation of one sample by different microorganisms.

This work is a continuation of these investigations and is dedicated to the study of the capacity of different microorganisms for oxidation of various types of pyrites as well as to the study of the processes occurring during the oxidation. Earlier, we studied the parameters of adaptation of two *A. ferrooxidans* strains, TFV-1 and TFBk, and the oxidation of two types of pyrites differing in the physical, chemical, and electrophysical properties (the n-type (type 1) and the p-type (type 2) conductivity) by these bacteria [3, 4]. In [5], the phenotypic and genotypic characteristics of representatives of phylogenetically remote groups of acidophilic chemolithotrophic microorganisms (*A. ferrooxidans*, *S. thermotolerans*, and *Ferroplasma acidiphilum*) were studied in the course of oxidation of three pyrite samples designated as 3, 4, and 5 under the optimum growth conditions for each microorganism.

The aim of this work was to investigate the electro-physical characteristics of three pyrite samples (3, 4, and 5) in the course of their chemical and bacterial-chemical oxidation by the strains of three microbial

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**Fig. 1.** Distribution of the  $K_{\text{thermoEMF}}$  values expressed in percent of (1) the grains of the initial pyrite 3, (2) the grains of pyrite 3 after chemical oxidation, and (3) after bacterial-chemical oxidation by strains: *A. ferrooxidans* TFBk (a), *F. acidiphilum* Y<sup>T</sup> (b), and *S. thermotolerans* Kr1<sup>T</sup> (c) at 28°C (a), 35°C (b), and 40°C (c).

species, namely, *A. ferrooxidans*, *S. thermotolerans*, and *F. acidiphilum*.

## MATERIALS AND METHODS

**Microbial strains and growth conditions.** The work was carried out using the strains *Acidithiobacillus ferrooxidans* TFV-1 and TFBk, *Sulfobacillus thermotolerans* Kr1<sup>T</sup>, and *Ferroplasma acidiphilum* Y<sup>T</sup> from the culture collection of the Laboratory of Chemolithotrophic Microorganisms of the Winogradsky Institute of Microbiology, Russian Academy of Sciences. The process of adaptation to pyrites and the con-

ditions for growth and cultivation of the microorganisms were described in [5].

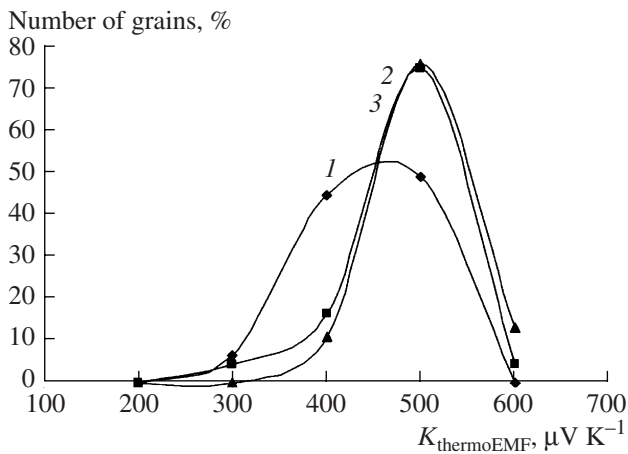
**Analysis of the properties of pyrites.** Analysis of the properties of the pyrites was carried out according to [3].

## RESULTS AND DISCUSSION

The mineral structure (deviation from the stoichiometric composition, the presence of electrically active admixtures of scattered elements) may have a significant effect on the kinetics of both chemical and bacterial-chemical pyrite oxidation. It is possible to assess the oxidation parameters by measuring the electrophysical characteristics of the samples: the type of conductivity, resistance, and the  $K_{\text{thermoEMF}}$ .

Pyrite 3 had type p conductivity; the  $K_{\text{thermoEMF}}$  values varied between 112 and 508  $\mu\text{V K}^{-1}$  ( $347 \pm 107 \mu\text{V K}^{-1}$ );  $\ln R$ , between 7 and 9.5 ( $8.3 \pm 0.6$ ). Pyrite 3 was the most heterogeneous by the  $K_{\text{thermoEMF}}$  value and had the greatest dispersion among all the three pyrites. Pyrite 4 also had type p conductivity; however, the  $K_{\text{thermoEMF}}$  values varied within a narrower range, from 275 to 480  $\mu\text{V K}^{-1}$  ( $399 \pm 49 \mu\text{V K}^{-1}$ );  $\ln R$ , varied between 5.3 and 9.2 ( $6.8 \pm 1.1$ ). Pyrite 5 had mixed conductivity (the n-p type), i.e., the grains with both the electronic and hole types of conductivity were present in the sample. Its  $K_{\text{thermoEMF}}$  values varied between  $-148$  and 240  $\mu\text{V K}^{-1}$  ( $-57 \pm 74 \mu\text{V K}^{-1}$ );  $\ln R$ , between 4.6 and 7.7 ( $5.6 \pm 0.8$ ). The n-type grains predominated in the pyrite 5 sample ( $-57 \pm 74 \mu\text{V K}^{-1}$ ).

On chemical oxidation of pyrite 3 at three temperatures (28, 35, and 40°C), no significant changes in the  $K_{\text{thermoEMF}}$  values were observed (Figs. 1a–1c). After bacterial-chemical oxidation by the *A. ferrooxidans* and *S. thermotolerans* strains, the  $K_{\text{thermoEMF}}$  values were in the same interval as in the initial sample; however, the grain ratios in the sample did change: the percentage of grains with a higher  $K_{\text{thermoEMF}}$  value increased (Figs. 1a, 1c). This may be due to two causes: (1) more active oxidation of iron than sulfur in pyrite and the release of cobalt and (2) the preferred bacterial oxidation of grains with a lower  $K_{\text{thermoEMF}}$  values. The second cause is more likely, because we did not observe a shift in the  $K_{\text{thermoEMF}}$  value range as in the case of pyrite 2; the  $K_{\text{thermoEMF}}$  of the latter was  $192 \pm 36 \mu\text{V K}^{-1}$  for the initial sample and  $119 \pm 24 \mu\text{V K}^{-1}$  for the treated sample [3]. After bacterial-chemical oxidation by the *F. acidiphilum* strain, the  $K_{\text{thermoEMF}}$  values remained within the same interval; however, we observed a shift in the maximum  $K_{\text{thermoEMF}}$  values towards decreasing (Fig. 1b) as distinct from the samples after bacterial-chemical oxidation by *A. ferrooxidans* and *S. thermotolerans* strains. *F. acidiphilum* is an archaeon with no cell wall. The cells of *F. acidiphilum* are surrounded only by the cytoplasmic membrane containing unique phospholipids  $\beta$ -D-glucopyranosyl caldarchaetidyl-

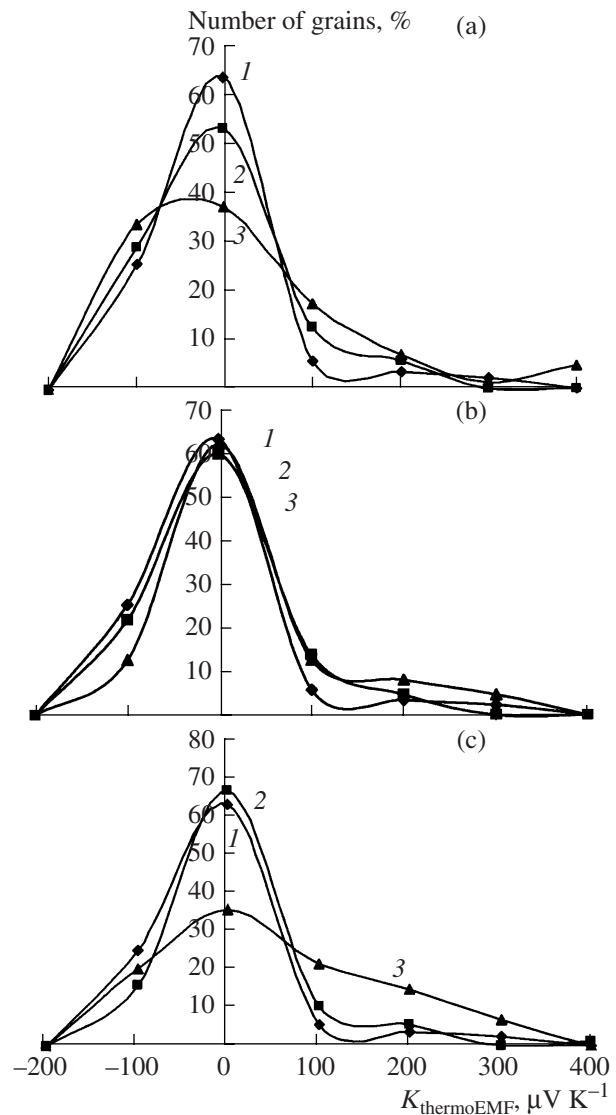


**Fig. 2.** Distribution of the  $K_{thermoEMF}$  values expressed in percent of the grains of the initial pyrite 4 (1), the grains of pyrite 4 after chemical oxidation (2), and after bacterial-chemical oxidation by *A. ferrooxidans* TFBk at 28°C (3).

glycerol and trihexosyl caldarchaetidylglycerol [6]. The characteristic features of pyrite 3 oxidation may be linked to the surface structure of the cells and their charge. The role of the cell charge and the charge of the oxidized inorganic substrate and their interaction were shown for *A. ferrooxidans* [7]. In the process of bacterial-chemical oxidation of pyrites, the electrophoretic mobility of the *A. ferrooxidans* cells significantly increased, whereas it was close to zero for pyrite particles. In the absence of a difference between these values, no oxidation occurred. Since the charge of *F. acidiphilum* cells was not investigated during the oxidation process, the data on the difference in the character of oxidation of this energy substrate by bacteria and archaea are difficult to explain.

In the case of pyrite 4, the changes in the electro-physical properties of the samples after chemical (28°C) and bacterial-chemical oxidation by *A. ferrooxidans* strains were similar, i.e., the  $K_{thermoEMF}$  values of pyrite grains remained within the same range as those in the initial sample; however, the number of grains with a higher  $K_{thermoEMF}$  value increased (Fig. 2). Pyrite 4 was the most difficult to oxidize for *A. ferrooxidans*. Since the  $K_{thermoEMF}$  changes in both cases were the same and both samples differed in this parameter from the initial sample, it is possible that this effect resulted from the release of admixtures of nickel and cobalt in the process of oxidation. This led to an increase in the number of grains with a higher  $K_{thermoEMF}$  in the samples.

During chemical oxidation of pyrite 5 at all experimental temperatures (28, 35, and 40°C) and its bacterial-chemical oxidation by the *F. acidiphilum* strain at 35°C, we observed no noticeable changes in the numerical distribution of the grains with varying  $K_{thermoEMF}$



**Fig. 3.** Distribution of the  $K_{thermoEMF}$  values expressed in percent of (1) the grains of the initial pyrite 5, (2) the grains of pyrite 5 after chemical oxidation, and (3) after bacterial-chemical oxidation by the strains: *A. ferrooxidans* TFBk (a), *F. acidiphilum* Y<sup>T</sup> (b), and *S. thermotolerans* Kr1<sup>T</sup> (c) at 28°C (a), 35°C (b), and 40°C (c).

values (Figs. 3a–3c). This is probably the result of the low activity of sample oxidation in these cases. Bacterial-chemical oxidation of pyrite 5 by *A. ferrooxidans* and *S. thermotolerans* resulted in a decreased number of grains with the  $K_{thermoEMF}$  values close to zero (Figs. 3a, 3c); the number of grains with positive  $K_{thermoEMF}$  values increased, and the number of grains with the  $K_{thermoEMF}$  values close to  $-100 \mu V K^{-1}$  remained at a level of the initial sample. Apparently, both microorganisms preferred to oxidize the fraction with the  $K_{thermoEMF}$  values close to zero and they proba-

bly oxidized preferably the ferrous rather than the sulfurous part of pyrite.

The  $\ln R$  value remained almost the same during chemical and bacterial-chemical oxidation of pyrite 3 by *A. ferrooxidans* ( $8.2 \pm 0.8$ ) (28°C). When the experimental temperature increased, we observed an increase in resistance ( $\ln R$  values): for chemical oxidation,  $\ln R = 8.5 \pm 0.9$ , for bacterial-chemical oxidation by *F. acidiphilum*,  $\ln R = 8.6 \pm 0.9$  (35°C); for chemical oxidation,  $\ln R = 8.8 \pm 0.8$ , for bacterial-chemical oxidation by *S. thermotolerans*,  $\ln R = 8.9 \pm 0.8$  (40°C). These changes were more markedly pronounced when more active bacterial-chemical oxidation occurred. This might be connected with increased solubility of the minerals at higher temperatures, which led to the release of admixtures and an increase in the stoichiometry of the samples (pure pyrite is an insulator).

Chemical and bacterio-chemical oxidation of pyrite 4 by *A. ferrooxidans* resulted in increased  $\ln R$  values, from initial  $\ln R = 6.8 \pm 1.1$  to  $\ln R = 7.2 \pm 0.5$  and  $\ln R = 7.3 \pm 0.7$ , respectively. This can also be explained by the dissolution of admixtures and the increased stoichiometry of the pyrite 4 samples.

During chemical oxidation of pyrite 5 at 28°C, the resistance almost did not change:  $\ln R = 5.7 \pm 0.8$ ; in the case of bacterial-chemical oxidation, it increased to  $\ln R = 6.3 \pm 1.1$ . When the experimental temperature increased, the logarithm of resistance increased as well, similar to the case of pyrite 3: for chemical oxidation,  $\ln R = 5.9 \pm 0.9$  (35°C), for bacterial-chemical oxidation by *F. acidiphilum*,  $\ln R = 6.1 \pm 1.0$  (35°C); for chemical oxidation,  $\ln R = 6.0 \pm 1.0$  (40°C), for bacterial-chemical oxidation by *S. thermotolerans*,  $\ln R = 6.6 \pm 1.1$  (40°C).

In the course of bacterial-chemical oxidation of pyrites 3 and 5 by *A. ferrooxidans* TFV-1, the character of changes in the pyrite samples was similar to the changes during oxidation by TFBk (data not shown).

Thus, it was shown that the electrophysical properties of natural pyrites are different both in the  $K_{\text{thermoEMF}}$  values (the n-type pyrite 1; the p-type pyrites 2, 3, 4; the n-p-type pyrite 5) and the logarithm of resistance [3; this work]. In the process of chemical and bacterial-chemical oxidation, the electrophysical properties of the pyrites may change. In the case of chemical oxidation, we observed either an extension of the  $K_{\text{thermoEMF}}$  range (pyrites 1 and 2, which were the most readily oxidized) or no changes in the  $K_{\text{thermoEMF}}$  values (pyrites 3 and 5, low activity of chemical oxidation). In the case of chemical oxidation of pyrite 4 (which is the most difficult to oxidize), the ratio of grains with different  $K_{\text{thermoEMF}}$  values in the sample changed: the number of grains with a higher  $K_{\text{thermoEMF}}$  value increased. The same changes were also observed for bacterial-chemical oxidation of pyrite 4; they were apparently linked to the release of nickel and cobalt admixtures. The changes in the course of chemical and bacterial-chemi-

cal oxidation of pyrites 1, 2, 3, and 5 were different [3, this work]. Of the five pyrites investigated, only chemical oxidation of pyrite 4 at 28°C resulted in a change in the logarithm of resistance towards increasing, which also supports the suggestion that admixtures are released and the sample's stoichiometry increases. With an increase in the experimental temperature, the logarithm of resistance increased. More active bacterial-chemical oxidation resulted in a more pronounced increase in the logarithm of resistance of chemical oxidation. In the course of bacterial-chemical oxidation by *A. ferrooxidans* (28°C), the  $\ln R$  increase was observed only when pyrites 4 and 5 were oxidized. In the case of bacterial-chemical oxidation of pyrite, proportionate oxidation of the sulfur and iron of pyrite 1 by *A. ferrooxidans*, more active oxidation of the sulfur of pyrite 2 by *A. ferrooxidans*, and more active oxidation of the iron of pyrites 3 and 5 by *A. ferrooxidans* and *S. thermotolerans* occurred. The preferred oxidation of certain fractions of pyrite samples was shown: of those with lower pyrite 1  $K_{\text{thermoEMF}}$  values, by *A. ferrooxidans* strains; with higher pyrite 3  $K_{\text{thermoEMF}}$  values, by the *F. acidiphilum* strain; of the medium fraction from the pyrite 5 sample, by *A. ferrooxidans* and *S. thermotolerans* strains. The comparative assessment of bacterial-chemical oxidation of pyrites by three microbial species showed that the direction of changes in the  $K_{\text{thermoEMF}}$  values was the same in the case of two bacteria, *A. ferrooxidans* and *S. thermotolerans*, and different in the case of the archaeon *F. acidiphilum*.

Thus, the physical, chemical, and electrophysical properties of pyrites influenced the character of their changes when they were oxidized by different microorganisms.

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