Research Article

Pyrite Biooxidation by Acidophilic Archaea AcidiplasmaSp. MBA-1 Under Varied Conditions

Aleksandr Bulaev

Winogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences. 33, bld. 2 Leninsky Ave., Moscow 119071,Russia

ORCID

Aleksandr Bulaev; 0000-0001-7914-9817

Abstract. The goal of this research was to study pyrite (FeS_2) bioleaching by a strain of the genus *Acidiplasma* under different conditions (temperature, pH) to evaluate the potential role of *Acidiplasma* representatives in biooxidation of this sulfide mineral. To compare the role of *Acidiplasma* archaea in pyrite biooxidation with other acidophilic microorganisms, the experiments were also performed with representatives of othergroups of microorganisms predominant in biohydrometallurgical processes.Pure and mixed cultures of moderately thermophilic acidophilic microorganisms, including strains *Acidithiobacillus caldus* MBC-1, *Sulfobacillusthermosulfidooxidans* VKMV 1269^T and *Acidiplasma*sp. MBA-1, were used. The experiments were carried out in flasks with 100 mL of mineral nutrient medium supplemented with 0.02% yeast extract and 1 g of pyrite on a rotary shaker for 20 days. Bioleaching was performed at 45, 55, and 60°C. The results demonstrated that the representatives of the genus *Acidiplasma*provided a comparatively higher rate of pyrite bioleaching at high temperatures (55 and 60°C) and low pH of the medium (1.0). Thus, according to the results, strains of the genus *Acidiplasma* may provide a high rate of pyrite bioleaching at low levels ofpH. Therefore, the results suggest that archaea of the genus *Acidiplasma* may be promising microorganisms to improve bioleaching processes with an increase in the operational temperature, which is usually maintained at 40–45°C in industrial scale reactors.

Keywords: biomining, bioleaching, acidophilic microorganisms, sulfide minerals, pyrite

Corresponding Author:
Aleksandr Bulaev; email:
bulaev.inmi@yandex.ru

Dates

Published 13 January 2022

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the 8th Scientific and Practical Conference Conference Committee.

1. Introduction

Biomining (biohydrometallurgy)is widely used for treatment of sulfide ores and concentrates. The principle upon which the biohydrometallurgical processes is based is oxidative disruption ofthe crystal lattice of sulfide minerals byvarious acidophilic iron- and sulfur-oxidizing microorganisms [1].

Biohydrometallurgical technologiesmay be applied toextract different non-ferrous and noble metals (e.g., copper, nickel, zinc, gold,and uranium) from sulfide ores and concentrates[1,2]. The most important area of biohydrometallurgy application is processing of refractory gold-bearing sulfide concentrates [1, 2].Pyrite (FeS_2), the most widespread sulfide mineral, is often a component ofrefractory gold-bearing ores and concentrates

[2, 3]. Therefore, pyrite biooxidation is of great importance for the processing refractory gold-bearing concentrates.

Mechanisms of pyritebioleaching [3, 4] and the effect of different factors on its biooxidation,including temperature, Eh, and properties of oxidizing microorganisms, have been actively studied [5,6].As during pyrite bioleaching, Fe^{3+}ion, which is produced during pyrite bioleaching by iron-oxidizing microorganism, is the main oxidizing agent, iron-oxidizers play a key role in its disruption[3, 4, 5]. For example, it was show that iron-oxidizing bacteria *Leptospirillum* and *Sulfobacillus* played the most important role in pyrite biooxidation by mixed cultures of iron- and sulfur-oxidizing microorganisms [5, 6, 7].

It should be noted that improvementofbiohydrometallurgical technologies may be based on the study of the microbe–mineral interactions and the effect of different factors on bioleaching of various sulfide minerals. One of the main factors affecting biooxidation of sulfide concentrates is temperature. As sulfide mineral oxidation is accompanied by heat generation,reactors of biooxidation plants are equipped with cooling systems to maintain the required temperature, while bioleaching processes on an industrial scale are usually performed at high temperature (40–45°C) [2]. As maintaining of the required temperature comprises a significant part of OPEX during biooxidation of sulfide concentrates, performing processes at elevated temperatures may provide the decrease in production cost [8].

As the temperature also affects the composition of microbial populations performing biooxidation, study of pyrite biooxidation by the microorganisms predominant at different temperatures may be of interest. In industrial bioleaching processes, thermotolerant and moderately thermophilic microorganisms including bacteria*Leptospirillum*, *Sulfobacillus* spp., and *Acidithiobacillus caldus* as well as archaea of the genera *Ferroplasma* and *Acidiplasma* of the family *Ferroplasmaceae* are predominant [2, 9, 10]. Representatives of the genus *Acidiplasma* are moderate thermophiles oxidizing iron and sulfur, whichpredominate in microbial populations performing bioleaching of different sulfide concentrates [9, 10]. These archaea have temperature optimum 45–55°C and are able to grow at temperatures up to 65°C. Thus,they are more resistant to the hightemperatures than other microorganisms predominant in biohydrometallurgical processes [11]. Therefore, performing bioleaching at elevated temperatures using these microorganisms may be promising approach to improve biooxidation processes. Therefore, bioleaching of different sulfide minerals by *Acidiplasma* archaea should be studied in detail.

The goal of the present work was to study pyrite bioleaching by the strain of the genus *Acidiplasma* underdifferent conditions (temperature, pH) to evaluate potential role of the representatives of the genus in biooxidation of this sulfide mineral. To compare the role of *Acidiplasma* archaea in biooxidation of this sulfide mineral with other acidophilic microorganisms, the experiments were also performed with representatives of other-groups of microorganisms predominant in biohydrometallurgical processes (strains of *A.caldus*and*S. thermosulfidooxidans*).

2. Materials and Methods

Sulfide mineral pyrite (FeS_2) (Akchatau mine, Karagandaregion, Kazakhstan) milled to a particlesize of not more than 75 μm was used in the study. Pure and mixed cultures of moderately thermophilic acidophilic microorganisms, including strains *Acidithiobacillus caldus* MBC-1, *Sulfobacillus thermosulfidooxidans* VKMV 1269^T, and *Acidiplasma* sp. MBA-1 previously isolated from samples of ores and pulp of biooxidation reactors were the subjects of the study. Properties of the strains are shown in Table 1. All strains were inoculated in the medium in such a way that initial cells number of each strain was about 1×10^8 cells/mL.The bioleaching was carried out in 250 mL Erlenmeyer flask with 100 mL of mineral nutrient medium containing salts of nitrogen and phosphorus ((g/L) $(NH_4)_2SO_4$– 3.0; KCl– 0.20; $MgSO_4 \times 7H_2O$ – 0.5; K_2HPO_4– 0.5) and 1 g of pyrite on a rotary shaker (200 rpm) for 20 days, the medium was supplemented with 0.02% yeast extract. Bioleaching experiments were performed at 45, 55, and 60°C. In some variants, initial pH was 1.5, while in other variants it was 1.0. The following combinations of the strains and conditions were used in the work: (1) mixed culture of *A. caldus* MBC-1, *S. thermosulfidooxidans* VKMV 1269^T, and *Acidiplasma* sp. MBA-1, 45°C, initial pH 1.5; (2) pure culture *S. thermosulfidooxidans* VKMV 1269^T, 45°C, initial pH 1.5; (3) pure culture *Acidiplasma* sp. MBA-1, 45°C, initial pH 1.5; (4) pure culture *Acidiplasma* sp. MBA-1, 55°C, initial pH 1.0; (5) pure culture *Acidiplasma* sp. MBA-1, 55°C, initial pH 1.5; (6) pure culture *Acidiplasma* sp. MBA-1, 60°C, initial pH 1.0; (7) pure culture *Acidiplasma* sp. MBA-1, 60°C, initial pH 1.5.

Table 1:

To estimate pyrite bioleaching under different conditions,parameters of the liquid phase reflecting activity of iron- and sulfur-oxidizing microorganisms (pH, Eh, concentrations of Fe^{2+} and Fe^{3+} ions) were determined. The concentrations of Fe^{2+} and Fe^{3+} ions were determined spectrophotometrically (at 475 nm) using the rhodanide method based

Table 1: Properties of the strains.

Microorganism	Oxidation		Optimum temperature / upper limit, ˚C	Optimum pH	Reference
	Fe^{2+}	S^0			
A. caldus MBC-1	-	+	45 / 53	2.0	[9]
S. thermosulfidooxidans VKMV 1269^T	+	+	45–48 / 60	2.0	[11]
Acidiplasma sp. MBA-1	+	+	50-55 / 63	1.0	[9]

on formation of a purple coordination complex of ferric iron ions Fe^{3+} and rhodanide SCN^{--} [13].

To compare the rate of pyrite leaching under different conditions, it was evaluated by the total concentration of iron ions in the medium, calculating the fraction of iron that was released into the solutionafter 20 days of the biooxidation.

3. Results

The results of the experiments under different conditions are shown in Figures 1, 2, and 3.Figure 1 demonstrates changes in liquid phase parameter during biooxidation pyrite by different microorganisms at 45˚C, while in Figure 2, the liquid phase parameters during pyrite bioleaching by the strain*Acidiplasma* sp. MBA-1 at 55 and 60˚C are shown. It should be noted that conditions of the experiment with the mixed culture and pure cultures of the strains *S. thermosulfidooxidans* VKMV 1269^T and*Acidiplasma* sp. MBA-1 at 45˚C corresponded to those in laboratory-scale reactors during the trials performed in the work [9]. This work demonstrated that at 45˚Cin laboratory reactors, biooxidation of pyrite-arsenopyrite gold bearing concentrate was performed by mixed microbial population, which formed during long-term biooxidation process and included strains*A. caldus*,*S. thermosulfidooxidans,* and*Acidiplasma.* Therefore, the results of the present work made it possible to evaluate the potential role of the strains of mixed population formed in bioleach experiment in pyrite bioleaching during model experiment.

It was shown that pyrite bioleaching was the most active in the variant with mixed culture of three strains (Figure 1) that was determined by the liquid phase parameters. While the pH values were similar in all variants of the experiments (Figure 1A), Eh values and iron ions concentrations differed in the experiments with different cultures (Figures 1B, 1C, and 1D). It was shown that by the end of the experiment, Eh value as well as ferric iron ion concentration was the highest in the experiment with the mixed culture (Figures 1B and 1C). It should be noted that ferrous iron ion was not detected

during the bioleaching (Figures 1D), which in turn demonstrated high iron-oxidizing activity of the culture. In the same time, in the experiment with the pure culture of *S. thermosulfidooxidans* VKMV 1269^T, Eh values and ferric iron concentrations did not differ significantly from those observed in the experiment with the mixed culture up to 15th day of the bioleaching (Figures 1B and 1C). After 15 days of the bioleaching, oxidizing activity of the strain *S. thermosulfidooxidans* VKMV 1269^T decreased that was reflected by the decrease in Eh and increase in ferrous iron concentration (Figures 1B and 1D). It may be explained by the fact that in the mixed cultures, autotrophic *A. caldus* may provide mixotrophic bacterium *S. thermosulfidooxidans* with organic exometabolites and maintain its activity during long-term experiments. In the same time, during pyrite bioleaching by the pure culture *S. thermosulfidooxidans*, its activity decreased by the end experiment due to thedepletion of added organic carbon source (yeast extract) [7].

In the variant with the pure culture of *Acidiplasma* sp. MBA-1, ferric iron ion concentrations were significantly lower than those in other variants (Figure 1C). In the same time, ferrous iron concentration (Figure 1D) were comparatively low and was detected only on the 15th day, while Eh value insignificantly lower than in the variant with the mixed culture (Figure 1B). Pyrite bioleaching rate at 45°C was the highest in the experiment with the mixed culture, while it was insignificantly lower in the experiment with the strain *S. thermosulfidooxidans* VKMV 1269^T(Figure 3, bars 1 and 2). In the experiment with *Acidiplasma* sp. MBA-1, leaching rate was comparatively low (Figure 3, bar 3).

Thus, it was shown that a 45°C strain *S. thermosulfidooxidans* VKMV 1269^T played the main role in pyrite bioleaching since in the experiment with its pure culture leaching rate was almost as high as in the experiment with the mixed culture, while the strain *Acidiplasma* sp. MBA-1 leached pyrite less actively.

Figure 2 demonstrated results of pyrite bioleaching by the strain *Acidiplasma* sp. MBA-1 at high temperatures (55 and 60°C) and different pH values(1.0 and 1.5).

It was shown that pH values changed insignificantly (Figure 2A) during the bioleaching. The Eh values (Figure 2B) were lower than those at 45°C, while ferrous iron concentrations were higher than in the experiment at 45°C (Figures 1D and 2D). Therefore, it may be assumed that at high temperatures iron oxidation activity was inhibited despite 55°C corresponded to the optimum temperature of the strain. In the same time, ferrous iron concentrations at higher temperatures might be higher due to the increase in the rate of chemical reactions of ferric iron ions with pyrite that in turn led to the accumulation of ferrous iron ions in the medium. It should be noted that at both temperatures, ferric iron concentrations in the medium (Figure 2C) were lower at pH 1.5 in comparison to

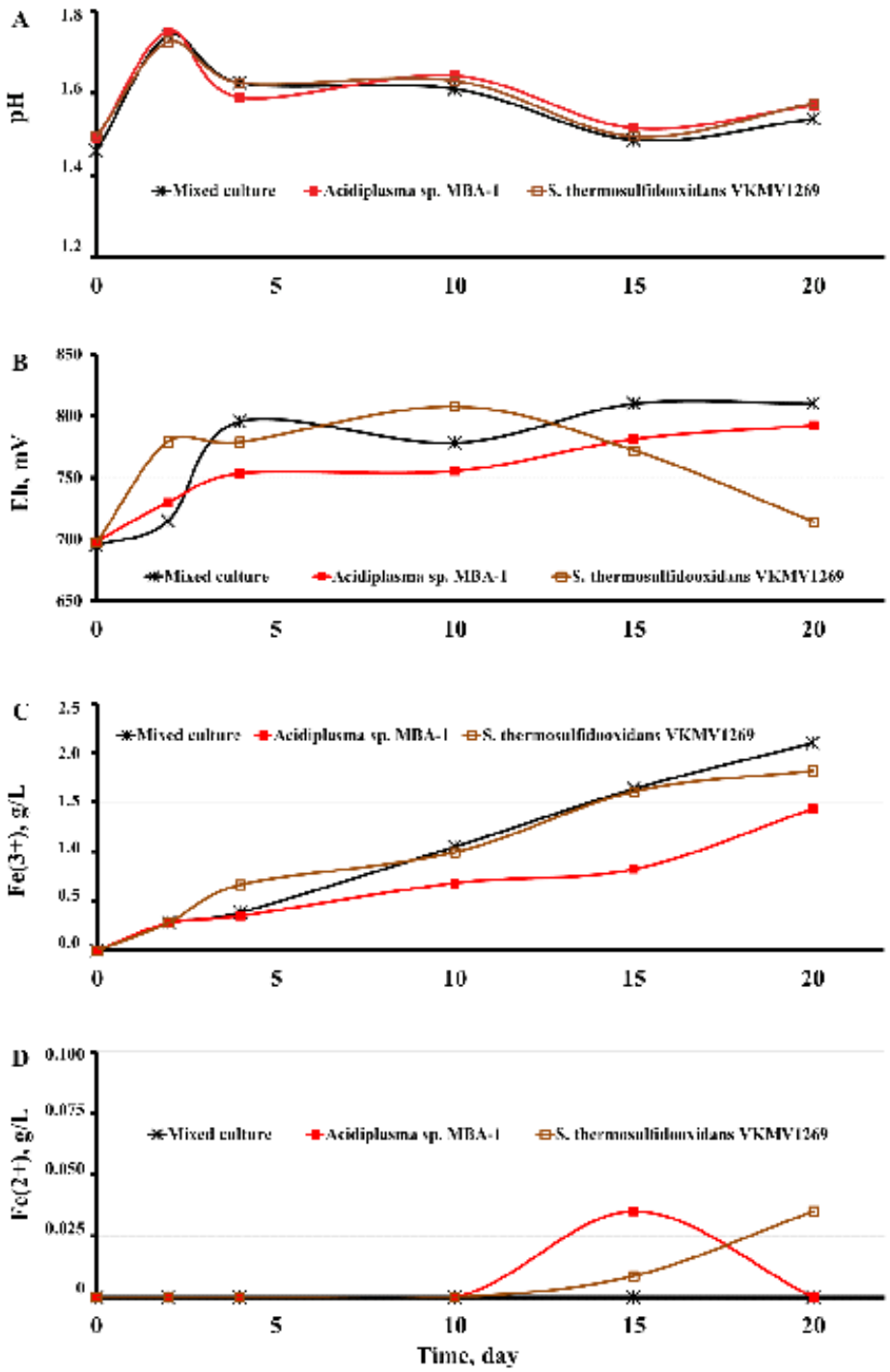

Figure 1: Changes in the liquid phase parameters during pyrite bioleaching at 45°C: A – pH; B – Eh; C – Fe^{3+} ion concentration (g/L); D– Fe^{2+} ion concentration (g/L).

the variants with initial pH of the medium of 1.0 corresponding to the optimum pH of the strain *Acidiplasma* sp. MBA-1.

Pyrite leaching rate was the lowest in the variant with pH 1.5 and temperature of 60°C (Figure 3, bar 7), while it was also comparatively low at pH 1.5 and temperature

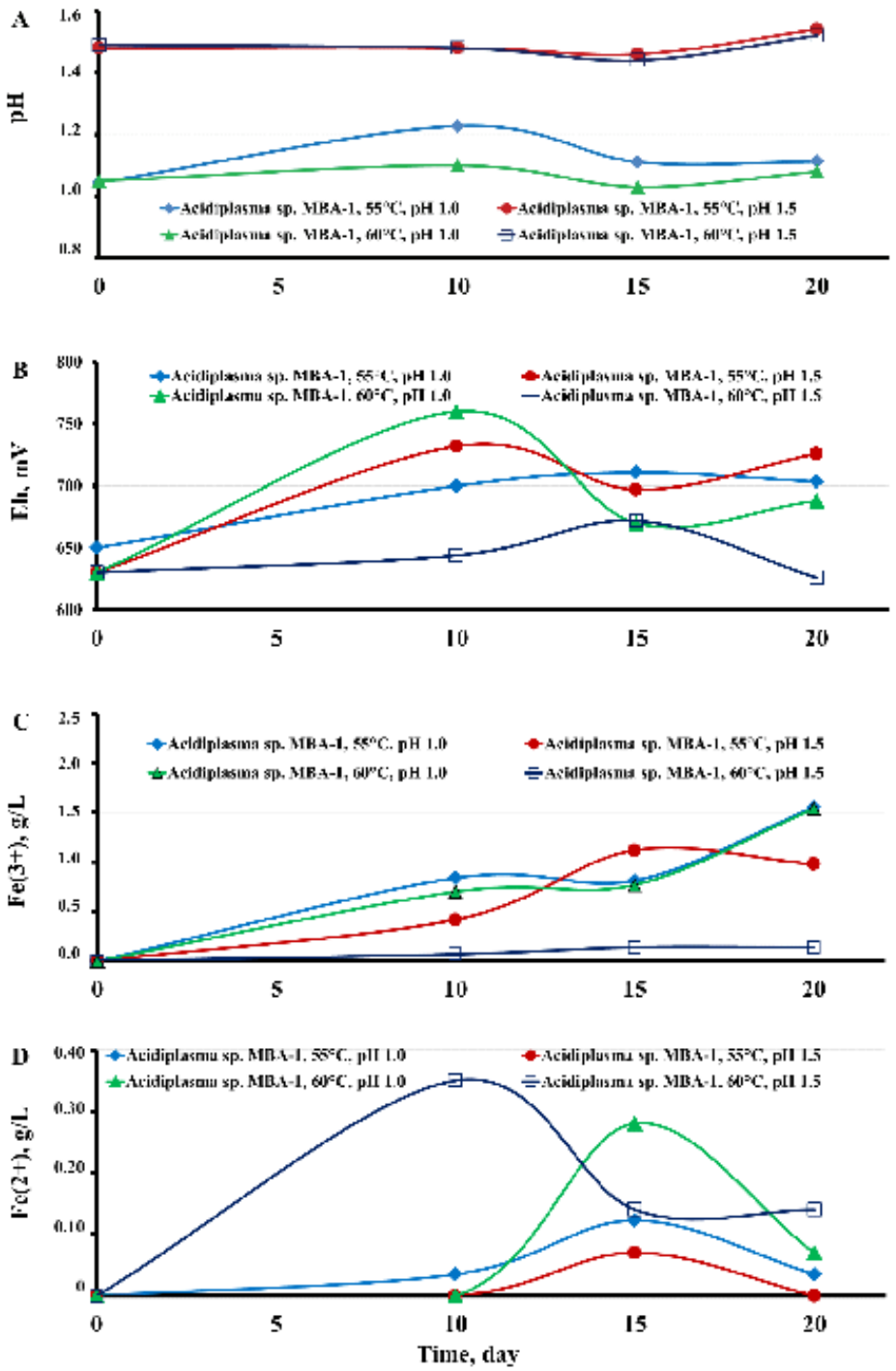

Figure 2: Changes in the liquid phase parameters during pyrite bioleaching by the strain *Acidiplasma* sp. MBA-1 at 55 and 60°C: A – pH; B – Eh; C – Fe^{3+} ion concentration (g/L); D – Fe^{2+} ion concentration (g/L).

of55°C (Figure 3, bar 5). In the same time, leaching rates were significantly higher at 55 and 60°C and at pH 1.0 (Figures 3, bars 4 and 6). In these variants, leaching rates were higher than in the experiment the strain *Acidiplasma* sp. MBA-1 at 45°C (Figure 3, bar 3). Thus, the increase in the temperature led to the decrease in bioleaching rate at

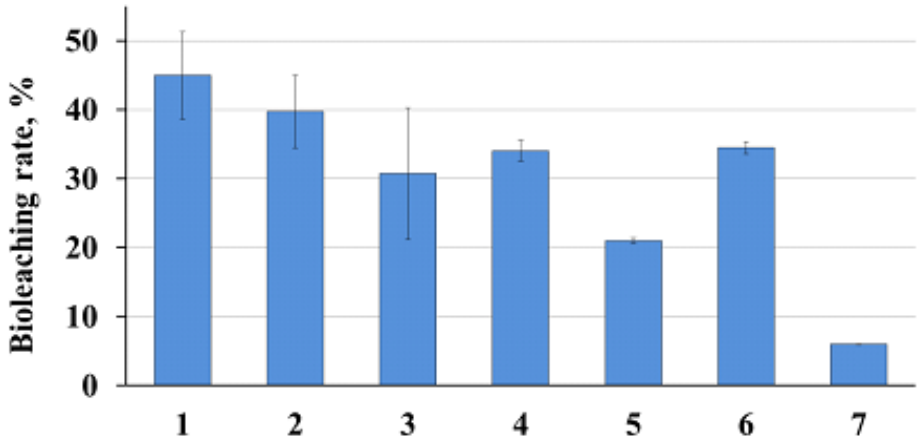

Figure 3: Rate of pyrite leaching by microorganisms under different conditions (%): (1) mixed culture of *A. caldus* MBC-1, *S. thermosulfidooxidans* VKMV 1269^T, and *Acidiplasma* sp. MBA-1, 45°C, initial pH 1.5; (2) pure culture *S. thermosulfidooxidans* VKMV 1269^T, 45°C, initial pH 1.5; (3) pure culture *Acidiplasma* sp. MBA-1, 45°C, initial pH 1.5; (4) pure culture *Acidiplasma* sp. MBA-1, 55°C, initial pH 1.0; (5) pure culture *Acidiplasma* sp. MBA-1, 55°C, initial pH 1.5; (6) pure culture *Acidiplasma* sp. MBA-1, 60°C, initial pH 1.0; (7) pure culture *Acidiplasma* sp. MBA-1, 60°C, initial pH 1.5.

pH 1.5, while decrease in pH to 1.0 made it possible to reach comparatively high rate of pyrite bioleaching.

4. Discussion

The results of the present work demonstrated the representatives of the genus *Acidiplasma* provided comparatively high rate of pyrite bioleaching at high temperatures (55 and 60°C). In the same time, the pH value should be maintained at low level to maintain high oxidizing activity of the strain of the genus *Acidiplasma*. Despite pyrite bioleaching rates reached by the mixed culture *A. caldus* MBC-1, *S. thermosulfidooxidans* VKMV 1269^T, and *Acidiplasma* sp. MBA-1 and pure culture of *S. thermosulfidooxidans* VKMV 1269^T were higher than those observed in the experiments with pure culture of *Acidiplasma* sp. MBA-1 under different conditions, application of the conditions close to the optimum of *Acidiplasma* sp. MBA-1 may provide some advantages. For example, biooxidation at higher temperatures may provide decrease of the costs of temperature maintaining in industrial-scale reactors. According to the results obtained, strain of the genus *Acidiplasma* may provide comparatively high rate of pyrite bioleaching in the case of pH maintaining at low level. Therefore, the results of the present work suggest that archaea of the genus *Acidiplasma*may be promising microorganisms to improve bioleaching processes by means of increase in operational temperature. In the same time, this assumption should be confirmed by the performing

of laboratory-scale trials, which may be planned based on the results obtained in the present work.

5. Conclusions

The results obtained in the present work may be used for planning further laboratory-scale trials to develop approaches allowing to improve biohydrometallurgical technologies by means of change operational conditions.

6. Funding

This work was supported by the Ministry of Science and Higher Education of the Russian Federation.

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