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Carbon Sources as a Factor Determining the Activity of Microbial Oxidation of Sulfide Concentrate at Elevated Temperature

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Abstract: The goal of the present work was to evaluate the possibility of improving the efficiency of the stirred tank reactor biooxidation of sulfide gold-bearing concentrate by means of addition of carbon sources required for the constructive metabolism of microorganisms. Biooxidation experiments were performed on gold-bearing pyrite-arsenopyrite concentrate in continuous mode at 45 °C to determine the influence of additional carbon sources (carbon dioxide and molasses) on sulfide mineral oxidation. The use of CO_2 allowed increasing the efficiency of the biooxidation and the extents of sulfide sulfur (Ss) oxidation and gold recovery were 79% and 84%, respectively. Biooxidation in a control experiment (without additional carbon sources) and when using molasses allowed achieving 39% and 66% oxidation of Ss as well as 73% and 81% of gold recovery. Analysis of the microbial populations formed in biooxidation reactors using NGS methods demonstrated that CO_2 application led to an increase in the relative abundance of the genus *Sulfobacillus*. Thus, it was determined that application of additional carbon source makes it possible to manage the biooxidation process, affecting both sulfide mineral oxidation and microbial population composition.

Keywords: biohydrometallurgy; acidophilic microorganisms; pyrite-arsenopyrite gold-bearing sulfide concentrates; carbon dioxide; molasses

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1. Introduction

Biooxidation in stirred tank reactors (STR biooxidation) has been successfully used at a commercial scale to extract gold and some non-ferrous metals (cobalt, nickel) from sulfide concentrates [1–6]. Biooxidation of sulfide minerals (mainly pyrite and arsenopyrite), which encapsulate fine gold particles, makes it possible to capture gold and enhance gold recovery by further cyanidation [1–4,7–9]. Currently, STR biooxidation may account for up to 5% of the global gold production [2]. The BIOX®, Bacox™, and BIONORD™ processes are examples of commercially used technologies based on STR biooxidation of sulfide gold-bearing concentrates [7–9].

All commercially used STR biooxidation technologies are based on the same microbially-mediated processes and use similar equipment design and operating conditions [3,4,7–9].

In general, STR biooxidation of sulfide concentrates is performed by aerobic acidophilic iron- and sulfur-oxidizing microorganisms, which oxidize sulfide gold-bearing minerals (arsenopyrite, pyrite, pyrrhotite) to obtain energy. In practice, these processes may be carried out in stirred tank reactors connected in series under controlled conditions (oxygen and mineral nutrition supply, pH, and temperature maintenance) that provide stable biooxidation performance [3,4,7–9]. Industrial STR biooxidation processes are usually carried out at temperatures of 40–45 °C as heat is generated during microbial sulfide mineral biooxidation. Temperature of industrial scale reactor pulp is maintained using cooling

system as over-heating may inhibit microbial activity [3,7]. In practice, STR biooxidation is always performed by mixed populations of different acidophilic microorganisms [1–23] (Table 1).

Table 1. Microbial populations formed in different STR biooxidation processes.

Composition	Concentrate Composition (Main Sulfide Minerals)	t, °C	Reference
Acidithiobacilluss caldus, Leptospirillum ferriphilum	Pyrite and arsenopyrite	40	[10,11]
A. caldus, L. ferriphilum, Sulfobacillus sp., *Ferroplasma* sp.	Pyrrhotite, chalcopyrite, sphalerite	45	[12]
A. caldus, Sulfobacillus thermosulfidooxidans, "Sulfobacillus montserratensis"	Pyrite, arsenopyrite, chalcopyrite	45	[13]
A. caldus, L. ferriphilum, F. acidiphilum, S. benefaciens	Pyrite	42	[5]
A. caldus, L. ferriphilum, S. thermosulfidooxidans, S. benefaciens	Chalcopyrite	42	[14]
A. caldus, L. ferriphilum, S. thermosulfidooxidans,	Chalcopyrite	42	[15]
A. caldus, L. ferriphilum, Sulfobacillus sp. TPY, *F. thermophilum*	Chalcopyrite	45	[16]
A. caldus, L. ferriphilum, Sulfobacillus sp., *Ferroplasma* sp., *Acidiplasma* sp.	Pyrite and arsenopyrite	40–50	[17]
A. caldus, A. ferrooxidans, L. ferriphilum, S. thermosulfidooxidans, F. acidiphilum	Pyrite, arsenopyrite and pyrrhotite	35	[18]
A. caldus, Sulfobacillus sp., *Acidiplasma* sp.	Pyrite and arsenopyrite	45	[19]
A. caldus, S. acidophilus, F. thermophilum	Chalcopyrite	45	[20]
A. caldus, L. ferriphilum, S. thermosulfidooxidans, S. benefaciens	Chalcopyrite	42	[21]
A. thiooxidans, Acidiphilium multivorum, Acidiferrobacter thiooxidans, L. ferriphilum, F. acidiphilum	Pyrite, arsenopyrite and pyrrhotite	39–42	[22]
A. caldus, S. benefaciens, F. acidiphilum, Cuniculiplasma divulgatum	Pyrite, chalcopyrite, tennantite, sphalerite	40	[23]

The analysis of microbial populations formed both in industrial scale processes and laboratory scale bioleach reactors modeling conditions of industrial processes has shown a predominance of different thermotolerant and moderately thermophilic acidophilic microorganisms, which may be explained by the maintenance of elevated temperature. In most cases, it was found that bacteria of the genera *Leptospirillum* and *Sulfobacillus*, moderately thermophilic bacteria of the genus *Acidithiobacillus* (*A. caldus*), as well as archaea of the family *Ferroplasmaceae* (genera *Acidiplasma* и *Ferroplasma*) were predominant microorganisms (Table 1). Thus, microbial populations of bioleach reactors usually include microorganisms with different properties:

- microorganisms capable of oxidizing either ferrous iron (Fe^{2+}) or reduced inorganic sulfur compounds (RISC), as well as ones oxidizing both Fe^{2+} and RISC;
- microorganisms with different type of carbon nutrition including autotrophs, which fix dissolved carbon dioxide using the energy obtained by the oxidation of ferrous iron and RISC, and mixo- and heterotrophs, which require organic carbon sources for stable growth, despite the fact that they also gain energy by the oxidation of inorganic compounds [1,24,25].

In this regard, interactions between microorganisms involved in sulfide concentrate biooxidation, which take place due to the differences in physiological properties, are of interest as they make it possible to understand patterns of biooxidation processes and manage their activity. One of the key factors, which may control activity of sulfide mineral biooxidation in both the reactors and natural ecosystems and which are partially determined by microbial interactions in the populations, is carbon supply of acidophilic microorganisms [3,7,12,26,27].

It is known that autotrophic acidophiles, oxidizing ferrous iron and sulfur, produce exometabolites, which are accumulated in the medium and in turn are consumed by

mixo- and heterotrophs as an organic carbon source [28–31]. The activity of autotrophic microorganisms in turn depends on the presence of dissolved CO_2 in the medium, which is supplied with air. Maintaining the concentration of dissolved CO_2 in the medium is important to reach the required rate of the biooxidation as has been shown by industrial practice [3,6,7]. For example, BIOX® process developers recommend either to use the concentrates, which contain at least 2% carbonate to provide sufficient CO_2 to promote growth of microorganisms, or to apply limestone or CO_2 that should be added to the primary reactors as a carbon source [7].

In our previous works, we studied the possibility to increase the rate of biooxidation of different sulfide concentrates using additional carbon sources (CO_2, yeast extract, molasses) [19,32]. Both organic and inorganic carbon sources were shown to enhance the biooxidation rate and affect the microbial population causing an increase in the total number of microorganisms and changes in the composition of the populations. In the same time, the biooxidation activity depended on the use of additional carbon sources to a greater extent at higher temperatures. For example, experiments on biooxidation of pyrite-arsenopyrite concentrate at 40 and 50 °C in batch mode using CO_2 demonstrated that at both temperatures the additional carbon dioxide supply affected the biooxidation rate, while at 50 °C, this had a more significant effect than at 40 °C. Thus, additional carbon supply may also be considered as a method to decrease the negative effect of bioleached reactor pulp over-heating.

Therefore, the goal of the present work was to evaluate the possibility to increase the efficiency of sulfide gold-bearing concentrate biooxidation in stirred tank reactors by means of addition of carbon sources required for the constructive metabolism of microorganisms. The results obtained in the present work were compared with those obtained at lower temperature (45 and 40 °C, respectively) in our previous work [33] to evaluate the effect of additional carbon nutrition on microbial population adaptation to temperature increase. In contrast to our previous work [32], where the possibility of CO_2 and molasses application was studied in batch mode, in the present work, experiments were performed in continuous mode that in turn allowed us to evaluate the effect of different carbon sources under conditions similar to those used in industrial scale processes.

2. Materials and Methods

2.1. Concentrate

The composition of the concentrate is shown in Table 2. The main sulfide minerals of the concentrate were pyrite (28.3%) and arsenopyrite (15.8%). In our previous work [33], we studied biooxidation of similar concentrate obtained from the same ore, which differed slightly in chemical composition from that used in the present article. The concentrate was refractory, since gold recovery by direct cyanidation did not exceed 43%, while biooxidation in continuous mode at 40 °C and residence time of 5 days made it possible to oxidize 79% of sulfide sulfur and to increase the gold recovery by cyanidation up to 92.5–93.5% [33]. In the present work, we have compared the results obtained with those shown in the work [33] as both concentrates were produced from the same ore and therefore were very similar both in chemical and mineral composition.

Table 2. Chemical composition of the concentrate.

Component	Content, %
SiO_2	36.7
Al_2O_3	15.9
CaO	2.9
S_{tot}	18.3
S_s	18.3
Fe_{tot}	20.0
As_{tot}	7.25
Au, g/t	62.35

2.2. Experimental Setup and Biooxidaton

Concentrate biooxidation was carried out in continuous mode in 2.5 L reactors under the following conditions: aeration—5 L/min, stirring rate—500 rpm, temperature—45 °C, the pulp density (solid to liquid ratio, S:L)—1.5:10 (150 g of the concentrate per 1000 mL of liquid medium), the residence time—6 days. Concentrate biooxidation was performed in a single-stage reactor. Temperature in the reactors was maintained using ELMI TW-2.03 circulating water baths (Elmi, Riga, Latvia) and U-shaped titanium heat exchangers; the stirring was performed using RW20 overhead stirrers (IKA, Staufen, Germany).

We used liquid mineral nutrient medium, which was previously used in sulfide concentrate biooxidation experiments [32,33], containing the following components (g/L): $(NH_4)_2SO_4$—0.75, KCl—0.05, $MgSO_4 \times 7H_2O$—0.125, K_2HPO_4—0.125, distilled water—1.0 L. The pH was adjusted by adding either concentrated sulfuric acid or $CaCO_3$ to the medium.

The effect of additional carbon sources (carbon dioxide and molasses) on the biooxidation was studied. A control experiment was performed without additional carbon sources and CO_2 supplied with air was the sole carbon source for microbial population of the reactor. The control experiment was performed in two reactors in parallel. After completing the control experiment, experiments with additional carbon sources were started in the same reactors. CO_2 was fed into the pulp of the first reactor (approximately 0.01 L/min that was about five times greater than amount of carbon dioxide supplied with air in the control) and molasses (final concentration in the pulp was of 0.02 % wt/vol by dry weight) was added to the pulp of the second reactor. The molasses (KDF, Moscow, Russia) was used in the form of 20% (wt/vol by dry weight) solution, which was prepared using sterile autoclaved distilled water and then sterilized using 0.22 μm membrane filter (Merck, Darmstadt, German y). The solution was stored at 4 °C. The molasses contained about 90% carbohydrates (mainly sucrose) and 35% carbon (by dry weight).

The amounts of supplied carbon dioxide and molasses were based on the results of previous works. In the article [12], CO_2 enriched air (0.2% CO_2) was successfully used in bioleaching experiments. In the present work, the amount of carbon dioxide supplied in the reactor corresponded to that used in the works (i.e., 0.2% CO_2 in air) [12,32]. In our previous works, it was also demonstrated that 0.02% molasses in the medium was appropriate for the cultivation several mixo- and heterotrophic iron-oxidizing strains [34] as well as may be used to increase sulfide concentrate biooxidation extent [32].

2.3. Sampling and Analysis

To evaluate the activity of the biooxidation, the parameters of the liquid phase of the pulp were analyzed. The pH and redox potential (Eh) were determined using pH-150MI pH meter (Izmeritelnaya Tekhnika, Moscow, Russia), ferrous and ferric iron and arsenic concentration were measured by trilonometric and iodometric titration, respectively [35,36].

The solid residues of biooxidation were separated from the liquid phase, dried, and analyzed to determine the extent of sulfide sulfur oxidation. As biooxidation process was performed in continuous mode, pulp samples to obtain solid residues were collected over several days when steady state conditions were reached. Averaged sample of the solid residue obtained in each experiment was used for the further analysis.

Determination of the content of iron, arsenic, and sulfur was carried out using phase analysis methods [37].

2.4. Cyanidation Test

The oxidized concentrate was treated by cyanidation for gold extraction. Cyanidation was performed under the following conditions: pulp density was 20% (w/v), sodium cyanide concentration was 2.0 g/L, process duration was 48 h, pH was 10.5–11.0, stirring rate was 250 rpm.

2.5. Microbial Population Analysis

Microbial population formed during continuous biooxidation of sulfide concentrate at 40 °C [33] was used in the present work. After experiments performed at 40 °C, the temperature in the reactors was increased and microbial population was adapted to elevated temperature (45 °C).

To evaluate the changes in the composition of microbial population under different conditions (temperature, carbon sources), it was analyzed using metabarcoding of V3–V4 16S rRNA gene fragments. The samples of the biomass were collected in the previous study (biooxidation at 40 °C) [33], at the end of the control experiment at 45 °C, as well as at the end of the experiments at 45 °C with additional carbon sources. For the analysis, pulp samples (50 mL) were collected from biooxidation reactors. To collect the biomass from the pulp sample, the solid phase was first separated by centrifugation at 1000 rpm (103 g) using an Allegra X-22 centrifuge (Beckman Coulter, Brea, CA, USA). Biomass was precipitated from the supernatant by centrifugation at 9500 rpm (9299 g). Biomass preparation, DNA isolation, library preparation based on the V3–V4 region of the 16S rRNA gene, amplicon preparation, sequencing using MiSeq system (Illumina, San Diego, CA, USA) were performed as described previously [22,38]. QIIME (version 1.9.1) software and Silva132 database [39,40] were used for data processing to perform the taxon-based assignment of operational taxonomic units (OTUs). In average, 10,000 fragments for each sample were analyzed.

3. Results

3.1. Biooxidtion

Figure 1 shows changes of pH and Eh values, as well as of the iron ion concentrations in the control experiments and after additional carbon supplementation. The curves demonstrate that both carbon dioxide and molasses influenced these parameters, which may be used for indirect evaluation of biooxidation activity. Average pH levels decreased (Figure 1a), while Eh values increased (Figure 1b). Total iron ion concentrations began to increase immediately after the start of additional carbon supplementation (Figure 1c). At the same time, the ferrous iron concentration sharply decreased from several grams per liter to the residual levels that indicated the increase in iron biooxidation activity (Figure 1d).

In Table 3, averaged values of the liquid phase parameters obtained in continuous experiments under different conditions are shown. The comparison of the data obtained in the present work (45 °C) with those from the previous work (40 °C) [33] demonstrated that at 40 °C liquid phase parameters corresponded to higher biooxidation activity, while the increase in the temperature up to 45 °C led to the increase in the pH and ferrous iron concentration. The Eh value as well as ferric iron and arsenic concentrations decreased after temperature increase. Despite at 40 °C no sulfuric acid addition was required to adjust the pH level, average pH level was lower than that in the control experiment at 45 °C in which sulfuric consumption was 90 kg/t of the concentrate. Additional carbon supplementation led to the change of liquid phase parameters. No sulfuric acid addition was required to maintain pH level, while average pH values were lower in both variants (with CO_2 and molasses addition).

It should be noted that CO_2 supplementation affected the liquid phase parameters of biooxidation pulp in a greater extent than molasses addition. Thus, the average pH level was lower when using CO_2 than that when using molasses. In comparison to the control, average iron and arsenic concentrations were 2 and 1.1 times and 5.2 and 1.9 times higher when using molasses and CO_2, respectively.

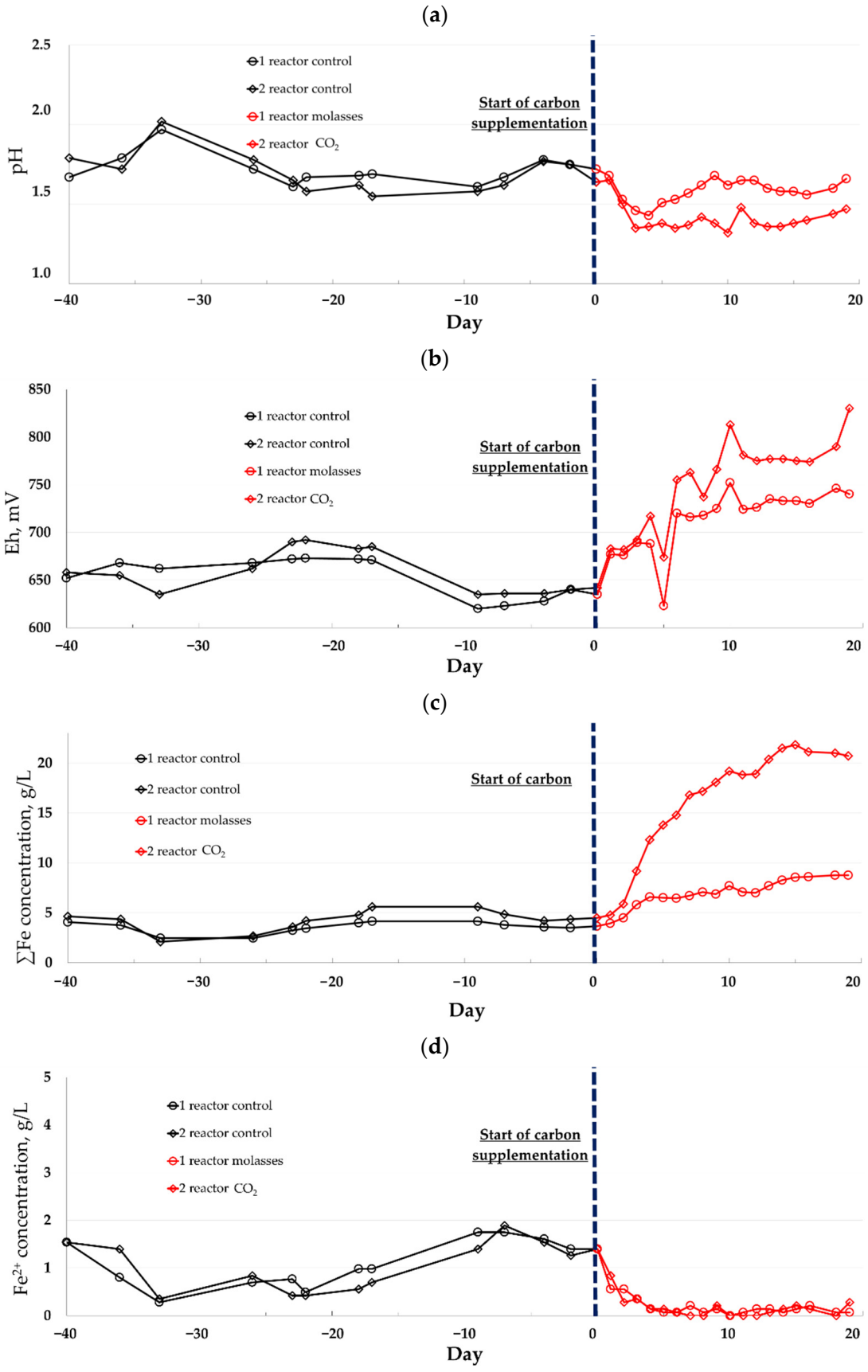

Figure 1. Changes in the pH (**a**) and Eh (**b**) values, total concentrations of Fe^{3+} and Fe^{2+} (**c**) and Fe^{2+} (**d**) ions (g/L) after the start of carbon supplementation.

Table 3. Parameters of the liquid phase of the pulp at the end of the experiment (steady state conditions).

Experiment		pH	Eh, mV	Concentration, g/L			H_2SO_4 Consumption, kg/t
Carbon Source	T, °C			Fe^{3+}	Fe_{total}	As	
Air (control) *	40	1.43 ± 0.02	799 ± 16	16.2 ± 0.8	16.2 ± 0.8	6.7 ± 0.2	0
Air (control) **	45	1.71 ± 0.1	656 ± 22	2.8 ± 0.7	3.9 ± 0.8	2.3 ± 0.2	90
Molasses **		1.61 ± 0.04	735 ± 9	7.9 ± 0.7	8.0 ± 0.7	2.6 ± 0.05	0
CO_2 **		1.40 ± 0.05	788 ± 20	20.3 ± 1.1	20.4 ± 1.1	4.5 ± 0.3	0

* [33], ** This study.

Solid residue analysis, which allows evaluating results of biooxidation experiments directly, corresponded to the analysis of liquid phase parameters. The highest sulfide sulfur (Ss) oxidation extent was reached in the experiment with CO_2 supplementation in which liquid phase parameters also suggested the highest biooxidation activity (Table 4). In turn, in control experiment at 45 °C, Ss oxidation extent was lower than that reached in the experiment at 40 °C as well as in the experiments with CO_2 and molasses.

Table 4. Results of biooxidation (mass yields and element content in the residues, sulfide sulfur oxidation extent).

Experiment		Mass Yield, %	Content, %			Ss oxidation, %
Carbon Source	T, °C		Fe	As	Ss	
Aeration (control) *	40	66.5	9.6	3.1	6.3	78.9
Aeration (control) **	45	77	17.4	5.2	14.2	39.1
Molasses **		73	14.6	4.6	8.5	66.0
CO_2 **		60	10.6	3.1	6.4	79.0

* [33], ** This study.

Thus, solid residue analysis supported suggestion that both additional carbon sources affected biooxidation and CO_2 influenced sulfide sulfur oxidation to a greater extent in comparison to molasses.

3.2. Cyanidation Tests

Cyanidation tests were used to evaluate the effect of additional carbon sources on gold extraction that in turn made it possible to compare efficiency of different carbon sources application as a method to increase the efficiency of sulfide concentrate treatment using biooxidation. Gold extraction from biooxidation residues was higher than from the concentrate (Figure 2). At the same time, it was shown that despite the difference between sulfide sulfur oxidation extents in the experiments with CO_2 and molasses (13%), difference between gold extraction extents in these experiments was 3.5% (Figure 3).

3.3. Microbial Population Analysis

Analysis of the microbial population showed that changes in the conditions of the biooxidation led to the change of the relative abundance of microbial genera in the populations (Figure 4). Composition of microbial population coincided to known properties of microorganisms (Figure 4 and Table 5).

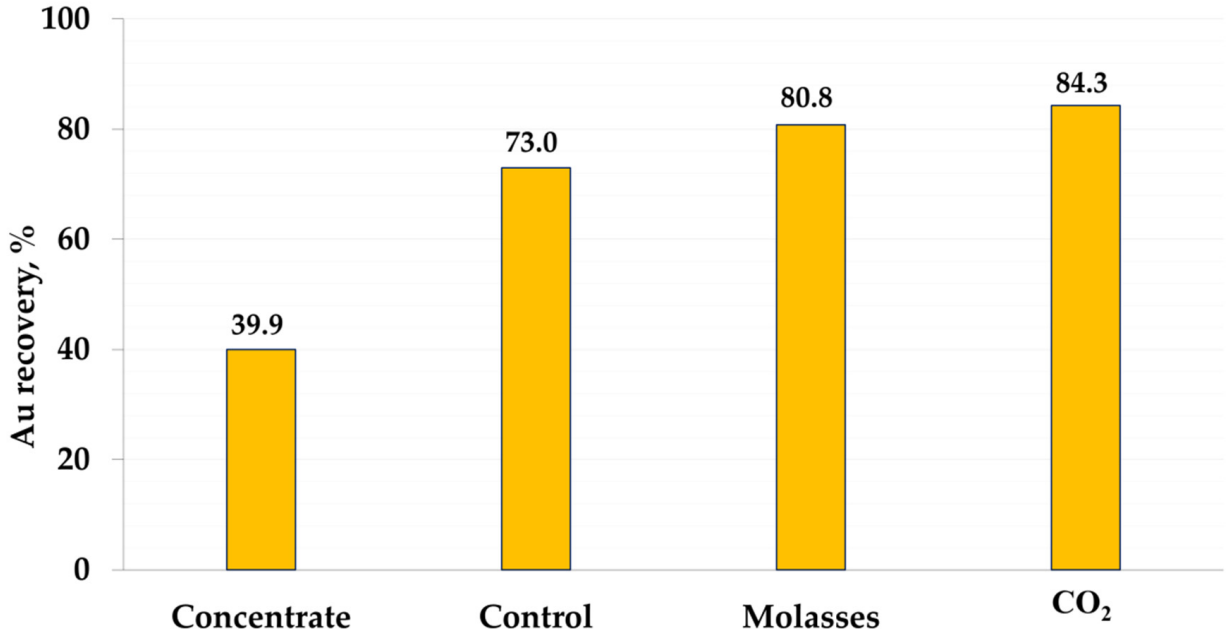

Figure 2. Gold recovery by cyanidation (%) from the concentrate as well as from the oxidation residues.

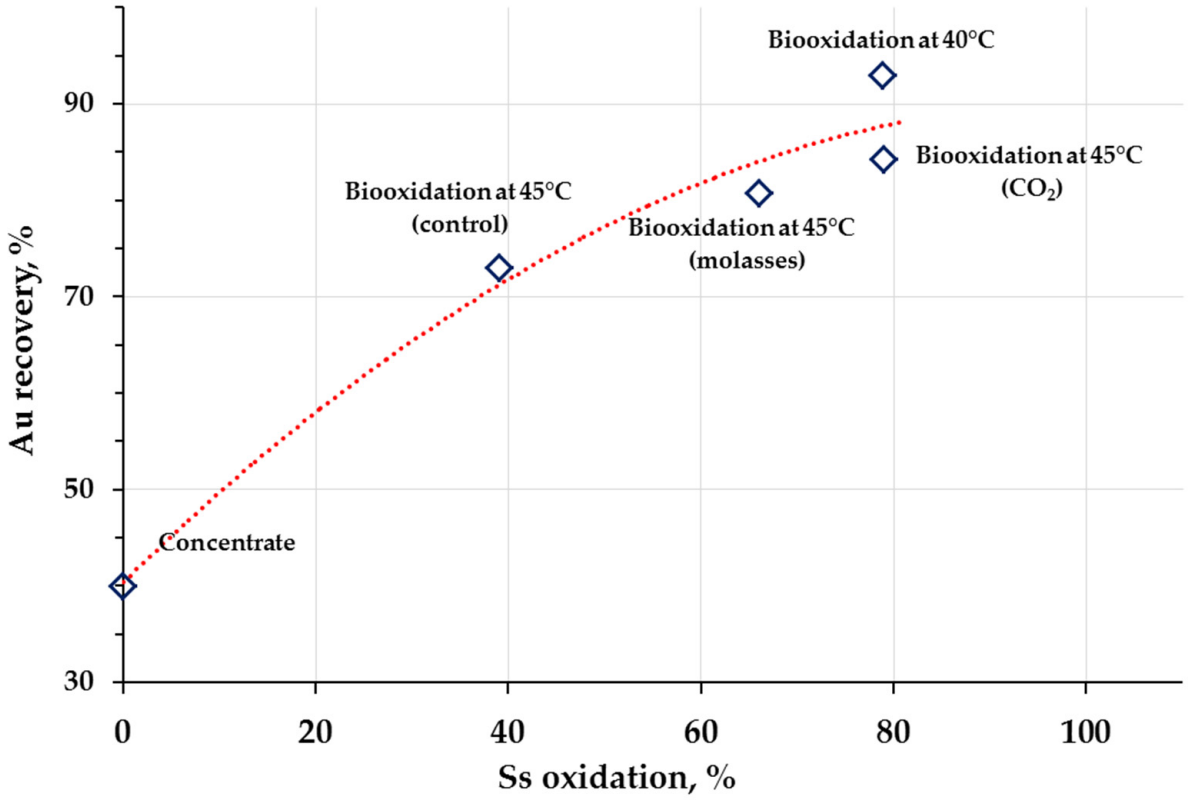

Figure 3. The dependence of gold recovery by cyanidation (%) on Ss oxidation extent.

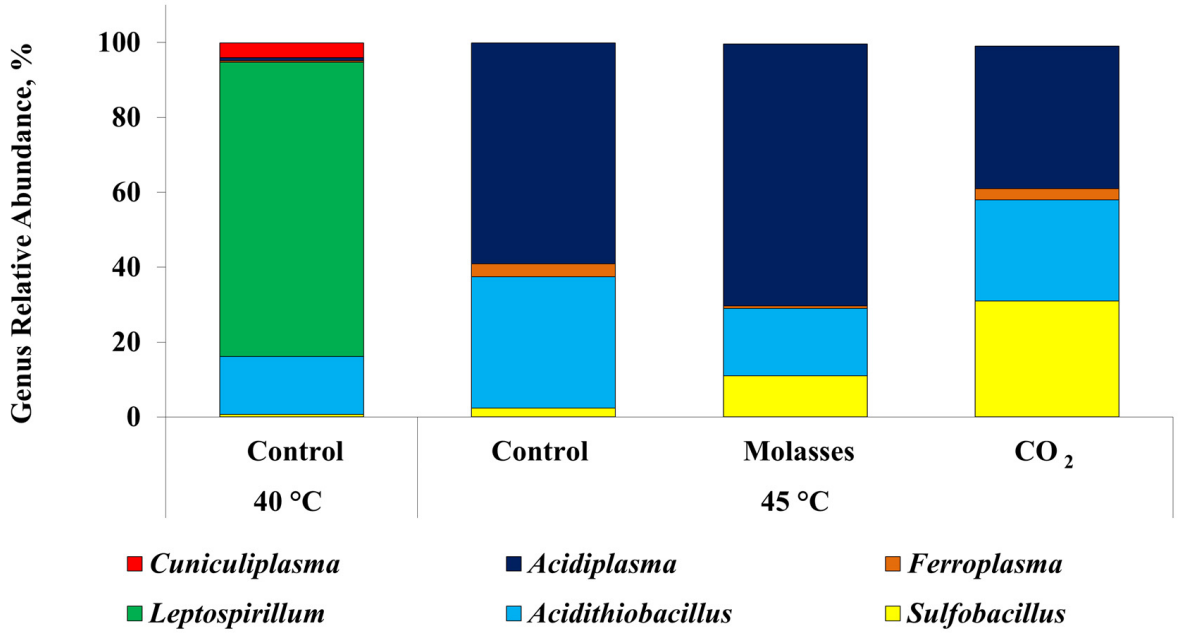

Figure 4. Results of molecular biological analysis of microbial populations–genus relative abundance (%).

Table 5. Physiological properties of microorganisms dominating in microbial communities of reactors for biooxidation of sulfide concentrates [1,19,25,40,41].

Microorganisms	Electron Donor	Temperature, °C (Optimum/Upper Limit)	Carbon Nutrition
Bacteria of the genus *Leptospirillum*	Fe^{2+}	28–50/45–60	Autotroph
Acidithiobacillus caldus	S^0	45/52	Autotroph
Bacteria of the genus *Sulfobacillus*	Fe^{2+}, S^0	38–55/55–60	Mixotroph
Archea of the genus *Ferroplasma*	Fe^{2+}	35–42/45–51	Mixotrophs, heterotrophs
Archea of the genus *Acidiplasma*	Fe^{2+}, S^0	45–54/60–65	Mixotrophs, heterotrophs
Archea of the genus *Cuniculiplasma*	Organic compounds	37–40/45–48	Heterotrophs

At 40 °C, bacteria of the genus *Leptospirillum* and *Acidithiobacillus* (closely related to *A. caldus*) were predominant. The increase in the temperature up to 45 °C, led to the disappearance of *Leptospirillum* sequences, which may be explained by the fact that most known studied strains of the genus *Leptospirillum* are mesophilic microorganisms, and their growth rate at temperatures exceeding 45 °C is usually significantly lower than that at 40 °C [11].

At the same time, the relative abundance of the genus *Acidithiobacillus* was high in all experiments at 45 °C, since the strains of *A. caldus* are active at 45 °C. In control experiments at 45 °C, sulfur-oxidizer *A. caldus* and moderately thermophilic archaea of the genus *Acidiplasma* were the most abundant, while archaea of the genus *Ferroplasma* and bacteria of the genus *Sulfobacillus* were also detected, but their relative abundance was comparatively low (Figure 4). Molasses addition led to the increase in the relative abundance of *Acidiplasma* and *Sulfobacillus*, which may be explained by the fact that representatives of these genera are able to consume organic compounds as carbon source (Table 5). Carbon dioxide supplementation led to the increase in the abundance of *Sulfobacillus* genus. Representatives of this genus are able to consume both carbon dioxide and organic compounds as carbon sources (Table 5). Therefore, application of both carbon sources resulted in the increase in *Sulfobacillus* relative abundance. At the same time, CO_2 supplementation increased *Sulfobacillus* abundance to a greater extent in comparison to the use of molasses.

Thus, it was revealed that application of additional carbon sources affecting the efficiency of the concentrate biooxidation also resulted in the change of microbial population composition.

4. Discussion

The results of the present work demonstrated possible approaches to cope with the negative effect of temperature rise on biooxidation of gold-bearing concentrate in laboratory scale stirred tank reactors. It was shown that temperature increase from 40 °C [33] to 45 °C led to the decrease in sulfide mineral biooxidation extent and gold recovery. Therefore, at 45 °C, experiments were performed to determine the possibility to increase the biooxidation efficiency using additional carbon sources (carbon dioxide and molasses) and decrease the inhibitory effect of the temperature rise.

It was shown that the use of both inorganic (carbon dioxide) and organic (molasses) additional carbon sources allowed to decrease inhibition of biooxidation due to the temperature rise and increase both sulfide sulfur oxidation extent and gold recovery by cyanidation. Therefore, the use of additional carbon nutrition may solve one of a relevant problem of industrial scale biooxidation processes, inhibition of biooxidation activity due to the overheating of bioleach reactor pulp.

The effects, which were revealed in the present work, may be explained by the influence of additional carbon sources on the composition of microbial population. The temperature increase from 40 to 45 °C led to the elimination of one of predominating microorganisms, the iron-oxidizer *Leptospirillum*, which plays a key role in iron and pyrite biooxidation. The increase in the temperature led to the change of microbial population composition and resulted in a predominance of mixo- and heterotrophic iron-oxidizing microorganisms (*Sulfobacillus* and *Acidiplasma*). These microorganisms require additional carbon sources (organic compounds and/or an atmosphere with enhanced CO_2 concentration) for cultivation (Table 5) [1,19,25,41,42]. In the experiments with CO_2 and molasses, the relative abundances of *Sulfobacillus* and *Acidiplasma* increased, respectively. As the highest mineral oxidation extent and significant increase in the relative abundance of *Sulfobacillus* was observed in the experiment with CO_2, it is suggested that additional carbon dioxide was the carbon source providing a competitive advantage for the mixotrophic bacteria *Sulfobacillus*, which in turn may play a key role in sulfide concentrate oxidation.

In our previous work, it was shown that *Sulfobacillus* representatives may be key pyrite oxidizers [43], but their activity depends on the carbon nutrition in the form of yeast extract added to the medium and/or exometabolites produced by autotrophic acidophiles (*A. caldus*). At the same time, in our study on the effect of carbon dioxide and molasses on the biooxidation of sulfide concentrate at 40 and 50 °C, we demonstrated that both CO_2 and molasses enhanced the activity of biooxidation and additional carbon sources affected the biooxidation rate in a greater extent at higher temperature [32]. At 50 °C, supplementation of carbon dioxide also led to an increase in abundance of *Sulfobacillus*.

Thus, summarizing the results of the present study and our previous work [32,43], we can propose that CO_2 supplementation leads to the prevalence of microorganisms playing a key role in sulfide mineral oxidation in the populations of bioreactors and therefore to the observed increase in biooxidation rate. As was shown, this may be critically important for the stabilization of biooxidation activity when increasing in temperature, which may be explained by the decrease in CO_2 solubility with temperature increase, which in turn led to the deficiency in dissolved CO_2 at 45 °C in the absence of additional carbon sources.

It should also be noted that despite the fact CO_2 made it possible to achieve a higher biooxidation efficiency, gold recovery values were similar in the experiments with molasses and carbon dioxide. Thus, it was shown that molasses application might provide comparable results with carbon dioxide in terms of gold recovery. As molasses are often considered as cheap food industry waste, their consumption in bioleaching processes may be relatively low, and its supply does not require modification of aeration and stirring system in the

reactors to perform effective mass transfer as in the case of carbon dioxide application, molasses usage in some cases may have certain advantages.

In this regard, the results obtained provide strong evidence in support of the hypothesis that additional carbon nutrition can significantly affect the biooxidation efficiency and decrease the negative effect of temperature rises. Thus, based on the results of the present work, further continuous laboratory and pilot tests may be designed to confirm the patterns revealed in the present and previous experimental works.

5. Conclusions

The results of the present work suggest that application of carbon sources required for the constructive metabolism of microorganisms may be used to manage the efficiency of stirred tank reactor biooxidation of sulfide pyrite-arsenopyrite gold-bearing concentratea. The influence of additional carbon sources (carbon dioxide and molasses) on the biooxidation of sulfide sulfur was determined. Carbon dioxide supplementation allowed increasing the efficiency of the biooxidation, while the extents of sulfide sulfur oxidation and gold recovery were 79% and 84%, respectively. Biooxidation without additional carbon sources and using molasses allowed achieving 39% and 66% of Ss oxidation as well as 73% and 81% of gold recovery. Analysis of the microbial populations of the biooxidation reactors demonstrated that the use of CO_2 led to an increase in the abundance of *Sulfobacillus* bacteria in the microbial population.

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References

1. Johnson, D.B. Biomining—Biotechnologies for extracting and recovering metals from ores and waste materials. *Curr. Opin. Biotechnol.* **2014**, *30*, 24–31. [CrossRef]
2. Johnson, D.B. The Evolution, Current Status, and Future Prospects of Using Biotechnologies in the Mineral Extraction and Metal Recovery Sectors. *Minerals* **2018**, *8*, 343. [CrossRef]
3. Mahmoud, A.; Cezac, P.; Hoadley, A.F.A.; Contaminea, F.; D'Hugues, P. A review of sulfide minerals microbially assisted leaching in stirredtank reactors. *Int. Biodeterior. Biodegrad.* **2017**, *119*, 118–146. [CrossRef]
4. Kaksonen, A.H.; Boxall, N.J.; Gumulya, Y.; Khaleque, H.N.; Morris, C.; Bohu, T.; Cheng, K.Y.; Usher, K.M.; Lakaniemi, A.-M. Recent progress in biohydrometallurgy and microbial characterization. *Hydrometallurgy* **2018**, *180*, 7–25. [CrossRef]
5. Morin, D.H.R.; d'Hugues, P. Bioleaching of a cobalt containing pyrite in stirred reactors: A case study from laboratory scale to industrial application. In *Biomining*; Rawlings, D.E., Johnson, B.D., Eds.; Springer: Berlin, Germany, 2007; pp. 35–55. [CrossRef]
6. Neale, J.; Seppala, J.; Laukka, A.; van Aswegen, P.; Barnett, S.; Gericke, M. The MONDO minerals nickel sulfide bioleach project: From test work to early plant operation. *Solid State Phenom.* **2017**, *262*, 28–32. [CrossRef]
7. van Aswegen, P.C.; van Niekerk, J.; Olivier, W. The BIOX process for the treatment of refractory gold concentrate. In *Biomining*; Rawlings, D.E., Johnson, B.D., Eds.; Springer: Berlin, Germany, 2007; pp. 1–35. [CrossRef]
8. Gericke, M.; Neale, J.W.; van Staden, P.J. A Mintek perspective of the past 25 years in minerals bioleaching. *J. S. Afr. Inst. Min. Metall.* **2017**, *109*, 567–585.
9. Belyi, A.V.; Chernov, D.V.; Solopova, N.V. Development of BIONORD® technology on Olimpiada deposit refractory arsenic–gold ores treatment in conditions of Extreme North. *Hydrometallurgy* **2018**, *179*, 188–191. [CrossRef]
10. Rawlings, D.E.; Coram, N.J.; Gardner, M.N.; Deane, S.M. *Thiobacillus caldus* and *Leptospirillum ferrooxidans* are widely distributed in continuous flow biooxidation tanks used to treat a variety of metal containing ores and concentrates. In *Biohydrometallurgy and the Environment: Toward the Mining of the 21st Century. Part A*; Amils, R., Ballester, A., Eds.; Elsevier: Amsterdam, The Netherlands, 1999; pp. 777–786. [CrossRef]
11. Coram, N.J.; Rawlings, D.E. Molecular relationship between two groups of the genus *Leptospirillum* and the finding that *Leptospirillum ferriphilum* sp. nov. dominates South African commercial biooxidation tanks that operate at 40 °C. *Appl. Environ. Microbiol.* **2002**, *68*, 838–845. [CrossRef] [PubMed]

12. Okibe, N.; Gericke, M.; Hallberg, K.B.; Johnson, D.B. Enumeration and characterization of acidophilic microorganisms isolated from a pilot plant stirred tank bioleaching operation. *Appl. Environ. Microbiol.* **2003**, *69*, 1936–1943. [CrossRef] [PubMed]
13. Dopson, M.; Lindstrom, E.B. Analysis of community composition during moderately thermophilic bioleaching of pyrite, arsenical pyrite, and chalcopyrite. *Microb. Ecol.* **2004**, *48*, 19–28. [CrossRef]
14. Spolaore, P.; Joulian, C.; Gouin, J.; Ibáñez, A.; Auge, T.; Morin, D.; d' Hugues, P. Bioleaching of an organic–rich polymetallic concentrate using stirred–tank technology. *Hydrometallurgy* **2009**, *99*, 137–143. [CrossRef]
15. Spolaore, P.; Joulian, C.; Gouin, J.; Morin, D.; d' Hugues, P. Relationship between bioleaching performance, bacterial community structure and mineralogy in the bioleaching of a copper concentrate in stirred–tank reactors. *Appl. Microbiol. Biotechnol.* **2010**, *89*, 441–448. [CrossRef] [PubMed]
16. Wang, Y.; Su, L.; Zhang, L.; Zeng, W.; Wu, J.; Wan, L.; Qiu, G.; Chen, X.; Zhou, H. Bioleaching of chalcopyrite by defined mixed moderately thermophilic consortium including a marine acidophilic halotolerant bacterium. *Bioresour. Technol.* **2012**, *121*, 348–354. [CrossRef]
17. van Hille, R.P.; van Wyk, N.; Froneman, T.; Harrison, S.T.L. Dynamic evolution of the microbial community in BIOX leaching tanks. *Adv. Mater. Res.* **2013**, *825*, 331–334. [CrossRef]
18. Kondrat'eva, T.F.; Pivovarova, T.A.; Bulaev, A.G.; Moshchanetskii, P.V.; Tsaplina, I.A.; Grigor'eva, N.V.; Zhuravleva, A.E.; Melamud, V.S.; Belyi, A.V. Selection of a community of acidochemolithotrophic microorganisms with a high oxidation rate of pyrrhotite–containing sulphide ore flotation concentrate. *Appl. Biochem. Microbiol.* **2013**, *49*, 495–501. [CrossRef]
19. Muravyov, M.I.; Bulaev, A.G. Two–step oxidation of a refractory gold–bearing sulfidic concentrate and the effect of organic nutrients on its biooxidation. *Miner. Eng.* **2013**, *45*, 108–114. [CrossRef]
20. Wang, Y.; Zeng, W.; Qiu, G.; Chen, X.; Zhou, H. A moderately thermophilic mixed microbial culture for bioleaching of chalcopyrite concentrate at high pulp density. *Appl. Environ. Microbiol.* **2014**, *80*, 741–750. [CrossRef]
21. Hedrich, S.; Guézennec, A.-G.; Charron, M.; Schippers, A.; Joulian, C. Quantitative monitoring of microbial species during bioleaching of a copper concentrate. *Front. Microbiol.* **2016**, *7*, 20441. [CrossRef] [PubMed]
22. Bulaev, A.; Belyi, A.; Panyushkina, A.; Solopova, N.; Pivovarova, T. Microbial population of industrial biooxidation reactors. *Solid State Phenom. Submitt.* **2017**, *262*, 48–52. [CrossRef]
23. Bulaev, A.; Melamud, V.; Boduen, A. Bioleaching of non-ferrous metals from arsenic-bearing sulfide concentrate. *Solid State Phenom.* **2020**, *299*, 1064–1068. [CrossRef]
24. Karavaiko, G.I.; Dubinina, G.A.; Kondrateva, T.F. Lithotrophic microorganisms of the oxidative cycles of sulfur and iron. *Microbiology (Mikrobiologiya)* **2006**, *75*, 512–545. [CrossRef]
25. Schippers, A. Microorganisms involved in bioleaching and nucleic acid-based molecular methods for their identification and quantification. In *Microbial Processing of Metal Sulfides*; Donati, E.R., Sand, W., Eds.; Springer: Dordrecht, The Netherlands, 2007; pp. 3–33. [CrossRef]
26. Gonzalez-Tori, E.; Llobet-Brossa, E.; Casamayor, E.O.; Amann, R.; Amils, R. Microbial ecology of an extreme acidic environment, the Tinto River. *Appl. Environ. Microbiol.* **2003**, *69*, 4853–4865. [CrossRef] [PubMed]
27. Druschel, G.K.; Baker, B.J.; Gihring, T.H.; Banfield, J.F. Acid mine drainage biogeochemistry at Iron Mountain. *Calif. Geochem. Trans.* **2004**, *5*, 13–32. [CrossRef]
28. Schnaitman, C.; Lundgren, D.G. Organic compounds in the spent medium of *Ferrobacillus ferrooxidans*. *Can. J. Microbiol.* **1965**, *11*, 23–27. [CrossRef] [PubMed]
29. Borichewski, R.M. Keto acids as growth-limiting factors in autotrophic growth of *Thiobacillus thiooxidans*. *J. Bacteriol.* **1967**, *93*, 597–599. [CrossRef]
30. Okibe, N.; Johnson, D.B. Biooxidation of pyrite by defined mixed cultures of moderately thermophilic acidophiles in pH-controlled bioreactors: Significance of microbial interactions. *Biotechnol. Bioeng.* **2004**, *87*, 574–583. [CrossRef]
31. Nancucheo, I.; Johnson, D.B. Production of glycolic acid by chemolithotrophic iron- and sulfur-oxidizing bacteria and its role in delineating and sustaining acidophilic sulfide mineral-oxidizing consortia. *Appl. Environ. Microbiol.* **2010**, *76*, 461–467. [CrossRef]
32. Bulaev, A.; Nechaeva, A.; Elkina, Y.; Melamud, V. Effect of carbon sources on pyrite-arsenopyrite concentrate bio-oxidation and growth of microbial population in stirred tank reactors. *Microorganisms* **2021**, *9*, 2350. [CrossRef]
33. Bulaev, A.G.; Boduen, A.Y.; Ukraintsev, I.V. Biooxidation of persistent gold-bearing ore concentrate of the Bestobe deposit. *Obogashchenie Rud* **2019**, *6*, 9–14. [CrossRef]
34. Bulaev, A.G.; Erofeeva, T.V.; Vorobeva, K.S.; Chelidze, G.G.; Ramonova, A.A. Effect of Organic Nutrients on the Activity of Archaea of the *Ferroplasmaceae* Family. *Moscow Univ. Biol. Sci. Bull.* **2018**, *73*, 146–152. [CrossRef]
35. Reznikov, A.A.; Mulikovskaya, E.P.; Sokolov, I.Y. *Metody Analiza Prirodnykh Vod (Methods for Analysis of Natural Waters)*; Nedra: Moscow, Russia, 1970; 140p. (In Russian)
36. Surovskaya, I.A.; Titov, V.I.; Brodskaya, V.M.; Vasil'ev, P.I.; Lipshits, B.M.; Elentukh, B.M. *Tekhnicheskii Analiz Tsvetnoi Metallurgii (Technical Analysis in Nonferrous Metallurgy)*; Metallurgizdat: Moscow, Russia, 1957; 567p. (In Russian)
37. Filippova, N.A. *Fazovyi Analiz Rud I Produktov Ikh Pererabotki (Phase Analysis of Ores and Products of Their Processing)*; Khimiya: Moscow, Russia, 1975; 280p. (In Russian)
38. Pimenov, N.V.; Merkel, A.Y.; Samylina, O.S.; Kanapatskii, T.A.; Tikhonova, E.N.; Vlasova, M.A.; Tarnovetskii, I.Y.; Malakhova, T.V. Structure of Microbial Mats in the Mramornaya Bay (Crimea) Coastal Areas. *Microbiology (Mikrobiologiya)* **2018**, *87*, 681–691. [CrossRef]

39. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Pẽa, A.G.; Goodrich, J.K.; Gordon, J.I.; et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335. [CrossRef]
40. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckneret, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **2012**, *41*, D590–D596. [CrossRef] [PubMed]
41. Golyshina, O.V.; Yakimov, M.M.; Lünsdorf, H.; Ferrer, M.; Nimtz, M.; Timmis, K.N.; Wray, V.; Tindall, B.J.; Golyshin, P.N. *Acidiplasma aeolicum* gen. nov., sp. nov., a euryarchaeon of the family *Ferroplasmaceae* isolated from a hydrothermal pool, and transfer of *Ferroplasma cupricumulans* to *Acidiplasma cupricumulans* comb. nov. *Int. J. Syst. Evol. Microbiol.* **2009**, *59*, 2815–2824. [CrossRef]
42. Golyshina, O.V.; Lünsdorf, H.; Kublanov, I.V.; Goldenstein, N.I.; Hinrichs, K.U.; Golyshin, P.N. The novel extremely acidophilic, cell-wall-deficient archaeon *Cuniculiplasma divulgatum* gen. nov., sp. nov. represents a new family, *Cuniculiplasmataceae* fam. nov., of the order. *Int. J. Syst. Evol. Microbiol.* **2016**, *66*, 332–340. [CrossRef]
43. Bulaev, A.G. Effect of organic carbon source on pyrite biooxidation by moderately thermophilic acidophilic microorganisms. *Microbiology* **2020**, *89*, 301–308. [CrossRef]