

Article

# Bioleaching for the Removal of Arsenic from Mine Tailings by Psychrotolerant and Mesophilic Microbes at Markedly Continental Climate Temperatures

Kuanysh N. Seitkamal <sup>1,2,\*</sup>, Nariman K. Zhappar <sup>1,2</sup>, Valentin M. Shaikhutdinov <sup>1</sup>, Aigerim K. Shibayeva <sup>1,\*</sup>, Sadia Ilyas <sup>3</sup>, Ilya V. Korolkov <sup>2,4</sup> and Hyunjung Kim <sup>3</sup>

- <sup>1</sup> LLP "Scientific-Analytical Center "Biomedpreparat", Stepnogorsk, 3 microdistrict-9, Stepnogorsk, Akmola Region 021500, Kazakhstan; nariman\_zhappar@mail.ru (N.K.Z.); 07valek@mail.ru (V.M.S.)
- <sup>2</sup> L.N. Gumilyov Eurasian National University, Satpaev str. 5, Nur-Sultan 010008, Kazakhstan; korolkovelf@mail.ru
- <sup>3</sup> Department of Mineral Resources and Energy Engineering, Jeonbuk National University, 567, Baekje-daero, Deokjin-gu, Jeonbuk 54896, Korea; ilyas.sadia24@gmail.com (S.I.); kshjkim@jbnu.ac.kr (H.K.)
- <sup>4</sup> The Institute of Nuclear Physics, Ibragimov str., 1, Almaty 050032, Kazakhstan
- \* Correspondence: mengoibyti@gmail.com (K.N.S.); ai\_gerim86@mail.ru (A.K.S.)

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**Abstract:** This study investigated the biological removal of heavy metals from mine tailings in Kazakhstan using acidophilic microorganism strains *Acidithiobacillus ferrivorans* 535 and *Acidithiobacillus ferrivorans* 377. The experiments were conducted in shake flasks at pH 1.6, various temperatures (28 °C, 18 °C, and 8 °C), and 10% solid concentration (*w*/*v*). The results of inductively coupled plasma optical emission spectroscopy and X-ray diffraction analyses showed that arsenic was particularly efficiently removed at 28 °C. At this temperature, *A. ferrooxidans* 377 was more efficient at removal than the other strain. Meanwhile, *A. ferrivorans* 535 was more efficient than *A. ferrooxidans* 377 at 8 °C. One of the more significant findings to emerge from this study is that arsenic can be removed at a low temperature and high solid concentration. The results of this study support the idea that microorganisms can be used for removing arsenic via a combination of biooxidation and chemical methods.

**Keywords:** *Acidithiobacillus ferrivorans; Acidithiobacillus ferrooxidans;* arsenic removal; bioleaching; mine tailings

# 1. Introduction

The control of hazardous waste, such as arsenic, is a major issue globally. Arsenic (As) is a hazardous waste product generated from the processing of various ores, such as copper, gold, nickel, lead, and zinc [1]. It is a ubiquitous element found at trace levels in all living matter, soil, rocks, natural waters, and the atmosphere. An increased level of arsenic can be mobilized through natural events such as volcanic emissions, especially the associated weathering products and ash, as well as anthropogenic activities. Humans have had significantly more influence on arsenic levels than natural events, through nonferrous metal mining and smelting, fossil fuel processing and combustion, wood preserving, pesticide production and application, and the disposal and incineration of municipal and industrial waste [2]. This has resulted in the pollution of soil and groundwater [3]. The majority of arsenic compounds are tasteless, odorless, and easily dissolve in groundwater, which presents a risk to health. Arsenic, as both inorganic and organometallic species, naturally occurs in the environment essentially in four oxidation states (–III, 0, +III, and +V). Arsenite (As(III)) is about 60 times more toxic than arsenate (As(V)). The exposure of individuals to arsenic mostly occurs



through drinking arsenic-contaminated water [4]. According to the World Health Organization, the consumption of water and food containing more than 0.01 mg/L inorganic arsenic is harmful to the body, whereas consuming that with a level exceeding 60 mg/L can be fatal [5]. It has been reported that, in India and Bangladesh, 60–100 million people are at risk of arsenic-related diseases from drinking arsenic-contaminated water [6]. This is a particular issue in Bangladesh, where thousands of people are dying of arsenicosis [7]. Many cases of arsenic poisoning have been reported from around the world, such as in the USA, Canada, Poland, Taiwan, China, Bangladesh, Chile, Vietnam, Japan, India, Mexico, and Argentina [8–10]. Kazakhstan is one of the largest producers of hazardous waste in the world. It has accumulated over 22 billion tons of waste, of which more than 16 billion tons is mining and processing waste, and about 6 billion tons is hazardous waste [11]. Mining and processing complexes in the Karaganda (29.4%), East Kazakhstan (25.7%), Kostanay (17.0%), and Pavlodar (14.6%) regions have produced the largest proportions of the waste in the country. These mine tailings are deposited in tailings dumps and exposed to the environment at low temperatures of about 0 °C for five or more months each year. The extraction of valuable minerals from the earth and mineral processing operations produce large amounts of waste, which are deposited as waste dumps or tailings [12]. In mine tailings, arsenic has been shown to occur in various forms, such as arsenopyrite (FeAsS), arsenian pyrite Fe(As,S)<sub>2</sub>, and arsenates, and is known to associate with iron oxyhydroxides. Since arsenic has been shown to be related to gold deposits, gold mining may contribute to arsenic pollution. Indeed, gold mining activities were acknowledged to be the key source of arsenic contamination in many regions [13]. Thus, to reduce the health risks arising from As, it is necessary to develop strategies that could reduce its toxicity.

Since As (V) is less soluble and is more effectively removed by physicochemical methods, it is important to oxidize As(III) to As(V) to achieve the satisfactory results of As removal [14]. Based on the analysis of data in the literature, using presently available technologies, especially physicochemical ones such as oxidation, adsorption, ion exchange, precipitation-coagulation, membrane filtration, permeable reactive methods, and biological techniques like phytoremediation and biological treatment with living microbes/bio-filtration, arsenic can be removed from contaminated water. However, all of these processes require an oxidation step to transform soluble As(III) to less soluble As(V), followed by the latter's separation. Since oxidation via a reaction with oxygen under atmospheric conditions takes a long time, this step is usually performed using chemical oxidants such as ozone, hydrogen peroxide, and chlorine [15]. That explains why bioleaching, using various microorganisms (acidophilic bacteria or fungi), can be an environmentally friendly and economical method providing an alternative to traditional methods. Biological leaching has been extensively studied over the past few years and is one of the bioremediation solutions used for the treatment of heavy metals (e.g., Cu, Co, Ni, Zn, and U) contained in sewage sludge, sediments, and contaminated soil [16]. Its use has been based on the ability of microorganisms to convert solid compounds into soluble elements that will be recovered. Recent studies have shown that high concentrations of arsenic can be leached from acid-contaminated soils by acidophilic iron- and sulfur-oxidizing bacteria. This has many advantages, such as low energy consumption, no emission of inorganic gaseous pollutants, increased leaching capacity, production of leaching agents in situ, and the formation of a microclimate around particles with high concentrations of leaching agents. The process also has certain disadvantages, such as longer reaction times, climate dependence, heavy metal toxicity, flotation, SX reagents for microbial activity, and the possibility of acid leakage [17]. The most familiar representatives of acidophilic bacteria used as bioleaching bacteria in leaching the metals in contaminated soils are bacteria from the genera Acidithiobacillus, Leptospirillum, Acidimicrobium, Sulfobacillus, and Sulfolobus [18]. Numerous studies have been conducted on bioleaching under mesophilic, moderately thermophilic, and extremely thermophilic conditions [19–21]. Acidithiobacillus is the most frequently used microorganism and studies on bioleaching mechanisms have focused mainly on Acidithiobacillus ferrooxidans (A. ferrooxidans) [22]. This species is a gram-negative acidophilic chemolithoautotroph, which requires atmospheric  $CO_2$  as a carbon source and obtains its energy for growth from the oxidation of ferrous ( $Fe^{2+}$ ) to ferric ( $Fe^{3+}$ ) iron [23–25]. *A. ferrooxidans* has been shown to be resistant to high concentrations of arsenic metalloid and is able to remove As in medium containing ferrous iron (Fe(II)) [22]. Hallberg et al. showed that, with several common phenotypic characteristics, *Acidithiobacillus ferrivorans* (*A. ferrivorans*) can grow at temperatures as low as 4 °C. They also found that *A. ferrivorans* can dominate in cold iron-rich environments with pH > 2.3. *A. ferrivorans* cells are gram negative, motile, and cannot form endospores, having an optimal temperature for growth of 27–32 °C [26]. It was found that ferrous iron can be more efficiently oxidized by the psychrotolerant mesophile *A. ferrivorans* in the bioleaching process of sulfide minerals at 4 °C than by the mesophilic *A. ferrooxidans* [27]. These oxidizing properties of bacteria are useful in arsenic removal processes. In the reaction with dissolved Fe(III) at low pH, As(III) can be oxidized to As(V) according to Reactions (Equations (1) and (2)) [28].

$$H_3AsO_3 + H_2O + 2Fe^{3+} \rightarrow H_2AsO_4^- + 3H^+ + 2Fe^{2+}$$
 (1)

$$2H_3AsO_4 + Fe_2 (SO_4)_3 \rightarrow 2FeAsO_4 + 3H_2SO_4$$
<sup>(2)</sup>

In previous studies on bioleaching, tests using *A. ferrivorans* were conducted [29,30]. However, in cold environments such as that in countries with a markedly continental climate, including Kazakhstan, low temperature becomes a limiting factor, reducing leaching speed and potentially making the process uneconomical. Nonetheless, the oxidation of sulfide minerals at a low temperature (<20 °C) has barely been studied. Bioleaching at a low temperature was reported in only a few papers [31–35]. However, bioleaching of arsenic-containing mine tailings at a low temperature (<10 °C), especially bioleaching by *A. ferrivorans*, has not been studied. Therefore, in the present work, *A. ferrivorans* strain 535 and *A. ferrooxidans* strain 377 were used for bioleaching in mine tailings containing As at temperatures of 8 °C, 18 °C, and 28 °C, which are common in countries with a markedly continental climate. The effects of ferric iron on the bioleaching kinetics of arsenic in a solution from waste were studied. During the bioleaching process, we investigated the relationships between the parameters of pH, oxidation–reduction potential (ORP), and As concentration. The study provides new information about the mechanisms of biological leaching of arsenic at low temperature.

#### 2. Materials and Methods

#### 2.1. Microorganisms and Samples

In this study, mine tailings were collected for testing from Bestobe Mine (52°36'18" N, 73°13'48" E), Akmola, Kazakhstan, which were kindly provided by Kazakhaltyn Mining-Metallurgical Concern JSC (Stepnogorsk, Akmola region, Kazakhstan). The 377 strain of *A. ferrooxidans* and the 535 strain of *A. ferrivorans* that were previously isolated from gold deposits at our laboratory were used in this study. These isolates were registered with the official potent deposit service at Republican State Enterprise "Republican collection of microorganisms" of the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (http://rcm.kz/) in Nur-Sultan, Kazakhstan. Microorganisms were incubated in 100 mL of Medium 9K-Fe [36] in 500 mL flasks under aerobic conditions at 28 °C and 200 rpm. The medium was adjusted to pH 1.8 at room temperature using 10 N H<sub>2</sub>SO<sub>4</sub> and autoclaved at 121 °C for 20 min.

### 2.2. Analytical Analysis

The mine tailings before and after bioleaching were analyzed mineralogically by X-ray diffraction (XRD) on a D8 ADVANCE (Bruker AXS GmbH, Billerica, MA, USA). Chemical phase analysis of the mine tailings for the arsenic and iron forms was carried out in accordance with the methods described by Filippova [37]. Minerallographic analyses of the mine tailings were conducted using the microscope OLYMPUS BX-51 Pol (Olympus Corporation, Shinjuku City, Tokyo, Japan) with a SIMAGIS 2P-2C video camera (SIAMS Ltd., Ekaterinburg, Russia) and SIAMS' Mineral S-7 image analysis software (SIAMS Ltd.). The minerallographic analyses were carried out in the accredited laboratory (ISO 17025:2007) of

Eastern Research Mining and Metallurgical Institute of Nonferrous Metals. The polished samples for XRD analyses were 70 microns. The volume of the samples were 0.2–0.5 cm<sup>3</sup>. It must also be stable and not decompose for at least 2 h. The method for preparing polished samples for microscopy was performed as described previously [38]. The mine tailings were also analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES, Thermo Scientific iCAP 7200 ICP Duo, Waltham, MA, USA). The chemical composition of the mine tailings was determined by adding 8 mL of 35% hydrochloric acid, 2 mL of 45% hydrogen fluoride, and 0.50 mL of 65% nitric acid to 0.25 g of the mine tailings. After that, the samples were heated at about 150 °C for 1 h, then kept at room temperature and diluted. Next, the suspension was introduced to ICP-OES for analysis of the target elements.

During the tests, 1 mL samples of the bioleaching solution were periodically taken (every 2 days and then every 4 days from 6 days after the bioleaching tests) from each flask under sterile conditions. The collected samples were each filtered and centrifuged for analysis of arsenic, ferric, and ferrous ion concentrations. The concentration of arsenic was analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES, Thermo Scientific iCAP 7200 ICP Duo). The concentrations of ferric and ferrous ions in the solution were determined by spectrometry (BioMate 3S UV-Visible, Thermo Scientific, Waltham, MA, USA). The pH and redox potential of the leaching solutions were periodically analyzed using a saturated calomel electrode (Mettler Toledo Seven Multi S47-K, Mettler Toledo AG, Schwerzenbach, Switzerland), while redox potential (Eh) was measured in units of mV by the combination of a platinum ring indicator and S7 screw head. All chemical analyses of ore samples and solutions were performed by the Biomedpreparat Scientic Analytical Center (Stepnogorsk, Akmola region, Kazakhstan), which is an accredited laboratory (ISO 17025:2009).

#### 2.3. Bioleaching Experiments

The bioleaching experiments were conducted in 250 mL Erlenmeyer flasks containing 10% mine tailings (*w*/*v*) and 10% inoculum (*v*/*v*) in a shaking incubator at 160 rpm and temperatures of  $8 \pm 1 °C$ ,  $18 \pm 1 °C$ , and  $28 \pm 1 °C$ . The initial pH of modified medium Fe-9K supplemented with filter-sterilized FeSO<sub>4</sub>·7H<sub>2</sub>O (22.1 g/L) was adjusted to 1.6 using 10 N H<sub>2</sub>SO<sub>4</sub>. During the As leaching tests, the pH of each flask decreased to the initial level of 1.6. The pH of each flask was periodically controlled and adjusted to the initial pH to prevent the formation of jarosite (KFe<sub>3</sub>(SO<sub>4</sub>)<sub>2</sub> (OH)<sub>6</sub>) [39]. The initial concentrations of the added inocula were determined by direct counting under a phase-contrast microscope (Standard 25, Carl Zeiss, Oberkochen, Germany) and by serial dilutions. Control tests without the microorganisms were also carried out under the same conditions. The final efficiency of As removal was calculated considering the mass balance of metal concentrations in solid and liquid phases. The particle size distribution of mine tailings used for bioleaching tests was meshed until 142 µm. During the bioleaching processe, a weight difference method was used by replacing lost water with distilled water. All bioleaching processes were carried out for 32 days.

### 3. Results and Discussion

## 3.1. Ore Sample Characterization

The chemical analysis on the sample showed that the total content of As was 0.045, that of S was 0.15, that of Fe was 3.1, and that of Zn was <0.05 (Table 1). XRD showed that the sample mainly contained quartz (SiO<sub>2</sub>), scorodite (FeAsO<sub>4</sub>·2H<sub>2</sub>O), albite, ordered (NaAlSi<sub>3</sub>O<sub>8</sub>), muscovite-2M1 glicolated [KAl<sub>2</sub>(Si,Al)<sub>4</sub>O<sub>10</sub>(OH)<sub>2</sub>], ferroan dolomite [Ca(Mg,Fe)(CO<sub>3</sub>)<sub>2</sub>], and ferroan clinoclore [Mg<sub>5</sub>Al(AlSi<sub>3</sub>O<sub>10</sub>)(OH)<sub>8</sub>] (Figure 1. As it was difficult to determine arsenic-containing minerals using XRD, minerallographic analysis and phase chemical analysis were performed. The results obtained from the minerallographic analysis are presented in Figure 2. Arsenopyrite was found in the free forms with a particle size of 0.05 mm and in the non-ore forms with a wide size range from 0.005 to 0.025 mm. Chalcopyrite and pyrite were found in the nonmetallic mass of the rock. According to the phase chemical analysis (Table 2), it was determined that the arsenic is for the most part represented by

arsenopyrite and scorodite minerals with a mass fraction (%) of 0.022. The mass fraction (%) of arsenic as oxide, elemental, and sulfide forms was not more than 0.005. The total mass fraction of Fe was 3.1%.

Table 1. The selected elemental content of mine tailings determined by ICP-OES analysis.



Figure 1. XRD pattern of mine tailings before bioleaching.





**Figure 2.** Optical microscopy (x200) images of the minerals before bioleaching: Polished section of mine tailings, (**a**) Single grain of arsenopyrite; (**b**) Arsenopyrite in non-ore forms; (**c**) Pyrite (light yellow) in a non-ore fragment; hematite (gray).

#### 3.2. Bioleaching Experiments

To examine the effects of microorganisms at a low temperature as well as other temperatures, arsenic bioleaching experiments were conducted at initial pH of 1.6 and pulp density of 10% with temperatures of 28 °C, 18 °C, and 8 °C by *A. ferrooxidans, A. ferrivorans*, and uninoculated control. Since the *A. ferrooxidans* and *A. ferrivorans* bacteria are acidophilic microorganisms active at an acidic pH, the pH level during the bioleaching process with different bacterial cultures did not exceed 1.8. Changes in the pH of all strains were reasonably similar. In the flasks with *A. ferrooxidans* 377 and *A. ferrivorans* 535 at 28 °C and 8 °C, respectively, the pH went down to 1.6. Meanwhile, the pH did not drop notably in the flasks without bacterial inoculation (data not shown). The As removal rates, reduction potential, and iron concentrates at 28 °C are shown in Figure 3. Since Bestobe Mine tailings contain arsenic in the form of arsenopyrite (FeAsS), arsenic removal kinetics in terms of the biological leaching of FeAsS was analyzed. In terms of the biological oxidation mechanisms, the bacteria oxidize ferrous iron to ferric iron and the As(III) can be oxidized to As(V) by ferric iron, according to Reactions (Equations (1), (3) and (4)) [40]:

$$4Fe^{2+} + 4H^{+} + O_2 \rightarrow 4Fe^{3+} + 2H_2O$$
(3)

$$FeAsS + 5Fe^{3+} + 3H_2O \rightarrow 2H_3AsO_3 + 6Fe^{2+} + S^0 + 3H^+$$
 (4)

In a study conducted by Zhang et al., it was shown that, during bioleaching tests, the oxidation–reduction potential (ORP) and pH values are the two most important factors, wherein at low pH and high ORP Fe(III) disturbs the surface of arsenopyrite by producing Fe(II) (Equation (4)). Thereafter, the accumulation of Fe(III), arsenate, and some sulfates forms several Fe(III)-containing precipitates (jarosites) [41]. The ferrous iron to ferric iron oxidation, which is described in the first chemical reaction, is assisted by bacteria. In the second reaction, the arsenopyrite is oxidized by free ferric ions with the dissolving arsenopyrite to form arsenic acid.

The As bioleaching rate was remarkably increased in the flasks with microorganisms for 14 days, but the As concentration in the solution subsequently increased slowly, changing by 2–3%. Meanwhile, in the control, the As removal efficiency of the reaction increased to about 20% in 26 days, but no subsequent As leaching was observed in that reaction. According to these results, the effect of microorganisms might have been inhibited in As leaching. In addition, the strain *A. ferrivorans* 535 achieved the highest redox potential after 10 days, while *A. ferrooxidans* 377 reached it in 4 days. Strains *A. ferrooxidans* 377 showed the highest activity, the active oxidation of iron in the medium was observed for 4 days, and the ORP values were above 550 mV. Although the strains *A. ferrivorans* 535 and *A. ferrooxidans* 377 oxidized 99% of Fe<sup>2+</sup> to Fe<sup>3+</sup> after 10 days, the bioleaching was continued till the end the experiments. After 18 days of bioleaching, the levels of Fe<sup>3+</sup> and ORP began to decrease. Meanwhile, Fe<sup>2+</sup> was increased, which indicates a decrease in cell activity due to an increase in the content of arsenic in the leaching solution above 31 mg/L (61%). The arsenic dissolution curves show that the efficiency of leaching As was directly related to the oxidation of Fe<sup>2+</sup> in solution. Throughout the experiment, in all flasks with microorganisms, the ORP was kept above 510 mV while the redox potential of the control flasks varied between 393 and 444 mV. The bioleaching efficiency of arsenic was 66–68%.

A long period of adaptation of strains to the mine tailings in all flasks at 18 °C was observed (Figure 4). Strains *A. ferrivorans* 535 showed better results regarding iron oxidation and arsenic recovery than strains *A. ferrivorans* 377. In the flask with the strains *A. ferrivorans* 535, the complete oxidation of Fe<sup>2+</sup> was achieved in 14 days. Along with the oxidation of the Fe<sup>2+</sup>, an increase in redox potential of the medium occurred, where the ORP value rose to 640 mV. In addition, strains *A. ferrivorans* 535 removed 64% of arsenic in 32 days. Meanwhile, strains *A. ferrooxidans* 377 was less active, as the complete oxidation of iron occurred in 19 days.



**Figure 3.** Ferric (Fe<sup>3+</sup>) concentration (**a**), solution redox potential, and As removal efficiency (**b**) during the bioleaching experiments with different strains at 28  $^{\circ}$ C.



**Figure 4.** Ferric (Fe<sup>3+</sup>) concentration (**a**), solution redox potential, and As removal efficiency (**b**) during the bioleaching experiments with different strains at 18  $^{\circ}$ C.

The changes in iron oxidation, ORP, as well as As concentration for the temperature of 8 °C are shown in Figure 5. *A. ferrivorans* 535 showed higher activity than *A. ferrooxidans* 377, in line with the differences observed between the two strains by other researchers [26–29]. The complete oxidation of iron by *A. ferrivorans* 535 occurred in 18 days, and the ORP value rose above 620 mV. The arsenic bioleaching within the 32 days of the experiment was 61.8% (30.9 mg/L). Meanwhile, in the flask with strain 377, the complete oxidation of iron was achieved in only 22 days.

Although most arsenic in the mine tailings presented as dissoluble minerals and our findings coincide with those of other researchers that the bioleaching rates of As and Fe depend on the temperature, the As removal efficiency was more than 50% at all temperatures. At the end of the experiment, As removal efficiency reached the highest value (66.5%) by *A. ferrivorans* 535 at 28 °C. After 14 days at 28 °C, As leaching did not change markedly. The same situation was observed at the other temperatures after 22 days. There are several possible reasons for the low As removal efficiency. First, the toxic effect of arsenic would increase to inhibit bacterial growth. However, several reports have described that, at As concentrations of 20 g/L or higher, bacterial growth was inhibited [40]. Second, according to Astudillo et al. [42], at a higher solid concentration, a limitation in the supply of gaseous nutrients such as  $CO_2$  or  $O_2$  could occur due to the accumulation of soluble iron and arsenic. Third, the formation of jarosite occurred at a pH of 1.8 or higher on the mineral surface. The formation of the passivation layer by jarosite (Equation (5)) could decrease the leached proportion [43]. According to the arsenic leaching in the control test, Deng et al. pointed out that the relatively high arsenic extraction

might be due to the easy solubility of arsenopyrite in acidic solutions and the buffer properties of the medium solution containing  $K_2$ HPO<sub>4</sub> [44].



$$K^{+} + 3Fe^{3+} + 2SO_{4}^{2-} + 6H_{2}O \rightarrow KFe_{3}(SO_{4})_{2} (OH)_{6} + 6H^{+}$$
(5)

**Figure 5.** Ferric (Fe<sup>3+</sup>) concentration (**a**), solution redox potential, and As removal efficiency (**b**) during the bioleaching experiments with different strains at 8  $^{\circ}$ C.

Table 2.	The results of	f the phase	chemical	analysis	of As	and	Fe in	the	mine	tailings	before	and
after bioleaching.												

As Phases	Before Leaching		8 °C		28 °C			
		377	535	Control	377	535	Control	
as oxide	< 0.005	ND **	ND **	ND **	ND **	ND **	ND **	
as elemental	< 0.005	ND **	ND **	ND **	ND **	ND **	ND **	
associated with Zn	< 0.005	ND **	ND **	ND **	ND **	ND **	ND **	
as sulfide	< 0.005	ND **	ND **	0.006	ND **	ND **	0.007	
as difficult to decompose *	0.022	0.022	0.019	0.023	0.015	0.013	0.022	
Total	0.045	0.022	0.019	0.039	0.015	0.013	0.034	
Fe Phase								
as oxide	3.1	2.11	2.03	1.59	1.71	1.86	1.4	
as sulfide	< 0.005	0.48	0.48	0.73	2.41	2.55	0.09	
as difficult to decompose *	3.1	0.012	0.007	0.014	0.026	0.008	0.02	
Total	0.045	2.59	2.51	2.32	4.12	4.41	1.49	

\* arsenopyrite, scorodite, jarosite (only in leach residue); \*\* not detected or <0.005.

Against this background, XRD analysis was performed to confirm the formation of jarosite on the mineral surface in mine tailings after bioleaching tests. It was shown that jarosite was not formed in low-temperature conditions (Figure 6b). The jarosite intensity was observed at 28 °C temperatures in the flasks with all strains (Figure 6a). From the experiments, we concluded that jarosite precipitation would not occur at a temperature of 8 °C at the initial pH of 1.6; consequently, As leaching efficiency was increased.



**Figure 6.** XRD pattern of mine tailings after bioleaching in the flasks with strains *A. ferrivorans* 535 at 28 °C (**a**) and 8 °C (**b**).

In addition to the XRD analysis, arsenic and iron phase analysis was conducted with some residues after biological leaching tests. The results of the phase chemical analysis are depicted in Table 2. In the Table 2, data of the leach residue can be compared with the data before leaching. What can be clearly seen in the table that the bioleaching process removed a significant part of the arsenic as dissoluble and difficult to dissolve minerals at 28 °C. Meanwhile, at the low temperature of 8 °C, the amount of arsenic as difficult to decompose hardly changed. All of the results support the mechanism of bioleaching proposed in Figure 7. To obtain more precise information on the stability and toxicity of the bioleaching residue, additional studies, including designing a pilot scale, will be carried out.

Despite the implications and significance of our research, there are some limitations in terms of the methods applied. One limitation is that we could not provide data on the growth phase of culture, or data from scanning electron microscopy. Another important limitation is that the leached arsenic needs to be removed by chemical methods such as sedimentation and filtration. To develop a comprehensive overview of the arsenic removal process, additional studies will be required on As(V) removal by chemical methods.



Figure 7. The mechanisms of bioleaching of arsenopyrite from the mine tailings for the removal of arsenic.

### 4. Conclusions

In this study, bioleaching for the removal of arsenic using biooxidation by pure cultures of A. ferrooxidans and A. ferrivorans was investigated using mine tailings containing arsenic. The results of this investigation show that at initial pH of 1.6, at different temperatures (8 °C, 18 °C, and 28 °C), and a solid concentration of 10%, a decrease in the temperature affected the given microorganisms activity and the period of adaptation to the mine tailings. After 32 days of cultivation, at temperature of 28 °C the A. ferrooxidans 377 was showed the highest leaching efficiency of As (up to 68%) with Fe<sup>2+</sup> iron oxidation of 4.7 g/L. In the bioleaching of mine tailings experiments at temperature of 8 °C, the highest As leaching (61%) was observed in the flask with the A. ferrivorans with Fe<sup>2+</sup> iron oxidation of 4.9 g/L. The As leaching behaviors in all the pure cultures according to the experiment temperatures showed that a higher temperature leads to a tendency to higher As leaching efficiency. The study contributes to our understanding of the impact of the psychrotolerant and mesophilic microbes in mine tailings during the removal of arsenic under low temperature conditions, and the influence of jarosite formation at low and mesophilic temperatures. Overall, this study strengthens the idea that the strains of A. ferrivorans can be more effective than strains of A. ferrooxidans in bioleaching of sulfide minerals and tailings at low temperature. Moreover, the results reported here shed new light on the application of A. ferrivorans in low temperature bioleaching of arsenic-containing mine tailings. Further research could also be conducted to determine the effectiveness of the use of a consortium of strains in markedly continental climatic conditions.

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