Hindawi
International Journal of Chemical Engineering
Volume 2024, Article ID 5611117, 8 pages
https://doi.org/10.1155/2024/5611117

Research Article

Acidithiobacillus ferrooxidans Leaching of Silica-Sulfide Gold Ores from May-Hibey Deposits, Tigray, Ethiopia

Goitom Gebreyohannes Berhe,[1] Desta Berhe Sbhatu,[2] Samuel Estifanos Gebre,[1] Kiros Hagos Abay,[2] Genet Gebryohannes Mhretu,[1] Gebrekidan Mebrahtu Tesfamariam,[1] Samuel Alemayehu Lapiso,[1] Mulugeta Sisay Cheru,[3] and Afewerk Gebre Meressa[4]

[1]*College of Natural and Computational Sciences, Mekelle University, P.O. Box 231, Mekelle, Ethiopia*
[2]*Mekelle Institute of Technology, Mekelle University, P.O. Box 1632, Mekelle, Ethiopia*
[3]*School of Materials Science and Engineering, Jimma University, P.O. Box 378, Jimma, Ethiopia*
[4]*Endowment Fund for the Rehabilitation of Tigray, P.O. Box 1234, Mekelle, Ethiopia*

Correspondence should be addressed to Goitom Gebreyohannes Berhe; goitom.gebreyohannes@mu.edu.et

Received 3 November 2023; Revised 30 January 2024; Accepted 27 February 2024; Published 8 March 2024

Academic Editor: Pratima Meshram

Oxidative leaching is an inexpensive alternative to using chemical cyanide extraction methods for gold from low-grade gold sulfide. Oxidation of finely ground gold-bearing ore by *Acidithiobacillus ferrooxidans* was evaluated in terms of cell density, pH, and leaching efficiency of Fe and Au in shake flask experiments. The compositional and elemental analyses of the beneficiated ore were analyzed using XRD and EDXRF spectroscopy. The ore's primary constituents are gold (4.356 mg/L), silicon, iron, and sulfur (62.456, 15.441, and 7.912 wt%, respectively). XRD spectra, the main phases of the concentrated ore, showed that the major components of the ore were quartz, syn, silicon sulfide, pyrite, and polymetallic elements such as silderenrite, gismondine, siderenikite, hematite, and syn. The experimental results, with *Acidithiobacillus ferrooxidans* bacteria and blank, were evaluated. The pH of the blank remained nearly constant, and the pH of the bioleached was occasionally lowered. The *A. ferrooxidans* strain always grew better throughout the bioleaching process. For the *A. ferrooxidans* strain, the cell density of cells reached a maximum of 90.00×10^6 cells/mL after the 11th week and decreased to 87.00×10^6 cells/mL after the 12th week. The decrease in cell density may be due to the presence of polymetallic elements such as Al, Cr, Ti, and Ni, leading to reduced metal tolerance of the *A. ferrooxidans* strain. In the *A. ferrooxidans* leaching process, the maximum total iron and gold extraction reached 92.16% (14.23 mg/L) and 99.97% (4.355 ppm), respectively, after the 11th week, and leaching tends to decrease up to 14 weeks, which may be due to the formation of secondary minerals. More research will be performed to optimize the procedure and leaching kinetic, examine the impact of metal content, and take into account the potential for bioleaching process pollution in addition to the amount of gold recovered.

1. Introduction

The application of bioleaching metal sulfides and its understanding have evolved over the past several decades. Bioleaching is typically a pretreatment process that involves the mobilization of metal cations from sulfide ores through biological processes including acidification, oxidation, and complexation, in addition, to mainly being used for the extraction of copper, cobalt, nickel, zinc, uranium, and gold ores from its sulfide ores [1–3]. Recently, precious metal mining production has increased significantly due to the development of advanced technology and global demands [1, 2], and new approaches will be needed to recover metals from low-grade ore deposits and mine tailings as a part of economic circular before further chemical treatment [4, 5]. The processes of mining gold, one of the most valuable and economical metals, produce large amounts of waste that still contain large amounts of valuable substances and in some

cases, the toxic chemicals cyanide and thiocyanide, often buried as landfill or waste near the mining areas [5]. It is important to develop better methods to avoid the use of these chemicals when extracting gold from its ores [6, 7].

Over the past decade, intensive research has focused on fundamental biochemical leaching reactions. Bioleaching typically involves in the removal of sulfide minerals by the action of chemolithotrophic acidophilic bacteria and is an economically viable competitive and respectful environmentally friendly biotechnology [2, 4, 8]. The environmental conditions of bioleaching are characterized by low pH values and high concentrations of reduced sulfur and iron compounds; in these ores, rocks and acid drainage waters from the mine represent prime places of iron-oxidizing *Acidithiobacillus* species [4, 7, 9–11]. Bioleaching is a promising and revolutionary method that uses environmentally friendly technologies, which are useful for the mining industry as well as for environmental detoxification [1–5]. Bacterial leaching may be enhanced by an increase in bacterial population. *A. ferrooxidans*, the most widely used bacterium in the bioleaching of sulfide minerals, was grown in an electrolytic bioreactor containing a ferrous medium [12].

According to the multiple subprocess mechanisms, the sulfide mineral is chemically oxidized by ferric iron. The overall chemical reactions for pyrite are [2, 3, 12] as follows:

$$\begin{aligned} 4\mathrm{MFeS}^2 + 11\mathrm{O}_2 + 6\mathrm{H}_2\mathrm{O} &\longrightarrow 4\mathrm{MSO}_4 + 4\mathrm{Fe(OH)}_3 + 4\mathrm{S}^0\,(+\,\text{Bacteria}) \\ 2\mathrm{S} + 3\mathrm{O}_2 + 2\mathrm{H}_2\mathrm{O} &\longrightarrow 2\mathrm{H}_2\mathrm{SO}_4\,(+\,\text{Bacteria}) \end{aligned} \tag{1}$$

where M represents metals.

Microorganisms that oxidize ferrous iron are responsible for converting the iron produced by these reactions into ferric iron. Sulfur-oxidizing microorganisms convert polysulfides, sulfur, or thiosulfates that are produced by the ferric leaching reactions to sulfate. By using bacteria to leach pyrite, a passivation layer is formed on the mineral surfaces, coating the unreacted materials and forming elemental sulfur in the process [1–3, 12]. This layer also prevents further leaching of the pyrite substrate coated in $Fe(OH)_3$. By adhering to the pyrite and chalcopyrite minerals in an ore containing gold, *A. ferrooxidans* helps break down the sulfide minerals, freeing up gold particles that facilitate further cyanidation.

The best-studied leaching bacteria are the genus *Acidithiobacillus*, which includes sulfur-oxidizing species and sulfur- and ferrous-oxidizing bacteria and which are common inhabitants of extremely acidic ecological sites, rich metals, has been extensively used in the bioleaching of concentrates, sludge, and metal-sulfide ores [13, 14]. Despite its high bioleaching efficiency, the genus *Acidithiobacillus* is often found at low densities in indigenous microbial consortia, which is still not fully understood and, conversely, *Acidithiobacillus* ferrooxidans bacterium is most commonly found in bioleaching [2, 3, 5]. Iron- and sulfur-oxidizing *Acidithiobacillus ferrooxidans* bacterium is capable of oxidizing the sulfur matrix before extracting cyanide, which contains gold particles encapsulated in gold-bearing sulfide ore [13, 14]. Biooxidation of gold ores is a less expensive and less polluting alternative to other oxidative pretreatment methods such as roasting and pressure oxidation. Recently, the biooxidation of gold ores has been researched worldwide to further apply to refractory gold ores [1, 3, 6, 15].

Acidophilic bacteria participate in the biooxidation of minerals and produce sulfuric acid or ferric ions that act as chemical lixiviants, and resilience to increasingly unfavorable environmental conditions that arise when minerals undergo biooxidation [3, 12, 15]. The dissolution of minerals is obtained by these bacteria, which alter the local environmental conditions such as pH, metal concentrations, oxygen availability, and redox potential [2, 4, 7, 15]. This bioleaching process is inhibited by the oxidation of Fe^{2+} and the presence of heavy metal ions [13–15]. Similarly, bioleaching efficiency varies significantly depending on mineral composition [16]. However, only a few studies have been reported on the bacterial sulfide oxidation, and the disadvantage of bioleaching is that moderate oxidation will slow down the bacterial growth cycle and thus slow down the turnover process [2, 8–10].

Effective bacterial growth and oxidation in bioleaching are influenced by many physical, chemical, and biological factors, including temperature, pH, oxygen, salt supply, and the amount of uptake and absorption [2, 6, 11]. In addition, it is important to find and maintain the optimal pH range for bioleaching. This can affect the quantity and quality of excreted bioacids, heavy metal accessibility, and tolerance during bioleaching, elemental composition and properties of the sediments, the density of the leach sludge, and the particle size of the ore [6, 8, 10]. Therefore, the possible impact of heavy metal tolerant on bioleaching with indigenous iron-oxidizing *Acidithiobacillus* bacteria is the main focus of this study, making gold-bearing sulfide ore suitable for the subsequent processes [2, 3, 8–10, 15, 16].

A resilient bacterium, most likely *Acidithiobacillus ferrooxidans*, was found to be effective in the bioleaching of soluble gold sulfide ore at low pH and in the determination of gold and iron concentrations in the presence of polymetals. This allowed the study to optimize the bioleaching process and avoid the use of fresh reagents, making the process more cost-effective and environmentally friendly. Despite being linked to silicon gold sulfide ore, this bacterium is not naturally suited to environments with elevated concentrations of heavy metals [3, 6, 10, 11].

To our knowledge, this is the first report on acidophilic consortia and bioleaching of gold-bearing sulfide ore from the May-Hibey deposit. Therefore, the aim of this work is to

elucidate the mineral composition of gold sulfide and the bioleaching of the indigenous *Acidithiobacillus ferrooxidans* bacteria as a single culture in the presence of metals' tolerance, and the cell density and concentrations of Fe and Au ions were quantified. The formation of liquid and solid-phase products over time/week is also characterized.

2. Materials and Methods

2.1. Mineralogy. The gold sulfide rock sample was collected from the May-Hibey deposit (Figure 1). May-Hibey is located in the northwestern zone of Tigray Regional State, Ethiopia [17]. The sample was ground in a disc mill and sieved to <74 μm (200 mesh size) in the Analytical Laboratory of Ezana Mining Share Company, Mekelle, Ethiopia. Compositional analyses of the beneficiated rock sample were conducted using energy-dispersive X-ray fluorescence (EDXRF 2800) (Table, Figure 2) and flame atomic absorption spectroscopy (FAAS) in the laboratories of the Ethiopian Ministry of Mines (Addis Ababa, Ethiopia) and Ezana Mining Share Company (Mekelle, Ethiopia), respectively. The crystallinity of each rock sample was determined by X-ray diffraction (XRD) at Adama Science and Technology University (Oromia, Ethiopia). The XRD output was processed, and pattern processing was identified using Jade 6 software (MD, USA) and plotted using Origin 8.0 software (OriginLab Corp., USA). Each trial was carried out three times, and the standard deviations were used to compute the error bars.

2.2. Microbial Agents and Chemicals

2.2.1. Culturing for Bacterial Bioleaching. The culturing and bioleaching analyses were conducted in the bioengineering laboratory of Mekelle Institute of Technology, Mekelle University, Mekelle, Ethiopia. *A. ferrooxidans bacteria culture was* obtained from the microbiology laboratory of the Department of Biology, College of Natural and Computational Sciences, Mekelle University, Mekelle, Ethiopia. Bacteria were maintained in a medium enriched with ferrous sulfate and sulfur mineral salts [6]. The culturing for the bioleaching process was carried out based on Xie et al., 2013 [6], and the 9 K liquid medium was used to culture the bacterial strain (initial density of bacteria: 1.1×10^6/mL). The filtered 9 K liquid medium constituted by 3.0 g/L of $(NH_4)_2SO_4$, 0.50 g/L of $MgSO_4{\cdot}7H_2O$, 0.50 g/L of K_2HPO_4, 0.10 g/L of KCl, and 0.01 g/L of $Ca(NO_3)_2$ as well as 0.01 g/L of $FeSO_4{\cdot}7H_2O$ is the source of energy.

The medium was sterilized at 121°C for 21 min before use. The bacterial load was inoculated into 100 mL of 9 K medium in a 250 mL flask, while their pH values were adjusted to 1.5 with drops of 5.0 mol/L of H_2SO_4. The flasks were then incubated at 40°C in a rotary shaker at 160 rpm. Care should be taken not to inhibit the bioleaching process due to changes in metal concentration, pH, and cell density compared to the first day of $1.1 \times 90 \times 10^6$ cells/mL. Cell density was determined by the hemocytometer counting method under an optical microscope (Olympus CX31, USA, 0.1 mm depth chamber) using the following equation:

Figure 1: Gold sulfide ore sample [17].

$$\frac{\text{cell}}{ml} = \left(\frac{\text{number of cells counted} * \text{dilution factor}}{\text{number of large squales counted}}\right) * 1000, \tag{2}$$

where the volume of the diluted sample (after dilution) divided by the volume of the original mixture in the sample (before dilution) is the dilution factor.

2.2.2. Metal Tolerance of A. ferrooxidans. The heavy metal tolerance of *A. ferrooxidans* was conducted in the microbiology laboratory of the Department of Biology, College of Natural and Computational Sciences, Mekelle University, Mekelle, Ethiopia.

For heavy metal tolerance studies, the stock solutions of heavy metals (mM/ml) were filter sterilized and added to sterile nutrient agar as follows: $HgCI_2$0.01-2; $CuCl_2.2H_2O$ 0.5, 1.0, 1.5, 2.0 and2.5; $CdCl_2$0.065, 0.125, 0.250, 0.500, and 1.0; $ZnSO_4.7H_2O$ 0.500, 1.0; AlK $(SO_4)_3.12H_2O$ (0.500, 1.0), $K_2Cr_2O_7$ (0.01-2), $CoCl_2$ (0.065, 0.125, 0.250, 0.500)and 2.0; $Pb(C_2H_3O_2)_2.3H_2O$ 0.5, 1.0 and 3.0. The pH range for these tests was 1–7, and the temperature range was 20–70 °C. All the chemicals (metal salts) were of analytical grade and were purchased from Sigma Aldrich (Addis Ababa, Ethiopia). Each water-soluble metal compound was dissolved in distilled sterile water, and those insoluble in water were dissolved in ethanol alcohol to prepare a 1000 ppm stock solution, from which solutions of lower concentrations were prepared by serial dilutions. Growth media, chemical solutions, and glassware were sterilized at 121°C for 15 min before use, and subsequent work was performed under sterile conditions in a laminar flow cabinet. The plates with each heavy metal to be tested were inoculated with about 10^8 cells, and the bacterial growth was evaluated after 7 days at 28°C [18, 19]. Isolates were considered resistant when growth occurred and sensitive when no growth was observed. The adaptation data were studied for a week.

2.2.3. Experiments

(1) Chemical Leaching. An experiment without the *A. ferrooxidans* bacteria was conducted as a blank to create a baseline and compare the efficacy of bacterial extraction. The sample was dissolved in sulfuric acid to a pH of 2.0, which was maintained during the extraction procedure [3].

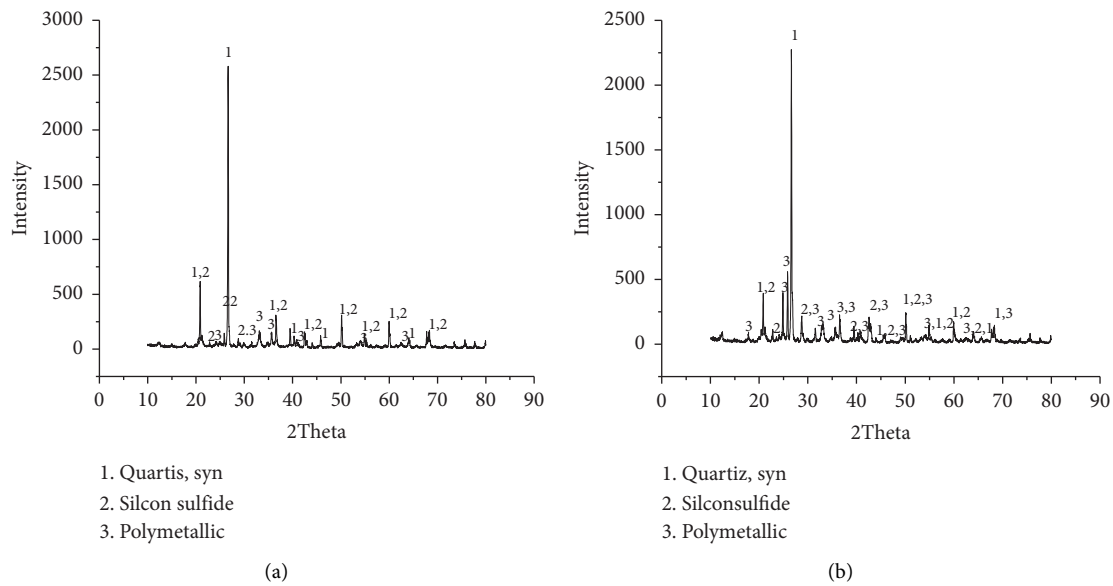

Figure 2: XRD patterns: (a) sulfide gold ore and (b) bioleached residue [17].

(2) Biological Leaching. Bioleaching experiments were carried out based on Xie et al., 2013 [6] and Artykova et al., 2022 [18] in 250 mL flasks, each containing 100 mL of the 9 K liquid growth medium which was inoculated with 10% (v/v) of *A. ferrooxidans' bacterium* dose (density of bacteria: 1.1×10^6 cells/mL) for a week. The pulp density of the silica-rich gold concentrate samples in each flask was 5% (w/v), and the initial pH of the culture was adjusted to 2.0 with 5 m/L of H_2SO_4. Flasks were incubated at 40°C in a rotary shaker at 160 rpm. Data on cell density, pH, concentrations of total gold, and total iron in the solutions were collected once/week for 14-week periods.

Losses of 9 K liquid medium due to sampling and water due to evaporation were compensated for by adding equivalent volumes of 9 K liquid medium and distilled water, respectively. Cell density was calculated based on equation (2), Fe and Au concentrations were determined by FAAS (AA240FC, Australia), and pH was measured using a pH meter (HI99130, Italy). EC was measured using a conductivity meter (JENWAY, Multi-3410, UK) from weeks 1–14. All experiments were performed in triplicate, and the average values were reported.

3. Results and Discussion

3.1. Mineralogical Analysis. The bioleaching of gold from gold ore is influenced by several factors, including ore type and particle size, type of leaching agent, temperature, time, pH of the medium (environment), and type of microbial agent. Before direct bioleaching, the <74 μm gold ore was concentrated by sequential beneficiations such as crushing, grinding, sieving, washing, and gravity separation to improve the gold grade from the parent ore (Figure 1) [17].

Composition and elemental analyses of the beneficiated ore show that the main elements in the ore were Si (62.456%), Fe (15.441%), and S (7.912%). The gold content was determined to be 4.356 mg/L (Table 1), which is similar to the author's study [17]. The XRD spectrum also indicates that quartz was the main fracture phase (Figures 2(a) and 2(b)). In addition, quantitative XRD analysis (after 14 weeks) of the ore and tailings showed that the main components of the ore were quartz, syn (major, SiO_2), silicon sulfide (SiS_2), pyrite (FeS_2), and other polymetallic complexes. As illustrated in Figures 2(a) and 2(b), the polymetallic complexes include pyrite (FeS_2), boron nitride (BN), gismondine ($CaAl_2Si_2O_8{\cdot}4H_2O$), siderenikite ($Na_3Mn(PO_4)$ (CO_3)), hematite, and syn (Fe_2O_3) [17], and studies on gold deposits at the Chambishi copper mine in south-central Zambia [19] and Lape-Sumbawa gold rocks in Indonesia [20] reveal similar ore compositions, with gold combined with Si, S, and Fe as pyrite. These findings show that the rock samples have high compositions and patterns of sulfur, iron, and gold. In fact, May-Hibey was known to have high gold deposits [17]. A sample containing a 200 g portion of ore concentrate was used for bioleaching studies.

3.2. Metal Tolerance. Due to their high toxicity, heavy metals, even at low concentrations, can pose serious threats to organisms, and the food chain transfer of metal ions has become a major public concern [21]. Moreover, heavy metals adversely influence microorganisms, which not only affect their growth but also their morphology and activities. Problems arising from heavy metal pollution can establish threats to human health and to ecosystems as well, as the metals exert a selective pressure on organisms, resulting in microbial populations with higher tolerance to metals, but with lower diversity, when compared to unpolluted neighboring areas [22].

Metal tolerance/resistance is a common property in many microorganisms that face toxic compounds in their habitats [22]. In this study, the effect of various metal ions such as, As, Cd, Co, Cr, Ni, Pb, and Zn and gold sulfide rock samples was studied, and it was found that both the bacteria

Table 1: Elemental analyses of concentrated ore (in % wt) using EDXRF and FAAS.

Elements	Ag*	Au*	Ti	Sb	Si	Cr	S	Fe	Al	Cu	Ni	Pb	Mn	As
Quantity	11.232	4.356	2.012	0.012	62.456	4.128	17.912	15.441	6.342	0.052	1.342	0.372	0.322	2.443

*Quantities are in mg/L.

and *A. ferrooxidans* were resistant to metal ions significantly. In addition, the cell density could not be affected by the chemical composition of the gold sulfide ore for further bioleaching processes [16, 22].

3.3. Bioleaching of Gold Concentrates. Results from sulfide bioleaching experiments with and without *A. ferrooxidans* were evaluated against changes in pH, cell density, and iron and gold extraction over 14 consecutive weeks.

3.3.1. Effect of pH

(1) Chemical Leaching. Sulfuric acid was used in the blank experiment to chemically extract the sediment at a fixed pH of 2.0. Figure 3(a) illustrates the comparatively tiny drop in pH and the subsequent return to 2.0, indicating a very low leaching efficiency on the metal ion contents of the solution from week 1 to week 14. Chemical extraction led to lower dissolution efficiencies for all elements than biological phase-enriched systems, as consistently noted by Ye et al. Dissolution in H_2SO_4 (pH 2.0) was the least effective for metal ions without *A. ferrooxidans* bacterium [3, 4, 23].

(2) Bioleaching. pH values for leaching effects were performed in the presence of *A. ferrooxidans* to study the change in pH during the leaching of metal ions of iron and gold from the sulfide ore. Bacterial bioleaching takes place in an acidic environment with pH values of 1.5–2.0 where most metal ions remain in solution. Lowering the pH value rapidly decomposes the gold ore as a sulfate solution [5, 21, 23–25]. *A. ferrooxidans* iron oxidants effectively oxidize sulfur and sulfides but grow at high pH values where metal ions are not retained in solution [5, 16, 21, 23–25]. As shown in Figure 3(a), the pH of the blank experiment (without bacteria) was nearly constant, but for the *A. ferrooxidans* leached sample, there was little change in pH from week 1 to 4, and this could be due to the leaching of metal ions into the sulfide ore. On the contrary, the result decreased sharply from week 11 and started to increase until week 14 in the bioleached sulfide ore (Figure 3(a)). These results point to the fact that the higher the metal ion concentrations, the lower the pH of the extraction solution. Furthermore, the lowering of the pH until the 11th week and the increase around the 14th week of the bioleaching process happen due to the activity of the *A. ferrooxidans* bacteria. It uses the H^+ ions for the growth and reproduction at the beginning of the process, as shown in equation (3), where M represents the polymetallic element [6]. The bioleaching process of the gold ore involves the oxidation of sulfur and a sulfide mineral which is represented by equations (3)–(6), leading to a decrease in pH [22].

Extensive research work has been carried out for the treatment of the complex gold-bearing sulfide ores [10, 22, 24]. The findings are consistent with those of Ioana et al. 2022 [4, 25]. With 84% pyrite oxidation in gold pyrite ore, 81% of the gold was recovered in solution. The gold recovery for these outcomes was enhanced by pyrite oxidation through bioleaching [3]:

$$4Fe^{2+} + 4H^+ + O_2 \longrightarrow 4Fe^{2+} + 2H_2O \ (+A.\ \text{ferooxidans}) \tag{3}$$

$$2FeMS + 2Fe^{3+} + 4H_2O + 6O_2 \longrightarrow 2H_3MO_4 + 4Fe^{2+} + 2H_+ + 2SO_4^{2-} \tag{4}$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \longrightarrow 2SO_4^{2-} + 15Fe^{2+} + 16H^+ \tag{5}$$

$$2S + 2H_2O + 3O_2 \longrightarrow 4H^+ + 2SO_4^{2-} \ (+A.\ \text{ferooxidans}) \tag{6}$$

This sulfuric acid in equation (5) maintains the pH at levels favorable to the growth of bacteria and also helps in the effective leaching of oxide minerals, and ferric sulfate is a strong oxidizing agent capable of dissolving a wide range of metal-sulfide minerals (3) [10, 22, 24]. An investigation was carried out on copper sulfide ore from Balaghat, Madhya Pradesh, India [2]. At pH values of 1.8 and from 2.0 to 2.5, the extraction of copper increases and decreases, respectively. Because of the lower oxidation potential of the leaching system and the decreased activity of the bacteria, the metal recovery was very low at low pH. The solubilization of copper was decreased when the pH was raised to 2.5, which may have been caused by a significant amount of iron (III) precipitating [2].

Since polymetallic sulfide ores contain structural Fe, oxidation releases iron into the solution, which acts as a redox shuttle in the bioleaching process. At pH values of >1.5, ferric iron forms pyrite ore, an acid-forming precipitation reaction with certain monovalent and divalent cations in the bioleaching solutions [10, 22, 24].

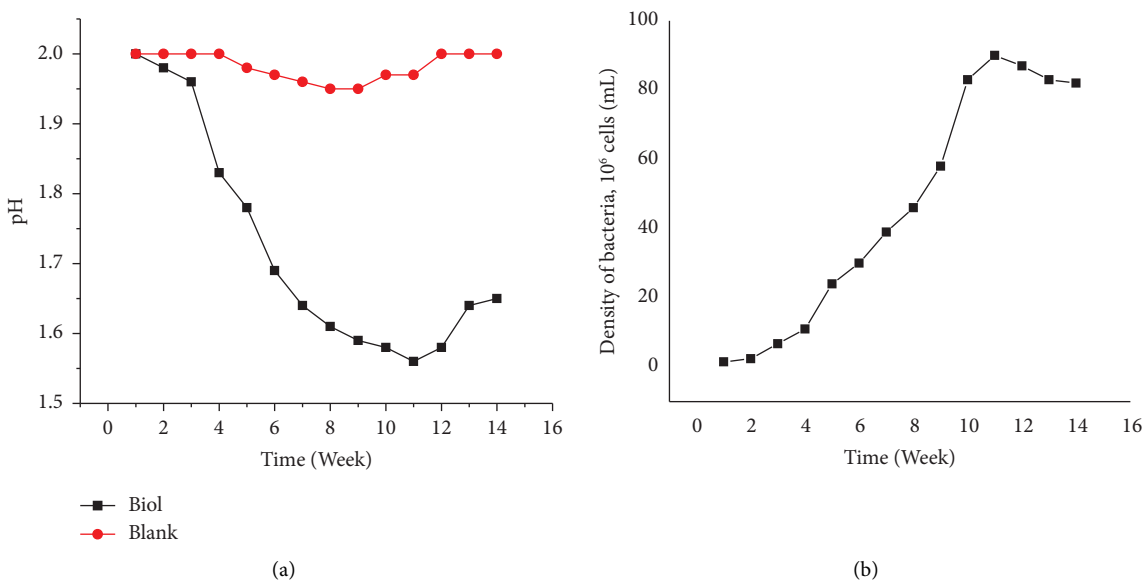

Figure 3: Growth patterns during bioleaching of sulfide gold concentrate: (a) effect of pH and (b) effect of density of the cell.

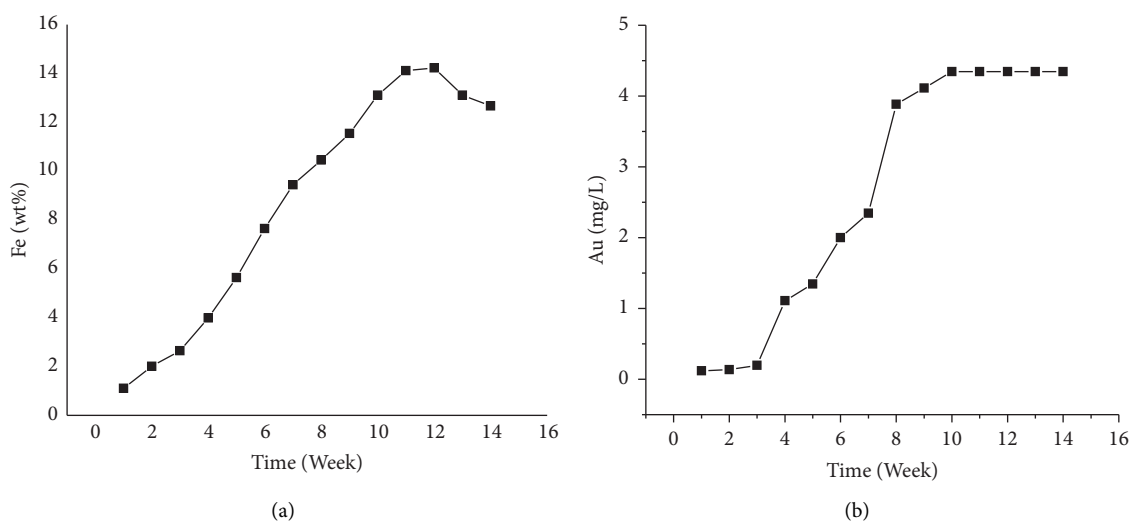

Figure 4: Sulfide gold concentrate bioleaching of (a) Fe and (b) Au.

3.3.2. Effect of Density of the Cells. As shown in Figure3(b), the cell density reached a maximum of 90×10^6 cells/mL at week 11, from the first week's cell density of 1.1×10^6 cells/mL started to decrease at the beginning of the week 12–14. Similarly, *A. ferrooxidans* grew gradually during the first 11 weeks of bioleaching, as observed in other studies [18, 22]. The decrease in cell density may be attributed to the reduced metal tolerance of bacteria due to the accumulation of polymetallic elements such as Al, Cr, Ti, and Ni.

3.3.3. Leaching Effect on the Concentration of Iron and Gold with A. ferrooxidans. The average concentrations of iron and gold bioleached by *A. ferrooxidans* from weeks 10 to 14 are shown in Figures 4 and 5, respectively. These results imply that the *A. ferrooxidans* bacteria promoted the dissolution of total iron and gold. Compared with the elemental composition of the sulfide ore (Table 1), the total iron and gold extraction reached maximums of 92.16% (14.23 mg/L) and 99.97% (4.355 ppm), respectively, by the 11th week. At approximately the 10th week of bioleaching, the total amount of iron and gold dissolution began to decrease, possibly due to the formation of secondary minerals (3)–(6). As reported in many studies, mineral bioleaching leads to the formation of a passive layer that is adsorbed on the surface of the mineral to prevent its further extraction by the bioleaching process [5.25]. In addition, a second simple physical gold recovery step will be performed to obtain the almost totally leached gold ions from the sulfide leached using the *A. ferrooxidans* bioleached process [8].

Observations in this study show that (Figures 3–5), in all experimental data, the degree of sulfide oxidation has a direct relationship with the rate of iron dissolution [11], which reduces the pH until the 11th week, and cell density reached

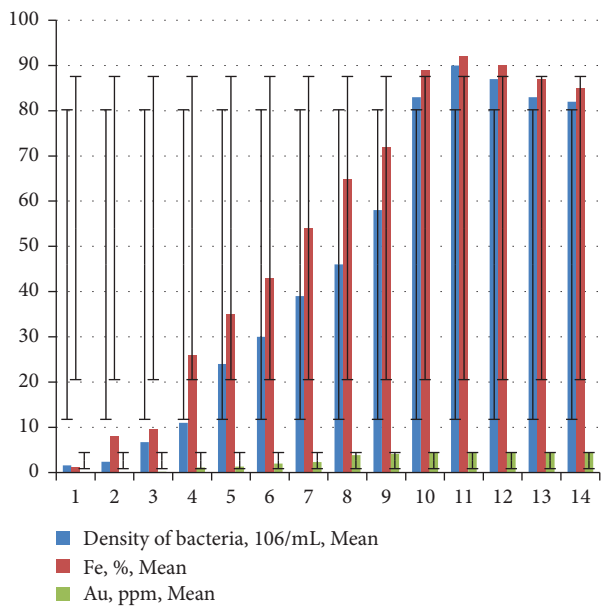

Figure 5: Error bars with a standard deviation of bioleaching gold sulfide on the density of *A. ferrooxidans,* Fe, and Au.

a maximum of 90×10^6 cells/mL compared to 1.1×10^6 cells/mL at the first day. The efficiency of oxidation can be detected by measuring the dissolution rate of iron and gold.

4. Conclusion

In summary, this study confirms the presence of high-grade gold in the form of concentrated gold sulfide ore in the May-Hibey deposit, Tigray, Ethiopia, and paves the way for further bioleaching of iron and gold. XRD spectrum, the dominant phases of the concentrated ore, shows that the main components of the ore are quartz, syn (major, SiO_2), silicon sulfide (SiS_2), pyrite (FeS_2), and polymetallic elements such as silderenrite, gismondine, siderenikite, hematite, and syn. The ore's primary constituents are gold (4.356 mg/L), silicon, iron, and sulfur (62.456, 15.441, and 7.912 wt%, respectively). For the *A. ferrooxidans* bacteria, the cell density reached a maximum of 90.00×10^6 cells/mL after the 11th week and decreased to 87.00×10^6 cells/mL from the 12th week onwards. This inhibition may be due to the presence of polymetallic elements in the gold sulfide ore. The pH of the blank sample (without bacteria) remains almost constant, and the pH of the bioleached sample drops from time to time. The *A. ferrooxidans* strain always grew better and better throughout the bioleaching process. Using *A. ferrooxidans*, the extraction efficiency of total iron and gold reached a maximum of 92.16% (14.23 mg/L) and 99.97% (4.355 ppm), respectively, after the 11th week. During the later stages of bioleaching, the extraction of total iron and sulfide tended to decrease, which may be due to the formation of secondary minerals. Further experiments will be carried out in studying, with the aim of optimizing the process and leaching kinetics, and the effect of the metal content takes into account not only the level of gold recovery but also the potential pollution of the process.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

GGB, DBS, and SAL conceived the problem of the study. GGB, DBS, SEG, KHA, AGM, GGM, GMT, and SAL prepared the research proposal and developed the design of the experiments. GGB prepared the first draft of the manuscript, and DBS and GMT reviewed it to produce the final draft.

Acknowledgments

The authors are thankful to Mekelle and Jimma University. The authors are also thankful to the administrators of labs of Mekelle and Adama Science and Technology Universities, the Ethiopian Ministry of Mines, and Ezana Mining Company Plc for allowing them to carry out the analyses. This study was supported by Mekelle University, PO Box 231, Mekelle, Ethiopia, through Grant no. CRPO/MU/CNCS/Rec/Medium/13/2019-2021.

References

[1] M. Vera, A. Schippers, S. Hedrich, and W. Sand, "Progress in bioleaching: fundamentals and mechanisms of microbial metal sulfide oxidation part A," *Applied Microbiology and Biotechnology*, vol. 106, no. 21, pp. 6933–6952, 2022.

[2] A. Abhilash, A. Ghosh, and B. D. Pandey, "Bioleaching of low grade granitic chalcopyrite ore by hyperthermophiles: elucidation of kinetics-mechanism," *Metallurgical Research & Technology*, vol. 112, no. 5, p. 506, 2015.

[3] D. Rouchalova, K. Rouchalova, I. Janakova, V. Cablik, and S. Janstova, "Bioleaching of iron, copper, lead, and zinc from the sludge mining sediment at different particle sizes, pH, and pulp density using Acidithiobacillus ferrooxidans," *Minerals*, vol. 10, no. 11, p. 1013, 2020.

[4] I. Tezyapar Kara, K. Kremser, S. T. Wagland, and F. Coulon, "Bioleaching metal-bearing wastes and by-products for resource recovery: a review," *Environmental Chemistry Letters*, vol. 21, no. 6, pp. 3329–3350, 2023.

[5] N. T. Quach, C. Pham-Ngoc, T. Bui et al., "Bioleaching potential of indigenous bacterial consortia from gold-bearing sulfide ore of Ta nang mine in vietnam," *Polish Journal of Environmental Studies*, vol. 31, no. 1, pp. 803–813, 2022.

[6] X. Xie, X. Yuan, N. Liu, X. Chen, A. Abdelgadir, and J. Liu, "Bioleaching of arsenic-rich gold concentrates by bacterial flora before and after mutation," *BioMed Research International*, vol. 2013, Article ID 969135, 10 pages, 2013.

[7] A. Valenzuela, J. L. Valenzuela, and J. R. Parga, "Effect of pretreatment of sulfide refractory concentratewith sodium hypochlorite, followed by extraction of gold by pressure cyanidation, on gold removal," *Advances in Chemical Engineering and Science*, vol. 3, no. 3, pp. 171–177, 2013.

[8] R. Ulloa, A. Moya-Beltrán, C. Rojas-Villalobos et al., "Domestication of local microbial consortia for efficient recovery of gold through top-down selection in airlift bioreactors," *Frontiers in Microbiology*, vol. 10, p. 60, 2019.

[9] J. Li, J. Wen, Y. Guo, N. An, C. Liang, and Z. Ge, "Bioleaching of gold from waste printed circuit boards by alkali-tolerant Pseudomonas fluorescens," *Hydrometallurgy*, vol. 194, Article ID 105260, 2020.

[10] G. H. Wang, J. P. Xie, S. P. Li et al., "Two-step oxidation of refractory gold concentrates with different microbial communities," *Journal of Microbiology and Biotechnology*, vol. 26, no. 11, pp. 1871–1880, 2016.

[11] K. Kudpeng, T. Bohu, C. Morris, P. Thiravetyan, and A. H. Kaksonen, "Bioleaching of gold from sulfidic gold Ore-Concentrate and electronic waste by roseovariustoleransand roseovariusmucosus," *Microorganisms*, vol. 8, no. 11, p. 1783, 2020.

[12] K. D. Abhilash, K. Mehta, and B. Pandey, "Bacterial leaching kinetics for copper dissolution from a low grade Indian chalcopyrite ore," *Rem: Revista Escola de Minas*, vol. 66, no. 2, pp. 245–250, 2013.

[13] K. Karimov, D. Rogozhnikov, E. Kuzas, O. Dizer, D. Golovkin, and M. Tretiak, "Deposition of arsenic from nitric acid leaching solutions of gold—arsenic sulphide concentrates," *Metals*, vol. 11, no. 6, p. 889, 2021.

[14] L. Castro, M. L. Blázquez, and J. Á Muñoz, "Leaching/bioleaching and recovery of metals," *Metals*, vol. 11, no. 11, p. 1732, 2021.

[15] R. Quatrini and D. B. Johnson, "Microbiomes in extremely acidic environments: functionalities and interactions that allow survival and growth of prokaryotes at low pH," *Current Opinion in Microbiology*, vol. 43, pp. 139–147, 2018.

[16] D. Bevilaqua, O. Garcia, and O. Tuovinen, "Oxidative dissolution of bornite by Acidithiobacillus ferrooxidans," *Process Biochemistry*, vol. 45, no. 1, pp. 101–106, 2010.

[17] G. G. Berhe, D. B. Sbhatu, and A. Gebremariam, "Study of holy water consumed to treat gastrointestinal ailments in gold deposit areas of May-Hibey, Northwestern Tigray, Ethiopia," *H2Open Journal*, vol. 6, no. 3, pp. 403–413, 2023.

[18] A. Artykova, Y. Elkina, A. Nechaeva, V. Melamud, A. Boduen, and A. Bulaev, "Options for increasing the rate of bioleaching of arsenic containing copper concentrate," *Microbiology Research*, vol. 13, no. 3, pp. 466–479, 2022.

[19] Z. Xiao-liang, K. Jue, S. Chun-bao, Z. Rui-yang, S. Min, and L. Shuo-fu, "Mineralogical characterization of copper sulfide tailings using automated mineral liberation analysis: a case study of the Chambishi Copper Mine tailings," *International Journal of Minerals*, vol. 51, 2021.

[20] E. Yanuar and Suprapto, "Leaching and adsorption of gold from lape-sumbawa rocks (Indonesia) by hypochlorite-chloride," *Procedia Chemistry*, vol. 17, pp. 59–65, 2015.

[21] C. B. Opara, R. Blannin, D. Ebert, M. Frenzel, K. Pollmann, and S. Kutschke, "Bioleaching of metal(loid)s from sulfidic mine tailings and waste rock from the Neves Corvo mine, Portugal, by an acidophilic consortium," *Minerals Engineering*, vol. 188, Article ID 107831, 2022.

[22] H. Z. Tavakoli, M. Bahrami-Bavani, Y. Miyanmahaleh, and P. Tajer-Mohammad-Ghazvini, "Identification and characterization of a metal-resistant Acidithiobacillus ferrooxidans as important potential application for bioleaching," *Biologia*, vol. 76, no. 4, pp. 1327–1337, 2021.

[23] M. Ye, P. Yan, S. Sun et al., "Bioleaching combined brine leaching of heavy metals from lead-zinc mine tailings: transformations during the leaching process," *Chemosphere*, vol. 168, pp. 1115–1125, 2017.

[24] H. B. Zhou, W. M. Zeng, Z. F. Yang, Y. J. Xie, and G. Z. Qiu, "Bioleaching of chalcopyrite concentrate by a moderately thermophilic culture in a stirred tank reactor," *Bioresource Technology*, vol. 100, no. 2, pp. 515–520, 2009.

[25] I. M. Sur, V. Micle, A. Hegyi, and A. V. Lăzărescu, "Extraction of metals from polluted soils by bioleaching in relation to environmental risk assessment," *Materials*, vol. 15, no. 11, p. 3973, 2022.