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Reuse of Acid Bioleachate in Bacterial Oxidation of a Refractory Gold Sulfide Concentrate

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Abstract: Bacterial pre-oxidation of refractory gold concentrates generates large volumes of leachate and requires a significant supply of nutrients to support bacterial growth. Therefore, bioleachate reuse reduces both water consumption and the nutrients required for the process. However, the efficiency of this method and its benefit need to be further explored. In the present study, two tests on the reuse of bioleachate in new cycles of bacterial oxidation were carried out to evaluate the efficiency and the benefit of bioleachate reuse. Our results showed that the reuse of bioleachates could reduce nitrogen and phosphorus requirements by 40% and 36%, respectively, after a 14-day biooxidation stage in a stirred tank bioreactor. We also showed that the reuse of bioleachate had a positive effect on the recovery of gold in a subsequent 48 h treatment by cyanidation. The gold recovery rate (initial concentration of 44 mg/kg) remained unchanged at 90% after the two bioleachate recirculation loops. The reuse of bioleachate also made it possible to increase the solubilization rates of other metals from the sulfide concentrate. Thus, the solubilization yields of copper (initial concentration of 3587 mg/kg) and zinc (initial concentration of 27,315 mg/kg) increased, respectively, from 14.8% and 40.2% to 37.5% and 99.6% after the two bioleachate recirculation loops.

Keywords: bioleachate; refractory gold concentrate; bacterial oxidation; biooxidation; leaching; reuse

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1. Introduction

Bacterial oxidation is one method used to pre-treat refractory gold sulfide concentrates [1]. The acid bioleachate resulting from the bacterial oxidation generates liquid effluent, concentrated in metals, which must be treated before being discharged [2]. The reuse of these bioleachates, although still little practiced, could help reduce costs and make the bacterial oxidation process even more helpful.

Bioleachates comprise metals/metalloids, such as iron and arsenic, as well as sulfuric acid. Iron is present in the ferric state (Fe^{3+}), as ferric sulfate ($Fe_2(SO_4)_3$). Arsenic is present in the arsenate state (As^{5+}), as arsenic acid (H_3AsO_4). One of the potential issues encountered with the reuse of bioleachate is the accumulation of some toxic oxidation products, such as heavy metals, in the reused bioleachate. Some of these metals can be highly toxic, and bacterial cells, responsible for the leaching activity, may lose their metabolic activity [3]. The accumulation of toxic oxidation products can progressively inhibit bacteria and is likely to lead to a decrease in the efficiency of mineral oxidation [4]. Tuovinen et al. [5] showed that *Acidithiobacillus ferrooxidans* is sensitive to zinc, copper, nickel, cobalt, manganese and aluminum ions at concentrations above 10 g/L. The inhibition effect of bacterial activity for tellurium, arsenic, silver and selenium anions may start at concentrations between 0.05 and 0.10 g/L [4].

During bacterial oxidation, nitrogen (N) and phosphorus (P) are the two main nutrients used by microorganisms and must be supplied to the pulp [6,7]. In addition to their consumption for the metabolic needs of bacterial cells, these nutrients can be lost through

the formation of the secondary precipitations of ammoniojarosite during the pre-oxidation step [8,9]. These precipitates can also act as physical barriers, slowing down the diffusion of leaching agents to the surface of sulfide particles where the main bacterial activity takes place [10]. These secondary precipitation products can also significantly increase the amount of metallic sludge to be managed, thus increasing the cost associated with its dewatering and disposal [11–13].

To reuse bioleachate in bacterial oxidation cycles, some solubilized heavy metals must also be removed to keep the bacteria active. The presence of ferric iron in the bioleachate can form a secondary precipitation, such as jarosite, which inhibits the bacterial oxidation of sulfide. The removal of heavy metals is normally performed by precipitation [14]. The molar ratio of iron and arsenic, as well as base metals, such as zinc, copper and cadmium in the bioleachate, determine the stability of the iron hydroxide precipitate [15]. For example, the higher the iron/arsenic ratio, the more stable the precipitate.

Bioleachate can be treated by precipitating metals as insoluble hydroxides using precipitating agents such as sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$), magnesium hydroxide ($Mg(OH)_2$) or alkali metal sulfides. The most used precipitating agents containing sulfur are CaS, Na_2S [16,17], hydrosulfide of sodium (NaHS) and sodium thiosulfate ($Na_2S_2O_3$) [17–19]. However, the precipitation of heavy metals using sulfur-containing agents poses an environmental hazard because of the risk of the emission of toxic hydrogen sulfide [18]. Using NaOH, although it is more expensive than hydrated lime ($Ca(OH)_2$), to treat bioleachate has some advantages [20]. Bioleachates treated with NaOH are easier to dehydrate compared to bioleachates treated with $Ca(OH)_2$, which also results in secondary precipitation products, such as gypsum and jarosite [8–10]. The formation of these two secondary products can negatively affect the process cost, including costly dewatering and sludge disposal steps [11–13].

In this study, bioleachates from the bacterial oxidation of a refractory gold sulfide concentrate are treated by removing ferric iron from the solution using sodium hydroxide (NaOH) as the precipitating agent and then reused twice in new cycles of bacterial oxidation. The effectiveness of this reuse of bioleachates is verified by evaluating the quantity of metals dissolved in the two reused bioleachates and in the yield of dissolution of the gold.

2. Materials and Methods

2.1. Preparation and Characterization of Refractory Gold Sulfide Concentrate

A sample of a refractory gold sulfide concentrate was obtained from a flotation operation, using potassium amyl xanthate (KAX) (40 mg/L) as a collector and methylisobutilcarbinol (MIBC) (2 mg/L) as a foaming agent. The pH was adjusted to 5.5 by adding NaOH or H_2SO_4 [21]. The concentrate comprised polymetallic sulfides. X-ray diffraction (XRD) was used to identify the main mineral phases present in the sample. The chemical characterization of the sample was carried out by ICP-AES. Elemental composition of gold sulfide concentrate is shown in Table 1. The particle size used in all bacterial oxidation tests was D_{80} at 12 μm.

Table 1. Elemental composition of gold sulfide concentrate.

Elements	Content (mg/kg)	Elements	Content (mg/kg)
Ag	18.9	Mn	1165
Al	8153	Mo	514
As	75	Na	97
Au	44	Ni	7338
Ba	77,519	P	96
Bi	<5	Pb	78,250

Table 1. *Cont.*

Elements	Content (mg/kg)	Elements	Content (mg/kg)
Ca	1006	S	112,422
Cd	306	Sb	30
Co	29.1	Sc	0.7
Cr	13,376	Si	201,138
Cu	3587	Sr	1230
Fe	112,517	Ti	358
K	1553	Th	<0.5
Mg	805	V	50
		Zn	27,315

2.2. Bacterial Pre-Oxidation

The bacterial pre-oxidation process was the same as that described to treat refractory gold by Andrianandraina et al. [21]. Briefly, a 5% inoculum of a mixed population of *Acidithiobacillus ferrooxidans* (ATCC19859) and *Acidithiobacillus thiooxidans* (ATCC19377) was used as the bacteria source. To maintain these microorganisms in an active state, 4.31 g/L $(NH_4)_2SO_4$ and 0.71 g/L K_2HPO_4 were added as a source of nitrogen and phosphorus. The initial pH was adjusted to 1.7 by adding sulfuric acid. The temperature was maintained at 30 ± 2 °C using a water bath system. The pulp was agitated at 120 rpm using an axial propeller to ensure that all particles remained in suspension. An aeration rate of two volumes of air per one volume of liquid per minute (vvm) was used in the biooxidation experiments. The experiments were carried out in batches and in a small transparent acrylic bioreactor (10 cm in diameter, 20 cm in height) with a capacity of 1 L. The pulp density was set at 20%.

2.3. Reuse of Bioleachates

The main steps in the reuse of bioleachate for bacterial oxidation are summarized in Figure 1. The first step (*Biooxidation 1*) comprised producing an initial bioleachate (*Bioleachate 1*) from a bacterial oxidation test. The solid part (*Concentrate 1*) was then separated from the liquid by filtration and kept for the cyanidation step. *Bioleachate 1* was treated at pH = 4.0 using sodium hydroxide (15 g/L) to precipitate the iron in the solution, i.e., *Bioleachate treatment 1*. The resulting liquid was used in the subsequent step (*Biooxidation 2*) to pre-oxidize the gold sulfide concentrate. After this bacterial oxidation, the solid fraction (*Concentrate 2*) was separated from the liquid fraction (*Bioleachate 2*). The solid residue was kept for the cyanidation test and the aqueous phase again retreated with NaOH (15 g/L) to precipitate the iron. The operation was repeated for the second reuse of the bioleachate, i.e., *Biooxidation 3*, which resulted in the production of *Bioleachate 3* and *Concentrate 3*. The cyanidation treatment made it possible to see the impact of the reuse of the treated bioleachate on bacterial oxidation performance and gold dissolution. Of the three bioleachates, 10 mL of each was taken for chemical analysis. The reuse of bioleachate saves the reagents used in the biooxidation of sulfides. To maintain the growth of microorganisms, it is sufficient to add only a few essential reagents, such as ammonium (NH_4^+), as a source of nitrogen, and phosphate (PO_4^{3-}), as a source of phosphorus.

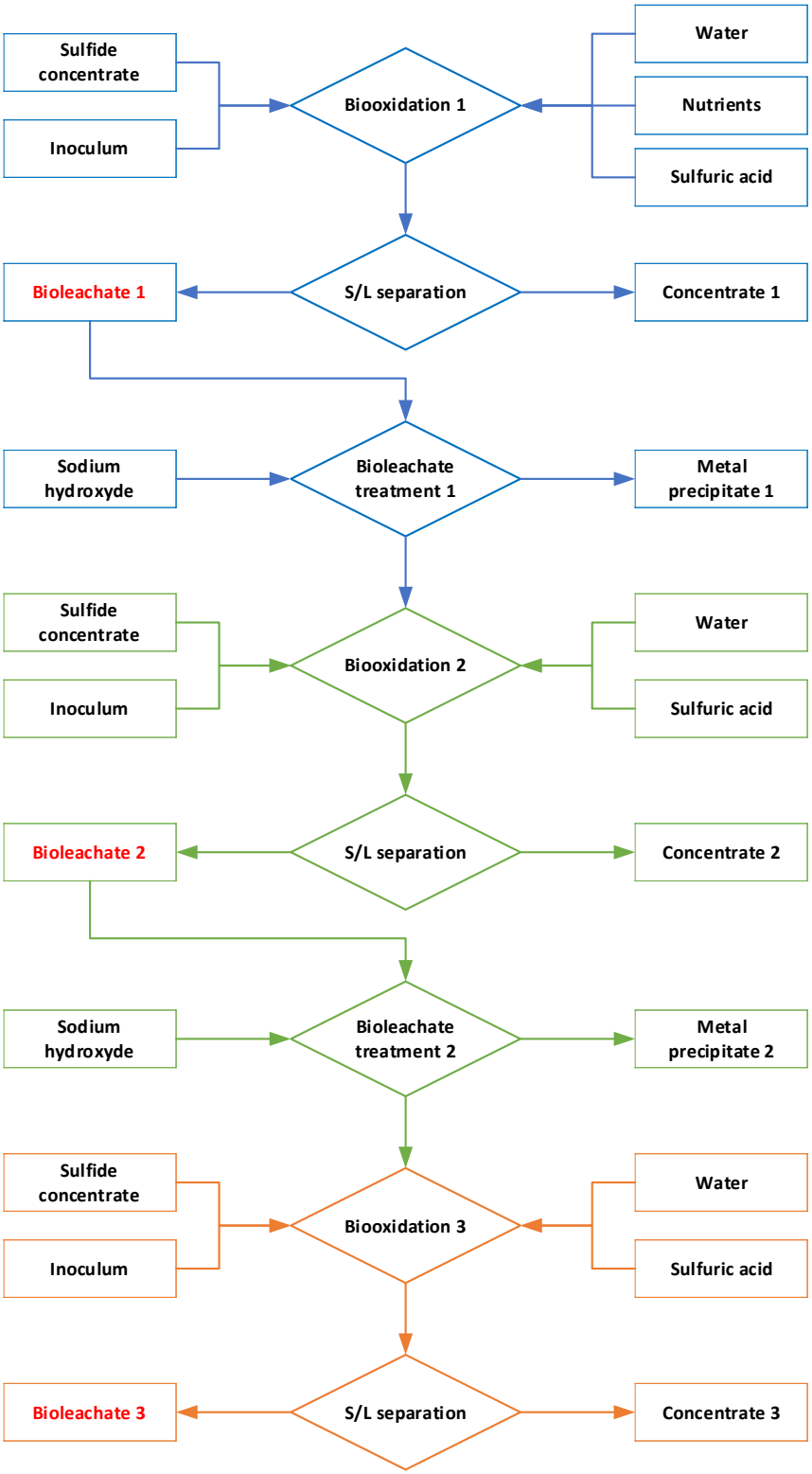


Figure 1. Flowsheet of the reuse of bioleachate in the biooxidation process.

2.4. Iron Precipitation

The presence of iron in the bioleachate at acidic pH (pH = 1.6) can form different precipitates, such as natrojarosite and ammoniojarosite. To minimize the formation of these different precipitates, iron should be precipitated before the reuse of bioleachate. To determine the optimum pH value for iron precipitation, eight precipitation tests were

performed using 50 mL of *Bioleachate 1* in 125 mL Erlenmeyer flasks with different pH values of 3.2, 3.4, 3.6 and 4.0. Sodium hydroxide (10–15 g NaOH/L) was used as a precipitating agent. The amount of NaOH necessary to precipitate the iron was calculated using the precipitation reaction between the sodium hydroxide solution and the Fe^{3+} ion Equation (1).

$$6\ NaOH + Fe_2(SO_4)_3 \rightarrow 2\ Fe(OH)_3 + 3\ Na_2SO_4 \quad (1)$$

To complete the precipitation reaction and avoid the overconsumption of hydroxyl ion (OH^-) by the sulfuric acid, NaOH was diluted with distilled water and then poured drop wise into an ampoule to settle during the precipitation reaction. After precipitation, the solutions were vacuum filtered using a Whatman G6 fiberglass membrane (porosity: 1–3 µm). The solid fraction was dried in an oven and weighed. The liquid fraction was acidified with 5% HNO_3 for its conservation until the analysis of the dissolved chemical elements by ICP-AES. Equation (2) was used to calculate iron removal efficiency in biological leachate.

$$\text{Iron removal efficiency (\%)} = 100 \times ([Fe]_{initial} - [Fe]_{final})/[Fe]_{initial} \quad (2)$$

where the $[Fe]_{initial}$ and $[Fe]_{final}$ are the initial and final concentrations of iron in the bioleachate.

Bioleachate 1 was treated for iron removal by using NaOH as the precipitating agent. Figure 2 shows the evolution of the iron concentration in *Bioleachate 1* as a function of the increase in pH because of the addition of a NaOH solution. The iron removal efficiencies at different pH values of 3.2, 3.4, 3.6 and 4.0 were 94.7 ± 0.7%, 98.1 ± 0.2%, 99.2 ± 0.2% and 99.9 ± 0.1%, respectively. The pH value of 4.0 was chosen as the best condition for iron removal, which resulted in an iron concentration of only 2.2 mg/L in the treated solution. The selection of pH = 4 is consistent with the work of Halder et al. [22] who used $Ca(OH)_2$ to precipitate iron. Santaolalla et al. [17] also showed that ferric iron precipitates efficiently at pH = 4.

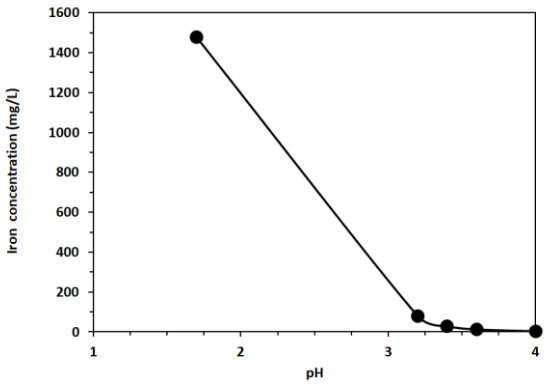


Figure 2. Soluble iron concentration in *Bioleachate 1* in function of the pH.

Bioleachate 1 was therefore treated for iron removal at pH = 4. The first treatment, i.e., *Bioleachate treatment 1*, was carried out using 1 L of bioleachate, where a concentration of 2.23 mg/L of iron was measured in the solution (Table 2). A precipitate sample with a dry mass of 3.76 g was also obtained per liter of bioleachate. The result obtained in this work agrees with those published by Choo et al. [23]. The ratio of the weight of the precipitate to the added sodium hydroxide was 0.3. This ratio is proportional to 1 mole of NaOH, which is equal to three times the number of moles of iron, since ferric iron is the main ionic form of the iron present in the bioleachate. A Fe^{3+} ion reacts with three OH^- ions to form an iron precipitate as ferric hydroxide ($Fe(OH)_3$).

The second treatment, i.e., *Bioleachate treatment 2*, was performed using 1 L of *Bioleachate 2*. The amount of NaOH used to precipitate iron and the pH value used were the same as in the first treatment test (Table 2). The sludge generated contained 6.37 g of precipitate per litter of bioleachate. The iron concentration remaining in *Bioleachate 2* after

the second precipitation test was 59.3 mg/L, which represents 98.2% iron removal. The weight ratio of the precipitate to sodium hydroxide added was 0.5. The amount of iron precipitated by the *Bioleachate 2* treatment was almost twice the amount of iron precipitated during the first treatment. This difference is explained by the different concentrations of iron in *Bioleachate 1* and *Bioleachate 2*, which were, respectively, 1477 mg/L and 3349 mg/L.

2.5. Batch Cyanidation

After the bacterial oxidation, the solid fraction was separated from the liquid fraction. The solid residue obtained was aerated for 1 h while maintaining a concentration of 8 mg/L of dissolved oxygen, then cyanidated at 20 °C. A sodium cyanide concentration of 706 mg/L was introduced to obtain a cyanide concentration of 500 mg/L CN^-. A pulp density of 10% solids was used for the cyanidation tests. This cyanidation work is more detailed in the work carried out on the treatment of refractory gold by Andrianandraina et al. [21].

2.6. Sample Analysis

The pH, temperature and oxidation reduction potential (ORP) were measured simultaneously using a Fisher Scientific XL 60D Accumet Excel. ORP measurements were performed by using Ag/AgCl reference electrode with an electrode potential of 199 mV vs. standard hydrogen electrode. The dissolved oxygen concentration was determined using a Hach HQ40d portable counter. To determine the metal content of the sulfide concentrate, a total digestion in hydrofluoric acid (HF) was first required. Analyses of the main metals constituting the solid concentrates were carried out using XRD and ICP-AES (Andrianandraina et al., 2021) [21]. A PANalytical-Aeris CuKα diffractometer was used for the analysis. Identification and semi-quantitative determination of the different crystalline phases were achieved by comparing the XRD diffractograms. The diffractogram peaks were interpreted using HighScore Plus software, and the major and minor phases of the sample were semi-quantified [24]. Semi-quantification of the identified species was performed using the Rietveld method [25]. The chemical characterization of the liquid samples was performed using ICP-AES (Vista-AX CCO, Palo Alto, CA, USA). NH_4-N and PO_4-P were measured using a Lachat Flow Injection Analyzer (model 8000 Series, Zellweger Analytics) (NH_4-N: QuikChem® Method 10-107-06-2-B; PO_4-P: QuikChem® Method 10-115-01-1-B). Analysis of dissolved gold during cyanidation was performed by the MP-AES microwave plasma atomic emission spectrometry technique (Agilent, model 4100+).

3. Results and Discussion

3.1. Initial Composition of the Gold Sulfide Concentrate

The initial composition of the refractory gold sulfide concentrate is shown in Table 1. The concentrate contains high levels of iron (11.3%), lead (7.83%), barium (7.75%) and zinc (2.73%). Chromium, nickel and copper are also found with respective concentrations of 1.34%, 0.73% and 0.36%. Precious metal contents are obviously high, with the initial contents of 44 mg/kg and 18.9 mg/kg for gold and silver, respectively.

XRD analyses revealed the major presence of silica (SiO_2), pyrite (FeS_2), galena (PbS), chalcopyrite ($CuFeS_2$), sphalerite (ZnS) and barite ($BaSO_4$).

3.2. Bioleachate 1

The concentrations of dissolved elements present in *Bioleachate 1* are presented in Table 2. The results show very high concentrations of sulfur (4277 mg/L), iron (1477 mg/L) and zinc (1097 mg/L). The high sulfur concentration is explained by the generation of sulfuric acid (H_2SO_4) by microorganisms as a by-product of bacterial oxidation of sulfides. The formation of sulfuric acid decreases the pH of the stock solution from 1.7 to pH = 1.6 (Table 2). This result is consistent with those reported by Leng et al. [26] on the bacterial pre-oxidation of a gold sulfide concentrate.

Table 2. Characteristics and chemical composition (mg/L) of the three bioleachates.

Parameters	DL	*Bioleachate 1*		*Bioleachate 2*		*Bioleachate 3*
		Non-Treated	**Treated**	**Non-Treated**	**Treated**	**Non-Treated**
pH		1.7	4	1.67	4	1.6
ORP (mV)		538	458	520	245	570
Al	0.01	29.4	7.71	47.4	10.2	43.4
Cr	0.02	36.5	0.5	50.6	5	55.8
Cu	0.01	53.3	21.8	111	71.5	178
Fe	0.01	1477	2.23	3349	59.3	3926
Mn	0.01	32.7	14.7	46.2	16.9	50.2
Na	0.03	269	3292	3637	4383	5176
Ni	0.01	19.4	8.67	53	29.4	53
Pb	0.04	2.86	1	135	68	135
S	0.2	4277	3254	10,489	4551	12,645
Zn	0.01	1097	523	3107	1046	3861

DL: Detection Limit.

The high ORP value of 530 mV (Table 2) shows that the iron in the solution is mainly ferric ion, following the oxidation of the ferrous ion by *Acidithiobacillus ferrooxidans* [27]. The high concentration of zinc in the solution is explained by the biooxidation of sphalerite (ZnS), a sulfide that is easily oxidized by microorganisms [28].

3.3. Metals' Solubilization

During the first bacterial pre-oxidation test, the solubilization percentages of iron (Fe), sulfur (S) and zinc (Zn) were, respectively, 21.9%, 38.0% and 40.2%. The solubilization of these three elements was calculated in relation to the concentration of the elements in the concentrate, i.e., the ratio between the mass of dissolved elements in the bioleachate and the mass of elements in the concentrate multiplied by 100. These solubilizations increased in *Bioleachate 2* with the respective values of 29.8%, 64.3% and 94.6% (Figures 3 and 4). These solubilization yields increased further in *Bioleachate 3* with the respective values of 36.4%, 89.1% and 99.6%. The increase in the activity of microorganisms producing sulfuric acid during the reuse of *Bioleachate 1* and *Bioleachate 2* caused these increases. The addition of 5% bacterial inoculation at each reuse may increase the quantity of microorganisms present in the bioleachate, which could explain the increase in bacterial activity. In addition, the microorganisms initially present in the bioleachate are already well acclimated to the sulfides, yielding the decrease in the latency time of the microorganisms. As shown in Table 2, a decrease in the pH value (pH = 1.6) in *Bioleachate 2* and *Bioleachate 3* was also noticed compared to *Bioleachate 1* (pH = 1.7). After treatment with NaOH, the bioleachate had a pH = 4 and then adjusted to pH = 1.7 before starting the reuse. After 14 days of bacterial oxidation, the pH of the bioleachate decreased to pH = 1.5.

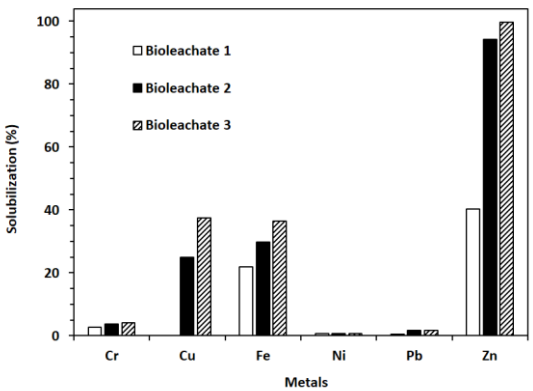
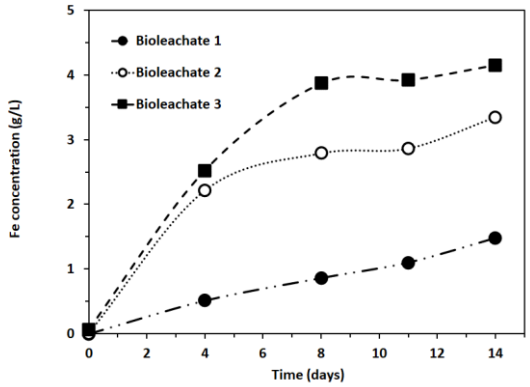
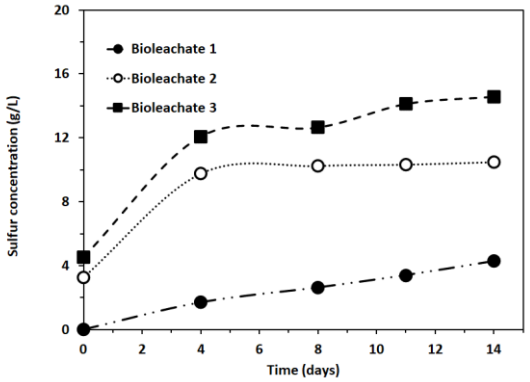


Figure 3. Metals' solubilization from the gold concentrate during the biooxidation process using new (*Bioleachate* 1) and reuse effluents (*Bioleachate* 2 and *Bioleachate* 3) as a culture medium.



(a)



(b)

Figure 4. Variation of iron (Fe) and sulfur (S) concentration in solution as a function of time during the bacterial oxidation process. (**a**): Iron; (**b**): Sulfur.

Figure 4 compares the dissolution progress of Fe and S of the refractory gold sulfide concentrate over 14 days for the three successive bacterial pre-oxidation tests. The iron concentration of *Bioleachate 1* increased steadily over 14 days to reach a final concentration of 1477 mg/L. The iron concentration in *Bioleachate 2* seemed to be stabilized after 4 days, while that of *Bioleachate 3* reached a plateau after 8 days. It is interesting to note that the final concentration of iron in bioleachates increased with their reuse, i.e., 1477 mg/L for *Bioleachate 1* compared to 3926 mg/L for *Bioleachate 3*. The addition of an extra 5% of bacterial inoculation in the bioleachate could explain this increase. It should also be emphasized that the subsequent bioleachates were treated to remove iron, and this is the reason the initial concentration of all bioleachates started at almost zero up to a final value of 3926 mg/L.

Similarly, an appreciable increase in the final concentration of soluble sulfur was noted during these tests, i.e., from 4277 mg/L for *Bioleachate 1* compared to 12,645 mg/L for *Bioleachate 3*. Figure 4 shows that the increase in the solubilization of iron and sulfur in the solution was notable after only 4 days of reaction. Thus, the reuse of bioleachate seemed to accumulate many sulfates. Other precipitates associated with the iron precipitates could reduce the accumulation of sulfur during the iron precipitation.

The increase in zinc solubilization could also be of electrochemical origin, i.e., by the galvanic interaction between pyrite and sphalerite particles [29]. Pyrite, less soluble than sphalerite, acts as a redox catalyst (i.e., an electron acceptor) by providing an additional surface for electronic transfer to ferric ions and establishes a galvanic coupling with sphalerite (the electron donor) to speed up its dissolution [30,31]. Indeed, the solubilization

of Zn depends on the amount of Fe^{3+} ions in the bioleachate because it is the Fe^{3+} ion that attacks zinc. In *Bioleachate 2* and *Bioleachate 3*, the concentration of dissolved iron was higher than in *Bioleachate 1*. This may show that the reuse of bioleachate could improve the solubilization of other metals as well.

The solubilization of chromium (Cr), nickel (Ni) and lead (Pb) was low and did not exceed 5% during the three successive bacterial pre-oxidation tests (Figure 3). Cr and Ni were probably associated with the silicate matrix, which was less attacked by the sulfuric acid produced by the bacteria. Regarding Pb, even if it is found mainly associated with galena (PbS), lead sulfate is poorly soluble, which makes it unfavorable to be dissolved by microbial leaching [2].

With copper (Cu), its solubilization was low (14.8%) in *Bioleachate 1*; then, it increased (24.9% and 37.5%) in the two bioleachates from the two reuse tests (Figure 3). The attack of Fe^{3+} ions, as a strong oxidizing agent generated by the bacterial oxidation of sulfides, can explain the increase in the solubilization of copper in the reused bioleachates [32].

Since the ferric iron precipitation at pH = 4.0 was selective, there were still some amounts of dissolved heavy metals left in the bioleachate. Repeated reuse of bioleachate builds up concentrations of dissolved metals in solution. In this study, the concentrations of metals, such as Al, Cr, Cu, Fe, Mn, Ni, Pb and Zn, in the bioleachate were analyzed by ICP-AES. The results are shown in Table 2. The highest concentrations of metals were found in Fe and Zn, whose quantity increased with the number of bioleachate reuse. For *bioleachate 1*, *bioleachate 2* and *bioleachate 3*, the concentrations of Fe and Zn were 1477 mg/L; 3349 mg/L; 3926 mg/L and 1097 mg/L; 3107 mg/L; 3861 mg/L, respectively. Excessive accumulation of these metals in bioleachate may limit the activities of microorganisms to dissolve metals. These dissolved metals, such as Zn, Pb, Ni, Mn, Cu, Cr, Co, Al and Ag, can become toxic to microorganisms if their concentration in the solution is high [5]. The results presented in Table 2 show, however, that the increase in dissolved metal concentrations was moderate and did not significantly affect the growth of microorganisms, given the very good sulfide oxidation activity noted during the second bioleachate reuse test. Zinc is a non-ferrous metal with the highest increase in concentration with a value of 3.86 g/L at the end of the second bioleachate reuse test. It would even be interesting to assess the economic profit in recovering this metal after a certain number of bioleachate reuse cycles. Solvent extraction techniques, combined with electrodeposition, would be potentially interesting to concentrate and purify this metal [33,34].

The high amounts of iron and sulfur in reused bioleachate resulted in a more rapid destruction of the sulfide matrix and exposure of the gold encapsulated in it [35]. Since the reuse of bioleach increases the amount of dissolved metals during the sulfide pre-treatment, it can also decrease the residence time of the pulp in the bacterial oxidation tanks and significantly reduce the operating and capital cost of equipment prior to cyanidation. In addition, the reuse of bioleachate is beneficial for the maintenance of bacterial activity during bacterial oxidation because of the strong sorption of certain toxic elements for microorganisms, such as arsenic, in the iron oxyhydroxide residue [35]. Indeed, the reuse of bioleachate in a bacterial oxidation cycle could decrease the adaptation time of the microorganisms with the sulfide concentrate before multiplying in the reactor.

3.4. Nutrients Availability

Table 3 shows the associated benefits of saving reagents (K, Mg, Ca) when reusing bioleachate. Reuse allows the addition of only a few essential reagents to maintain the growth of microorganisms. The two essential reactive components envisaged to be added to bioleachate for all reuses are ammonium (NH_4^+), as a source of nitrogen, and phosphate (PO_4^{3-}), as a source of phosphorus, to ensure the optimal growth of microorganisms [36]. The decrease in the ammonium and phosphate concentrations during biooxidation treatment can be explained by the formation of jarosite precipitate $XFe_3(SO_4)_2(OH)_6$ where X is a variable and can comprise Na^+ (Natrojarosite), K^+ (Potasium jarosite), NH_4^+ (Ammoniojarosite). Phosphate can also be lowered by the precipitation of ferric phosphate.

Table 3. Concentration (mg/L) of the soluble nutrients in the initial culture media and bioleachates.

Medium		Nutrients				
		NH_4-N	PO_4-P	K	Mg	Ca
Synthetic 9K media		914	126	140	710	10.0
Bioleachate 1	Non-treated	1882	42.6	150	123	169
	Treated	429	3.0	71.3	57.7	76.6
Bioleachate 2	Non-treated	1638	284	398	126	188
	Treated	543	143	248	47.7	44.8
Bioleachate 3	Non-treated	2004	284	510	114	205

The treated bioleachates initially contained most of the nutritional elements necessary to maintain the survival of the microorganisms. Only the addition of nitrogen and phosphorus to the bioleachate is necessary before its reuse. The obtained results presented in Table 3 show that the NH_4-N value in untreated bioleachates 1, 2 and 3 was increased. This increase could be explained by the addition of 5% bacterial inoculum during the reuse of each of these bioleachates. However, after treatment of the bioleachates, a significant decrease in NH_4-N of bioleachates 1 and 2 was observed. This decrease could be explained by the formation of ammoniojarosite. For the PO_4-P value in bioleachate 1, there was a noticeable decrease in its value from 126 to 42.6 mg/L. This decrease could be explained by the precipitation of ferric phosphate in the biooxidation 1 test. The addition of nitrogen and phosphorus and the 5% bacterial inoculation at each bioleachate reuse test could, however, explain the increase in PO_4-P in the second and third biooxidation tests. In addition, the sulfide concentrate used initially contained 96 mg/kg phosphate.

Regarding potassium (K), this comes both from the ore and from the supply of KH_2PO_4, used as a source of phosphate. The concentration of this element in the solution increased during the three bacterial oxidation tests. This can be explained by a high content of K in the concentrate, which can be solubilized under acidic conditions. However, a decrease in K during the treatment of the bioleachates was observed, which could be caused by the formation of the potassium jarosite. The concentration of magnesium (Mg) in the three bioleachates was relatively unaffected by the reuse process. This element is solubilized from the carbonate magnesium minerals present in the processed ore [21]. As for calcium (Ca), there was a progressive increase in the bioleachates, which could be explained by a high concentration of Ca in the concentrate.

3.5. Cyanidation Test

Figure 5 illustrates the kinetics of gold solubilization during the cyanidation tests of the three concentrates, having undergone the bacterial pre-oxidation step. The solubilization curves are practically similar, and the final yields after 48 h of cyanidation are very similar, i.e., 90.5%, 90.2% and 89.8% for *Concentrate 1*, *Concentrate 2* and *Concentrate 3*, respectively.

Some ions, such as iron, copper, cobalt, nickel, zinc, can consume cyanide during gold cyanidation. Mubarok et al. [37] reported that the high consumption of sodium cyanide (NaCN) during cyanidation of bioleached residue is due to the redissolution of sulfur and iron that are precipitated during bacterial oxidation and reacted with the cyanide ion to form thiocyanate and ferrocyanide complexes. If these heavy metals can be minimized before cyanidation, the cyanide consumption can be reduced. In this study, the result of reuse of bioleachates in new cycles of bacterial oxidation of a refractory gold sulfide concentrate showed a significant increase in some dissolved heavy metals from the sulfide concentrate. Indeed, the high amounts of certain dissolved heavy metals in the bioleachates means that the concentration of heavy metals in the bioleachate residue (solid fraction intended for cyanidation) also decreases. This could explain the decrease in cyanide consumption during bioleachate reuse.

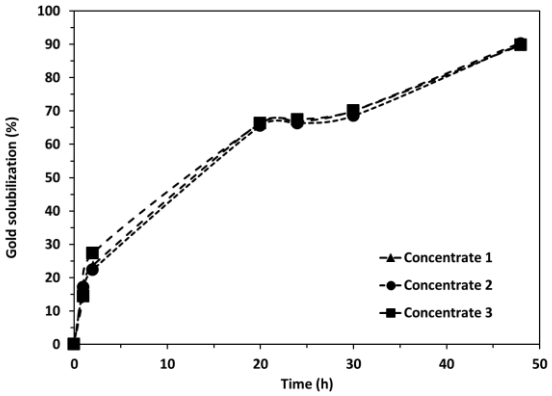


Figure 5. Dissolution kinetic of gold by cyanidation of the concentrates treated by the bacterial oxidation process.

The works of Ciftci and Akcil [38] on the bacterial oxidation of refractory gold with the extreme thermophilic bacterium produced similar results, and they obtained 92% of the dissolved gold after cyanidation with a consumption of 17.5 kg per ton of sodium cyanide. However, the cost associated with the optimal growth temperature of this bacteria may decrease the efficiency of its use in a refractory gold bacterial oxidation process. Pulp density may also limit the use of this extreme thermophilic bacterium because its oxidation reaction is exothermic. Since the dissolution of refractory gold sulfide is a slow process, it is therefore necessary to find a technique to speed up it. The contribution of Ahna et al. [39] to the improvement of bacterial oxidation using the sand farming process has yielded significant results, with 96% of refractory gold recovery. This reduces the costs associated with grinding and reagents. However, the pre-oxidation time before cyanidation is long, including 83 days in a batch reactor. The works of Carvalho et al. [1] on pretreatment of refractory gold by bacterial oxidation also showed a long pre-oxidation time of 40 days, with 85% gold recovery after cyanidation with a D_{100} particle size of 37 µm. Our results show bioleachate reuse may contribute to improving the recovery of refractory gold. After 14 days of bacterial pre-oxidation in a batch reactor, about 90% of the gold was dissolved after 48 h of cyanidation, while the direct cyanidation was only 68% [21]. This result could be further improved by extending the bacterial pre-oxidation time from 16 to 20 days.

4. Conclusions

The reuse of bioleachates during the bacterial oxidation of refractory gold sulfide concentrate was evaluated in this study. The results revealed that the reuse of bioleachates accumulates the quantities of dissolved elements despite the application of a ferric iron precipitation treatment at pH = 4.0. The microorganisms responsible for dissolving metal sulfides are more active when reusing bioleachates than when using a new culture medium. The presence of certain dissolved metals in the reused bioleachate exerts a positive influence on the dissolution of other metals. Nitrogen and phosphorus are the only two nutrients to be added to bioleachate when it is reused. In addition, we showed that the gold dissolution yield by cyanidation of microbially treated sulfide is 90%, with or without the reuse of bioleachates. Through this study, we showed that the reuse of bioleachate has positive impacts on the overall performance of bacterial oxidation, representing a great potential to save water and chemical reagents.

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