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Bioleaching for Copper Extraction of Marginal Ores from the Brazilian Amazon Region

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Abstract: The use of biotechnology to explore low-grade ore deposits and mining tailings is one of the most promising alternatives to reduce environmental impacts and costs of copper extraction. However, such technology still depends on improvements to be fully applied in Brazil under industrial scale. In this way, the bioleaching, by *Acidithiobacillus ferrooxidans*, in columns and stirred reactors were evaluated regarding to copper extraction of a mineral sulfide and a weathered ore from the Brazilian Amazon region. Samples (granulometry of 2.0/4.75 mm) were characterized by X-ray diffraction (XRD), energy dispersive X-ray fluorescence (EDXRF) spectrometry and scanning electrons microscopy (SEM). The pH and Oxidation-reduction potential (Eh) were daily monitored and leachate samples were collected for copper extraction determination by EDXRF. After 47 days, the columns bioleaching efficiency was 1% (1298 mg $Cu{\cdot}L^{-1}$) and 0.95% (985 mg $Cu{\cdot}L^{-1}$) for 2.00/4.75 mm sulfide ore, respectively, whereas the stirred reactors bioleaching resulted in 4% (348 mg $Cu{\cdot}L^{-1}$) for the mineral sulfide and 47% (295.5 mg $Cu{\cdot}L^{-1}$) for the weathered ore.

Keywords: biomining; bioleaching; mining tailings; copper; waste management

1. Introduction

Brazil is one of the biggest mineral producers in the world (1st in niobium, 3th in iron ore and 3th in bauxite/alumina) however, regarding copper it is not even in the top ten ranking biggest producer [1]. In 2016, Brazilian's mining companies processed 89 million tons of copper containing ores, with an copper average of 0.61%; and Pará State, in the Amazon region, concentrated 87% of this processing [2]. During the same period, Brazil imported 1.7 billion dollars on copper containing materials (raw materials, manufactured products and chemicals) mainly from Chile, Peru, United States and China [1]. New strategies will be necessary to improve Brazilian's copper production through the exploration of low-grade ore deposits and mining tailings, reducing the external dependences of this metal in a future scenario of a high-grade ores' depletion and environmental conservation pressure against new pits opening. Bioleaching is a well-stablished economical and a more environmentally friendly alternative to the processing of low-grade copper ores (Cu < 0.5%) and mining tailings, responding for 7% of the world's copper production (17 million tons) [3], however this technology has not been commercially applied in Brazil yet. Bioleaching has also gaining force for metal concentrates, electronic wastes, spent industrial catalysts, contaminated soils and wastewater valuable metals recovery and can improve Brazilian's economical circularization [4].

The concept behind bioleaching is the microbial production of primary products responsible to the oxidation/solubilization of mineral sulfides, releasing the metals of interest in the leaching solution before a further recuperation by electrochemical/biosulfidation methods [5,6]. The most studied microorganism involved in sulfide ores bioleaching is the aerobic, mesophilic and extremophile acidic bacteria *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) [5]. This chemolithotrophic bacterium oxidizes reduced forms of iron (Fe^{2+}) and sulfur as electrons and energy source, using carbon dioxide (CO_2) as a carbon source [7,8]. During the chalcopyrite oxidation process (Equation (1)), *A. ferrooxidans* oxidizes both the ferrous ions (Fe^{2+}) (Equation (2)) as well as the elemental sulfur (S^0) (Equation (3)) generated as a byproduct, resulting in the formation of ferric ions (Fe^{3+}) and H^+, that attack the chalcopyrite ($CuFeS_2$) in a loop effect [7–9].

$$CuFeS_2 + 4H^+ + O_2 \rightarrow Cu^{2+} + Fe^{2+} + 2S^0 + 2H_2O, \tag{1}$$

$$4Fe^{2+} + 4H^+ + O_2 \rightarrow 4Fe^{3+} + 2H_2O, \tag{2}$$

$$2S^0 + 3O_2 + 2H_2O \rightarrow 2SO_4^{2-} + 4H^+, \tag{3}$$

$$CuFeS_2 + 4Fe^{3+} \rightarrow Cu^{2+} + 2S^0 + 5Fe^{2+}, \tag{4}$$

There is a gradient complexity of biohydrometallurgy methods according to ore pre-processing efforts, such as dump (without ore size reduction), heap (crushed/agglomerated ores), "in situ" (underground leaching) and stirred reactors bioleaching (crushed ore/concentrate) [10]. Heap bioleaching is the most widespread commercial method being applied to nickel, zinc, cobalt and copper containing ores processing [10]. Heap bioleaching can also be applied for the detoxication of tailings containing high concentrations of heavy metals, such as arsenic [11]. The main advantages of heap bioleaching is the lower investments with infrastructure and operational costs due to the technological simplicity and lower energy requirement [12] when comparing with traditional pyrometallurgical and hydrometallurgical methods that annually consumes 6.13×10^8 GJ of energy for copper extraction [13]. Heap bioleaching of crushed secondary sulfide ores (chalcocite and covellite) comprises almost 80% of all industrial scale projects [3]. Despite its high abundance (70% of the world's copper reserves [4]) chalcopyrite is very recalcitrance to bioleaching resulting in slow copper recovery [14] and only few pilot plants have been testing the bioleaching of primary copper ores [10]. Bench-scale column tests are a useful tool to optimize variables such as temperature, pH, Eh, granulometry, aeration rate and bacterial species, among others, that directly affect sulfide ores bioleaching efficiency before the commercial heap implementation [15,16].

Weathered ores containing high clay levels and sulfide ores containing high levels of fine particle are unsuitable to heap leaching (unless it pass by an agglomeration step) compromising the permeability and solution's diffusion through the column, resulting in lower efficiency [12] and can alternatively be processed in stirred reactor. Due to its higher costs when comparing to heap leaching, stirred reactors bioleaching have been commercially used mainly for refractory gold concentrates processing [17]. As the major advantages when comparing with heap leaching, the use of stirred reactors allows for the total control of variables such as pH, redox potential, O_2 and CO_2 mass transfer and temperature, maximizing the bioleaching performance [18,19]. The stirred reactors bioleaching of copper concentrates have also been tested [20–22] but some variables optimization such as pulp density, agitation rate, temperature and microbial diversity are still necessary to enhance the primary recalcitrant ores bioleaching efficiency [23–25].

The aim of this work was to evaluate the bioleaching potential of copper ores from the Brazilian Amazon region, in replacement to pyrometallurgical/hydrometallurgical methods traditionally applied for copper recovery. To achieve that, we evaluated the effect of ore granulometry in column leaching tests and the leaching efficiency of weathered and copper sulfide ores in stirred reactors, by the bacteria *Acidithiobacillus ferrooxidans*.

2. Materials and Methods

2.1. Sampling

Copper mineral sulfide and weathered copper ore samples were collected at a copper mine located at Pará State, in the Brazilian Amazon region. Before chemical and mineralogical analysis, 100 g of samples were pulverized in a mortar (RM200, Retsch GmbH, Haan, Germany) and sieved to reach granulometry <0.15 mm. For the columns leaching tests, the mineral sulfide was milled and sieved resulting in two granulometric fractions: 2.0 mm and 4.75 mm. The mineral sulfide and weathered ore samples were manually milled and sieved until reaching a granulometry <1.68 mm prior to stirred reactor bioleaching test.

2.2. Chemical, Mineralogical and Morphological Characterization

Ores samples mineralogical composition was determined by X-ray diffraction—XRD in an diffractometer (Empyrean, PANalytical, Almelo, The Netherlands) with a Cu kα radiation, 40 kV/40 mA, scan range from 4 to 75°2θ, 0.02°2θ step size, pixCell 1D detector. Powder samples were prepared by backloading method in a sample holder. Chemical composition was determined in an energy dispersive X-ray fluorescence spectrometer - EDXRF (Epsilon 3^{XLE}, PANalytical, Almelo, The Netherlands) equipped with a rhodium tube and 15 W maximum potency.

For the morphological studies, ore fragments were mounted in an aluminum stub under an adhesive carbon tape. The particles were covered with a thin gold layer under vacuum (Desk V metallizer, Denton Vacuum, Moorestown, NJ, USA) before scanning electrons microscopy—SEM (Vega 3, Tescan, Kohoutovice, Czech Republic) and back-scattered electrons—BSE visualization and energy-dispersive spectroscopy—EDS (Oxford Instruments, Abingdon-on-Thames, Reino Unido) elemental analysis.

2.3. Assembling of the Bioleaching Columns

Acidithiobacillus ferrooxidans LR [26] was cultivated in 0.4 L of T & K8 liquid medium in 1 L Erlenmeyer flasks incubated in a rotatory shaker at 150 RPM and 30 °C, for 72 h, using iron sulfate ($FeSO_4{\cdot}7H_2O$) as electrons donor and pH 1.8. Bacterial inoculum was added to 3.6 L of modified T & K8 liquid medium (without $FeSO_4{\cdot}7H_2O$) composing the leaching solution used for columns irrigation. Two acrylic tubes (0.5 m height and 0.09 m diameter) were filled with 3 kg of 2 mm (column 1) and 4.75 mm (column 2) particle size mineral sulfide ore. Leaching solution was irrigated at the top of the column using a peristaltic pump and collected by gravity in a 10 L plastic container. Leaching solution temperature was constantly maintained at 30 °C by a submersed electric heater (5 W) installed inside the tank and forced column aeration was supplied at a 0.05 $L{\cdot}s^{-1}$ rate by an air compressor. A scheme of the column bioleaching assemble is show in Figure 1.

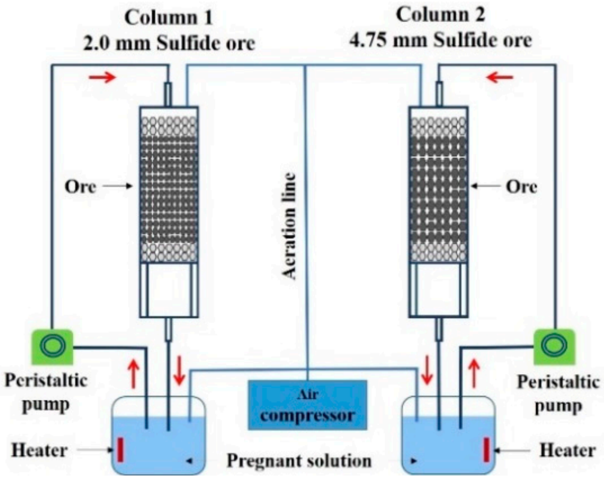

Figure 1. Columns bioleaching representative scheme.

2.4. Assembling of the Bioleaching Stirred Reactors

Bacterial inoculum (2.5 L) was cultivated as described above and added to 22.5 L of modified T & K8 medium (without $FeSO_4{\cdot}7H_2O$) composing the stirred reactors leaching solution. Two 50 L PVC cylindric tanks (0.56 m height × 0.4 m diameter) were used for the bioleaching test. Leaching solution (25 L) and 1250 g (5% m/v pulp density) of the sulfide ore (reactor 1) or weathered ore (reactor 2) were transferred to the reactors, being the pH adjusted to 1.8 by the addition of concentrated sulfuric acid. Both reactors were continuously stirred by a mechanical stirrer (450 RPM) equipped with a four-bladed style propeller. Reactor's temperature (30 °C) was maintained by a submerged electric heater (25 W) and air injection ($0.05\ L{\cdot}s^{-1}$) was supplied by an air compressor. Figure 2 shows the stirred reactors scheme.

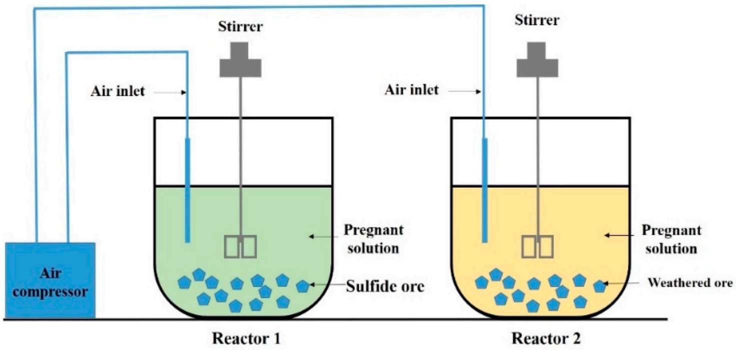

Figure 2. Stirred bioleaching reactors scheme.

2.5. Bioleaching Assessment

Both bioleaching experiments were conducted along 47 days. Redox potential (Eh) (FerMac 290 redox meter, Ag/AgCl/KCl probe) and pH (FerMac 260 pHmeter, EasyFerm Plus PHI K8 160 probe) were daily monitored. The leaching solution pH was maintained below 2.0 in both experiments to avoid passivating layers formation by the addition of concentrated sulfuric acid when necessary. Pregnant solution samples were collected once a week for soluble copper quantification by EDXRF. *A. ferrooxidans* population was quantified once a week by cells counting using a Neubauer chamber in an optical microscope (DM3000 400×, Leica, Wetzlar, Alemanha). Bacterial viability was also checked once a week by transferring 20 mL of leaching solution to 180 mL of T & K8 fresh medium in a 500 mL Erlenmeyer flask and incubating it in a rotatory shaker (150 RPM), at 30 °C, for 72 h. Bacterial activity was confirm by visual color change of T & K8 medium from green to rust-brown indicating bacterial iron oxidation. The leaching system's contamination by ore's derived bacteria was periodically checked by leaching solution sample's dilutions plating into Petri dishes containing solid T & K medium, followed by incubation at 30 °C, for 72 h and colonies visual evaluation.

3. Results and Discussion

3.1. Mineralogical, Chemical and Morphological Composition

Figure 3 shows the mineralogical composition of the investigated samples. The predominant minerals identified in the sulfide ore sample were: chalcopyrite (3.03 and 1.85Å), quartz (3.34 and 4.25 Å), magnetite (2.53 and 1.48 Å), chlorite (14.7 and 3.5 Å) and ferrous magnesiohornblende (3.12 and 8.40 Å). Regarding the weathered sample, the predominant minerals identified were: quartz (3.34 and 4.25 Å), aluminous goethite (4.17 Å), hematite (2.69 and 2.53 Å), illite (10 and 5 Å) and chlorite (14, 7 and 3.5 Å).

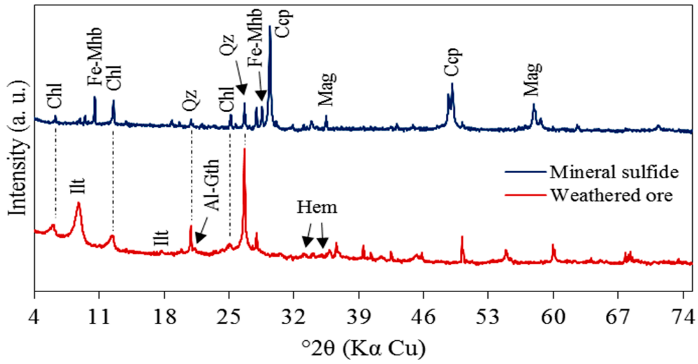

Figure 3. X-ray diffraction (XRD) diffractograms of mineral sulfide (blue line) and weathered ore (red line) samples. Chl: chlorite; Fe-Mhb: ferrous magnesiohornblende; Ccp: chalcopyrite; Mag: magnetite; Ilt: Illite; Al-Gth: aluminous goethite; e Hem: hematite.

Table 1 shows the chemical composition of the evaluated samples. The mineral sulfide samples showed the prevalence of sulfur, iron, copper and silicon, being the first three elements associated to chalcopyrite. An increase in copper and sulfur and a decrease in silicon, aluminum, calcium and magnesium concentrations were observed with the reduction of the mineral sulfide particles size. Silicon was associated to quartz and ferrous magnesiohornblende ($Ca_2(Mg,Fe^{+2})_4Al(Si_7Al)O_{22}(OH,F)_2$) being the later also the probable source of minor elements identified, such as aluminum, calcium and magnesium. Iron was also associated do magnetite (Fe_3O_4).

Table 1. Chemical composition (%) of the ore samples determined by energy dispersive x-ray fluorescence (EDXRF).

Sample	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	CaO	CuO	MgO	NiO	K_2O	P_2O_5	SO_3	Na_2O	Others
Mineral sulfide 4.75 mm	20.3	0.2	4.2	25.2	2.1	13.9	2.3	0.1	0.3	0.3	31.1	-	-
Mineral sulfide 2 mm	14.1	0.1	2.9	26.8	2.0	16.1	1.9	0.2	0.2	0.4	35.2	-	0.1
Mineral sulfide <1.68	11.4	0.1	2.5	26.1	1.6	17.4	1.5	0.1	0.2	0.4	38.6	-	0.1
Weathered ore <1.68	47.4	1.0	16.8	24.6	1.2	1.3	2.8	-	3.6	0.7	-	0.3	0.3

The weathered ore sample was composed mainly by the elements: silicon (47.77%), aluminum (16.66%) and iron (24.63%). The predominance of silicon was associated to the high level of quartz found in the sample as well as associated to the clay minerals illite and chlorite. Iron was single associated to the mineral hematite (Fe_2O_3) or in combination with aluminum in the structure of the minerals: aluminous goethite (FeAlO(OH)) and chlorite. The weathered ore sample contained 1.23% of copper, however, the source mineral could not be identified.

EDS-SEM morphological analysis allowed the visualization of gangue coupled chalcopyrite (Figure 4A). The weathered ore agglomerate was composed basic by heterogenous clay small fragments

(Figure 4B) with only few bighting hematite particles. The copper presence was only identified over the surface of a clay mineral together with the elements O, Si, Al, Fe and Mg in very few spots (Figure 4C). No other copper associated minerals were identified confirming the XRD results and the low contents of copper in the sample.

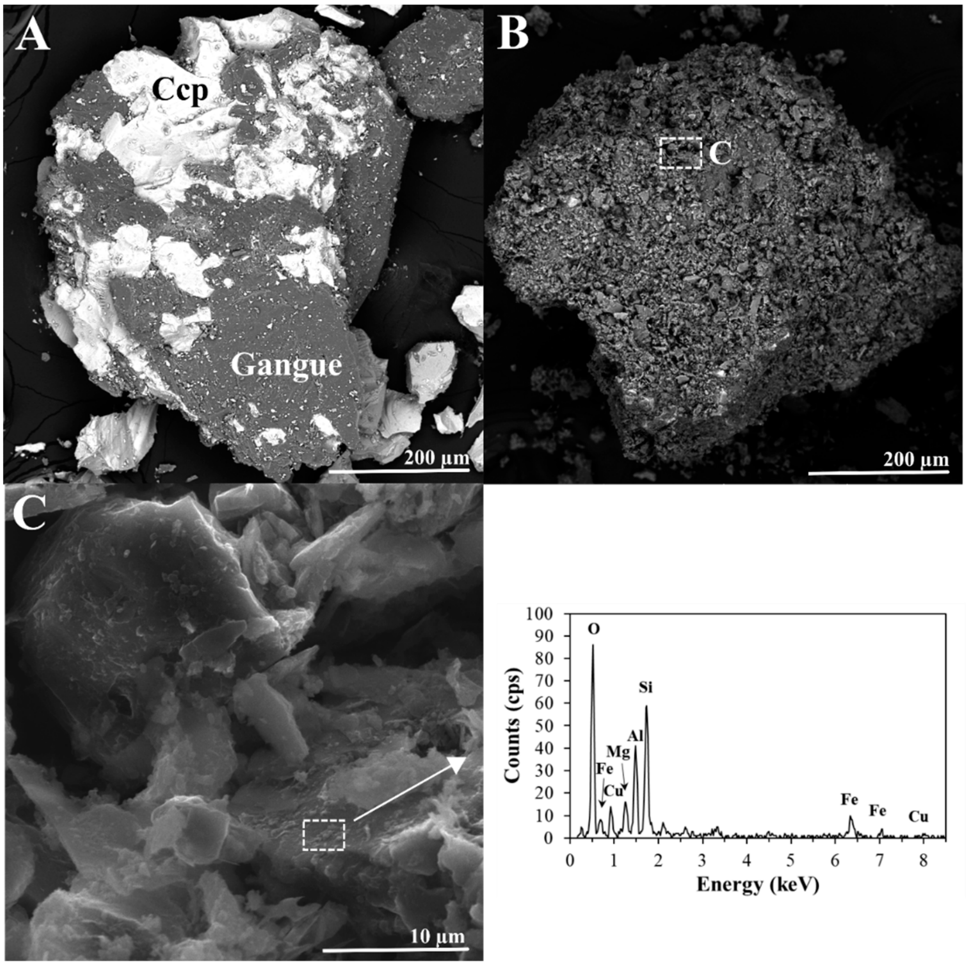

Figure 4. Back-scattered electrons-Scanning electrons microscopy (BSE-SEM) micrograph (HV: 20 kV): (**A**) Sulfide ore highlighting the presence of the chalcopyrite (Ccp) mineral and (**B**) weathered ore agglomerate showing the heterogenous small particles composition. Scanning electrons microscopy-Energy dispersive spectroscopy (SEM-EDS) (HV: 20 kV) micrograph: (**C**) Elemental composition of a clay mineral surface highlighting the presence of Cu with the elements O, Fe, Mg, Al, Si.

3.2. Shifts in *A. ferrooxidans* Population

Figure 5 shows the *A. ferrooxidans* population shifts along the column and stirred reactor bioleaching experiments and metabolic viability after 47 days. An increase in planktonic *A. ferrooxidans* population was observed in both columns and in the reactor 1 that received the mineral sulfide ore whereas it behaves stable on rector amended with weathered ore. *A. ferrooxidans* metabolic viability was also confirmed by visual iron oxidation in T & K8 medium after inoculation, as showed in Figure 5B,C.

The maximum *A. ferrooxidans* population increment was observed in reactor 1, growing from 7.8×10^6 cells·mL^{-1} after inoculation to 6.6×10^7 cells·mL^{-1} after 47 days. A common problem regarding stirred reactor bioleaching is the mechanical cell lysis caused by the collision against ore particles under agitation [27]. The proper adjustment of agitation speed, impeller type and ore pulp concentration is primordial to an efficient metal recovery [28]. In the present work the ore pulp applied in the reactors was reduced to 5% (*w*/*v*) in detriment of 12% previously used by Nascimento et al. [25]

evaluating the same ore samples, resulting in a greater copper recovery. No cell debris were observed during microscopic counting indicating bacterial integrity by the applied pulp density.

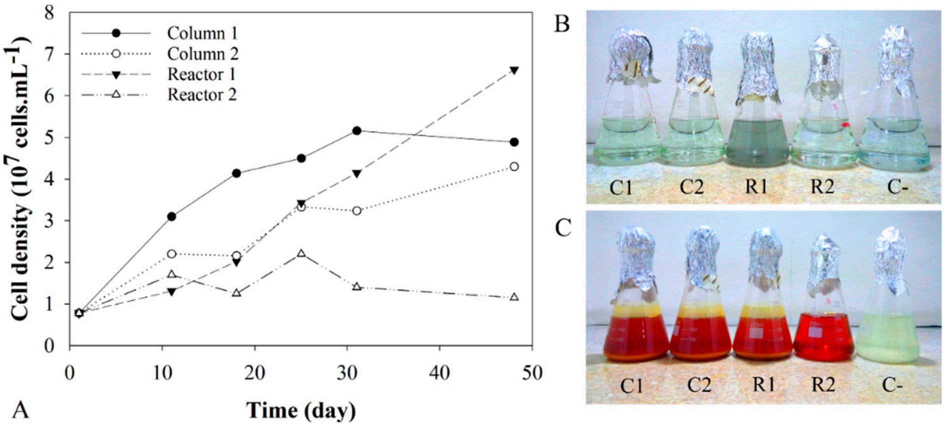

Figure 5. *A. ferrooxidans* population dynamics (**A**) along the columns and stirred reactors bioleaching experiments. Viability test of *A. ferrooxidans* cells collected from the columns/reactors leaching solution after the 50th day bioleaching showing the T & K8 medium color before (**B**) and after iron oxidation (**C**). C1: Column 1. C2: Column 2. R1: Reactor 1. R2: Reactor 2. C-: Negative control.

Planktonic bacterial population on columns leaching solution reached a final density around 4.9×10^7 cells·mL^{-1} (column 2) and 4.3×10^7 cells·mL^{-1} (column 2), after 47 days experiment (Figure 5A). The lower planktonic bacterial density on columns leaching solution is expected in function to bacterial adhesion and biofilm formation over the ore particles, as observed by SEM micrograph (Figure 6). Bacterial adhesion is mediated by the secretion of extra-cellular polymeric substances (EPS) allowing the microorganism to be close to the energy source [7]. Beyond EPS secretion, bacterial cell attachment to ore's surface showed to be affected by bacterial shape, with a trend of linear chains attachment for rod-shaped bacteria whereas filament-shaped ones tend to appear individually attached [4]. Other biological and physicochemical interactions, such as cell-cell and cell-quartz interactions were demonstrated in model microorganisms, such as *E. coli*. O157:H7 [29,30] and may be further investigated under bioleaching conditions. The presence of natural organic matter (NOM) in process waters negatively affect bioleaching efficiency by reducing bacterial attachment to mining tailings due to unfavorable interactions [31]. The attached bacteria contributes with the so called "contact leaching" mechanism by oxidizing the ferrous iron (Fe^{2+}) ions into ferric iron (Fe^{3+}) ions within the biofilm that attacks chalcopyrite (Equation (4)) [7,32]. A complete description about all the bioleaching mechanisms can be viewed in Vera et al. [32].

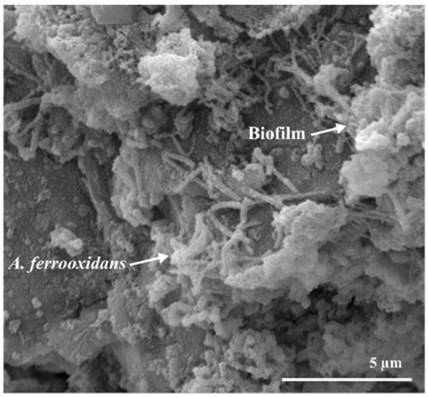

Figure 6. Scanning electron microscope (SEM) micrograph (HV:10.0 kV) showing *A. ferrooxidans* adhesion and biofilm formation over the sulfide ore particle during column bioleaching.

By the other side, *A. ferrooxidans* planktonic population few variated in reactor 2 along the experiment, reaching maximum density after 22 days (2.2×10^7 cells·mL^{-1}) and a final population of 1.1×10^7 cells·mL^{-1} (Figure 5A). The observed effect was probably due to the lack of reduced forms of iron and sulfur as an energy source for the bacterial growth in the oxidized weathered ore. Hematite was the main iron constituting mineral in the weathered sample and different from chalcopyrite and magnetite, it is composed only by oxidized Fe^{3+} ions, with no available energy to sustain bacterial growth.

3.3. pH and Eh Monitoring

The leaching solution pH was daily monitored and adjusted to 1.8–1.9, when necessary, in order to prevent the formation of passivating agents, such as jarosite, favored under pH values above 2.0 [33] and to promote optimal condition to bacterial growth [8] (Figure 7A). Different from pyrite, chalcopyrite is susceptible to protons attack, being dissolved under pH < 4.0. The sulfur moiety from chalcopyrite is oxidized to S^0 via the polysulfide mechanism [32]. *A. ferrooxidans* is also able to oxidize the elemental sulfur generating sulfuric acid (Equation (3)), however, proton attack seems to be less important than Fe^{3+} mechanism for chalcopyrite leaching [34].

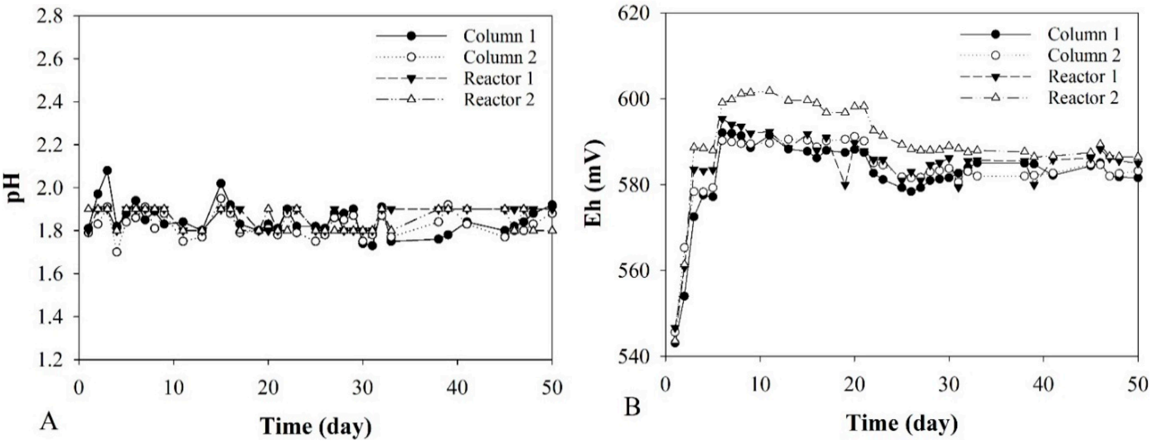

Figure 7. pH (**A**) and Eh (**B**) behavior of the columns and stirred reactors leaching solution.

Redox potential started around 540 mV in all experiments raising to 590–600 mV after the 6th day operation (Figure 7B). The observed initial increase in Eh was due to the ferrous iron oxidation from T & K8 ($FeSO_4 \cdot 7H_2O$) culture medium incorporated to the system with the bacterial inoculum. An available initial source of energy is essential during bacterial adaptation stage before the ore bioleaching start. After the 6th day, Eh in the both leaching columns and reactor 1 became stable between 580–590 mV until the end of the experiment. An increase to 600 mV was observed after the 6th day until the 21th day in the reactor 2 amended with the weathered ore probably due to the accumulation of Fe^{3+} in the absence of reduced mineral sulfides, reaching a final value of 586 mV at the 50th day (Figure 7B). Santos et al. also observed an increase from 300–400 mV to 570–600 mV (Ag/AgCl/KCl) in leaching solution Eh during the seven first days of chalcopyrite bioleaching by *A. ferrooxidans*, under different ferrous ions concentrations [35]. Redox potential of leaching solution is recognized as one of the most important issues on chalcopyrite bioleaching and it is mainly controlled by the $[Fe^{3+}]/[Fe^{2+}]$ ratio dynamics along the process [35]. One of the proposed mechanisms is that Eh must allow the initial reduction of chalcopyrite to chalcocite (Cu_2S) and its subsequent oxidation and releasing the soluble copper which is achieved under 400–450 mV (Ag/AgCl/KCl) [10]. Above this Eh range, Fe^{3+} ions can precipitate in the form of ferric salts, such as jarosite, passivating the leaching process [36]. In this case, $[Fe^{3+}]/[Fe^{2+}]$ ratio must be balanced by increasing Fe^{2+} concentration, resulting in higher Cu dissolution rates by avoiding passivation layers formation [37]. However, Nguyen and co-workers [37] observed a decrease on Cu leaching efficiency when the initial Fe^{2+}

concentration was greater than 10 g·L^{-1} when evaluating the chalcopyrite bioleaching using adapted mesophilic bacteria. The balancing of [Fe^{2+}]/[Fe^{3+}] ratio (~1) and Eh control is primordial for efficient chalcopyrite leaching rates obtention [37].

3.4. Copper Extraction

3.4.1. Columns Bioleaching

Figure 8 shows the Cu extraction efficiency (A) and Cu concentration (B) in the pregnant solution along the columns and stirred reactors bioleaching experiments.

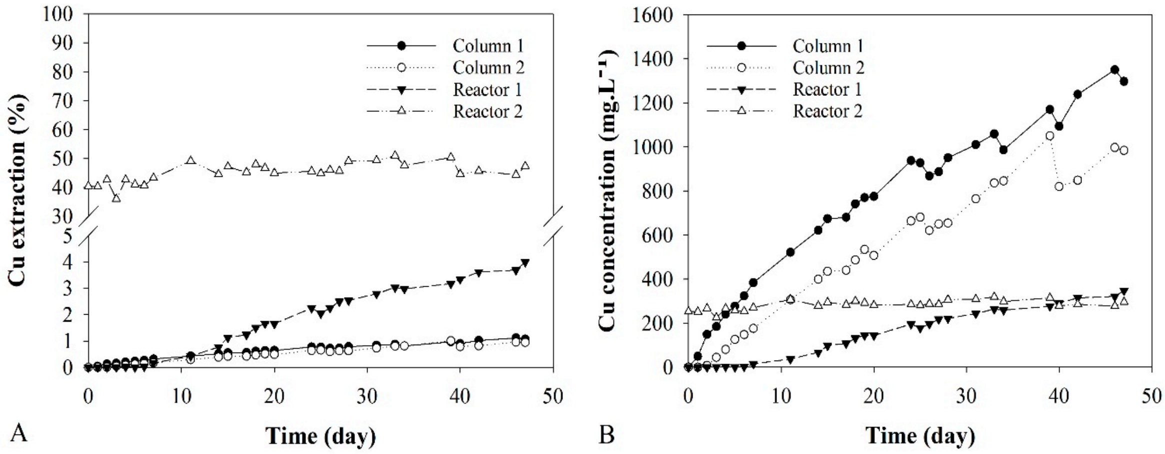

Figure 8. Copper extraction (%) efficiency (**A**) and soluble copper concentration (mg·L^{-1}) in the pregnant solution (**B**) along the sulfide (reactor 1) and weathered (reactor 2) ores bioleaching experiment using *A. ferrooxidans*.

The copper extraction efficiency obtained after 47 days bioleaching in column 1 (2.0 mm) and column 2 (4.75 mm) were 1.07% and 0.95%, respectively (Figure 8A). Despite the low extraction efficiency, a linear growing trend was observed indicating that the bioleaching process was not passivated after 47 days and higher rates could be obtained in a longer experiment. A higher copper concentration in the pregnant solution (Figure 8B) was obtained in column 1 (1298 mg·L^{-1}) when compared to column 2 (985 mg·L^{-1}) after 50 days, probably due to the higher initial copper content in the sample. Chalcopyrite heap bioleaching can take years in contrast of secondary sulfide ores, such as chalcocite and covellite that requires months, making its commercial application more difficult [7,12]. In general, bioleaching efficiency is inversely proportional to the ore particles size, increasing with the particle size decrease [12,15,36]. However for commercial proposals, the increment on copper recovery cannot be surpassed by the energy costs expend on ore gridding [7]. In the present work, a substantial increment on copper recovery efficiency was not observed when comparing the particle sizes of 2.0 mm and 4.75 mm in a bench-scale, however, it may be significant under a higher scale. Wang and co-workers obtained 93.11%, 91.04% and 80.45% copper recovery from a low-grade sulfide ore (0.99% of total Cu; 35% chalcopyrite, 60% bornite and 5% chalcocite) with particle sizes varying from 5–8 mm, 5–15 mm and 5–20 mm, respectively, confirming the particle size effect [23]. Beyond the ore particles size, in general, heap bioleaching may also be affected by aeration rates, additives use (e.g., chloride and silver ions), temperature, microbial diversity and cells pre-adaptation to ores bleaching [3,14,16,36–38]. These parameters may be tested in future columns bioleaching experiments in order to increase the Amazon region sulfide ores bioleaching efficiency.

3.4.2. Stirred Reactors Bioleaching

Reactor 1 showed a maximum copper extraction efficiency of 4% (Figure 8A) corresponding to a final soluble Cu concentration of 348 mg·L^{-1} in the pregnant solution (Figure 8B), after 47 days bioleaching. The Cu solubilization from the sulfide ore started only after the 6th day, followed by a linear trend increment (Figure 8B) in agreement with the observed lag phase on *A. ferrooxidans* growth in the same reactor (Figure 5A). A previous study evaluating the stirred tank bioleaching potential of mineral sulfides from Carajás region [25] obtained an extraction efficiency of 0.47% (65 $mg \cdot L^{-1}$ of Cu) after 50 days. The copper bioleaching rate obtained in the present work was 8.5 times higher than those obtained by Nascimento et al. [25] in response mainly to the pulp density reduction from 12% to 5% and the constant temperature control (30 °C). The increase in copper solubilization was probably correlated to the lower bacterial mechanical damage due to the pulp density reduction, as discussed above and increase of *A. ferrooxidans* metabolic efficiency due to the constant optimum temperature maintenance.

Comparing with the columns approach, stirred reactors bioleaching of the sulfide ore sample showed a higher Cu recovery efficiency, however this system is limited by the ore pulp density which resulted in an average three times lower Cu concentration on the pregnant solution (Figure 8B). Stirred reactors bioleaching has a much higher operational cost and requires an extra capital investment when compared to heap bioleaching that restrict its use mainly to of high-value metals concentrates [14]. Recent works obtained higher efficiency during stirred bioreactor bioleaching of copper concentrates containing chalcopyrite applying moderated thermophilic bacterial consortium including *Leptospirillum ferriphilum*, *Acidithiobacillus caldus*, *Sulfobacillus* sp. under temperatures above 42 °C [20,21,24]. Zeng et al. [24] obtained 62% copper extraction (12.6 $g \cdot L^{-1}$) from chalcopyrite (6% pulp density) bioleaching in a 3 L stirred bioreactor inoculated with moderate thermophilic bacteria at 48 °C and 500 RPM. Recently, Hedrich et al. [21] have been proposed a dual step reactors bioleaching process where the first step comprised for the growth of the bacteria followed by a 4 °C increasing in reactors temperature during the chalcopyrite bioleaching step increasing copper recovery [21]. The authors obtained 96% (approximately 15 $g \cdot L^{-1}$) copper recovery from a copper concentrate from black shale processing containing chalcopyrite setting the initial temperature to 46 °C during the first three days for bacterial growth and 50 °C during the metal bioleaching step [21]. Another strategy to increase metal recovery efficiency is the bacterial pre-adaptation to bioleaching by successive replacement of the energy substrate in the culture medium by the mineral sample along subcultures [38]. This technique showed to be more efficient during biooxidation of refractory gold concentrates for arsenic removal, when compared to unadapted microbial cultures [39]. Adapted mesophilic microorganisms, such as *A. caldus* and *L. ferriphilum* were also used for chalcopyrite bioleaching in Erlenmeyer flasks, resulting in 64% Cu bioleaching efficiency after 14 days [37]. The use of pre-adapted moderate thermophilic bacterial consortia might be evaluated in the future as a strategy to increase the stirred reactor bioleaching rates of sulfide ores from Amazon region, in Brazil.

Regarding the weathered ore sample bioleaching (reactor 2), a constant leaching efficiency with an average of 45% was obtained along 47 days (Figure 8A). The obtained rate was higher than those found by Nascimento and co-workers [25] of 17.5% of copper extraction after 50 days reactor bioleaching of an weathered ore (1.4% Cu) from Carajás region. Since the first day experiment we observed a copper concentration in the pregnant solution of 250 $mg \cdot L^{-1}$, suggesting a chemical leaching effect rather than the bioleaching (Figure 8B). No copper associated mineral was observed in the weathered ore after XRD (Figure 2) not allowing us to track the copper origin in this sample. One hypothesis is that oxidized copper released from matter rock weathering process along soil formation history or soluble copper leached from metallic sulfide ores were adsorbed by the clay fraction, specially illite, as identified by XRD. Turan et al. [40] confirmed the capacity of illite to adsorb copper ions leached from industrial effluents reaching 93% Cu^{2+} adsorption. Illite copper adsorption, complexation and precipitation increases with increasing pH [41]. Alvarez-Puebla et al. [41] evaluated the surface speciation of Cu retention on natural illite clays, observing a prevalence of hydroxy copper precipitates on clay surface under higher pH values (>5.79) in detriment of a preferred electrostatic copper ions sorption between pH 2.53 and 3.01. It is possible that copper hydroxides may be chemically leached by the sulfuric acid present in the in leaching solution, releasing the cupper ions (Cu^{2+}) from the clay surface. However, part of the copper may be still electrostatically sorted to the mineral surface at pH 1.8, explaining the remaining insoluble fraction on the reactor. A deeper evaluation might be conducted in the future to confirm the proposed hypothesis.

Despite the evidences of a chemical leaching instead of a bioleaching process, the use of bacteria for weathered ores stirred reactors copper recovery is still valid. Sulfur oxidizing bacteria, such as *Acidithiobacillus thiooxidans* can be used for biogenic sulfuric acid production, reducing the processing costs [42,43]. Weathered copper ores containing high amounts of clay minerals are not suitable heap leaching causing the solution percolation clogging[12], so the reactors leaching by using bacterial made sulfuric acid is a viable alternative to high copper leaching rates in a short time incubation.

4. Conclusions

Chalcopyrite was the main copper associated mineral in the sulfide ore whereas no copper associated mineral was observed in the weathered ore sampled from Brazilian Amazon region. A higher copper leaching efficiency was obtained in the stirred reactors bioleaching in response to the better pH and temperature control, higher *Acidithiobacillus ferrooxidans* density and surface area to Fe^{3+} and H^+ attack due to the lower particles' sizes. However, stirred reactors bioleaching is constrained by the higher costs and pulp density limitations making heap bioleaching more suitable of sulfide ore processing, based on columns bioleaching tests. Higher copper leaching efficient may be achieved using pre-adapted moderate thermophilic bacteria consortia. Copper recovery from the weathered ore was mainly caused by chemical leaching rather than bioleaching. Biotechnological approaches, such as biogenic sulfuric acid production, can potentially be used to reduce the processing costs because of the small copper amount.

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