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Bioleaching of a Chalcocite-Dominant Copper Ore from Salta, Argentina, by Mesophilic and Thermophilic Microorganisms

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Abstract: The study and development of new sustainable mining methods to exploit low-grade ores and secondary metallic resources are essential to meet global demand and contribute to caring for the environment. Copper is one of the most widely used metals and chalcocite is the main secondary sulfide of this metal. Therefore, the study of copper recovery from chalcocite-dominant minerals could have a great impact on the industry. In this study, we assess at bench scale the feasibility of applying biohydrometallurgical processes to extract copper from chalcocite-rich minerals from Taca Taca, Argentina, using native mesophilic microorganisms (30 °C) and thermophiles (45, 65 °C). The indigenous mesophilic consortium was dominated by *Acidithiobacillus ferrooxidans* and could solubilize all the copper present in the systems (113 mg/L) within three weeks without any change in the pH of the solution. Notably, by increasing the temperature up to 45 and 65 °C, copper leaching was enhanced, completing the recovery in 7–14 days. The oxidizing microorganisms active in these conditions were *Ferroplasma* sp. and *Acidianus copahuensis*, respectively. An increase in the abiotic copper recovery was also observed as temperature rose; as well as a slight acidification of the solution. This study constitutes the first assessment for the bioleaching of Taca Taca ores.

Keywords: chalcocite; bioleaching; copper sulfides; hydrometallurgy; Taca Taca project; mesophiles; thermophiles

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1. Introduction

Copper is a metal broadly used in everyday life. It is essential for constructions, electric power transmission, electronic communications, the aerospace industry, and scientific equipment, among other diverse fields of applications. Due to the increasing high-levels of global copper demand and the continuous depletion of high-grade copper deposits, the mining industry requests techniques for the exploitation of low-grade primary copper reservoirs and secondary copper resources (electronic and other copper-containing waste materials, scrap copper) [1,2].

Chalcocite (Cu_2S) is the main secondary copper sulfide mineral having the highest copper content. This sulfide is usually formed by oxidation, reduction, dissemination, and migration of primary sulfides such as chalcopyrite ($CuFeS_2$) [3]. Chalcocite is an important low-grade mineral ore for copper extraction; therefore, the copper mine industry faces the need to develop suitable technologies to exploit this type of low-grade ores in order to meet the global demand [4–6].

In the last decades, there has been much interest in the development of hydrometallurgical methods for metal extraction from sulfide minerals. Bioleaching, a branch of hydrometallurgy, utilizes the activity of microorganisms in aqueous extractive metallurgy for the recovery of metals from ores, concentrates, and recycled or residual materials [7].

Compared to traditional methods, bioleaching offers attractive alternatives with advantages such as low investment, mild operation conditions, energy efficiency, and sustainability [8,9]. It has been proven that bioleaching may be applied as an alternative to increasing copper recoveries, particularly, from low-grade ores and refractory concentrates which are difficult to process using conventional technologies. Many studies have shown the feasibility of the microbial leaching of metal sulfide including secondary copper sulfides as chalcocite [4,10–13]. Numerous physical and chemical variables affect the leaching process, among them pH, temperature, redox potential, mineralogical composition, and particle size distribution. Microorganisms are one of the key factors that influence bioleaching efficiency. Acidophilic bacteria and archaea capable of metabolizing iron and reduced inorganic sulfur compounds (RISC) are the main players involved in the bioleaching process. These microorganisms contribute to the mineral dissolution by generating the oxidizing agent iron(III), and by subsequently oxidizing to sulfuric acid the released sulfur compounds arising from the mineral [14]. Mixed and pure cultures of acidophilic mesophiles, moderate, and extreme thermophiles have been used in bioleaching processes, but the use of native communities, inherently adapted to the conditions of the ore, seems to be more efficient than the application of exogenous strains.

Bioleaching processes are already commercially used, particularly for the recovery of copper [15,16]. Although copper recovery through bioleaching has increased worldwide, Argentina has no commercial experience in bioleaching operations and only a few regional ores have been studied at bench scale [17–21]. This work contributes to the knowledge for the application of this technology to regional ores, particularly, Taca Taca mineral ores. The Taca Taca mining project is a high-potential copper–gold–molybdenum porphyry deposit located at 3560 m.a.s.l. in Los Andes Department, Northwest Salta Province, Argentina. Currently, First Quantum Minerals Ltd. (Vancouver, Canada) is exploring the natural resources of this area. Taca Taca constitutes one of the main copper projects in Argentina and, given the characteristics of the deposit, it is suitable for conventional open pit, grinding, and flotation methods. As with most open pit mining operations, it is expected to accumulate low-grade minerals in stocks, since the amount of the metal contained is too low for profitable extraction by conventional methods. Thus, for efficient exploitation of this natural resource, it is relevant to develop sustainable technologies to increase metal recoveries from low-grade mineral deposits.

The purpose of this study is to assess the feasibility of applying biohydrometallurgical processes to extract copper from chalcocite-rich minerals from Taca Taca using native microorganisms. Leaching experiments were set up to evaluate the copper recovery in biotic and abiotic conditions. In addition, experiments on Taca Taca minerals at different temperatures, from mesophilic to thermophilic conditions, were performed. Factors that may influence the bioleaching yield of the ore are discussed. This study aims to be a useful reference for the optimization of biohydrometallurgical processes for the recovery of copper from Taca Taca, as well as from other chalcocite-rich deposits.

2. Materials and Methods

2.1. Site and Mineral Description

The Taca Taca mining project is located in the Puna region of Salta Province, Argentina, at 3560 m.a.s.l. (24°53′33″ S 67°65′ W) (Figure 1).

Core samples from the deposit, provided by the mining company, were used throughout this study. The main mineralogical species detected by X-ray diffraction analysis were pyrite (FeS_2) and chalcocite (Cu_2S) (Bruker D8 Advance A25), which makes the mineral suitable for studying its bioleaching feasibility. Other minerals detected in the ore matrix were quartz (SiO_2), muscovite ($KAl_2(AlSi_3O_{10})(OH)_2$), alunite ($KAl_3(SO_4)_2(OH)_6$), and pyrite ($FeS_2$). Different layers of the core samples were analyzed and the one with the highest copper content was selected for the bioleaching assays. The main chemical composition of this layer, detected by X-ray Fluorescence (TXRF Bruker S2 picofox) was (*w*/*w*): 2.33% S, 1.64% Fe, 1.13% Cu, 0.34% Mg, 0.33% Na, 0.25% Cl and 0.19% Ti. Fresh groundwater of the site, available for mining processes, was also sampled and characterized by X-ray fluorescence, being the main chemical components Ca (32 mg/L), Cl (10 mg/L), K (4 mg/L), Zn (0.15 mg/L), Mn (0.32 mg/L), Ti (0.15 mg/L), and Fe (0.14 mg/L).

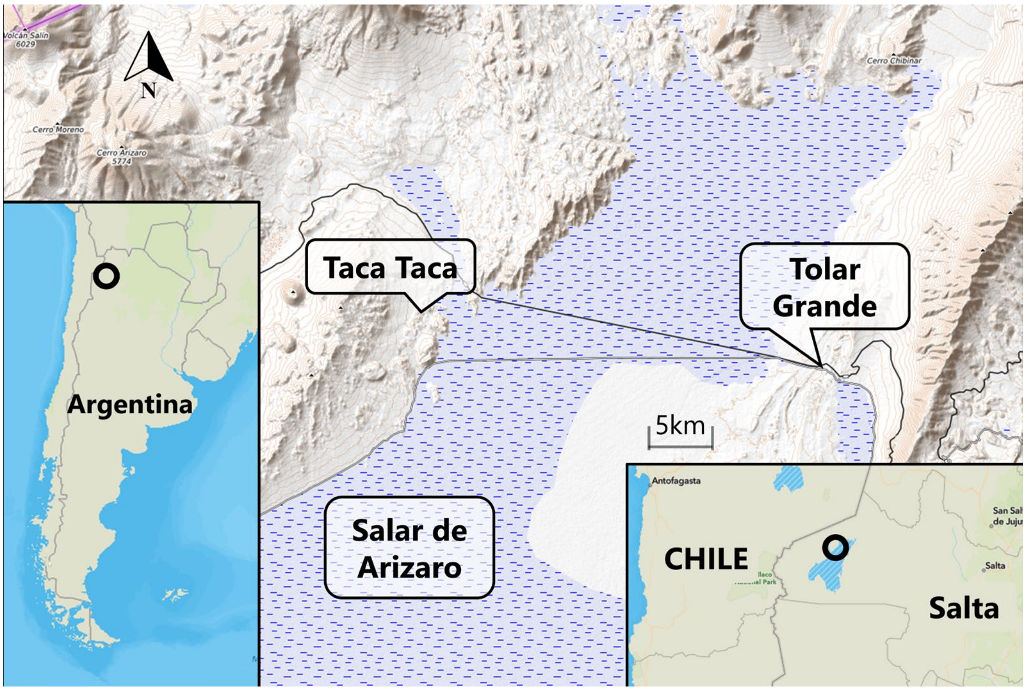

Figure 1. Location of Taca Taca mining project, Northwest Salta province, Argentina. The project (highlighted with black circles) is situated on the western side of Salar de Arizaro, 35 km away from the village of Tolar Grande.

2.2. Microorganisms and Culture Conditions

To obtain native acidophilic iron- and/or sulfur-oxidizing microbial consortia, mineral samples from Taca Taca were cultivated in 100 mL of Mackintosh basal salt solution (MAC) pH 1.8 [22] supplemented with 9 g/L ferrous iron and 10 g/L elemental sulfur (S°) powder. Growth media components were obtained from Biopack, Argentina. Enrichment cultures were incubated in agitation at 150 rpm and different temperatures: 30, 45, or 65 °C. Culture samples were taken periodically, and microbial growth was followed through pH measurements and ferrous iron titration with potassium permanganate. The obtained consortia were then subcultured and maintained under the same conditions.

For moderate thermophilic experiments, two different consortia obtained from the natural extreme environment of Caviahue–Copahue Neuquén, Argentina, were used [23,24]. Consortium 45_Fp is mainly composed of archaea *Ferroplasma*, while consortium 45_Ac has *Acidithiobacillus* as the predominant genus. Both genera of bacteria and archaea have demonstrated their capacity to bioleaching sulfide minerals [25–31]. Consortia 45_Fp and 45_Ac were grown in MAC medium pH 2.4, supplemented with ferrous iron (4 g/L) and S° (10 g/L) incubating at 45 °C in an orbital shaker at 150 rpm.

Assays at 65 °C were carried out using the thermoacidophilic iron and sulfur-oxidizing archaea *Acidianus copahuensis* (DSM 29038), isolated from the same extreme region, Caviahue–Copahue [32,33]. This strain was grown in MAC medium pH 2, supplemented with S° (10 g/L) and yeast extract (1 g/L), at 65 °C and 150 rpm.

2.3. DNA Extraction and Amplicon Sequencing

Genomic DNA was extracted from the mesophilic consortium obtained from Taca Taca following the CTAB method [34]. The DNA sample was sent to MR DNA (Molecular Research, USA) for amplicon sequencing. In total, 250 bp paired-end reads of the hypervariable region V4 of the 16S rRNA gene were sequenced using pair of primers

515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACNVGGGTWTCTAAT) on Illumina MiSeq sequencer platform (Illumina, San Diego, CA, USA). The data were deposited with links to BioProject accession number PRJNA542136 in the NCBI BioProject database. Sequencing data were processed using R package DADA2 [35,36] to analyze amplicon sequence variants (ASVs) following the procedure recommended by the authors. Taxonomic assignment was performed using Silva non-redundant training set (v138). Later, species were assigned based on the exact match between the ASVs and the reference strains in the Silva database.

2.4. Bioleaching Assays

For bioleaching assays, mineral samples were reduced in size through consecutive steps of crushing and grinding until particle diameters were smaller than 100 µm.

The mesophilic leaching experiments were carried out in glass flasks containing 150 mL of the aqueous phase with initial pH adjusted to 2.0 with sulfuric acid (96–98% purity Merck, United States), and with 1.0% (*w*/*v*) of mineral. The conditions tested included inoculated and non-inoculated systems in MAC medium, with and without the addition of ferrous iron (1 g/L) and S° (1 g/L). Sterile controls were made by sterilizing the mineral with heat pulses in a microwave and replacing the inoculum with the same volume of paraformaldehyde. Similarly, inoculated assays replacing MAC medium for distilled water and using Taca Taca groundwater were performed. Each bioleaching condition was conducted in duplicate.

A consortium of indigenous bacteria cultivated in MAC medium pH 1.8 with ferrous iron (9 g/L) and S° (1 g/L) was used as inoculum in the assay. At the end of the exponential growth, the culture was filtered to remove iron and sulfur solid compounds, then, cells were harvested by centrifugation (Supra 22K, Hanil, Gimpo, Korea) at 8000 rpm for 10 min. Cell pellets were washed twice with acidic water in order to remove any trapped ions, and then resuspended with the corresponding aqueous phase. Cells were counted using an improved Neubauer chamber to calculate the necessary volume of inoculum to reach an initial cell population of 1×10^8 cell/mL in the final suspension.

Flasks were kept at 30 °C with shaking at 120 rpm on an orbital shaker and, periodically, distilled water was added to the flasks to compensate for evaporation losses.

Leaching efficiency was assessed by analyzing soluble copper, total and ferrous iron, redox potential, and pH at regular periods of time. Copper and total iron concentrations in the solution (released from the mineral during the assays) were determined by atomic absorption spectrophotometry (AAS) (VARIAN Spectra AA-240), while ferrous iron concentration was quantified by spectrophotometry using the o-phenanthroline method [37].

Bioleaching assays at 45 and 65 °C were conducted later in order to evaluate the impact of increasing temperatures. Consortia 45_Fp and 45_Ac were used as inoculum in bioleaching assays at 45 °C, while inoculation of bioleaching systems at 65 °C was performed using *A. copahuensis* pure cultures (65_Cop). All bioleaching systems were carried out in MAC medium following the aforementioned protocol.

3. Results and Discussion

3.1. Native Microorganisms

The feasibility of applying biohydrometallurgical processes to extract copper from chalcocite-containing minerals from Taca Taca mining project, Salta, Argentina, was assessed. Samples from the region were cultured to select native acidophilic chemolithotrophic microorganisms. Some of these microorganisms have the ability to grow on iron as electron donors catalyzing the bioleaching process of mineral sulfides [38–40]. Furthermore, acidophilic prokaryotes are well known for their high tolerance to heavy metals. Indigenous microorganisms are expected to be better adapted to the mineral composition of the site than exogenous strains, and therefore, achieve higher leaching efficiencies and metal recoveries. This relies on the presence of toxic trace elements in the deposit that shall have modulated a community with specific and improved resistance strategies [41].

A mesophilic iron/sulfur-oxidizing consortium was obtained, while thermophilic enrichments at 45 and 65 °C showed a poor response and slow growth in subsequent subcultures. The microbial composition of the obtained native consortium at 30 °C was analyzed through 16S rRNA amplicon sequencing. A total of 95% of the sequences processed (594,035 of 625,927 clean sequences) belonged to ASV 1, which was later classified as *Acidithiobacillus ferrooxidans* using SILVA database (v 138). *A. ferrooxidans* is an extremophilic Gram-negative bacteria able to grow in mesophilic conditions fixing CO_2 and oxidizing iron and/or sulfur compounds, this species is usually found worldwide in acidic environments and has been extensively studied through the years [42,43]. Moreover, its applications in biomining are well reported [29–31].

Most of the remaining sequences from the Taca Taca consortium (4.7%) were also assigned to the *Acidithiobacillus* genus; however, they could not be classified at the species level. Other genera present, though representing less than 0.05% of the consortium, were *Ralstonia*, *Pelomonas*, *Pseudomonas*, *Acidianus*, and *Acidiphilium*, among others. In summary, 76 ASVs were inferred, 44 of which were classified as *Acidithiobacillus*, representing 99.8% of the consortium.

The considerably low diversity of the consortium obtained through culturing techniques suggests a specialized indigenous community. This may be related to the specific extreme conditions of the site: the predominance of pyrite-like iron sulfide minerals, scarcity of carbon sources, heavy metal presence, high UV exposition, and low pH; conditions to which the *Acidithiobacilli* are well adapted and, therefore, in which they usually prevail.

3.2. Bioleaching at 30 °C

The mesophilic consortium was used to evaluate its capacity to bioleach copper from Taca Taca minerals under different conditions. Considering the FRX analysis and the amount of mineral added to each glass flask (see Materials and Methods, Section 2.4), the maximum recovery of copper was estimated at 113 mg/L. Figure 2 shows the kinetics of copper solubilization in the seven systems evaluated. All four systems inoculated with the consortium (A–D) showed higher and faster copper solubilization than the non-inoculated systems (E–G). Similar studies carried out with chalcocite-bearing minerals and iron and/or sulfur-oxidizing microorganisms have reported similar recoveries of copper [3,44]. Furqan and co-workers [44] observed that about 80–90% of the total Cu content present in the ore matrix was solubilized after 30 days of the leaching process mediated by *A. ferrooxidans* at 30 °C with supplementation of iron(II). Comparable copper recoveries were also obtained in bioleaching experiments performed at 30 °C with pure chalcocite and a microbial consortium mainly consisting of *Acidithiobacillus* and *Sulfobacillus* [3]. In the present study, the maximum recovery was reached after 14–21 days of incubation at 30 °C. At this point, three groups with distinctive bioleaching efficiencies were identified (p-val < 0.01 in an ANOVA). (The complete statistical analysis is shown in the Supplementary Materials Tables S1–S3).

The first group includes the four inoculated systems (A–D), with no significant differences in metal recovery among them. The addition of external iron(II) and sulfur (C) did not enhance the metal recovery suggesting that these metabolic substrates were already in excess in relation to the microbial mass, i.e., the amount of iron(II) and sulfur in the solution due to the acidic solubilization of the mineral (see below, Equations (1) and (2)) were enough to reach the peak of the oxidation activity of the microorganisms present.

Three different aqueous matrices were tested: distilled water, basal salts solution (MAC), and Taca Taca local groundwater. Distilled water may lack some required micronutrients and thus limit microbial growth; conversely, the local groundwater is a complex matrix with high conductivity and may contain toxic compounds such as heavy metals and carbon acids. Nonetheless, these three systems (A, B, and D) exhibited similar copper recovery. This is a remarkable result for the feasibility of developing copper bioleaching processes in Taca Taca. Water management is one of the most critical aspects in biohydrometallurgy, the possibility to use untreated local groundwater represents lower operational cost and environmental impact.

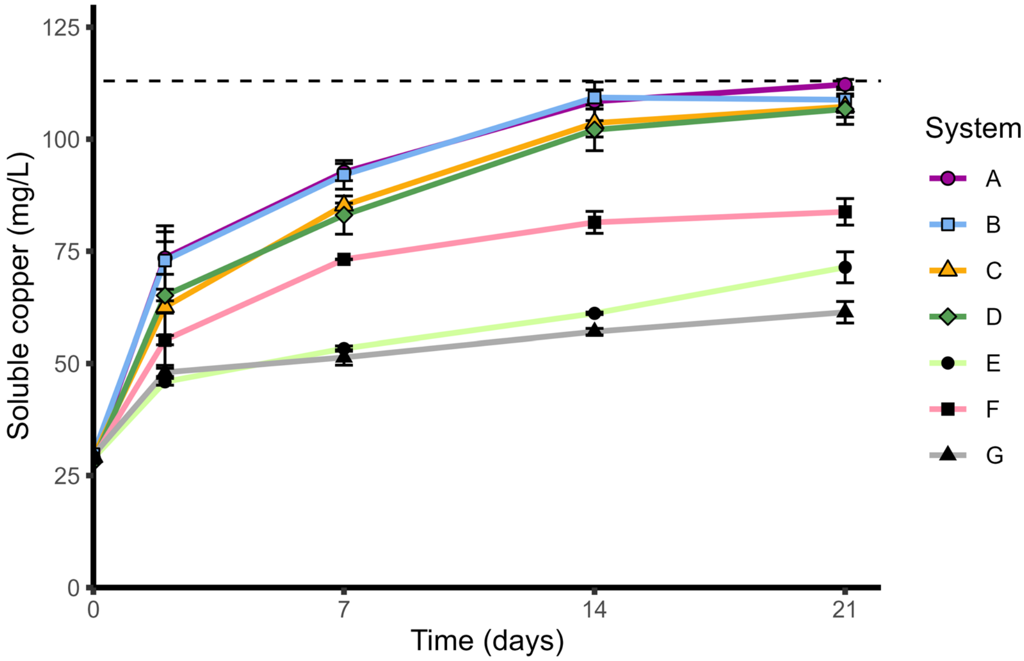

Figure 2. Kinetics of copper solubilization from Taca Taca ore by the native consortium incubated at 30 °C for 21 days. Dashed line represents the maximum copper recovery estimated. Bioleaching systems (A–D) were inoculated with the consortium. Three aqueous phases with initial pH adjusted to 2.0 were tested: distilled water (A), MAC medium (B), and local groundwater from Taca Taca (D). System (C) was performed in MAC medium with exogenous supplementation of ferrous iron and sulfur. Non-inoculated controls were performed in MAC medium (E) and with sulfur and ferrous iron supplementation (F). Sterile control (G) was performed in MAC medium using a sterilized mineral and paraformaldehyde.

The second group in Figure 2 consisted in the flasks not inoculated but with exogenous iron(II) and sulfur supplementation (F). The copper leached in this system reached 80 mg/L (70%) after 21 days of incubation. Conversely, the third group, represented by the non-inoculated systems with non-sterile (E) or sterilized mineral (G)—both using MAC medium as aqueous solution—leached around 60 and 70 mg/L after the same period of time (50–60% of the total copper present).

Some metal sulfide minerals, such as chalcocite, are soluble in acid and oxygenated conditions [45,46]. Equation (1) generally describes the process.

$$Cu_2S + 0.5O_2 + 2H^+ \rightarrow CuS + Cu^{2+} + H_2O \quad (1)$$

According to this chemical reaction, one mole of chalcocite produces one mole of soluble copper and one mole of covellite (CuS) (a non-acid soluble sulfide mineral). Therefore, from two moles of copper present in chalcocite, only one is chemical solubilized. This abiotic process could explain the ~50% solubilization exhibited in both non-inoculated systems (E and G).

Chalcocite can also be leached by ferric iron according to Equation (2) [47,48].

$$Cu_2S + 2Fe^{3+} \rightarrow 0.125\ S_8 + 2Cu + 2Fe^{2+} \quad (2)$$

Taca Taca mineral samples are rich in pyrite; however, in acidic solutions, the solubilized iron from this ore is present almost solely as ferrous iron. Unless some iron-oxidizer is added to the system, the concentration of ferric iron is negligible and Equation (2) does not occur. In the inoculated bioleaching systems of this study, *A. ferrooxidans* can oxidize the soluble ferrous iron supplying ferric iron to the solution and feeding Equation (2). Moreover, ferric iron can "attack" covellite releasing another mole of copper, as is represented in Equation (3), fulfilling the solubilization of the copper present in the chalcocite.

$$CuS + 2Fe^{3+} \rightarrow 0.125\ S_8 + Fe^{2+} \quad (3)$$

Lastly, ferric iron can also contribute to the solubilization of the pyrite, supplying ferrous iron to the system and to the microorganisms who catalyze the global process.

Figure 3 shows the kinetics of soluble iron in relation to the concentration of ferrous iron and total iron. In all the systems total iron increased or remained constant. As mentioned before, the chemical leaching of pyrite-like minerals releases ferrous iron with the negligible production of ferric iron in acidic solutions in accordance with the equilibrium ratio. This can be seen in System G (not inoculated and using a sterilized mineral), where most to all the iron present was ferrous iron as a result of the absence of an oxidative agent. In the inoculated systems (A–D), where *A. ferrooxidans* uses ferrous iron as an electron donor and produces ferric iron, the iron ratio closes to zero immediately, meaning that most of the iron in the solution was present as the ferric species due to the microbial activity.

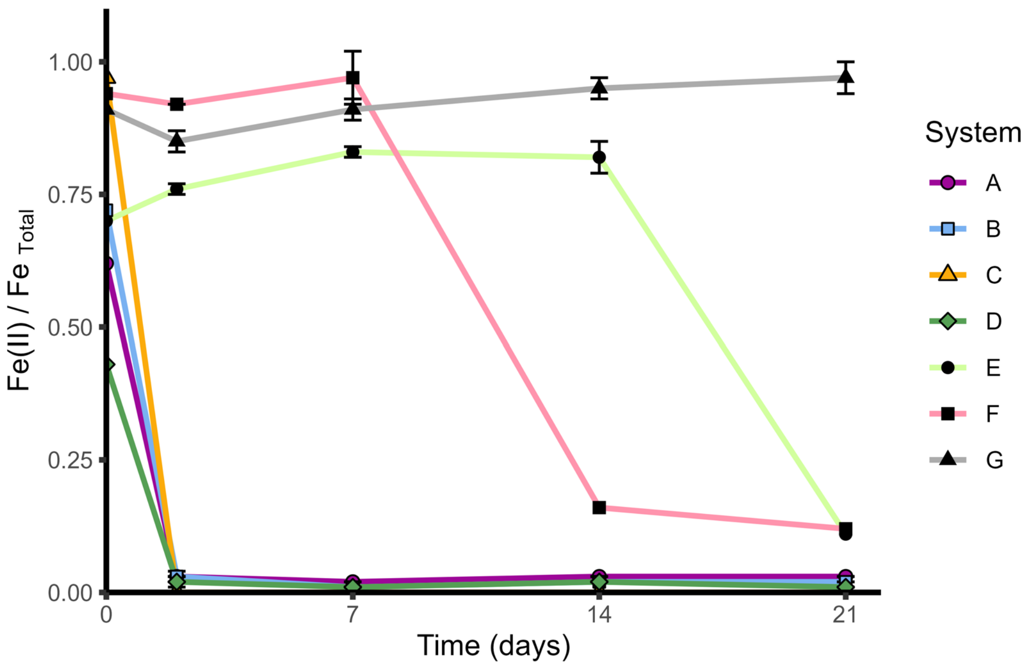

Figure 3. Evolution of Fe(II)/FeTotal ratio during bioleaching assay at 30 °C. Inoculated systems were performed in distilled water (A), MAC medium (B), and local groundwater from Taca Taca (D) with initial pH adjusted to 2.0. System (C) was performed in MAC medium with exogenous supplementation of ferrous iron and sulfur. Non-inoculated controls were performed in MAC medium (E) and with sulfur and ferrous iron supplementation (F). Sterile control (G) was performed in MAC medium using a sterilized mineral and paraformaldehyde.

In systems E and F, which were not inoculated, but the mineral used was not sterilized, the iron speciation ratio kinetics in these systems showed a ratio near 1 during the first days, behaving as an abiotic system where most of the total iron is ferrous iron; but then, the ratio tended to zero, meaning that an oxidative process was in progress (since total soluble iron remained constant). These results may suggest the activation of some iron-oxidizing microorganisms attached to the mineral. The addition of exogenous iron and sulfur (both potential substrates for these microorganisms) could have favored their activation and growth, and hence, explain why system F was activated faster than system E.

In accordance with the iron ratio results, the redox potential (ORP) of the inoculated systems exhibited a rapid increase from ~400 to ~650 mV in the first two days (Figure 4), indicating oxidative processes. Conversely, the sterilized control (system G) held a steady potential of 400 mV throughout the assay, while non-sterilized controls (systems E and F) showed a late rise of the ORP, which could be associated with the growth of iron-oxidizing microorganisms attached to the mineral mentioned before.

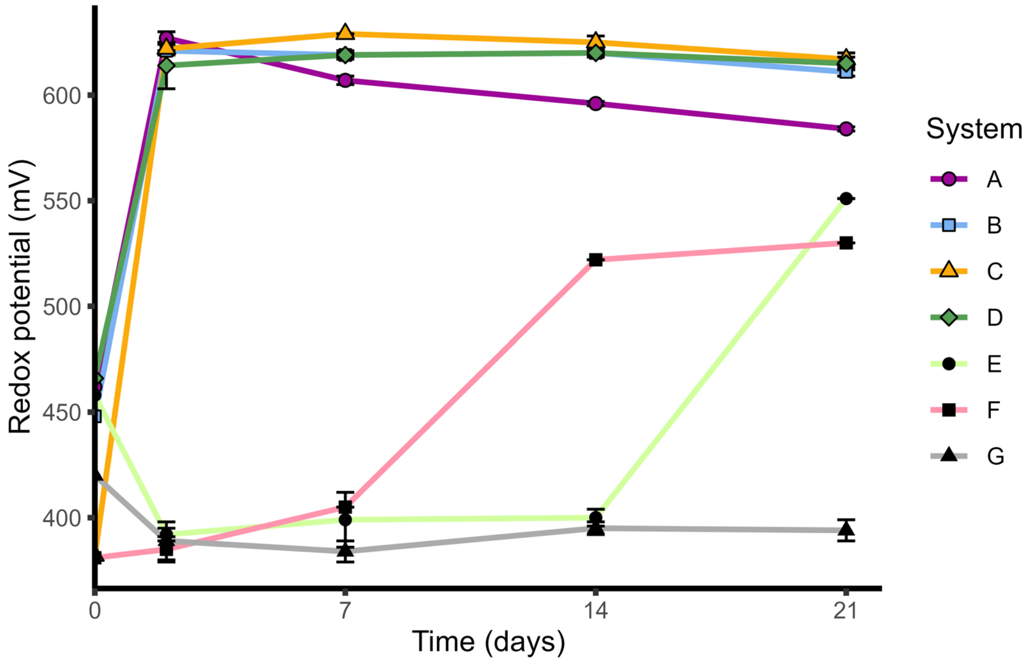

Figure 4. Evolution of ORP values during bioleaching tests at 30 °C with native consortium. Inoculated systems were performed in (A) distilled water, (B) MAC medium, (C) MAC medium + Fe(II) + S(0), (D) local groundwater with initial pH adjusted to 2.0. Non-inoculated controls were performed in MAC medium (E), MAC medium with Fe(II) and S(0) supplementation (F), and using a sterilized mineral with the addition of paraformaldehyde (G).

The evolution of pH during the bioleaching process is an important aspect regarding its feasibility. If the global pH changes significantly, then it will be necessary to adjust it with external agents regularly during the operation of the facility implying economic costs. Figure 5 shows the evolution of pH during the assays. Notably, in most systems, global pH remained steady at around 2 units, with the exception of systems C and F which exhibited an acidification of the medium. These two systems had an external addition of sulfur which could have disrupted the equilibrium, the production of protons observed may be related to the biological oxidation of the excess sulfur to sulfuric acid. As has been mentioned before, the delay in system F to oxidize the excessive sulfur could be related to the activation and growth of the microorganisms attached to the mineral, while system C had an already active consortium of sulfur-oxidizing bacteria.

In summary, it was possible to completely solubilize the copper present in chalcocite-containing minerals from Taca Taca in three weeks of incubation at 30 °C using a native consortium dominated by *A. ferrooxidans*. The process could be carried out using local groundwater without an effect on the global pH of the solution.

3.3. Bioleaching at 45 and 65 °C

Later, we analyzed if the copper recovery could be enhanced by increasing the temperature using other recognized bioleaching microorganisms able to grow under these conditions. Temperature is an important factor in industrial bioleaching processes since higher operational temperatures would increase the rate of metal dissolution and decrease passivation phenomena over mineral surfaces [49]. According to this, the use of thermophiles could be advantageous. The thermophiles most relevant for biomining applications are those able to grow at low pH, high temperature and tolerate high concentrations of heavy metals [50].

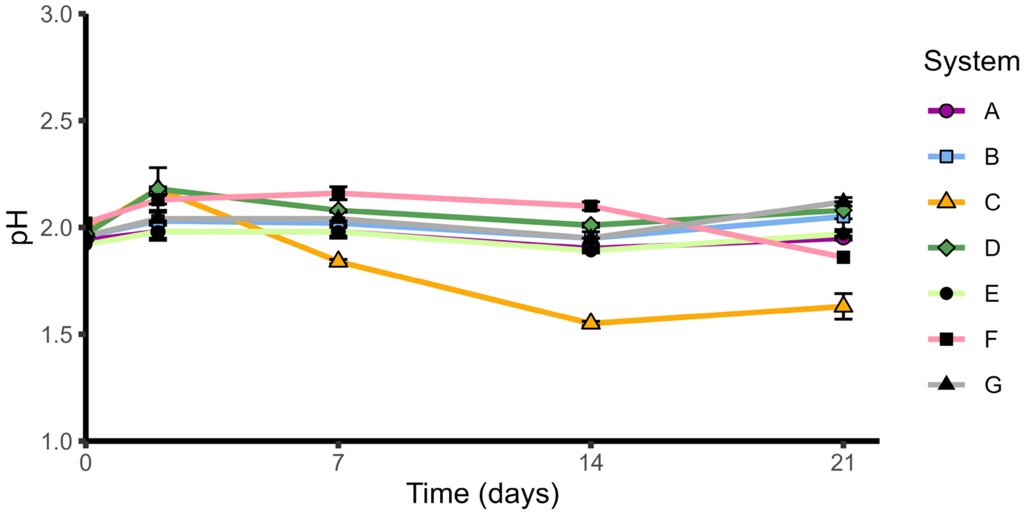

Figure 5. Evolution of pH values during bioleaching tests at 30 °C with the native consortium. Bioleaching systems (A–D) were performed with inoculation, in MAC medium with (C) and without ferrous iron and sulfur addition (D), in distilled water (A) or Taca Taca groundwater (D) with initial pH adjusted to 2.0. Systems (E–G) correspond to non-inoculated assays, performed in MAC medium, with (F) and without ferrous iron and sulfur supplementation (E) or with sterilized mineral and paraformaldehyde (G).

Most of the bioleaching heaps and bioreactors in the world are operated at mesophilic conditions, reaching temperatures of 40 °C; however, it has been reported that some bioleaching heaps could reach temperatures higher than 80 °C as the exothermic oxidation reactions increase the temperature [51]. When using microbial communities or consortia, as the temperature increases, microorganisms succeed each other and the composition of the community changes; as a result, the optimal temperature of the process is defined by the microbial composition of the inoculated community in accordance to their optimal growth temperature; i.e., a bioleaching community dominated by mesophilic bacteria will perform better at 30 °C, while a bioleaching community rich in thermophiles is expected to have optimum recovery at >60 °C [52–56].

In order to evaluate the bioleaching of Taca Taca ore at different temperatures, indigenous thermophilic microbial consortia were attempted. However, both thermophilic enrichment cultures (incubated at 45 and 65 °C) showed a weak growth capacity under these conditions. In view of the difficulty to obtain native thermophilic consortia, two moderate thermophilic consortia, with an optimum temperature of 45 °C, and an isolated thermophilic archaeon growing at 65 °C, were used to assess the influence of temperature in the copper recovery from Taca Taca mineral ore.

Figure 6 shows the kinetics of copper recovery for the moderate thermophilic consortia and the thermophilic archaeon *A. copahuensis*. Similar to the native consortium assay, the maximum copper recovery was estimated at 113 mg/L (dashed line). In all three inoculated systems, 100% of the copper present was solubilized in less than two weeks. These performances are faster than the one obtained using the native consortium at 30 °C. Particularly, moderate thermophilic consortia 45_Fp could recover all the copper in approximately one week (Figure 6a). This consortium, obtained from a hot spring in the volcanic and geothermal area of Caviahue Copahue, Argentina [23,24], was dominated by *Ferroplasma*, an extensively studied archaeal genus that grows chemoorganotrophically in acidic conditions. The ability of *Ferroplasma* spp. to oxidize iron and its applicability in the bioleaching of copper has been already reported [25–28].

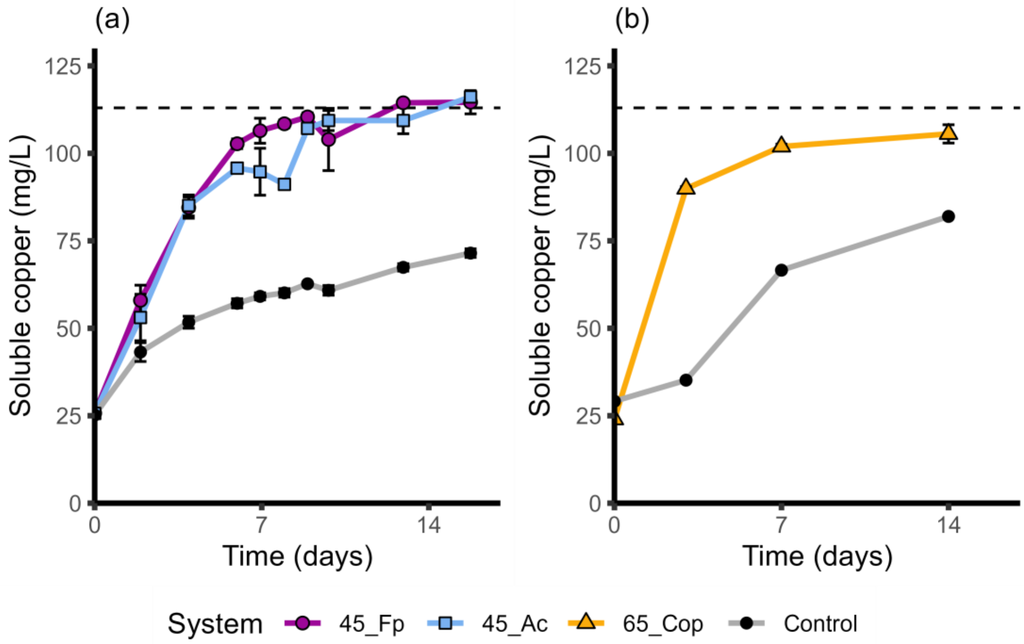

Figure 6. Copper recovery during bioleaching assays at 45 (**a**) and 65 °C (**b**). Sterile controls performed in MAC medium using a sterilized mineral and paraformaldehyde were included at both temperatures.

On the other hand, the thermophilic system, inoculated with a pure culture of *A. copahuensis*, also exhibited fast solubilization of the copper present in the mineral (Figure 6b). This extremophilic archaeon is indigenous from Caviahue Copahue, Argentina [32,33]. Interestingly, *A. copahuensis* has been recently studied for its capacity to recover copper from chalcopyrite (a refractory copper iron sulfide mineral) and zinc [18,19,57].

At both thermophilic temperatures, sterile controls exhibited higher copper recovery than at 30 °C (Figure 2, System G), in accordance with previous reports regarding the effect of increasing temperatures over chalcocite dissolution, particularly, on the thermal catalysis of the covellite dissolution (Equation (3)) [58].

The iron ratio analysis showed that the predominant species in the inoculated systems was ferric iron, indicating that an iron oxidation process was being carried on. Consequently, the redox potential in these systems increased from ~350 mV at the start of the assay to >500 mV after two weeks, while the increase in the control systems was null or less significant (Figure S1, Supplementary Materials).

Finally, similar to the native consortium, the moderate thermophilic consortia did not change the global pH of the medium holding a value of 2.2 units throughout the 16 days of the bioleaching assays (Figure S3). Conversely, in the thermophilic system, the global pH descended consistently from 2.2 to 2.0 units, indicating a slight acidification of the medium (Supplementary Materials, Figure S2). The global proton production could be related to a rise in the pyrite oxidation rate (Equation (4)) and, eventually, to the hydrolysis of ferric iron to produce jarosite (Equation (5), where M is a monovalent cation). Both chemical reactions may be catalyzed by the higher temperature of the system.

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 2SO_4 + 16H^+ + 15Fe^{2+} \quad (4)$$

$$3Fe^{3+} + M^+ + 2SO_4 + 6H_2O \rightarrow MFe_3(SO_4)_2(OH)_6 + 6H^+ \quad (5)$$

All three thermophilic systems were able to recover 100% of the copper present in the Taca Taca mineral sample. It is worth noticing that while moderate thermophilic consortium

45_Fp seems to be the most efficient, the other moderate thermophilic consortium, 45_Ac, dominated by Acidithiobacilli, exhibited a similar recovery to the thermophilic archaeon *A. copahuensis*. This result, in accordance with the previous reports [52–56], suggests that the microbial composition (or the bioleaching agent) is more relevant than the optimal temperature of the process.

4. Conclusions

This study presents the first assessment for the recovery of copper through biohydrometallurgical processes from chalcocite-containing minerals from Taca Taca mining project. The feasibility of these processes was demonstrated using native microorganisms from the region as well as other known bioleaching bacteria and archaea. Copper could be completely solubilized in less than three weeks at mesophilic conditions and using local groundwater without any supplements. The recovery could be sped up to one week at moderate thermophilic conditions; however, increasing the temperature further led to an acidification of the medium—possibly due to a rise in the rate of secondary chemical reactions—which would imply higher operational costs harming the feasibility of the process.

Further studies assessing the copper recovery using a mixed consortium of mesophilic, moderately thermophilic, and thermophilic microorganisms will be carried out. This study is relevant since temperatures in bioleaching heaps, piles, and reactors variates as a consequence of microbial activity. Therefore, a mixed consortium could enhance the efficiency of the process by allowing microbial succession of leaching microorganisms.

Supplementary Materials: The following are available online at https://www.mdpi.com/xxx/s1, Figure S1: Evolution of ORP values during bioleaching assays at 45 (**a**) and 65 °C (**b**); Figure S2: Evolution of pH values during bioleaching assays at 45 (**a**) and 65 °C (**b**); Figure S3: Evolution of Fe(II)/FeTotal ratio during bioleaching assays at 45 (**a**) and 65 °C (**b**); Table S1: Analysis of variance of copper recovery by the indigenous community; Table S2: Tukey matrix of pairwise comparison for copper recovery after 14 days; Table S3: Pairwise comparison of copper recovery after 21 days in Tukey test.

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