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# New approaches for extracting and recovering metals from mine tailings

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ABSTRACT

Waste materials from metal mining, such as mineral tailings, often contain significant amounts of potentially valuable metals particularly where, as in many historic operations, the efficiency of flotation technologies used to concentrate target minerals was not as good as those currently available. A two-stage mineral leaching and metal recovery protocol was developed to extract copper from tailings generated as waste materials in two mines currently operating in Spain and Serbia. The most effective extraction of copper (84 to >90%) was achieved by bioleaching the tailings at 45 °C, using a defined microbial consortium, where elemental sulfur was added to the tailings and the pH of leach liquors allowed to fall to ~pH 1, at which point anaerobic conditions were imposed. The thermo-tolerant acidophiles *Acidithiobacillus caldus* and *Sulfobacillus thermosulfidooxidans* emerged as the dominant bacteria present in both tailings leachates under these conditions. Copper present in the pregnant leach solutions (PLS) produced were next precipitated as a sulfide phase using hydrogen sulfide generated in a low pH (4.0) sulfidogenic bioreactor. The off-line system used allowed the copper present in PLS to be precipitated selectively without the need to adjust the pH of the PLS, though small amounts of silver present in PLS from one of the tailings samples co-precipitated with copper sulfide. Experimental data also suggested that it would be possible to extract silver from bioleached solid residues (where it was mostly found) using a simple chemical extractant. The results suggested that bio-processing these waste materials would have economic as well as environmental benefits.

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## 1. Introduction

Mining of metals generates considerably amounts of waste materials. These generally have very little economic value, making their exploitation not profitable, though they often have the potential to pose a long-term threat and cause damage to the environment. Mine wastes vary depending on their physical and chemical composition, the type of mining and the way the mineral is processed. Millions of tonnes of ore are processed every year by the mining industry, >95% of which is disposed of in the form of waste rocks and mine tailings. The latter are finely ground rock particles generated during the processing of ore materials and separation of target metal minerals, and are highly reactive because of their small particle size and content of reactive minerals, such as pyrite ($FeS_2$). Mine tailings may contain base transition metals, such as iron, copper, nickel and zinc, in relatively high concentrations, and also occasionally precious metals such as gold and silver, in minerals (and native metals) that have been not been separated by froth flotation (e.g. Bryan et al., 2006; Ahmadi et al., 2015). Toxic elements, such as arsenic, may also be present in elevated concentrations (e.g. Nguyen et al., 2015). Exposure of mine tailings to both oxygen and water facilitates the (microbially-catalysed) oxidative dissolution of the sulfide minerals they contain, generating acidic waste waters that contain elevated concentrations of dissolved metals and sulfate. Acid mine drainage (AMD) is a widely-reported environmental pollutant, with global distribution.

Storage of waste rocks and mineral tailings at, and in the vicinity of, mine sites represents a long-term engineering and environmental challenge. Occasionally, catastrophic environmental pollution has occurred due to the failings of the system used to store mine wastes. One such example is the failure of the retaining dam of a tailings impoundment at the Aznalcóllar-Los Frailes mine (in south-west Spain) which resulted in the release of vast amounts of acidic waters and tailings slurries into the catchment of a local major river nearby the Doñana National Park in April 1998 (Grimalt et al., 1999; Ericksson and Adamek, 2000). However, mine tailings may also be recycled and reused, e.g. manganese-rich tailings could be used in agro-forestry, building and construction materials, coatings, cast resin products, glass, ceramics, and glazes (Lottermoser, 2011). Increasing demands for metals, coupled with

depleting reserves of high-grade primary ores, has meant that waste materials from historic mining operations may have greater metal contents than primary ores currently being excavated. Since mine tailings have already been part-processed (by comminution, to produce fine-grain particles) the cost of extracting residual metals from them is often economically more attractive than mining a deep-buried primary ore body. In addition, mine wastes may contain metals (such as rare earth elements) that were not considered worth extracting when the ores were initially processed, but which have since increased in value and use. This was the case with cobalt-rich tailings produced at a former copper mine in Kasese, Uganda, which were reprocessed by bioleaching decades after mining at the site ended, both to extract cobalt and to remove the environmental threat posed by the tailings deposits (Morin and D'Hugues, 2007).

Harnessing the abilities of some species of prokaryotic microorganisms to catalyse the oxidative dissolution of sulfide minerals and thereby to facilitate the extraction of metals is a global biotechnology, used mostly to recover some base including copper, nickel, cobalt and zinc, and precious metals such as gold. The geochemical processes and the microorganisms involved in biomining have been widely researched and are well understood (e.g. Vera et al., 2013; Johnson, 2014). However, not all sulfide minerals are readily bioleached such as chalcopyrite. This mineral (the most abundant copper mineral) is notoriously difficult to effectively oxidise using conventional bioleaching approaches (reviewed in Watling, 2006). Recently, extraction of metals from oxidised ores using reductive, rather than oxidative, bioleaching has been described (Johnson et al., 2013). In contrast to conventional biomining, this requires the addition of an extraneous material, such as elemental sulfur, to act as the electron donor for bacteria that reduce ferric iron minerals.

In this work, we describe results from bioleaching mine tailings generated at two operational copper mines, carried out at different temperatures (with mesophilic and moderately thermophilic acidophilic microorganisms) and using a combination of oxidative and reductive bioleaching.

## 2. Materials and methods

### 2.1. Mineral tailings

Mineral tailings were obtained from two copper mines, Bor (Serbia; operated by RTB Bor), and Cobre Las Cruces (CLC; Sevilla, Spain; operated by First Quantum Minerals Ltd.).

### 2.2. Bioleaching microbial consortia

Three different consortia of acidophilic microorganisms were used in bioleaching experiments. These were: (i) a thermo-tolerant consortium, which included the autotrophic iron-oxidizer *Leptospirillum* (*L.*) *ferriphilum* (both the type strain and strain MT63), the autotrophic sulfur-oxidizer *Acidithiobacillus* (*At.*) *caldus*[T], facultatively autotrophic and obligately heterotrophic iron/sulfur oxidizing and iron-reducing bacteria (*Sulfobacillus* (*Sb.*) *thermosulfidooxidans*[T] and *Acidibacillus* (*Ab.*) *sulfuroxidans*[T], and two heterotrophic iron-oxidizing archaea: *Ferroplasma* (*F.*) *acidiphilum* (strain BRGM4) and *Acidiplasma* sp. (strain FV); (ii) a second thermo-tolerant consortium, used in bioreactors where pH was not controlled, that included *L. ferriphilum* strain MT63, *At. caldus*[T], *Sb. thermosulfidooxidans*[T], "*Ab. sulfurooxidans*[T]", *F. acidiphilum* strain BRGM4, *Acidiplasma* strain FV, and two strains of *Sulfobacillus* that had been isolated from the Bor tailings (*Sb. thermosulfidooxidans* strain BOR3 and *Sb. acidophilus* strain BOR1); (iii) a mixed mesophilic/thermo-tolerant consortium, that included six species of mesophilic acidophiles (autotrophic iron/sulfur-oxidizing and iron-reducing *Acidithiobacillus* spp. (*At. ferrooxidans*[T], *At. ferridurans*[T], *At. ferriphilus*[T] and *At. ferrivorans* strain CF27), "*Ab. ferrooxidans*[T]" (a heterotrophic iron-oxidizer/reducer), *Acidiphilum* (*A.*) *cryptum* (strain SJH; a heterotrophic iron-reducer)), and three thermo-tolerant species (*Sb. thermosulfidooxidans*[T], *F. acidiphilum* strain BRGM4 and *Acidiplasma* strain FV). The consortia were grown in iron/yeast extract media pH 2.0, at 30 (mesophilic/thermo-tolerant consortium) or 45 °C (thermo-tolerant consortia) for 2–3 weeks previously to inoculation of the reactors.

### 2.3. Bioleaching experiments and processing of mineral residues

Bioleaching of mineral tailings was carried out in 2.3 L bioreactor vessels coupled to temperature- and pH-control units (Electrolab, UK). Temperatures were maintained at either 30° (for the mesophilic/thermo-tolerant consortium) or 45 °C (for the two thermo-tolerant consortia), and the reactors were stirred continuously at 150 rpm. Control of pH (where used) was by automated addition of 0.5 M sulfuric acid or 0.5 M sodium hydroxide. For aerobic conditions, the reactor vessels were gassed with sterile air, and when anoxic conditions were required this was replaced by oxygen-free nitrogen (OFN), both at flow rates of ~1 L/min. A basal salts/trace elements solution (Ñancucheo et al., 2016) was put into each reactor vessel (1.4 L for aerobic bioleaching, and 1.9 L for combined aerobic/anaerobic bioleaching), followed by mineral tailings (5% pulp densities; w/v) and 100 mL of pre-grown microbial inocula.

Six bioleaching experiments were carried out in total, three with Bor tailings and three with CLC tailings. These were: (i) aerobic bioleaching, carried out at 30 °C and pH 1.7; (ii) aerobic bioleaching, carried out at 45 °C and pH 1.7; (iii) alternated aerobic/anaerobic bioleaching, carried out at 45 °C with an initial pH of 1.8 but with pH control removed and elemental sulfur added to the tailings (at 2%, w/v) to act as an electron donor under both aerobic (for acid production) and anaerobic (for ferric iron reduction) conditions. In the last set-up, the bioreactors were aerated up to the point until the pH had declined to 1.0, at which point the air was replaced with OFN. Samples were withdrawn from the bioreactors at regular intervals to measure redox potentials and pH values (off-line), concentrations of soluble transition metals (iron, copper, zinc, manganese), and also iron speciation.

When the bioleaching phase was considered to be complete, mineral slurries were removed from the bioreactor vessels, and the pregnant leach solutions (PLS) separated from the mineral residues. Tests were carried out to selectively precipitate copper from PLS by sulfide precipitation (Section 2.4). Copper and silver were extracted both from mineral residues and fresh mineral tailings by mixing 0.5 g of representative samples with 10 mL of a solution containing 3.42 M NaCl and 0.2 M HCl (Romero et al., 2003), and heating for 1 h at 90 °C.

### 2.4. Sulfidogenic bioreactors and selective recovery of copper from PLS

Low pH, continuous-flow sulfidogenic bioreactors (Fig. 1) were used as sources of hydrogen sulfide ($H_2S$) for precipitating copper present in PLS generated in the bioleaching experiments described above. Details of the design and operation of these biosulfidogenic reactors are given elsewhere (e.g. Ñancucheo and Johnson, 2012). In brief, these were 2.2 L (working volume) bioreactors coupled to FerMac 310/60 control units (Electrolab, UK), maintained at pH 4.0 and fed with an acidic (pH ~ 2.5) liquid medium containing 5 mM glycerol (the electron donor used by the sulfidogenic bacteria in the bioreactor). The $H_2S$ generated in the reactor vessels was transported in a continuous flow of OFN into off-line gas bottles that contained samples of PLS samples.
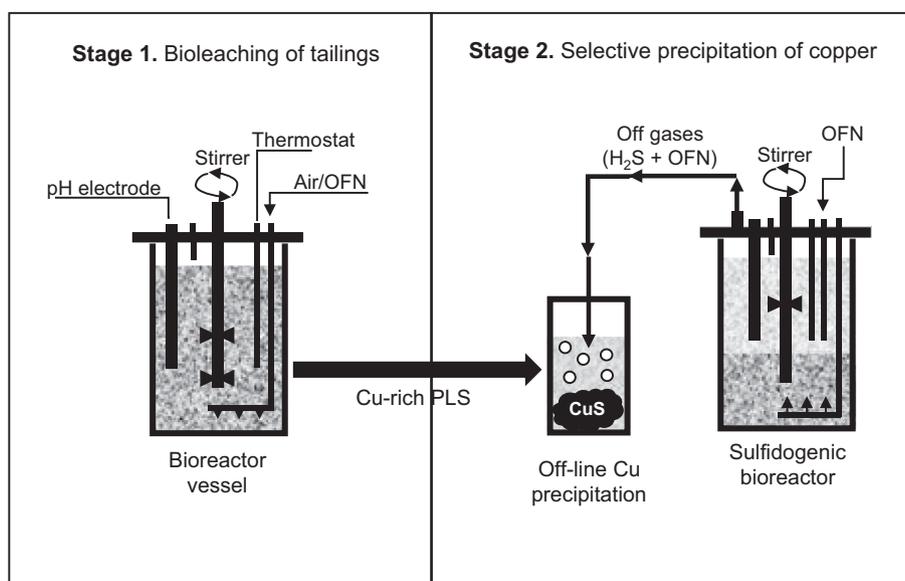
**Fig. 1.** Schematic representation (not in scale) of the reactors used during the bioleaching experiments (stage 1) and the offline precipitation of copper using a low pH sulfidogenic bioreactor (stage 2).

## 2.5. Biomolecular analyses

Liquid samples used to determine the compositions of microbial consortia using a biomolecular approach were collected at the beginning and the end of the tailings leaching experiments. Solutions were filtered through 0.2 µm size pore membrane filters until saturation, and DNA extracted using MoBio "ultraclean soil DNA isolation kits", following the manufacturer's instructions. Terminal restriction enzyme fragment length polymorphism (T-RFLP) analysis of bacterial and archaeal 16S rRNA genes was used to assess the microbial compositions (Kay et al., 2014). Terminal restriction fragment (T-RFs) were identified by reference to those in the databank maintained at Bangor University.

## 2.6. Analytical methods

Ferrous iron concentrations were determined using the Ferrozine assay (Stookey, 1970). Total iron concentrations were measured reducing all the ferric iron present to ferrous with an excess of ascorbic acid, and ferric iron concentrations determined from the differences in ferrous and total iron concentrations. Transition metals were measured with a Dionex-320 ion chromatograph fitted with an IonPAC® CS5A column and an AD absorbance detector and/or atomic absorption spectrometry (AAS) using a Varian SpectrAA 220 FS. Total concentrations of copper was also determined using the colorimetric method described by Anwar et al. (2000). Redox potentials and pH were measured off-line using combination electrodes coupled to an Accumet® 50 pH-meter, and redox values corrected to $E_H$ (i.e. relative to a hydrogen reference electrode). Tailings samples (fresh samples and leached samples) were gently hand ground in a mortar and pestle to ensure a sample size of <100 µm, and minerals present identified using a Siemens D5000 X-ray Diffractometer (Cu Kα radiation,

40 kV and 30 mA). The samples were scanned at room temperature over 2θ angles of 2–70°, on a 0.02° step with a step time of 1 s, taking about 60 min. The samples were rotated during measurement to minimise the effects of preferential mineral orientation. The profiles produced by the scan were compared to peaks of reference minerals using the JCPDS PDF-2 (2004) database and EVA software V.18.0.0.0.

## 3. Results

### 3.1. Compositions of mineral tailings

XRD analysis identified quartz, pyrite and gypsum, but no copper minerals, in CLC tailings. Other data, provided by Francisco Sánchez Ruiz (Cobre Las Cruces, S.A.), showed that most (60%) of copper in these tailings was deported in chalcopyrite, with smaller amounts present in covellite (18%), tetrahedrite (12.5%), enargite (8%) and bornite (1.5%). Quartz, kaolinite, pyrite, gypsum and alunite were identified in Bor tailings by XRD analysis. The copper minerals present were not identified, but elsewhere (Antonijević et al., 2008) Bor tailings were reported to contain <1% copper sulfides, deported in covellite (44%), chalcopyrite (26%), enargite (20%) and chalcocite (10%). Table 1 lists the abundance of the major elements in the two tailings samples. Copper was the only transition metal (apart from iron) present in significant concentrations in the Bor tailings, while the CLC tailings also contained lead and zinc.

### 3.2. Bioleaching of Cobre Las Cruces tailings and recovery of metals from solid residues

When bioleached aerobically at 30 °C, ~35% of the copper was extracted from CLC tailings during the first two weeks, but after

**Table 1**
Elemental composition of Bor and Cobre Las Cruces tailings, shown as % or (∗) as mg/kg; < below detection limit.

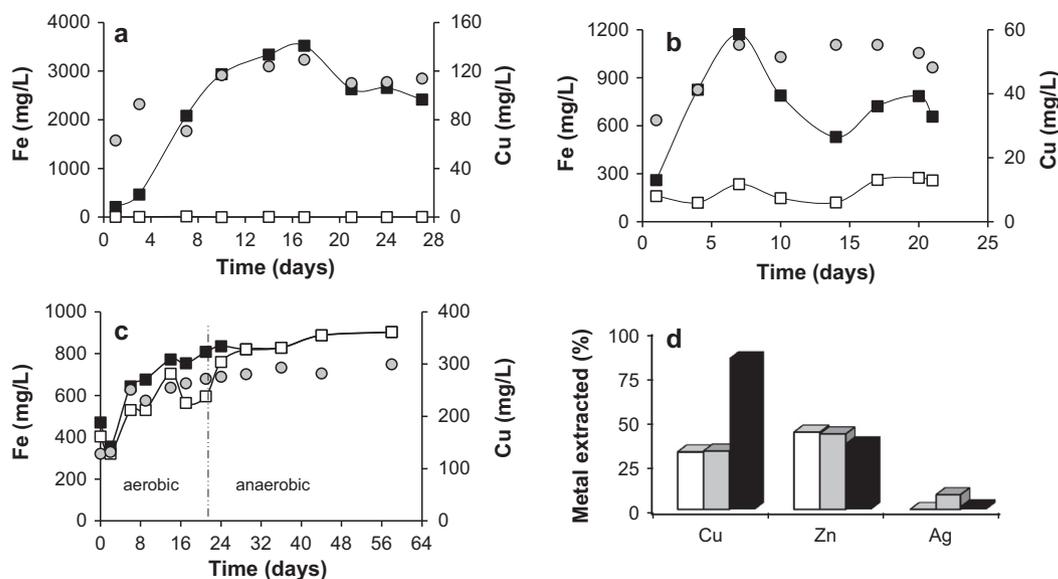| Element (%) | Cu | Fe | S | Mg | Si | Ca | Zn | As | P | Ti | Pb | Sr | Ag* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bor | 0.13 | 7.72 | 6.66 | 10.41 | 16.55 | 1.78 | 0.04 | < | 0.24 | 0.21 | < | 0.10 | 3[a] |
| Cobre Las Cruces | 0.72 | 28.05 | 22.04 | 12.42 | 7.22 | 1.87 | 0.13 | 0.23 | 0.19 | 0.17 | 0.76 | < | 30 |

[a] Data from Antonijević et al. (2008).

**Fig. 2.** Bioleaching of CLC tailings at (a) 30 °C, pH 1.7 and constant aeration; (b) 45 °C, pH 1.7 and constant aeration; (c) 45 °C, no pH control, and aerobic/anoxic conditions. Key: (●) copper; (■) total soluble iron, (□) ferrous iron. Figure (d) shows comparative percentages of copper, zinc and silver extracted by bioleaching at 30 °C, pH 1.7 and constant aeration (white bars), 45 °C, pH 1.7 and constant aeration (grey bars) and 45 °C, pH 1.8–1.0 and aerobic/anoxic conditions (black bars).

that time copper concentrations decreased alongside those of soluble iron (Fig. 2a), and the final copper concentration in the leach liquor was equivalent to 32% extraction from the tailings. Although slightly higher concentrations of soluble copper were found during the early stages of CLC tailings aerobically at 45 °C (Fig. 2b), these subsequently declined, so that the final percentage of copper extracted (32.5%) was similar to that obtained at 30 °C. Concentrations of soluble iron were consistently much lower in 45 °C (and decreased notably after day 8) than in 30 °C leachates (Fig. 2b). The most effective bioleaching of copper from CLC tailings was, however, obtained at 45 °C in the presence of added sulfur, pH control removed, and aerobic/anaerobic phases combined (Fig. 2c). Just before the gas supply was changed from air to OFN (at day 21) the copper concentration was already twice that obtained at the same temperature when the pH was maintained at 1.7. After switching to anoxic conditions, copper concentrations continued to increase, but to a small extent, reaching a maximum of 304 mg/L (corresponding to 84.4% copper extraction from the tailings). Zinc was also bioleached from CLC tailings, but very little lead (∼0.1%) was solubilised (data not shown).

As expected, there were major differences in iron speciation under the three bioleaching regimes, which were reflected in $E_H$ values of the leachates. Virtually all of the soluble iron was present as oxidised ferric iron in 30 °C leachates, and $E_H$ values between days 10 and 27 varied between +888 and +881 mV. Ferric iron was also the dominant soluble species present in aerobic 45 °C leachates, though significant concentrations of ferrous iron were also

found throughout the bioleaching period, corresponding to lower $E_H$ values of between +718 and +816 mV. In complete contrast, ferrous iron was always the dominant species present (and $E_H$ values less positive) when sulfur was added to the mineral slurry mix and pH was allowed to decline under aerobic conditions. Some soluble ferric iron was present in the leach liquor when this reactor was aerated, but this was rapidly reduced to ferrous when anoxic conditions were imposed (Fig. 2c) with corresponding falls in $E_H$ values, from +652 mV to +580 mV. It is interesting to note that total soluble iron concentrations increased (from 808 mg/L to a maximum value of 952 mg/L) during the anoxic bioleaching phase, indicating that solid phase, as well as soluble ferric iron, was reduced in this period.

Total percentages of copper, zinc and silver extracted from CLC tailings by bioleaching under different conditions, are shown in Fig. 2d. Significant amounts of silver, and to a lesser extent of copper, were extracted chemically from mineral residues (i.e. following bioleaching), as shown in Table 2. The total amount of copper extracted from CLC tailings by a combination of bioleaching under aerobic/anoxic conditions and chemical treatment of solid residues was ∼86%. However, far less silver was recovered under this regime than in both situations where the bioreactors were continuously aerated and maintained at pH 1.7. Table 2 also shows that 99% of the silver and 61% of the copper appeared to be extractable from non bio-processed tailings using NaCl/HCl. The latter also extracted ∼9% of the lead present in the fresh tailings, and 6.6–7.5% of that in the bioleached mineral residues.

**Table 2**
Percentages of copper and silver extracted from CLC tailings by bioleaching and chemical extraction from leach residues and fresh tailings (mean values ± standard deviations).

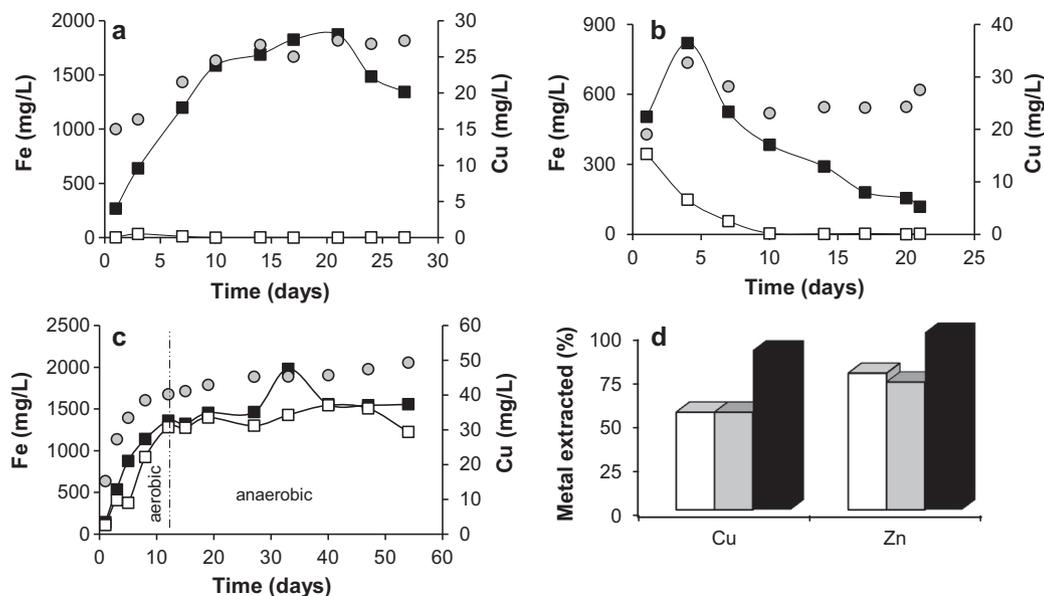| | Temperature/pH/aeration | Bioleaching | Extraction from residues | Combined | Extraction from fresh tailings |
|---|---|---|---|---|---|
| Copper | | | | | 61 ± 1.0 |
| | 30 °C/1.7/aerobic | 32 | 19.5 ± 0.9 | 51.5 | |
| | 45 °C/1.7/aerobic | 32.5 | 12.3 ± 0.3 | 44.8 | |
| | 45 °C/1.8–1.0/aerobic → anoxic | 84.4 | 1.2 ± 0.4 | 85.6 | |
| Silver | | | | | 99 ± 2.4 |
| | 30 °C/1.7/aerobic | <detectable | 99 ± 4.6 | 99 | |
| | 45 °C/1.7/aerobic | 8 | 98 ± 5.3 | 99 | |
| | 45 °C/1.8–1.0/aerobic → anoxic | 2 | 50 ± 2.8 | 52 | |

**Fig. 3.** Bioleaching of Bor tailings at (a) 30 °C, pH 1.7 and constant aeration; (b) 45 °C, pH 1.7 and constant aeration; (c) 45 °C, pH 1.8–1.0 and aerobic/anoxic conditions. Key: (●) copper; (■) total soluble iron, (□) ferrous iron. Figure (d) shows comparative percentages of copper and zinc extracted by bioleaching at 30 °C, pH 1.7 and constant aeration (white bars), 45 °C, pH 1.7 and constant aeration (grey bars) and 45 °C, pH 1.8–1.0 and aerobic/anoxic conditions (black bars).

### 3.3. Bioleaching of Bor tailings and recovery of metals from solid residues

Extraction of copper (~55%) was similar when Bor tailings were bioleached at 30° or 45 °C at pH 1.7 and in constant aerobic conditions (Fig. 3a and b). As with CLC tailings, concentrations of soluble iron declined after an initial rapid increase (at 45 °C), and there was greater evidence of co-precipitation of copper than in the case of CLC tailings. Again, the highest percentage of copper extraction was obtained when Bor tailings were bioleached under conditions of no pH control and phases of oxic and anoxic conditions. The pH in this bioreactor fell more rapidly than in the case of CLC tailings, to <1.0 within 10 days. Redox potentials declined subsequently from +630 mV to +588 mV, and concentrations of soluble copper continued to increase. In contrast to bioleaching of Bor tailings carried out at the same temperature by under constant aeration and pH 1.7, concentrations of soluble iron and copper did not decrease at any stage when Bor tailings were bioleached under "freefall pH" conditions. In total >90% of the copper was extracted from Bor tailings when bioleached at extremely low pH and alternating aerobic/anoxic conditions (Fig. 3d). Other metals were also more effectively (e.g. ~99% in the case of zinc) extracted by low pH and combined oxidative/reductive leaching (Fig. 3d). Essentially all the manganese (~99%) present in Bor tailings was extracted in all three of bioleaching tests (data not shown).

As was the case with chemical extraction of CLC bioleached solids, significant amounts of silver, and to a lesser extent of cop-

per, were extracted chemically from mineral residues, as shown in Table 3. The total amount of copper extracted from Bor tailings by a combination of bioleaching at low pH and aerobic/anoxic conditions and extraction from solid residues was ~99%. However, silver was not leached under any of the bioleaching experiments, but was extracted from both non bio-processed tailings and bioleached solids (Table 3).

### 3.4. Offline precipitation of copper from PLS

Copper was precipitated from the leach liquors produced by bioleaching CLC and Bor tailings by contacting with $H_2S$ generated in low pH sulfidogenic bioreactors. Over 99% of the copper present in 200 mL of leachates was removed from solution within 6 h. The other major metals (iron, manganese and zinc) present in the leachates remained in solution, but all of the silver present was co-precipitated with copper (Fig. 4). During the formation of copper/silver sulfides in the off-line gas bottle, the pH of the PLS declined slightly (from 0.94 to 0.89 for CLC PLS, and from 0.82 to 0.81 for Bor PLS).

### 3.5. Compositions of microbial bioleaching consortia

Bacteria, but no archaea, were detected in PLS at the end of the bioleaching stage of the experiments. *Sb. thermosulfidooxidans* was present in all bioleachates (with both mineral tailings bioleached at 30° and at 45 °C) and *At. caldus* in all experiments carried out

**Table 3**
Percentages of copper and silver extracted from Bor tailings by bioleaching and chemical processing of leached residues (mean values ± standard deviations).

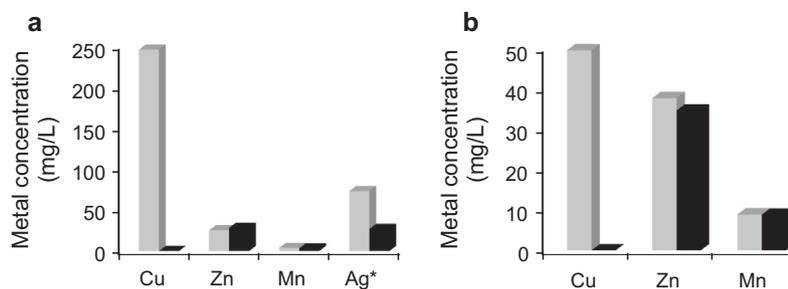|  | Temperature/pH/aeration | Bioleaching | Extraction from residues | Combined | Extraction from fresh tailings |
|---|---|---|---|---|---|
| **Copper** |  |  |  |  | 51.1 ± 2.7 |
|  | 30 °C/1.7/aerobic | 55 | 20.5 ± 1.0 | 75.5 |  |
|  | 45 °C/1.7/aerobic | 55 | 11.7 ± 2.2 | 66.7 |  |
|  | 45 °C/1.8–1.0/aerobic → anoxic | 90 | 9.2 ± 1.7 | 99.2 |  |
| **Silver** |  |  |  |  | 76.1 ± 7.3 |
|  | 30 °C/1.7/aerobic | <detectable | 92.4 ± 13.7 | 92.4 |  |
|  | 45 °C/1.7/aerobic | <detectable | 88.9 ± 5.0 | 88.9 |  |
|  | 45 °C/1.8–1.0/aerobic → anoxic | <detectable | 77.4 ± 10.0 | 77.4 |  |

**Fig. 4.** Concentrations of metals in PLS produced by bioleaching (a) CLC and (b) Bor tailings, at 45 °C, with no pH control and alternating aerobic/anoxic conditions. The grey bars depict metal concentration before, and the black bars after, contacting with biogenic $H_2S$. *Silver concentrations are shown as µg/L.

at 45 °C but not at 30 °C. The mesophilic consortium in CLC tailings bio-processed at 30 °C was dominated by *At. ferrooxidans* both at the beginning and at the end of the experiment, and *Sb. thermosulfidooxidans* was also detected at the end. This was also the case for the parallel Bor tailings reactor, although in this case *At. ferrivorans* was also detected at the start of the experiment. The 45 °C (aerobic) CLC bioreactor was dominated by *At. caldus* and *Sb. thermosulfidooxidans* throughout, while the microbial community in the corresponding Bor tailings reactor was dominated by *At. caldus* at the start, but by *Sb. thermosulfidooxidans*, *Sb. acidophilus* and *Ab. sulfuroxidans* by the end of the experiment. The 45 °C CLC tailings bioreactor operated with no pH control and alternating oxic/anoxic conditions was dominated by *Sb. thermosulfidooxidans* and *At. caldus* by the end of the experiment (CLC tailings). The corresponding Bor tailings reactor was dominated by *At. caldus* and *Sb. acidophilus*; *Sb. thermosulfidooxidans*, *Ab. sulfuroxidans*, and *L. ferriphilum* were also detected, though in relatively small relative abundance, at the end of the bioleaching period (data not shown).

## 4. Discussion

Bioleaching of copper from CLC tailings was more effective at higher temperature (45 °C) than at 30 °C, and was further enhanced by allowing the pH to fall to from 1.8 to 1.0 (due mostly to oxidation of added elemental sulfur to sulfuric acid) and combining phases of oxidative and reductive bioleaching. Similar results were obtained with the Bor tailings where the pH fell more rapidly (and to <1.0) during the oxidative phase of bioleaching. Similar copper concentrations were recovered during the 30 and 45 °C bioleaching. However, in the chemical extraction of the processes tailings more copper was extracted from those tailings bioleached at 30 °C. Therefore, it can be assumed that less copper was extracted from the tailings leached at 30 °C than at 45 °C, but somehow copper was precipitated in a non-soluble for that is not susceptible to chemical extraction by the method used.

More than 80% and 90% of the copper present was extracted from CLC and Bor tailings, respectively, by leaching under optimised conditions, which was 30–40% greater than that achieved by bioleaching under constant aeration and a fixed pH of 1.7. The rationale of imposing an anoxic bioleaching phase was to determine whether this would induce additional extraction of metals by promoting the bacterially-catalysed reductive dissolution of oxidised minerals either present in the tailings or generated during the oxidative phase of bioleaching (e.g. jarosites), as previously demonstrated for limonite ores (Johnson et al., 2013). It has also been suggested that reduced inorganic sulfur oxyanions (such as thiosulfate), which might also enhance silver extraction, might be produced as transient intermediates by sulfur-metabolising acidophiles under anoxic conditions (Hedrich et al., 2013). In the event, there was no evidence of the latter in the current experiments, and the main reason for the greatly enhanced extraction

of copper from both CLC and Bor tailings under the most successful experimental regime was the lower pH achieved.

The relatively high efficiency of copper bioleaching from the CLC tailings was unexpected given that much of this metal (68%) was deported in two minerals (chalcopyrite and enargite) that are usually regarded as recalcitrant, at least to conventional bioprocessing (Watling, 2006). Low redox potentials (~+630 to +650 mV vs SHE) favour the leaching of chalcopyrite (Gericke et al., 2010), during the oxidative/reductive bioleaching low redox potential were kept, which may have had favoured the leaching of the chalcopyrite present in the tailings. In addition, other reason for the relatively high percentage of copper extracted may well be the significant (30 mg/kg) silver content of CLC tailings. Silver has long been recognised to enhance the bioleaching of chalcopyrite, and has in the past been added to facilitate this process (e.g. Romero et al., 2003). Copper concentrates sometimes contain sufficient silver to catalyse the oxidative dissolution of chalcopyrite, even at relatively low temperatures (Johnson et al., 2008). Relatively little silver was solubilised by bioleaching CLC tailings under any condition, though more was detected in aerobic 45 °C leachates, which also generated more secondary jarosite minerals than other protocols used, suggesting that argentojarosite (Sasaki et al., 1995) was not a significant by-product.

259080121794905The data obtained indicated that most of the silver present in fresh CLC tailings could be extracted using a hot acidic saline solution (Romero et al., 2003) and that the same amount was extractable from tailings that had been bioleached at 30 °C. Much less silver was extracted from CLC tailings that had been bioleached at 45 °C, which may have been due to more being solubilised at this higher temperature. Mass balance calculations showed a shortfall in silver accounting however, particularly in the 45 °C reactor that had been operated without pH control. One possible reason for this was that concentrations of silver in bioleach liquors were mostly at the level of sensitivity of the analytical technique used to measure them, and were therefore subject to some degree of inaccuracy.

The compositions of the microbial consortia that developed in these bioleaching experiments showed some degree of variation with temperature, pH and type of tailings. The bacteria that were detected all have roles in mineral bio-processing, either (i) in catalysing the oxidation of ferrous to ferric iron, the main chemical oxidant of sulfide minerals, or (ii) the oxidation of sulfur coupled either to molecular oxygen, forming sulfuric acid, or (iii) the reduction of soluble or mineral-phase ferric iron. Some of the microbial consortia data obtained were much as had been anticipated, e.g. the dominance of the thermo-tolerant species *Sb. thermosulfidooxidans* and *At. caldus* in the 45 °C bioreactors, and of mesophilic iron-oxidizing *Acidithiobacillus* spp. in 30 °C bioreactors. Other results were more unexpected such as the occurrence of *Sb. thermosulfidooxidans* in all 30 °C, although as minority, as well as 45 °C bioreactor consortia. The most phylogenetically diverse consortia were

found in the 45 °C bioreactors leaching Bor tailings (operated both with and without pH control) where three other species (*Ab. sulfuroxidans*, *Sb. acidophilus* and *L. ferriphilum*) were found in addition to *Sb. thermosulfidooxidans* and *At. caldus*. The reason for this greater biodiversity is not known, though it could relate to the fact that concentrations of copper (which is more toxic to some acidophiles than others) in Bor leachates were far less than in than those in CLC leachates.

Copper present in PLS from both CLC and Bor tailings was readily precipitated as a sulfide phase by contacting with biogenic $H_2S$, as demonstrated previously (Ñancucheo and Johnson, 2012; Hedrich and Johnson, 2014). By doing this with no pH adjustment to the leachates, copper was selectively removed, as other metals present in significant concentrations in the leach liquors (iron and zinc) do not form sulfide phases at pH values <3. This approach would therefore facilitate the recovery and recycling of copper from both mineral tailings wastes. The only metal present that did co-precipitate (from CLC tailings leachates) was silver (which was present in much smaller concentrations), as $Ag_2S$ has a smaller solubility product than CuS.

More than 1.5 million tonnes of mineral tailings are produced each year at the Cobre Las Cruces mine. Given 85% extraction of copper (the maximum achieved in the current bioleaching tests), 9.2 kilotonnes of metal could be produced (with a current value ~$44 million US) from what is currently classed as a waste material. Additional revenue would come from the zinc that could also be recovered by bioleaching (~$1.3 million US/annum). The question of how much of the silver could be extracted and recovered economically needs to be examined further. Data from the current study suggests that chemical extraction is a feasible option, but that the process that is best for bioleaching copper produces a silver-depleted (or at least, less extractable) mineral residue.

Stankovic et al. (2015) estimated that between 54,000 and 81,000 t of copper is present in the old flotation tailings at the Bor mine. These authors used the acidic ferric iron-rich water in the nearby Lake Robule to leach copper from these tailings, and extracted ~80% of that present. More effective extraction (>90%) was achieved by bioleaching in the current experiments, and the estimated total value of copper that could be extracted and recovered from these old tailings using such an approach is between $225 million (US) and $337 million (US). Again there would be some additional value from co-bioleaching and recovering zinc (estimated as between $30 and $45 million US). In addition to these potential direct financial benefits, bio-processing the tailings wastes would help to remove some, at least, of the environmental hazard that these tailings represent (protracted release of metals and acid into the wider environment), much in the same way that bio-processing cobalt-rich tailings at Kasese in Uganda facilitated the production of a more environmentally-benign waste product (Morin and D'Hugues, 2007).

## 5. Conclusions

A modified approach, involving a combination of oxidative and reductive bioleaching carried out at extremely low pH values and at 45 °C, was shown to be highly effective in extracting copper from mineral tailings generated as waste materials in two mines currently operating in Spain and Serbia. Soluble copper in leach liquors was selectively precipitated as CuS by contacting with biogenic $H_2S$.

However, whether the combination of oxidative and reductive phases during the bioleaching or the low pH and/or low redox potential conditions are the main factors enhancing the extraction of copper is still needed to be clarified in future experiments.

A combined approach for extracting and recovering metals from mineral waste materials could be used at pilot- and full-scale operations at both mine sites, where the projected economic benefits are considerable.

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