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## New approaches for extracting and recovering metals from mine tailings

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### Minerals Engineering

DOI:

[10.1016/j.mineng.2016.10.008](https://doi.org/10.1016/j.mineng.2016.10.008)

Published: 15/05/2017

Peer reviewed version

[Cyswllt i'r cyhoeddiad / Link to publication](#)

*Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):*

Falagan Rodriguez, M. D. C., Grail, B., & Johnson, D. (2017). New approaches for extracting and recovering metals from mine tailings. *Minerals Engineering*, 106, 71-78.  
<https://doi.org/10.1016/j.mineng.2016.10.008>

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1 **New approaches for extracting and recovering metals from mine**  
2 **tailings**

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21 **Abstract**

22

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

42

43

44 *Keywords:* bioleaching, acidophile, tailings, copper, silver.

45

46

## 47 **1. Introduction**

48 Mining of metals generates considerably amounts of waste materials. These generally  
49 have very little economic value, making their exploitation not profitable, though they often have  
50 the potential to pose a long-term threat and cause damage to the environment. Mine wastes  
51 vary depending on their physical and chemical composition, the type of mining and the way  
52 the mineral is processed. Millions of tonnes of ore are processed every year by the mining  
53 industry, >95% of which is disposed of in the form of waste rocks and mine tailings. The latter  
54 are finely ground rock particles generated during the processing of ore materials and  
55 separation of target metal minerals, and are highly reactive because of their small particle size  
56 and content of reactive minerals, such as pyrite ( $\text{FeS}_2$ ). Mine tailings may contain base  
57 transition metals, such as iron, copper, nickel and zinc, in relatively high concentrations, and  
58 also occasionally precious metals such as gold and silver, in minerals (and native metals) that  
59 have been not been separated by froth flotation (e.g. Bryan et al., 2006, Ahmadi et al., 2015).  
60 Toxic elements, such as arsenic, may also be present in elevated concentrations (e.g. Nguyen  
61 et al., 2015). Exposure of mine tailings to both oxygen and water facilitates the (microbially-  
62 catalysed) oxidative dissolution of the sulfide minerals they contain, generating acidic waste  
63 waters that contain elevated concentrations of dissolved metals and sulfate. Acid mine  
64 drainage (AMD) is a widely-reported environmental pollutant, with global distribution.

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

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

86       Harnessing the abilities of some species of prokaryotic microorganisms to catalyse the  
87 oxidative dissolution of sulfide minerals and thereby to facilitate the extraction of metals is a  
88 global biotechnology, used mostly to recover some base including copper, nickel, cobalt and  
89 zinc, and precious metals such as gold. The geochemical processes and the microorganisms  
90 involved in biomining have been widely researched and are well understood (e.g. Vera et al.,  
91 2013; Johnson, 2014). However, not all sulfide minerals are readily bioleached such as  
92 chalcopyrite. This mineral (the most abundant copper mineral) is notoriously difficult to  
93 effectively oxidise using conventional bioleaching approaches (reviewed in Watling, 2006).  
94 Recently, extraction of metals from oxidised ores using reductive, rather than oxidative,  
95 bioleaching has been described (Johnson et al., 2013). In contrast to conventional biomining,  
96 this requires the addition of an extraneous material, such as elemental sulfur, to act as the  
97 electron donor for bacteria that reduce ferric iron minerals.

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

102

## 103 2. Materials and Methods

104

### 105 2.1. Mineral tailings

106

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

109

### 110 2.2. Bioleaching microbial consortia

111

112 Three different consortia of acidophilic microorganisms were used in bioleaching  
113 experiments. These were: (i) a thermo-tolerant consortium, which included the autotrophic  
114 iron-oxidizer *Leptospirillum (L.) ferriphilum* (both the type strain and strain MT63), the  
115 autotrophic sulfur-oxidizer *Acidithiobacillus (At.) caldus*<sup>T</sup>, facultatively autotrophic and  
116 obligately heterotrophic iron/sulfur oxidizing and iron-reducing bacteria (*Sulfobacillus (Sb.)*  
117 *thermosulfidooxidans*<sup>T</sup> and *Acidibacillus (Ab.) sulfuroxidans*<sup>T</sup>, and two heterotrophic iron-  
118 oxidizing archaea: *Ferroplasma (F.) acidiphilum* (strain BRGM4) and *Acidiplasma* sp. (strain  
119 FV); (ii) a second thermo-tolerant consortium, used in bioreactors where pH was not  
120 controlled, that included *L. ferriphilum* strain MT63, *At. caldus*<sup>T</sup>, *Sb. thermosulfidooxidans*<sup>T</sup>,  
121 “*Ab. sulfuroxidans*<sup>T</sup>”, *F. acidiphilum* strain BRGM4, *Acidiplasma* strain FV, and two strains of  
122 *Sulfobacillus* that had been isolated from the Bor tailings (*Sb. thermosulfidooxidans* strain  
123 BOR3 and *Sb. acidophilus* strain BOR1); (iii) a mixed mesophilic/thermo-tolerant consortium,  
124 that included six species of mesophilic acidophiles (autotrophic iron/sulfur-oxidizing and iron-  
125 reducing *Acidithiobacillus* spp. (*At. ferrooxidans*<sup>T</sup>, *At. ferridurans*<sup>T</sup>, *At. ferriphilus*<sup>T</sup> and *At.*  
126 *ferrivorans* strain CF27), “*Ab. ferrooxidans*<sup>T</sup>” (a heterotrophic iron-oxidizer/reducer),  
127 *Acidiphilum (A.) cryptum* (strain SJH; a heterotrophic iron-reducer)), and three thermo-tolerant  
128 species (*Sb. thermosulfidooxidans*<sup>T</sup>, *F. acidiphilum* strain BRGM4 and *Acidiplasma* strain FV).  
129 The consortia were grown in iron/yeast extract media pH 2.0, at 30 (mesophilic/thermo-tolerant

130 consortium) or 45 °C (thermo-tolerant consortia) for 2 to 3 weeks previously to inoculation of  
131 the reactors.

132

### 133 **2.3. Bioleaching experiments and processing of mineral residues**

134

135 Bioleaching of mineral tailings was carried out in 2.3 L bioreactor vessels coupled to  
136 temperature- and pH-control units (Electrolab, UK). Temperatures were maintained at either  
137 30° (for the mesophilic/thermo-tolerant consortium) or 45°C (for the two thermo-tolerant  
138 consortia), and the reactors were stirred continuously at 150 rpm. Control of pH (where used)  
139 was by automated addition of 0.5 M sulfuric acid or 0.5 M sodium hydroxide. For aerobic  
140 conditions, the reactor vessels were gassed with sterile air, and when anoxic conditions were  
141 required this was replaced by oxygen-free nitrogen (OFN), both at flow rates of ~ 1 L/min. A  
142 basal salts/trace elements solution (Ñancucheo et al., 2016) was put into each reactor vessel  
143 (1.4 L for aerobic bioleaching, and 1.9 L for combined aerobic/anaerobic bioleaching), followed  
144 by mineral tailings (5% pulp densities; w/v) and 100 mL of pre-grown microbial inocula.

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

155 When the bioleaching phase was considered to be complete, mineral slurries were  
156 removed from the bioreactor vessels, and the pregnant leach solutions (PLS) separated from  
157 the mineral residues. Tests were carried out to selectively precipitate copper from PLS by

158 sulfide precipitation (section 2.4). Copper and silver were extracted both from mineral residues  
159 and fresh mineral tailings by mixing 0.5 g of representative samples with 10 mL of a solution  
160 containing 3.42 M NaCl and 0.2 M HCl (Romero et al., 2003), and heating for 1 h at 90°C.

161

#### 162 **2.4. Sulfidogenic bioreactors and selective recovery of copper from PLS**

163

164 Low pH, continuous-flow sulfidogenic bioreactors (Fig. 1) were used as sources of  
165 hydrogen sulfide (H<sub>2</sub>S) for precipitating copper present in PLS generated in the bioleaching  
166 experiments described above. Details of the design and operation of these biosulfidogenic  
167 reactors are given elsewhere (e.g. Nancucheo and Johnson, 2012). In brief, these were 2.2 L  
168 (working volume) bioreactors coupled to FerMac 310/60 control units (Electrolab, UK),  
169 maintained at pH 4.0 and fed with an acidic (pH ~ 2.5) liquid medium containing 5 mM glycerol  
170 (the electron donor used by the sulfidogenic bacteria in the bioreactor). The H<sub>2</sub>S generated in  
171 the reactor vessels was transported in a continuous flow of OFN into off –line gas bottles that  
172 contained samples of PLS samples.

173

#### 174 **2.5. Biomolecular analyses**

175

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

184

#### 185 **2.6. Analytical methods**



186

187 Ferrous iron concentrations were determined using the Ferrozine assay (Stookey, 1970).  
188 Total iron concentrations were measured reducing all the ferric iron present to ferrous with an  
189 excess of ascorbic acid, and ferric iron concentrations determined from the differences in  
190 ferrous and total iron concentrations. Transition metals were measured with a Dionex-320 ion  
191 chromatograph fitted with an IonPAC® CS5A column and an AD absorbance detector and/or  
192 atomic absorption spectrometry (AAS) using a Varian SpectrAA 220 FS. Total concentrations  
193 of copper was also determined using the colorimetric method described by Anwar et al. (2000).  
194 Redox potentials and pH were measured off-line using combination electrodes coupled to an  
195 Accumet® 50 pH-meter, and redox values corrected to  $E_H$  (i.e. relative to a hydrogen reference  
196 electrode). Tailings samples (fresh samples and leached samples) were gently hand ground  
197 in a mortar and pestle to ensure a sample size of  $<100\ \mu\text{m}$ , and minerals present identified  
198 using a Siemens D5000 X-Ray Diffractometer (Cu  $K\alpha$  radiation, 40 kV and 30 mA). The  
199 samples were scanned at room temperature over  $2\theta$  angles of  $2^\circ$  to  $70^\circ$ , on a  $0.02^\circ$  step with  
200 a step time of 1 second, taking about 60 minutes. The samples were rotated during  
201 measurement to minimise the effects of preferential mineral orientation. The profiles produced  
202 by the scan were compared to peaks of reference minerals using the JCPDS PDF-2 (2004)  
203 database and EVA software V.18.0.0.0.

204

### 205 **3. Results**

206

#### 207 **3.1 Compositions of mineral tailings**

208

209 XRD analysis identified quartz, pyrite and gypsum, but no copper minerals, in CLC tailings.  
210 Other data, provided by Francisco Sánchez Ruiz (Cobre Las Cruces, S.A.), showed that most  
211 (60%) of copper in these tailings was deported in chalcopyrite, with smaller amounts present  
212 in covellite (18%), tetrahedrite (12.5%), enargite (8%) and bornite (1.5%). Quartz, kaolinite,  
213 pyrite, gypsum and alunite were identified in Bor tailings by XRD analysis. The copper minerals

214 present were not identified, but elsewhere (Antonijević et al., 2008) Bor tailings were reported  
215 to contain <1% copper sulfides, deported in covellite (44%), chalcopyrite (26%), enargite  
216 (20%) and chalcocite (10%). Table 1 lists the abundance of the major elements in the two  
217 tailings samples. Copper was the only transition metal (apart from iron) present in significant  
218 concentrations in the Bor tailings, while the CLC tailings also contained lead and zinc.

219

### 220 **3.2. Bioleaching of Cobre Las Cruces tailings and recovery of metals from solid** 221 **residues**

222

223 When bioleached aerobically at 30°C, ~ 35 % of the copper was extracted from CLC tailings  
224 during the first two weeks, but after that time copper concentrations decreased alongside  
225 those of soluble iron (Fig. 2a), and the final copper concentration in the leach liquor was  
226 equivalent to 32% extraction from the tailings. Although slightly higher concentrations of  
227 soluble copper were found during the early stages of CLC tailings aerobically at 45°C (Fig.  
228 2b), these subsequently declined, so that the final percentage of copper extracted (32.5%)  
229 was similar to that obtained at 30°C. Concentrations of soluble iron were consistently much  
230 lower in 45°C (and decreased notably after day 8) than in 30°C leachates (Fig. 2b). The most  
231 effective bioleaching of copper from CLC tailings was, however, obtained at 45°C in the  
232 presence of added sulfur, pH control removed, and aerobic/anaerobic phases combined (Fig.  
233 2c). Just before the gas supply was changed from air to OFN (at day 21) the copper  
234 concentration was already twice that obtained at the same temperature when the pH was  
235 maintained at 1.7. After switching to anoxic conditions, copper concentrations continued to  
236 increase, but to a small extent, reaching a maximum of 304 mg/L (corresponding to 84.4%  
237 copper extraction from the tailings). Zinc was also bioleached from CLC tailings, but very little  
238 lead (~0.1%) was solubilised (data not shown).

239 As expected, there were major differences in iron speciation under the three bioleaching  
240 regimes, which were reflected in  $E_H$  values of the leachates. Virtually all of the soluble iron  
241 was present as oxidised ferric iron in 30°C leachates, and  $E_H$  values between days 10 and 27

242 varied between +888 and +881 mV. Ferric iron was also the dominant soluble species present  
243 in aerobic 45°C leachates, though significant concentrations of ferrous iron were also found  
244 throughout the bioleaching period, corresponding to lower  $E_H$  values of between +718 and  
245 +816 mV. In complete contrast, ferrous iron was always the dominant species present (and  
246  $E_H$  values less positive) when sulfur was added to the mineral slurry mix and pH was allowed  
247 to decline under aerobic conditions. Some soluble ferric iron was present in the leach liquor  
248 when this reactor was aerated, but this was rapidly reduced to ferrous when anoxic conditions  
249 were imposed (Fig. 2c) with corresponding falls in  $E_H$  values, from +652 mV to +580 mV. It is  
250 interesting to note that total soluble iron concentrations increased (from 808 mg/L to a  
251 maximum value of 952 mg/L) during the anoxic bioleaching phase, indicating that solid phase,  
252 as well as soluble ferric iron, was reduced in this period.

253 Total percentages of copper, zinc and silver extracted from CLC tailings by bioleaching  
254 under different conditions, are shown in Fig. 2d. Significant amounts of silver, and to a lesser  
255 extent of copper, were extracted chemically from mineral residues (i.e. following bioleaching),  
256 as shown in Table 2. The total amount of copper extracted from CLC tailings by a combination  
257 of bioleaching under aerobic/anoxic conditions and chemical treatment of solid residues was  
258 ~ 86%. However, far less silver was recovered under this regime than in both situations where  
259 the bioreactors were continuously aerated and maintained at pH 1.7. Table 2 also shows that  
260 99% of the silver and 61% of the copper appeared to be extractable from non bio-processed  
261 tailings using NaCl/HCl. The latter also extracted ~9% of the lead present in the fresh tailings,  
262 and 6.6 – 7.5% of that in the bioleached mineral residues.

263

### 264 **3.3. Bioleaching of Bor tailings and recovery of metals from solid residues**

265

266 Extraction of copper (~ 55%) was similar when Bor tailings were bioleached at 30° or 45°C  
267 at pH 1.7 and in constant aerobic conditions (Fig. 3a and 3b). As with CLC tailings,  
268 concentrations of soluble iron declined after an initial rapid increase (at 45°C), and there was  
269 greater evidence of co-precipitation of copper than in the case of CLC tailings. Again, the

270 highest percentage of copper extraction was obtained when Bor tailings were bioleached  
271 under conditions of no pH control and phases of oxic and anoxic conditions. The pH in this  
272 bioreactor fell more rapidly than in the case of CLC tailings, to <1.0 within 10 days. Redox  
273 potentials declined subsequently from +630 mV to +588 mV, and concentrations of soluble  
274 copper continued to increase. In contrast to bioleaching of Bor tailings carried out at the same  
275 temperature by under constant aeration and pH 1.7, concentrations of soluble iron and copper  
276 did not decrease at any stage when Bor tailings were bioleached under “freefall pH” conditions.  
277 In total >90 % of the copper was extracted from Bor tailings when bioleached at extremely low  
278 pH and alternating aerobic/anoxic conditions (Fig. 3d). Other metals were also more effectively  
279 (e.g. ~ 99% in the case of zinc) extracted by low pH and combined oxidative/reductive leaching  
280 (Fig. 3d). Essentially all the manganese (~99%) present in Bor tailings was extracted in all  
281 three of bioleaching tests (data not shown).

282 As was the case with chemical extraction of CLC bioleached solids, significant amounts of  
283 silver, and to a lesser extent of copper, were extracted chemically from mineral residues, as  
284 shown in Table 3. The total amount of copper extracted from Bor tailings by a combination of  
285 bioleaching at low pH and aerobic/anoxic conditions and extraction from solid residues was ~  
286 99 %. However, silver was not leached under any of the bioleaching experiments, but was  
287 extracted from both non bio-processed tailings and bioleached solids (Table 3).

288

### 289 **3.4. Offline precipitation of copper from PLS**

290

291 Copper was precipitated from the leach liquors produced by bioleaching CLC and Bor  
292 tailings by contacting with H<sub>2</sub>S generated in low pH sulfidogenic bioreactors. Over 99% of the  
293 copper present in 200 mL of leachates was removed from solution within 6 hours. The other  
294 major metals (iron, manganese and zinc) present in the leachates remained in solution, but all  
295 of the silver present was co-precipitated with copper (Fig. 4). During the formation of  
296 copper/silver sulfides in the off-line gas bottle, the pH of the PLS declined slightly (from 0.94  
297 to 0.89 for CLC PLS, and from 0.82 to 0.81 for Bor PLS).

298

### 299 **3.5. Compositions of microbial bioleaching consortia**

300

301 Bacteria, but no archaea, were detected in PLS at the end of the bioleaching stage of the  
302 experiments. *Sb. thermosulfidooxidans* was present in all bioleachates (with both mineral  
303 tailings bioleached at 30° and at 45°C) and *At. caldus* in all experiments carried out at 45°C  
304 but not at 30°C. The mesophilic consortium in CLC tailings bio-processed at 30°C was  
305 dominated by *At. ferrooxidans* both at the beginning and at the end of the experiment, and *Sb.*  
306 *thermosulfidooxidans* was also detected at the end. This was also the case for the parallel Bor  
307 tailings reactor, although in this case *At. ferrivorans* was also detected at the start of the  
308 experiment. The 45°C (aerobic) CLC bioreactor was dominated by *At. caldus* and *Sb.*  
309 *thermosulfidooxidans* throughout, while the microbial community in the corresponding Bor  
310 tailings reactor was dominated by *At. caldus* at the start, but by *Sb. thermosulfidooxidans*, *Sb.*  
311 *acidophilus* and *Ab. sulfuroxidans* by the end of the experiment. The 45°C CLC tailings  
312 bioreactor operated with no pH control and alternating oxic/anoxic conditions was dominated  
313 by *Sb. thermosulfidooxidans* and *At. caldus* by the end of the experiment (CLC tailings). The  
314 corresponding Bor tailings reactor was dominated by *At. caldus* and *Sb. acidophilus*; *Sb.*  
315 *thermosulfidooxidans*, *Ab. sulfuroxidans*, and *L. ferriphilum* were also detected, though in  
316 relatively small relative abundance, at the end of the bioleaching period (data not shown).

317

### 318 **4. Discussion**

319

320 Bioleaching of copper from CLC tailings was more effective at higher temperature (45°C)  
321 than at 30°C, and was further enhanced by allowing the pH to fall to from 1.8 to 1.0 (due mostly  
322 to oxidation of added elemental sulfur to sulfuric acid) and combining phases of oxidative and  
323 reductive bioleaching. Similar results were obtained with the Bor tailings where the pH fell  
324 more rapidly (and to <1.0) during the oxidative phase of bioleaching. Similar copper  
325 concentrations were recovered during the 30 and 45 °C bioleaching. However, in the chemical

326 extraction of the processes tailings more copper was extracted from those tailings bioleached  
327 at 30 °C. Therefore, it can be assumed that less copper was extracted from the tailings leached  
328 at 30 °C than at 45 °C, but somehow copper was precipitated in a non-soluble for that is not  
329 susceptible to chemical extraction by the method used.

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

343 The relatively high efficiency of copper bioleaching from the CLC tailings was unexpected  
344 given that much of this metal (68%) was deported in two minerals (chalcopyrite and enargite)  
345 that are usually regarded as recalcitrant, at least to conventional bio-processing (Watling,  
346 2006). Low redox potentials (~+630 to +650 mV vs SHE) favour the leaching of chalcopyrite  
347 (Gericke et al., 2010), during the oxidative/reductive bioleaching low redox potential were kept,  
348 which may have had favoured the leaching of the chalcopyrite present in the tailings. In  
349 addition, other reason for the relatively high percentage of copper extracted may well be the  
350 significant (30 mg/kg) silver content of CLC tailings. Silver has long been recognised to  
351 enhance the bioleaching of chalcopyrite, and has in the past been added to facilitate this  
352 process (e.g. Romero et al., 2003). Copper concentrates sometimes contain sufficient silver  
353 to catalyse the oxidative dissolution of chalcopyrite, even at relatively low temperatures

354 (Johnson et al., 2008). Relatively little silver was solubilised by bioleaching CLC tailings under  
355 any condition, though more was detected in aerobic 45°C leachates, which also generated  
356 more secondary jarosite minerals than other protocols used, suggesting that argentojarosite  
357 (Sasaki et al., 1995) was not a significant by-product.

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

367 The compositions of the microbial consortia that developed in these bioleaching  
368 experiments showed some degree of variation with temperature, pH and type of tailings. The  
369 bacteria that were detected all have roles in mineral bio-processing, either (i) in catalysing the  
370 oxidation of ferrous to ferric iron, the main chemical oxidant of sulfide minerals, or (ii) the  
371 oxidation of sulfur coupled either to molecular oxygen, forming sulfuric acid, or (iii) the  
372 reduction of soluble or mineral-phase ferric iron. Some of the microbial consortia data obtained  
373 were much as had been anticipated, e.g. the dominance of the thermo-tolerant species *Sb.*  
374 *thermosulfidooxidans* and *At. caldus* in the 45°C bioreactors, and of mesophilic iron-oxidising  
375 *Acidithiobacillus* spp. in 30°C bioreactors. Other results were more unexpected such as the  
376 occurrence of *Sb. thermosulfidooxidans* in all 30°C, although as minority, as well as 45°C  
377 bioreactor consortia. The most phylogenetically diverse consortia were found in the 45°C  
378 bioreactors leaching Bor tailings (operated both with and without pH control) where three other  
379 species (*Ab. sulfuroxidans*, *Sb. acidophilus* and *L. ferriphilum*) were found in addition to *Sb.*  
380 *thermosulfidooxidans* and *At. caldus*. The reason for this greater biodiversity is not known,

381 though it could relate to the fact that concentrations of copper (which is more toxic to some  
382 acidophiles than others) in Bor leachates were far less than in than those in CLC leachates.

383 Copper present in PLS from both CLC and Bor tailings was readily precipitated as a sulfide  
384 phase by contacting with biogenic H<sub>2</sub>S, as demonstrated previously (Ñancucheo and Johnson,  
385 2012; Hedrich and Johnson, 2014). By doing this with no pH adjustment to the leachates,  
386 copper was selectively removed, as other metals present in significant concentrations in the  
387 leach liquors (iron and zinc) do not form sulfide phases at pH values <3. This approach would  
388 therefore facilitate the recovery and recycling of copper from both mineral tailings wastes. The  
389 only metal present that did co-precipitate (from CLC tailings leachates) was silver (which was  
390 present in much smaller concentrations), as Ag<sub>2</sub>S has a smaller solubility product than CuS.

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

400 Stankovic et al. (2015) estimated that between 54,000 and 81,000 t of copper is present in  
401 the old flotation tailings at the Bor mine. These authors used the acidic ferric iron-rich water in  
402 the nearby Lake Robule to leach copper from these tailings, and extracted ~ 80% of that  
403 present. More effective extraction (>90%) was achieved by bioleaching in the current  
404 experiments, and the estimated total value of copper that could be extracted and recovered  
405 from these old tailings using such an approach is between \$225 million (US) and \$337 million  
406 (US). Again there would be some additional value from co-bioleaching and recovering zinc  
407 (estimated as between \$30 and \$45 million US). In addition to these potential direct financial  
408 benefits, bio-processing the tailings wastes would help to remove some, at least, of the



409 environmental hazard that these tailings represent (protracted release of metals and acid into  
410 the wider environment), much in the same way that bio-processing cobalt-rich tailings at  
411 Kasese in Uganda facilitated the production of a more environmentally-benign waste product  
412 (Morin and d'Hugues, 2007).

413

## 414 **5. Conclusions**

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416 A modified approach, involving a combination of oxidative and reductive bioleaching  
417 carried out at extremely low pH values and at 45°C, was shown to be highly effective in  
418 extracting copper from mineral tailings generated as waste materials in two mines currently  
419 operating in Spain and Serbia. Soluble copper in leach liquors was selectively precipitated as  
420 CuS by contacting with biogenic H<sub>2</sub>S.

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

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

427

## 428 **Acknowledgement**

429

430 The authors are grateful to Carlos Frías, at Cobre Las Cruces S.A., and Srdjan Stankovic at  
431 the Faculty of Mining and Geology, University of Belgrade for providing the tailings materials,  
432 and to Francisco Sánchez Ruiz at Cobre Las Cruces S.A., for providing data on the  
433 composition of CLC tailings. We also would like to acknowledge the analytical work performed  
434 (i) at the Camborne School of Mines, University of Exeter UK, by Malcolm Spence (Lab XRF)  
435 and Rob Fitzpatrick (Handheld XRF); (ii) at Cobre Las Cruces S.A., by Natalia Moreno

436 Bermejo; and (iii) at the Biohydrometallurgy and Mining and Metallurgy Institute Bor, by Vesna  
437 Conic. This work was supported by the *Natural Environment Research Council*, UK (Grant ref.  
438 NE/L014076/1) as part of its *Resource Recovery from Waste* programme.

439

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505 **Table 1.** Elemental composition of Bor and Cobre Las Cruces tailings, shown as % or (\*) as

506 mg/kg; < below detection limit

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

508 (\*\*data from Antonijević et al., 2008)

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511 **Table 2.** Percentages of copper and silver extracted from CLC tailings by bioleaching and  
 512 chemical extraction from leach residues and fresh tailings (mean values  $\pm$  standard  
 513 deviations).

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

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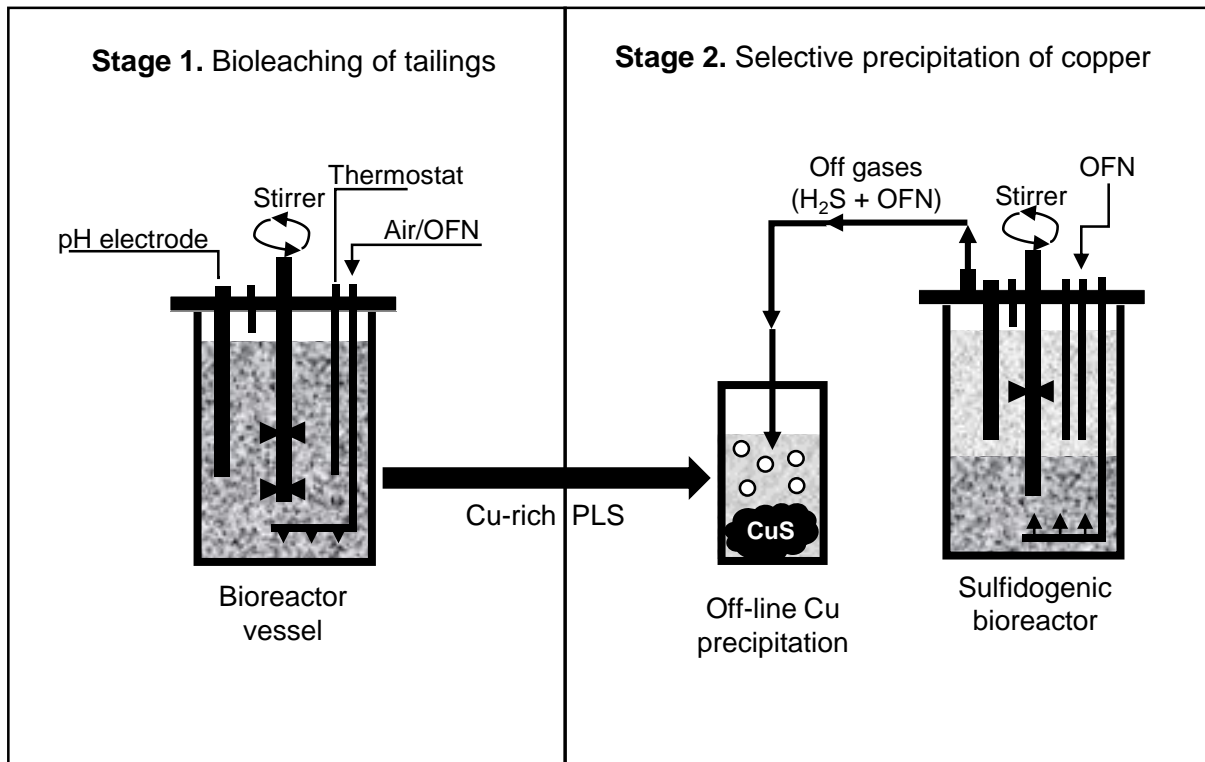
516 **Table 3.** Percentages of copper and silver extracted from Bor tailings by bioleaching and  
 517 chemical processing of leached residues (mean values  $\pm$  standard deviations).

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

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523 **Figure 1.** Schematic representation (not in scale) of the reactors used during the bioleaching  
524 experiments (stage 1) and the offline precipitation of copper using a low pH sulfidogenic  
525 bioreactor (stage 2).

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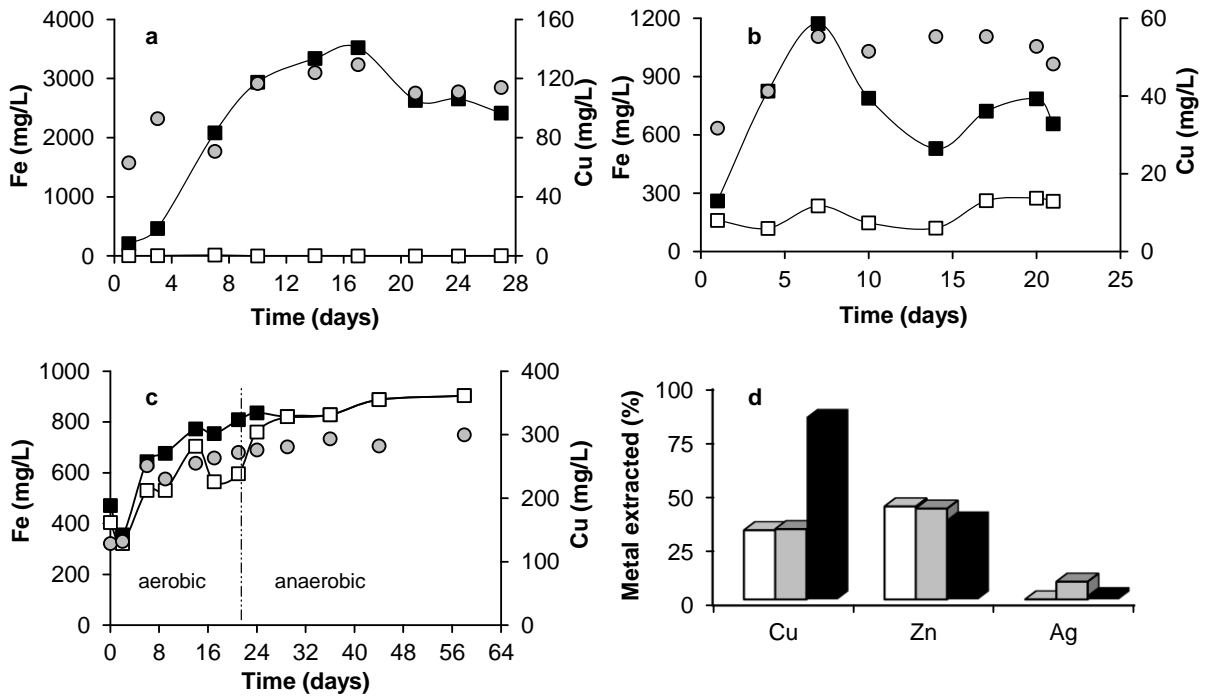
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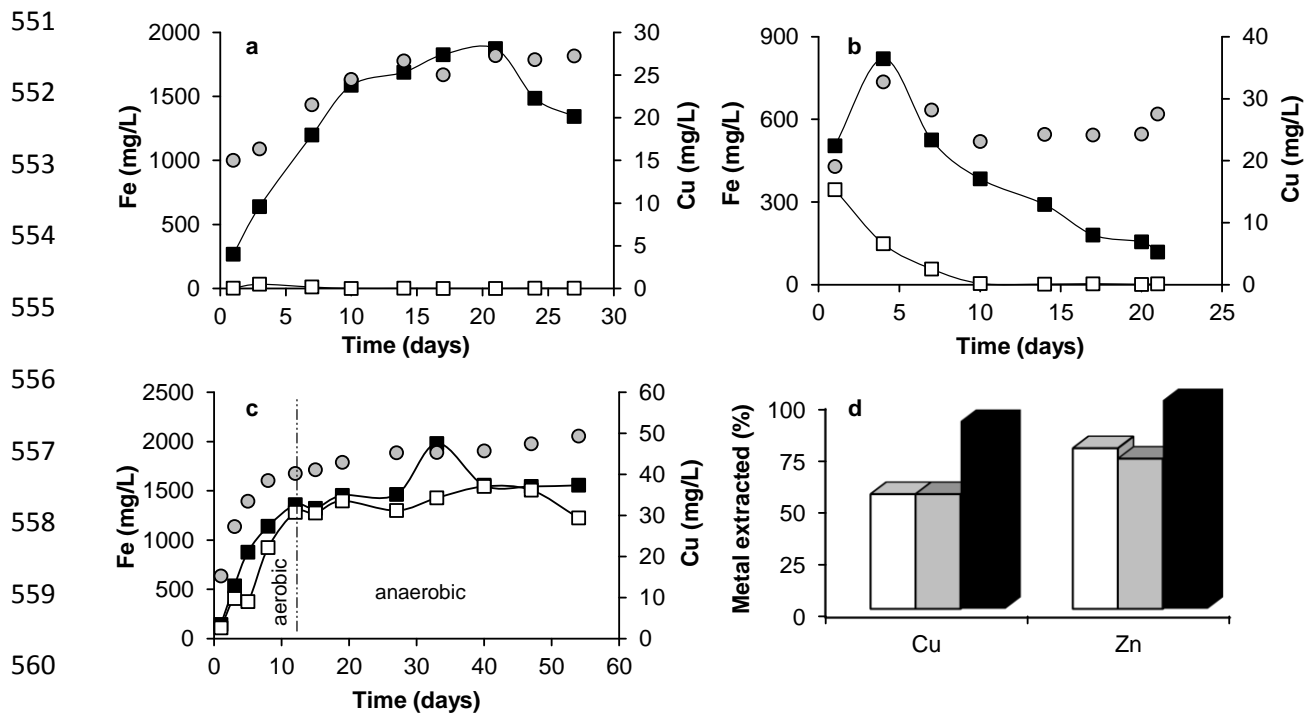
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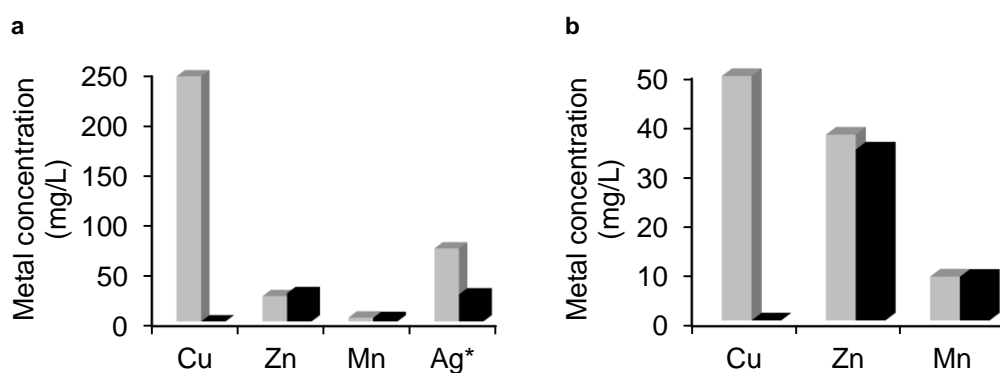


**Figure 2.** Bioleaching of CLC tailings at (a) 30°C, pH 1.7 and constant aeration; (b) 45°C, pH 1.7 and constant aeration; (c) 45°C, no pH control, and aerobic/anoxic conditions. Key: (○) copper; (■) total soluble iron, (□) ferrous iron. Fig. 2d shows comparative percentages of copper, zinc and silver 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).



563 **Figure 3.** Bioleaching of Bor tailings at (a) 30°C, pH 1.7 and constant aeration; (b) 45°C, pH  
 564 1.7 and constant aeration; (c) 45°C, pH 1.8 – 1.0 and aerobic/anoxic conditions. Key: (○)  
 565 copper; (■) total soluble iron, (□) ferrous iron. Fig. 3d shows comparative percentages of  
 566 copper and zinc extracted by bioleaching at 30°C, pH 1.7 and constant aeration (white bars),  
 567 45°C, pH 1.7 and constant aeration (grey bars) and 45°C, pH 1.8 – 1.0 and aerobic/anoxic  
 568 conditions (black bars).

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**Figure 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<sub>2</sub>S. \*Silver concentrations are shown as µg/L.