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1 **Bioleaching of secondary and critical raw materials from Red Mud by a mixed**  
2 **culture in a semi-continuous reactor**

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## 19 **ABSTRACT**

20 Red Mud (RM) is a byproduct of the Bayer Process, used to extract alumina from bauxite. This  
21 residue contains valuable metals and rare earth elements (REEs), which can be bioleached under  
22 acidic conditions and later recovered, eventually reducing the RM pollution potential. The focus of  
23 this study was to extract metals through bioleaching using biostimulated biomass. A sequencing batch  
24 reactor (SBR) was operated at two different solid-to-liquid ratios (S/L, 2% and 1.5%) under aerobic  
25 conditions, using a feeding strategy based on pH evolution over time. The lowest pH obtained at 2%  
26 S/L was 3.8. Metals extraction efficiencies were  $68\pm 18\%$  for Mn,  $58\pm 4.5\%$  for Mg,  $51\pm 2.9\%$  for Al,  
27  $0.6 \pm 0.07\%$  for Ti and  $< 0.1\%$  for Fe. At 1.5% S/L, a slightly lower pH was achieved (3.4), with  
28 metals extraction efficiencies similar to those obtained at 2% S/L, but more stable over time. At 1.5%  
29 S/L, REEs (i.e., Nd, Ce, Y, Sc and La) extraction efficiencies were also evaluated, and the highest  
30 value was obtained for Nd (around 65%). The results were promising and provided useful information  
31 for further enhancing the efficiency of metals extraction, in the perspective of process scale-up.

32

33 **KEYWORDS:** biohydrometallurgy, metals, rare earth elements, red mud, secondary raw materials,  
34 sequencing batch reactor

35

## 36 **1. Introduction**

37 Over the past two centuries, aluminium (Al) has played a pivotal role in industrial development,  
38 primarily due to the Bayer process, the alkaline extraction of alumina from bauxite. However, as the  
39 demand for aluminium has increased, so has the generation of waste associated with its production  
40 process (Power *et al.*, 2011). The main waste produced through the Bayer process is called Red Mud  
41 (RM), because of its typical colour caused by its chemical and mineralogical composition, which  
42 includes iron (Fe), silicon (Si), aluminium (Al), calcium (Ca), titanium (Ti) and other minor elements  
43 (Hind *et al.*, 1999).

44

45 Due to population growth and technological development, the demand for minerals and their  
46 consumption have increased in recent years. Metallurgical wastes can be sources of these minerals,  
47 as they are still rich in valuable elements (Sethurajan *et al.*, 2018; Jain and Sharma, 2004) which may  
48 be recovered, thus fostering the transition from a linear to a circular economy (EIT 2020; ERA-MIN  
49 2013). The last CRM final report has included bauxite/aluminium as critical materials (CRMs final  
50 report, 2023). Numerous approaches have been explored for the utilization of RM or its disposal,  
51 which include its use as an adsorbent for removing heavy metals and toxic substances from  
52 contaminated wastewater, as a building material such as bricks, concrete, and road foundations, as a  
53 catalyst for oxidizing organic compounds and reducing nitrates, and as a soil amendment (Zhang *et al.*,  
54 2020).

55 In the last decades, RM has been recognised as an outstanding source of secondary and critical raw  
56 materials such as rare earth elements (REEs), which are characterized by both a high risk of supply  
57 disruption and high economic importance to the industry (CRMs final report, 2023; Rasoulnia *et al.*,  
58 2021). REEs are essential in diverse industries, including metallurgy, petroleum, textiles, and high-  
59 tech applications (Charalampides *et al.*, 2015). The non-homogeneous global distribution of these  
60 elements has prompted countries without sufficient land-based resources to seek alternative sources  
61 (Abhilash *et al.*, 2021). China accounts for more than 90% of REEs world production, while other  
62 countries' supply is covered by their purchase (Giese, 2020). As a matter of fact, the demand for  
63 alternatives is growing, and secondary sources such as waste streams are now of great interest. Access  
64 to REEs is strategically important for economic growth and technological advancements. Efforts are  
65 underway to develop advanced extraction technologies and secure a stable supply of these valuable  
66 metals (Abhilash *et al.*, 2021).

67 A way to recover valuable substances from RM is to leach metals into solution via chemical reagents.  
68 A possible option could be biohydrometallurgy, which exploits the metabolism of microorganisms to  
69 achieve the leaching of metals from solid wastes, ores or minerals (Jain and Sharma, 2004; Sethurajan  
70 *et al.*, 2018). This eco-friendly approach offers advantages over known chemical methods, including

71 lower energy consumption, reduced use of chemicals and generation of hazardous fluids, and  
72 enhanced cost-effectiveness. Bioleaching, a key process in biohydrometallurgy, can be operated by  
73 autotrophic or heterotrophic microorganisms. Autotrophic bioleaching employs bio-  
74 oxidation/reduction cycles with inorganic acids, while heterotrophic leaching relies on the microbial  
75 production of organic metabolites and the formation of soluble chelates to mobilize elements (Pedram  
76 *et al.*, 2020). One option to leach metals contained in non-sulfidic ores, such as RM, may be to use  
77 metabolites produced by heterotrophic bacteria and fungi sustained by a suitable organic substrate  
78 (Jain and Sharma, 2004).

79 Up to now, most of the studies concerning the bioleaching of valuable elements from RM were  
80 conducted in batch mode with the addition of an external and/or preliminarily enriched inoculum  
81 (Ghorbani *et al.*, 2008; Qu *et al.*, 2019; Vakilchap *et al.*, 2016). This study aims at exploring possible  
82 strategies for process optimization, shifting the biomass enrichment from a batch scale to a semi-  
83 continuous one. Furthermore, it also considers the feasibility of selecting the existing heterotrophic  
84 biomass on RM, thus reducing the operational costs due to an external inoculum.

85 The overall objective of this work was therefore to investigate the possibility to exploit the biomass  
86 initially present in the RM and verify its bioleaching potential in the long-term using a sequencing  
87 batch reactor (SBR), without any preliminary selection and inoculum. An operational strategy based  
88 on pH evolution over time was applied, and the process performance was evaluated in terms of  
89 extraction efficiencies of major and minor elements such as Al, Fe, Ti, Mg, Mn, Nd, Y, Ce, La and  
90 Sc. Results were promising and gave useful information in the perspective of a future process scale-  
91 up.

92

## 93 **2. Materials and Methods**

### 94 **2.1 Red Mud characterization**

95 RM was characterized in terms of pH, electric conductivity (EC), acid neutralization capacity (ANC),  
96 metals content and mineral composition. RM samples were previously mixed and ground to 2 mm.  
97 The sample was collected from a plant storage site in Vitrolles (France), where it had been exposed  
98 to the atmosphere since the 1960s when the plant was closed (Cozzolino et al., 2023).

99

### 100 **2.2 Bioleaching experiments**

101 The experiments were carried out in a 5-litre sequencing batch reactor (SBR) equipped with an  
102 external water jacket for temperature control. To preserve the autochthonous biomass possibly  
103 colonizing RM, the material was not dehydrated before the SBR start-up. A synthetic leaching  
104 medium (LM) consisting of 30 g/l of ethanol 96%, 10 g/l of glucose, and 10 g/l of yeast extract (YE),  
105 according to Qu *et al.* (2019), was used. During the experimentation, the concentration of YE was  
106 reduced due to issues related to foam formation. Different solid-to-liquid ratios (S/L) were used: 2%  
107 and 1.5%, referred to the dry mass. More specifically, the S/L ratio was reduced at 1.5% in the second  
108 part of the experiment based on the hypothesis that a lower S/L ratio may reduce the potential  
109 inhibiting effect of RM on biomass. All the experiments were carried out at room temperature. A  
110 mechanical stirrer was used to provide mixing (rotating speed, 100-120 rpm). To avoid oxygen  
111 limitation, the reactor was aerated, maintaining the dissolved oxygen concentration above 1 ppm. The  
112 air was flushed through porous stones inside the reactor, and the flowrate was kept in the range 1-2  
113 l/min with a flowmeter. In a typical working cycle, pH increased soon after the feeding, then rapidly  
114 decreased due to biological activity and reached a plateau. Each new feeding was added at the end of  
115 the plateau, when signals of pH rise were detected. According to the observed pH trend over time, 2  
116 litres of fresh LM and RM with the desired S/L ratio were periodically replaced in the reactor to start  
117 a new working cycle. Depending on the duration of working cycles, the resulting HRT (hydraulic  
118 residence time) varied between 12.5 and 17.2 days. The experimentation lasted 170 and 58 days at

119 2% and 1.5% S/L, respectively. Samples were taken at the beginning, during and at the end of each  
120 working cycle.

121

### 122 **2.3 Analytical methods**

123 The pH and EC of untreated (raw) RM were determined after mixing and stirring the RM suspension  
124 in distilled water ( $v/w= 5$  l/kg) for 16 h (Qu and Lian, 2013), using a benchtop meter (HI5522, Hanna  
125 Instruments). RM chemical composition was analysed by ICP-OES (Optima 7000, PerkinElmer) after  
126 total digestion according to EPA method 3052. REEs content in raw RM was evaluated by X-ray  
127 fluorescence analysis (Bruker D8 Advance).

128 The mineralogical composition of raw and treated RM was carried out by X-Ray powder diffraction  
129 (XRD) technique, on samples dried at 40 °C. The samples were analysed using a Rigaku Geiger Flex  
130 diffractometer equipped with a Cu X-Ray tube, operating at 30 kV and 30 mA. All XRD analyses  
131 were performed in the range from 4 to 70° of  $2\theta$ .

132 The RM water content was determined after drying the sample at 105 °C. The ANC was assessed by  
133 mixing several subsamples of dry RM with distilled water (10:1  $v/w$ ) containing pre-selected amounts  
134 of acid ( $\text{HNO}_3$  65%) and stirring the suspension for 48 h (UNI CEN/TS 15364). The ANC values  
135 were determined considering a target pH of 4.5 ( $\text{ANC}_{4.5}$ ).

136 During bioleaching tests, 15 ml samples were periodically taken from the reactor and centrifuged at  
137 5000 rpm for 15 minutes to remove the solid part. The supernatants were used to evaluate pH, EC,  
138 metals and organic acids concentration. For metals analysis, part of the supernatant was filtered at  
139 0.45  $\mu\text{m}$ , acidified (1%  $v:v$  of nitric acid 65%), diluted with grade-I water, and then analysed with  
140 ICP-OES (Varian 710-ES). The remaining part of the samples was filtered at 0.45  $\mu\text{m}$ , diluted with  
141 grade-I water and used to evaluate organic acid concentrations through HPLC (LC 4000, Jasco).

142

143

144

## 145 **2.4 Microbiological analysis**

146 Samples of the slurry were axenically collected two and five days after the beginning of the 18<sup>th</sup> cycle  
147 and used for the enumeration of cultivable aerobic microorganisms. The viable titres were determined  
148 as colony-forming units (CFU) by plating serial 10-fold dilutions of each sample onto three different  
149 culture media. The Horikoshi Medium (yeast extract 1 g/l, K<sub>2</sub>HPO<sub>4</sub> 0.2 g/l, MgSO<sub>4</sub> 7H<sub>2</sub>O 0.2 g/l,  
150 Na<sub>2</sub>CO<sub>3</sub> 10 g/l, glucose 10 g/l, agar 17 g/l, pH 10.0) was used for enumeration of alkalophilic  
151 microorganisms (Horikoshi, 1999). The medium was modified by omitting the addition of sodium  
152 carbonate and adjusting the pH at 7.0 and 3.0 for the enumeration of neutrophilic and acidophilic  
153 microorganisms, respectively. After five days incubation at 25 °C bacteria and moulds were  
154 separately counted.

155

## 156 **2.5 Calculations**

157 The cumulative metals extraction efficiency was calculated according to equation (1):

$$158 \quad \text{Extraction efficiency [\%]} = \frac{\text{Metal in leachate at a specific time [mg]}}{\text{Metal content in the RM [mg]}} \cdot 100 \quad (1)$$

159 The process efficiency was evaluated also with the following equation (2):

$$160 \quad \text{Daily extraction capacity} \left[ \frac{mg}{day \cdot l} \right] = \frac{\text{Metal concentration} \left[ \frac{mg}{l} \right] \cdot V_o [l]}{\text{cycle duration [day]} \cdot V_R [l]} \quad (2)$$

161 Where “metal concentration” is the concentration for each metal used to evaluate the highest  
162 extraction for each S/L (2%, 1.5%), the “cycle duration” is the number of days of the considered  
163 cycle, V<sub>o</sub> is the volume of slurry discharged at the end of the cycle and V<sub>R</sub> is the reactor volume.

164 The bioleaching process kinetics was calculated as the slope of the steepest part of pH profile over  
165 time.



166 **3 Results**

167 **3.1 Red Mud characterization**

168 The pH, EC and ANC<sub>4.5</sub> values assessed for RM were 11.6, 2.5 mS/cm and 5.2 mmol H<sup>+</sup>/g,  
169 respectively. Table 1 shows the concentrations of the most relevant chemical elements in RM, metals  
170 and REEs. A more detailed characterization is described in Cozzolino *et al.* (2023).

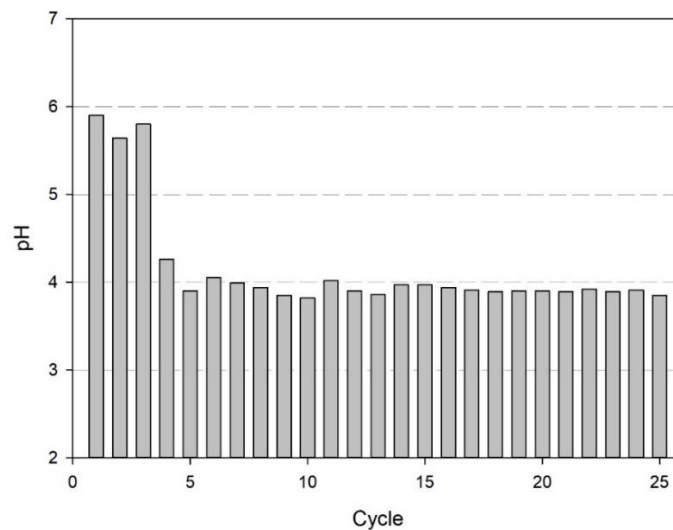
171 *Table 1. Main metals (weight percentage) and some rare earth elements (mg/kg) contained in RM*

<b>Fe<sub>2</sub>O<sub>3</sub> [%]</b>	43.55 ± 7.26	<b>TiO<sub>2</sub> [%]</b>	6.98 ± 1.70	<b>Nd [mg/kg]</b>	190
<b>Al<sub>2</sub>O<sub>3</sub> [%]</b>	20.7 ± 3.78	<b>CaO [%]</b>	4.08 ± 0.1	<b>Ce [mg/kg]</b>	651
<b>SiO<sub>2</sub> [%]</b>	9.7 ± 2.35	<b>Na<sub>2</sub>O [%]</b>	6.68 ± 0.02	<b>Y [mg/kg]</b>	199
<b>MgO [%]</b>	0.31 ± 0.02	<b>K<sub>2</sub>O [%]</b>	0.1 ± 0.01	<b>Sc [mg/kg]</b>	97
<b>MnO [%]</b>	0.103 ± 0.03			<b>La [mg/kg]</b>	280

172

173 **3.2 Bioleaching experimentation**

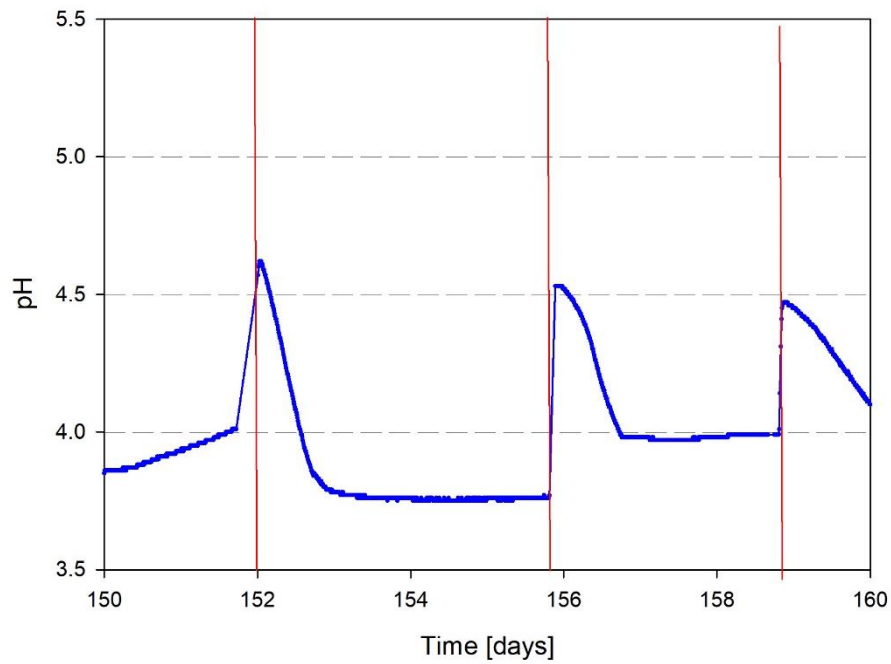
174 Process performance at 2% S/L. Figure 1 illustrates the minimum pH values achieved in each cycle  
175 over time. Initially, the reactor maintained a neutral pH similar to that observed in batch tests at the  
176 same operational conditions (Cozzolino *et al.*, 2023). However, after the first cycles, the minimum  
177 pH value decreased and gradually stabilized.



178

179 *Figure 1. Lowest pH values for each cycle over time at 2% S/L*

180 Figure 2 shows the pH trends of some of the most representative cycles under stable operating  
181 conditions. The pH increase corresponds to a new feeding and the beginning of a new working cycle.



182

183 *Figure 2. pH trends of some representative cycles at 2% S/L*

184

185 Table 2 shows the minimum pH values and the kinetics for some of the most representative cycles  
186 performed at 2% S/L.

187

*Table 2. Process kinetics and pH plateaux at 2% S/L*

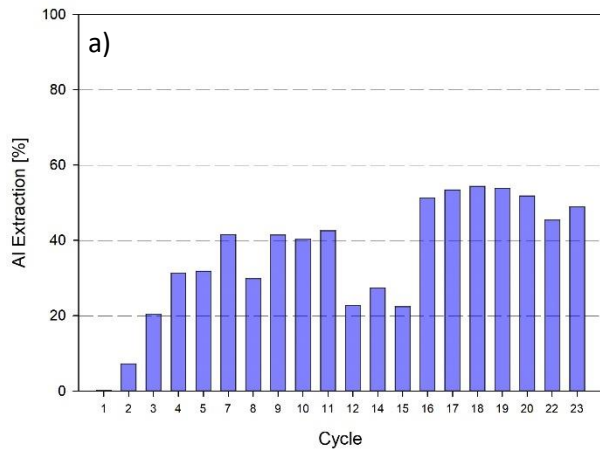
<b>Cycle</b>	<b>Kinetics [pH units/ day]</b>	<b>Plateau [pH]</b>
<b>13</b>	0.73	3.86
<b>15</b>	0.55	3.97
<b>16</b>	0.3	3.94
<b>17</b>	0.3	3.91
<b>18</b>	0.25	3.89
<b>19</b>	0.27	3.9
<b>21</b>	0.3	3.89
<b>22</b>	0.37	3.92
<b>23</b>	0.66	3.89
<b>24</b>	0.26	3.91
<b>25</b>	0.23	3.85

189

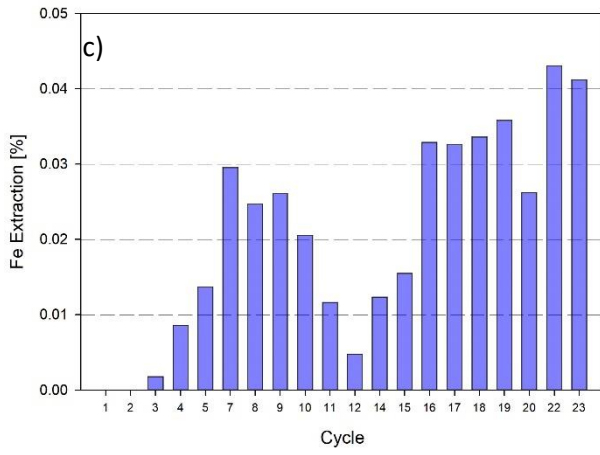
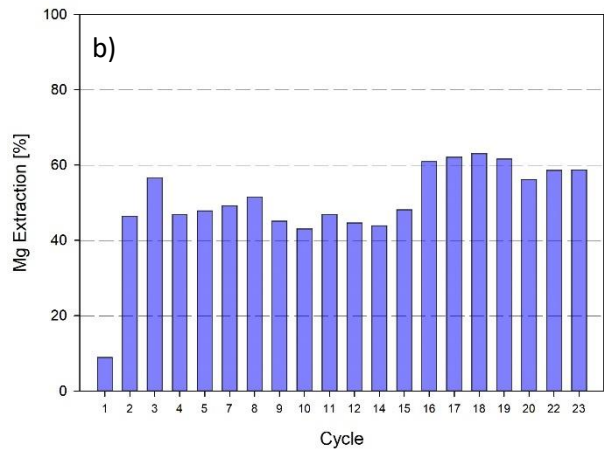
190 The kinetics was in a range between 0.25 and 0.73 pH units/day. A lower variation is observed in the  
191 minimum pH obtained among these cycles set.

192 Figure 3 shows the trends of the metals extraction efficiencies over time from the reactor start-up.

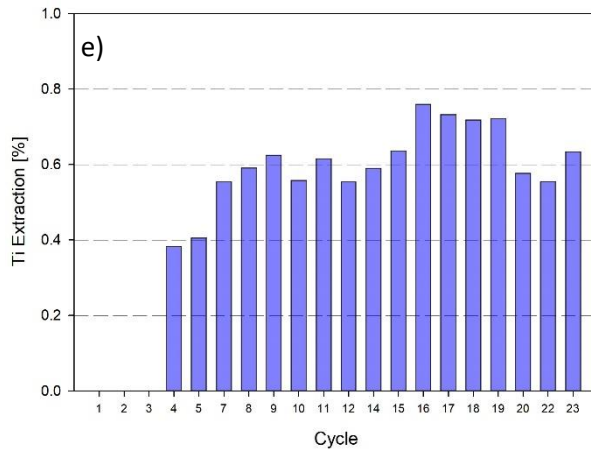
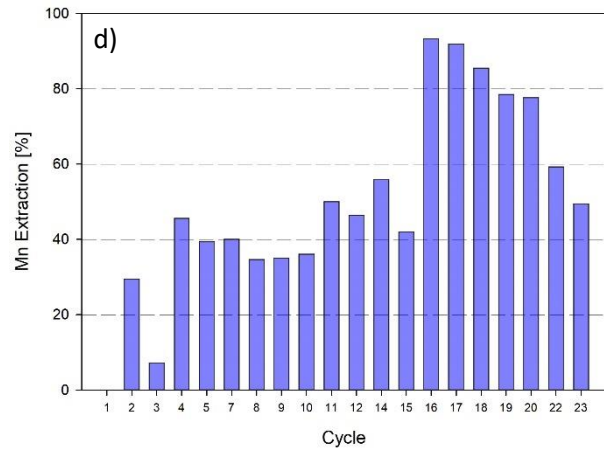
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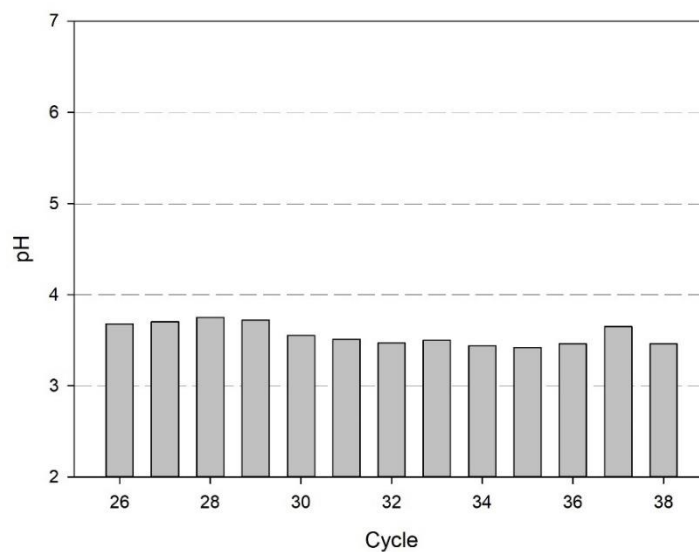
196

197 *Figure 3 Metals extraction efficiencies during the first 23 cycles at 2% S/L: Al (a), Mg (b), Fe (c),*

198 *Mn (d), Ti (e)*

199 In general, it can be observed that the lower the pH, the higher the metals concentration in the liquid  
200 phase, but there was an upward trend in terms of extraction for some metals from around cycle 5,  
201 even if the pH showed slight variations. Aluminium showed an increasing trend, with an average  
202 extraction of  $51.3 \pm 2.9$  %. Magnesium and manganese also increased over time ( $58 \pm 4.5$  % for Mg and  
203  $68 \pm 18.4$  % for Mn). Iron had an odd and fluctuating tendency and showed a low extraction: despite  
204 its high concentration in the RM, a low concentration in the liquid phase was found and therefore a  
205 low extraction percentage (average extraction of  $0.03 \pm 0.01$  %). Finally, Ti showed the same growing  
206 trend, though the highest extractions achieved were still lower than 1%. The averages were measured  
207 considering the cycles from the 15<sup>th</sup>, when the metals extraction efficiencies started to stabilize.

208 Process performance at 1.5% S/L. The applied S/L ratio was reduced from 2 to 1.5, to check if lower  
209 amounts of RM would have an effect on process performance. In particular, the possibility to reduce  
210 the potential inhibiting effects and extend the duration of working cycles at low pH conditions was  
211 investigated, supposing that longer exposures at low pH would enhance metals extraction. Figure 4  
212 shows the minimum pH values for each cycle over time at 1.5% S/L. Table 3 reports the kinetics of  
213 pH drop and the pH plateaux values.



214

215

Figure 4. Lowest pH values for each cycle over time at 1.5% S/L

Table 3. Process kinetics and pH plateaux at 1.5% S/L

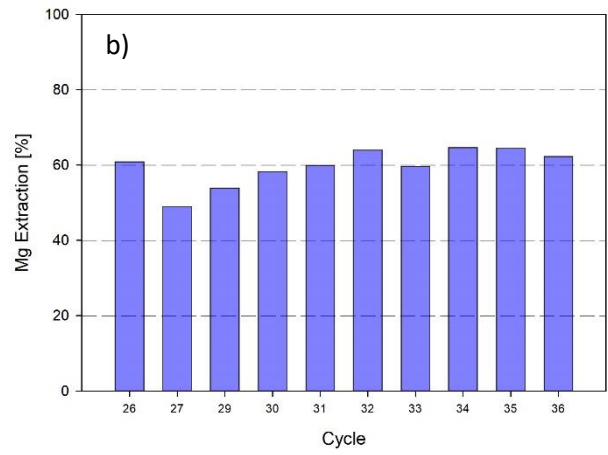
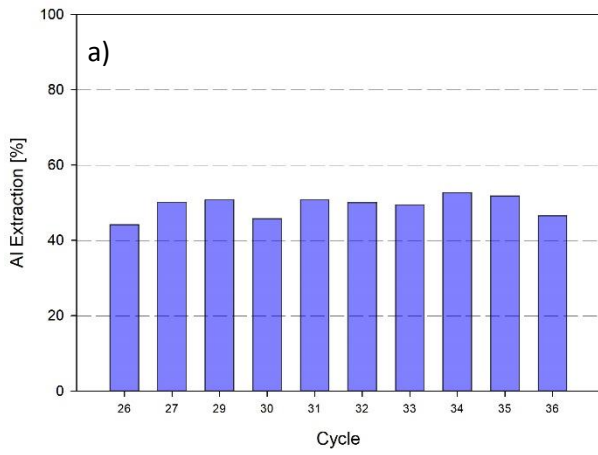
Cycle	Kinetics [pH units/days]	Plateau [pH]
31	0.23	3.51
32	0.15	3.47
34	0.13	3.44
35	0.08	3.42
36	0.07	3.46
37	0.07	3.65
38	0.09	3.46

217

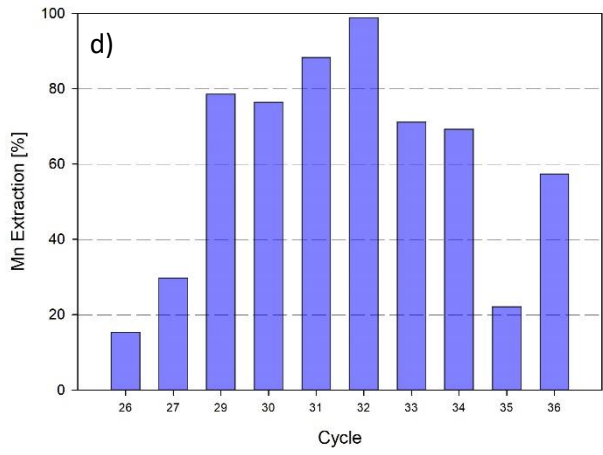
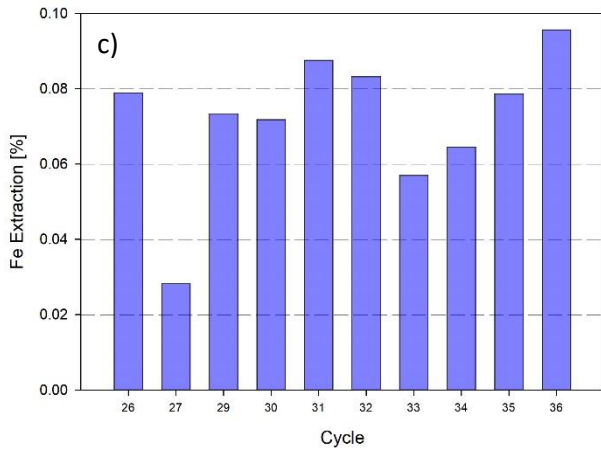
218 As expected, longer cycles were achieved, probably connected to a lower amount of RM fed into the  
 219 reactor. However, the lower RM concentration may correspondingly reduce the amount of biomass  
 220 concentration entering the reactor.

221 Figure 5 shows the trend of metal extraction efficiencies. The aluminium extraction was around  
 222  $49 \pm 2.6$  %, and remained constant during the test at S/L 1.5%. A very low iron extraction was obtained  
 223 ( $<0.1$ %). A very fluctuating extraction efficiency was observed for Mn ( $60 \pm 27$  %). Mg extraction  
 224 efficiency reached an average of  $59 \pm 4$  %. Finally, the extraction of titanium increased, as well, but  
 225 remained lower than 1%. Given the relatively stable operation of the SBR at 1.5 S/L, all the cycles  
 226 have been considered to calculate the average extraction.

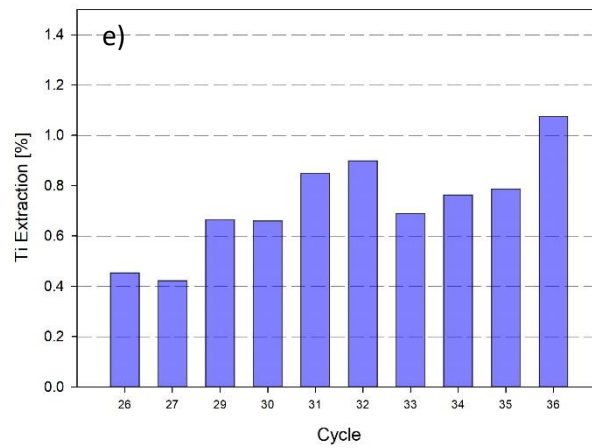
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228



229



230

Figure 5. Metals extraction efficiencies at 1.5% S/L: Al (a), Mg (b), Fe (c), Mn (d), Ti (e)

231

232 The daily extraction capacity was also calculated. Table 4 summarizes the values obtained for each  
233 metal, using the results from cycles 18 (2% S/L) and 31 (1.5% S/L), as examples of the best results  
234 obtained.

235 *Table 4. Estimated daily extraction capacity [mg/(day·l)] at 2% and 1.5% S/L*

	<b>Al</b>	<b>Fe</b>	<b>Ti</b>	<b>Mg</b>	<b>Mn</b>
<b>2% S/L</b>	66.4	0.18	0.16	1.71	0.09
<b>1.5% S/L</b>	48	0.21	0.16	1.18	0.1

236

237 It is relevant to remark that Al high extraction efficiencies correspond to a high extraction capacity,  
238 however this is not the case for Mn because of its low concentration in RM, as shown in Table 1.

239 The results for Ti and Mn at 1.5% S/L are quite similar to those achieved at 2% S/L, whereas Fe is  
240 higher because of the higher extraction at 1.5%. Al showed a lower value at 1.5%: since less RM was  
241 fed, longer cycles were obtained with a consequent smaller daily extraction capacity. This behaviour  
242 is less evident with the other metals. At 1.5% S/L, some rare earth elements (REEs) were measured  
243 in the liquid phase at pH 3.5, and the extraction percentages are reported in Table 5.

244 *Table 5. REEs extraction efficiencies from the RM using 1.5% S/L*

<b>ELEMENT</b>	<b>EXTRACTION [%]</b>
<b>Neodymium (Nd)</b>	65.2
<b>Cerium (Ce)</b>	19.5
<b>Yttrium (Y)</b>	30.2
<b>Scandium (Sc)</b>	34.4
<b>Lanthanum (La)</b>	21.4

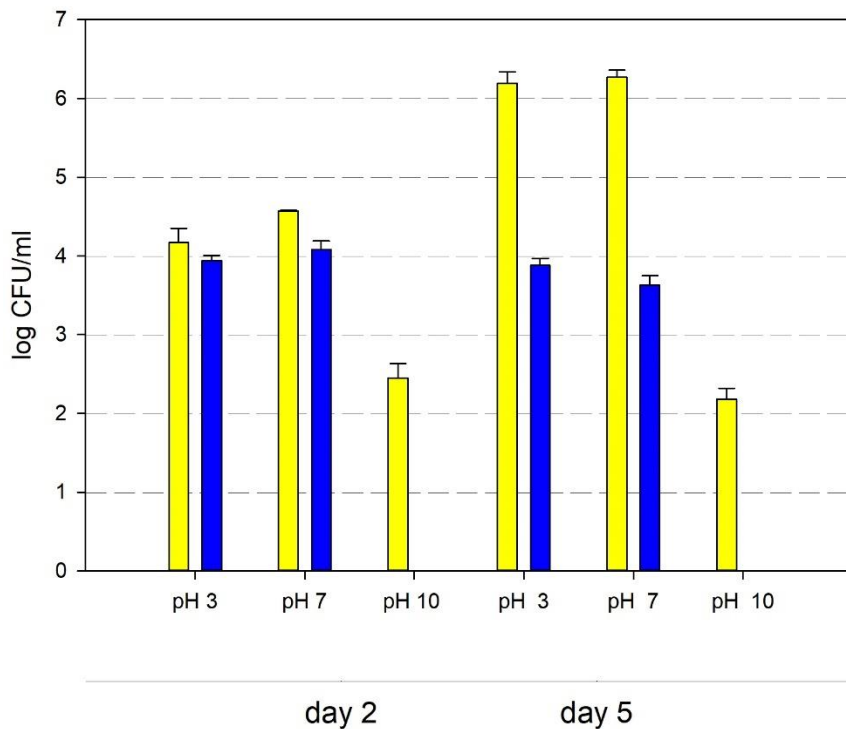


245 Acid production. From the HPLC analysis, it was found that acetic acid was the one present at the  
246 highest concentrations (20 g/l). This is an average value among the cycles at 2% S/L shown in this  
247 study. Because of this very high concentration in the leached solution, it was not possible to detect  
248 the presence of other acids such as oxalic, which may, however, be present (Cozzolino *et al.*, 2023).  
249  
250 Mineralogical analysis results. Figure S1 shows the XRD analysis for untreated and treated RM  
251 (cycle 18). Untreated RM composition was based on hematite (Fe<sub>2</sub>O<sub>3</sub>), calcite (CaCO<sub>3</sub>), sodalite  
252 (KNa<sub>3</sub>Al<sub>3</sub>Cl(SiO<sub>4</sub>)<sub>3</sub>), boehmite (AlO(OH)), gibbsite (Al(OH)<sub>3</sub>), rutile (TiO<sub>2</sub>). After bioleaching, there  
253 was no evidence of sodalite, gibbsite, boehmite and calcite, so indicating the transfer of metals  
254 associated with these components into the liquid phase. One component clearly present in the residue  
255 after the treatment was hematite, in agreement with the low concentration of Fe in the liquid phase.  
256 Weddellite (Ca(C<sub>2</sub>O<sub>4</sub>)-2(H<sub>2</sub>O)) was formed, probably as the result of Ca<sup>2+</sup> precipitation due to the  
257 reaction with oxalic acid (Qu *et al.*, 2019, Cozzolino *et al.*, 2023).

258

### 259 **3.3 Selective enumeration of microorganisms**

260 The viable titres of heterotrophic alkaliphiles, neutrophiles, and acidophiles are shown in Figure 6.  
261 At the beginning of the cycle, the viable titres of neutrophilic and acidophilic microorganisms were  
262 comparable and in the order of 10<sup>4</sup> CFU/ml for both bacteria and moulds. Moreover, two orders of  
263 magnitude lower viable titre was found for alkaliphilic bacteria. At the end of the cycle, the titre of  
264 alkaliphilic bacteria was almost stable. On the contrary, an enrichment in acidophilic and neutrophilic  
265 bacteria was observed, as highlighted by a two-order-of-magnitude increase in the viable titres. The  
266 alkaliphilic moulds were not detected at all, both at the beginning and at the end of the analysed  
267 cycle.



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*Figure 6. Viable titres (log-transformed) of alkaliphilic (pH 10), neutrophilic (pH 7), and acidophilic (pH 3) microorganisms. Yellow: bacteria. Blue: moulds.*

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#### **4 Discussion**

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At 2% S/L, a pH below 4 (3.9) was reached on cycle #5 for the first time. This value is quite low

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compared to the minimum values obtained in batch tests performed with the same operational

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conditions, i.e., 5.9 (Cozzolino *et al.*, 2023). Such behaviour can be linked to the progressively

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enhanced biological activity, compared to the initial one. In line with the pH values, also the

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extractions observed in the first SBR cycles were similar to those achieved with batch tests conducted

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at the same operating conditions and LM, then the extracted quantity progressively increased. For

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instance, Al in SBR reached an extraction 5-6 times higher than the batch tests conducted at the same

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S/L and temperature (51% vs 10%, respectively). Also, Fe and Ti extraction efficiencies were a few

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times higher than those achieved in batch tests, though still lower than 1%. Mg and Mn extraction

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efficiencies in SBR and batch tests were 58% and 68% vs 18% and 9%, respectively. Such an increase

282 in metals extraction can be related to the lower pH achieved in the SBR due to a more intense  
283 biological activity developed in the reactor.

284 From the comparison between pH values at 2% (Figure 1) and 1.5% S/L (Figure 4), it is possible to  
285 observe that pH reached slightly lower values at 1.5% S/L (i.e., about 3.4), probably due to the lower  
286 percentage of RM, which is very alkaline. However, the process kinetics was also lower compared to  
287 the operation at 2% S/L.

288 Regarding the extraction efficiencies, around 50% were achieved for Al at both 2% and 1.5% S/L.  
289 Magnesium, iron and titanium showed slightly higher extraction efficiencies at 1.5% S/L, compared  
290 with those achieved at 2% S/L. A different behaviour was observed in the case of manganese: despite  
291 the higher extraction efficiencies observed at 2% S/L, it must be considered that Mn results showed  
292 a high standard deviation.

293 The pH plateaux obtained during this work have been compared to some studies from the literature  
294 carried out using selected and enriched cultures, in batch mode (Qu *et al.*, 2013, 2019; Vakilchap *et*  
295 *al.*, 2016). The results obtained in this study using the sequencing batch reactor (SBR) demonstrated  
296 a reduction in pH, creating a more acidic environment. This finding aligns with previous studies that  
297 have reported similar outcomes. Fe and Ti extractions are still low compared to the ones obtained by  
298 Qu *et al.* (2019) with *Acetobacter* (around 30% and 45%, respectively) for a one-step process at 2%  
299 (w/v) and for a duration of 20-30 days. However, Al extraction was more in a range similar to some  
300 studies in the literature: 55% (Qu *et al.*, 2019) and 69% (Vakilchap *et al.*, 2016) extraction were  
301 obtained for a one-step process at 2% (w/v), at 28-30 °C and after 20-30 days of treatment.

302 Considering the extraction of REEs, our study has shown better results for Nd compared to Qu *et al.*  
303 (2019) and Qu and Lian (2013) (65.2% vs almost 25% and 30%, respectively), who reported a similar  
304 Ce extraction to our result (19.5% vs 22% and 25%, respectively), while Y extraction was quite lower  
305 in our results (30.2%) than in their ones (60% and 80%, respectively). Comparable Nd extractions  
306 were obtained by Abhilash *et al.* (2021), who treated RM from India by using *Gluconobacter oxydans*

307 with higher RM concentrations. Qu *et al.* (2019) reported an Sc extraction of 50%, and Qu and Lian  
308 (2013) more than 70%, both higher than our results (34.4.%). Campo Pedram et al. (2020) achieved  
309 similar results in Sc extraction, in which the ability of the mould *Aspergillus niger* in bioleaching was  
310 tested. Lanthanum extraction was similar to the study performed by Qu and Lian (2013). La and Sc  
311 extraction efficiencies in our study were comparable to those obtained by Qu et al. 2015 using a  
312 continuous set-up (i.e., 27% for La, and 30% for Sc). The difference in extraction results can be linked  
313 to the wide spectrum of microorganisms used in the literature and connected to the metabolites  
314 produced. Moreover, the mineralogical phases, in which these minor components are present, can  
315 affect the results.

316

## 317 **5 Conclusions**

318 A sequencing batch reactor was started-up to investigate the possibility of exploiting the biomass  
319 initially present in RM and verify its bioleaching potential in the long-term operation, without any  
320 preliminary selection and enrichment. An operating strategy based on pH evolution over time was  
321 adopted to set optimal cycle duration. All the elements of interest were released, and the highest  
322 extraction efficiency was achieved for Al, Mn and Mg. Some of them were better released with a  
323 lower S/L. Promising results were also obtained for minor elements with higher economic interest  
324 such as REEs. The metals extraction was higher if compared to batch tests conducted at the same  
325 operational conditions, demonstrating the biological activity enhancement and the possibility of not  
326 using an external inoculum. After the bioleaching treatment, the reactor produces a slurry containing  
327 a liquid phase with dissolved metal ions and a residual RM. In a hypothetical scale-up of the  
328 treatment, it would be necessary to separate the liquid phase to recover all the leached metals. To  
329 ensure proper disposal or potential reuse, a physico-chemical characterization is required to assess  
330 the suitability of the treated RM. Moreover, the possibility to replace the synthetic organic leaching

331 medium with organic waste as the carbon and energy source worths of being investigated, in the  
332 perspective of further reducing the operating costs.

333

334 **Supplementary Materials:** The following supporting information can be downloaded at xxx, Figure  
335 S1: XRD analysis of raw RM and bioleached one at 2% S/L

336

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353

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