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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, circular economy, metals, red mud, rare earth elements,  
34 secondary raw materials, 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, calcium (Ca), titanium (Ti) and other minor elements  
43 (Hind *et al.*, 1999).

44

45 Due to the population growth and the technologies 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; Vidal *et al.*,  
49 2013). The last CRM (Critical Raw Materials) final report has included bauxite/aluminium as critical  
50 materials (CRMs final report, 2023). Numerous approaches have been explored for the utilization of  
51 RM or its disposal, which include its use as an adsorbent for removing heavy metals and toxic  
52 substances from contaminated wastewater, as a building material such as bricks, concrete, and road  
53 foundations, as a catalyst for oxidizing organic compounds and reducing nitrates, and as a soil  
54 amendment (Zhang *et al.*, 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 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 to explore possible  
82 strategies for process optimization, shifting the biomass enrichment from a batch- to a semi-  
83 continuous scale. Furthermore, the possibility of achieving bioleaching of RM in SBR without any  
84 preliminary biomass selection and enrichment is investigated, thus reducing the operational costs due  
85 to an external inoculum.

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

93

## 94 **2. Materials and Methods**

### 95 **2.1 Red Mud characterization**

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

100

### 101 **2.2 Bioleaching experiments**

102 The experiments were carried out in a 5-litre sequencing batch reactor (SBR) equipped with an  
103 external water jacket for temperature control, maintaining the process at  $22 \pm 2$  °C. To preserve the  
104 autochthonous biomass possibly colonizing RM, the material was not de-hydrated before the SBR  
105 start-up. A synthetic leaching medium (LM) consisting of 30 g/l of ethanol 96%, 10 g/l of glucose,  
106 and 10 g/l of yeast extract (YE), according to Qu *et al.* (2019), was used. During the experimentation,  
107 the concentration of YE was reduced due to issues related to foam formation. Different solid to liquid  
108 ratios (S/L) were used: 2% and 1.5%, referred to the dry mass. More specifically, the 2% ratio was  
109 adopted on the basis of our previous study which investigated the bioleaching of metals from red mud  
110 in batch conditions using non-enriched biomass (Cozzolino *et al.*, 2022). The S/L ratio was reduced  
111 at 1.5% in the second part of the experiment based on the hypothesis that a lower S/L ratio may reduce  
112 the potential inhibiting effect of RM on biomass, without significantly reducing the amount of red  
113 mud treated per cycle. A mechanical stirrer was used to provide mixing (rotating speed, 100-120  
114 rpm). To avoid oxygen limitation, the reactor was aerated, maintaining the dissolved oxygen  
115 concentration above 1 ppm. The air was flushed through porous stones inside the reactor, and the  
116 flowrate was kept in the range 1-2 l/min with a flowmeter. A reactor scheme is shown in  
117 supplementary materials (Figure S1). In a typical working cycle, pH increased soon after the feeding,  
118 then rapidly decreased due to biological activity and reached a plateau. Each new feeding was added  
119 at the end of the plateau, when signals of pH rise were detected. According to the observed pH trend

120 over time, 2 litres of fresh LM and RM with the desired S/L ratio were periodically replaced in the  
121 reactor to start a new working cycle. Samples were taken at the beginning, during and at the end of  
122 each working cycle. The experimentation lasted 170 and 58 days at 2% and 1.5% S/L, respectively.  
123 A schedule of the experimental campaign and main operating conditions are resumed in Table 1.

124 *Table 1. Schedule of the experimental campaign and main operating parameters.*

Phase	S/L (%)	Duration (days)	Cycles	Average HRT (days)
I	2.0	170	From 1 to 25	12.1±6.3
II	1.5	58	From 26 to 38	14.6±4.1

125 The reactor was sterilized before the experimentation started. Neither the air nor the LM were  
126 sterilized during the process, in a scale-up perspective.

127

### 128 **2.3 Analytical methods**

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

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

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

142 During bioleaching tests, 15 ml samples were periodically taken from the reactor and centrifuged at  
143 5000 rpm for 15 minutes to remove the solid part. The supernatants were used to evaluate pH, EC,

144 metals and organic acids concentration. For metals analysis, part of the supernatant was filtered at  
145 0.45  $\mu\text{m}$ , acidified (1% v:v of nitric acid 65%), diluted with grade-I water, and then analysed with  
146 ICP-OES (Varian 710-ES; Perkin Elmer Optima 2100 DV) (Cozzolino *et al.*, 2023). The remaining  
147 part of the samples was filtered at 0.45  $\mu\text{m}$ , diluted with grade-I water and used to evaluate organic  
148 acid concentrations using a LC4000 HPLC (Jasco) equipped with a Water – Fast fruit juice analytical  
149 column (7.8x150 mm, 50 angstrom, 7  $\mu\text{m}$ ) and run at T=60 °C, H<sub>3</sub>PO<sub>4</sub> 0.01N eluent solution,  
150 isocratic mode, flow 0.8 ml/min.

151

## 152 **2.4 Microbiological analysis**

153 Samples of the slurry were axenically collected two and five days after the beginning of the 18<sup>th</sup> cycle  
154 and used for the enumeration of cultivable aerobic microorganisms. The viable titres were determined  
155 as colony forming units (CFU) by plating serial 10-fold dilutions of each sample onto three different  
156 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,  
157 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  
158 microorganisms (Horikoshi, 1999). The medium was modified by omitting the addition of sodium  
159 carbonate and adjusting the pH at 7.0 and 3.0 for enumeration of neutrophilic and acidophilic  
160 microorganisms, respectively. After five days incubation at 25 °C bacteria and moulds were  
161 separately counted.

162

## 163 **2.5 Calculations**

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

$$165 \text{ Extraction efficiency } [\%] = \frac{\text{Metal in leachate at a specific time [mg]}}{\text{Metal content in the RM [mg]}} \cdot 100 \quad (1)$$

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

$$167 \text{ Daily extraction capacity } \left[ \frac{\text{mg}}{\text{day} \cdot \text{l}} \right] = \frac{\text{Metal concentration } \left[ \frac{\text{mg}}{\text{l}} \right] \cdot V_o [\text{l}]}{\text{cycle duration } [\text{day}] \cdot V_R [\text{l}]} \quad (2)$$



168 Where “metal concentration” is the concentration for each metal used to evaluate the highest  
 169 extraction for each S/L (2%, 1.5%), the “cycle duration” is the number of days of the considered  
 170 cycle,  $V_o$  is the volume of slurry discharged at the end of the cycle and  $V_R$  is the reactor volume.  
 171 The bioleaching process kinetics was calculated as the slope of the steepest part of pH profile over  
 172 time.

### 173 3 Results

#### 174 3.1 Red Mud characterization

175 The pH, EC and  $ANC_{4.5}$  values assessed for RM were 11.6, 2.5 mS/cm and 5.2 mmol  $H^+$ /g,  
 176 respectively. The ANC plot was reported in our previous study and showed a buffering capacity  
 177 around pH 6.5, potentially caused by high carbonates content, as confirmed by total limestone  
 178 analysis (Cozzolino *et al.*, 2023). Table 2 shows the concentrations of the most relevant chemical  
 179 elements in RM, metals and REEs. A more detailed characterization is described in Cozzolino *et al.*  
 180 (2023).

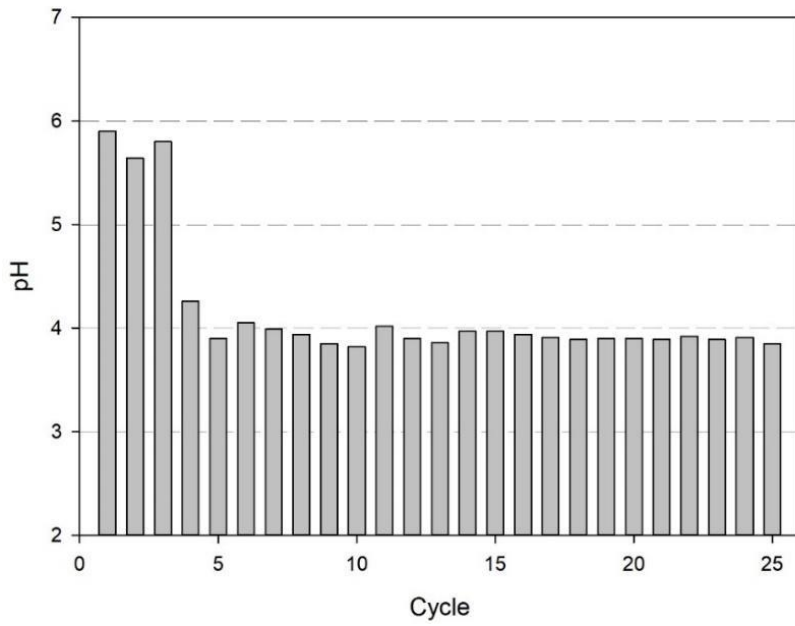
181 *Table 2. Main metals (weight percentage) and some rare earth elements (mg/kg) contained in RM*

<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

182

#### 183 3.2 Bioleaching experimentation

184 Process performance at 2% S/L. Figure 1 illustrates the minimum pH values achieved in each cycle  
 185 over time. Figure 1 illustrates the minimum pH values achieved in each cycle over time. During the  
 186 first cycles, the minimum pH progressively decreased to neutral values similar to those observed in  
 187 batch tests at the same operational conditions (Cozzolino *et al.*, 2023). However, from cycle 5 onward,  
 188 the minimum pH achieved in each cycle stabilized at around 4, as reported in the figure.



189

190

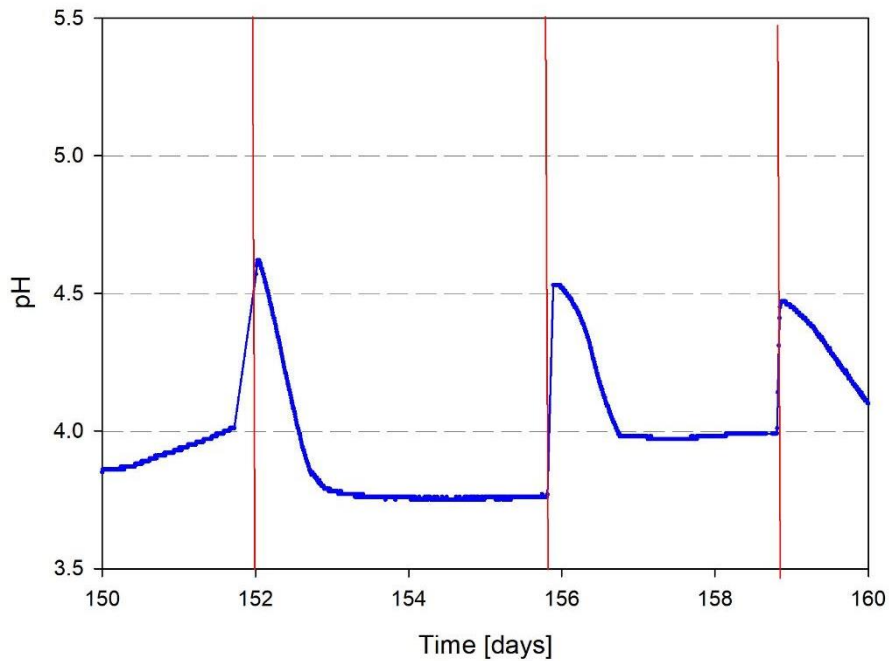
*Figure 1. Lowest pH values for each cycle over time at 2% S/L*

191

Figure 2 shows the pH trends of some of the most representative cycles under stable operating shows

192

conditions. The pH increase corresponds to a new feeding and the beginning of a new working cycle.



193

194

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

195

196 Table 3 shows the minimum pH values and the kinetics for some of the most representative cycles  
197 performed at 2% S/L.

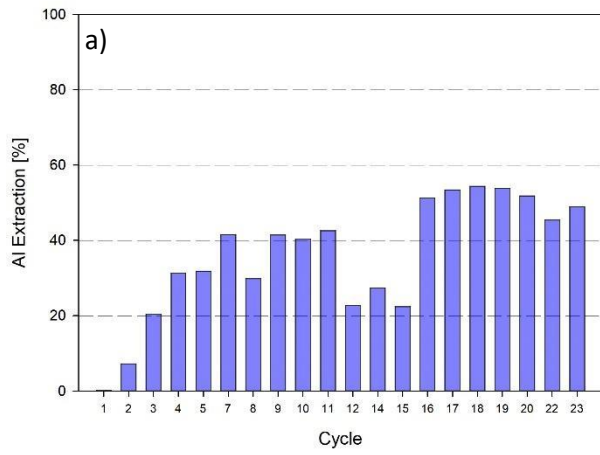
198 *Table 3. Process kinetics and pH plateaux at 2% S/L*

<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

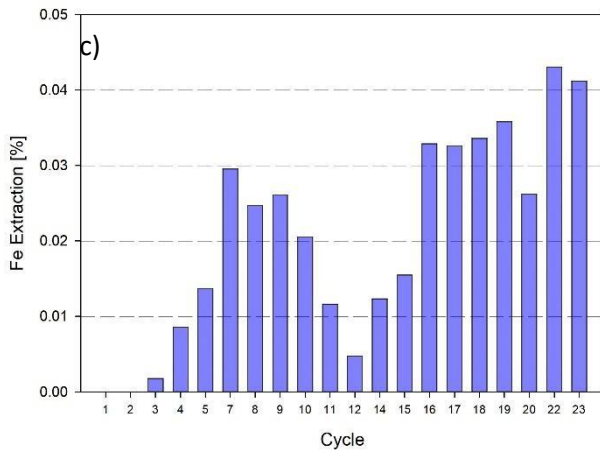
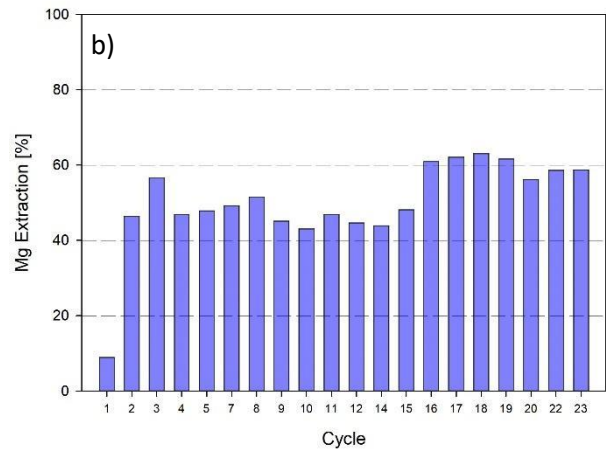
199  
200 The kinetics was in a range between 0.25 and 0.73 pH units/day. A lower variation is observed in the  
201 minimum pH obtained among these cycles set.

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

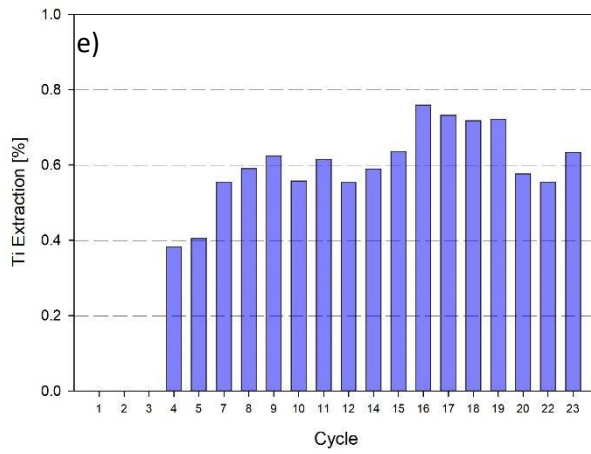
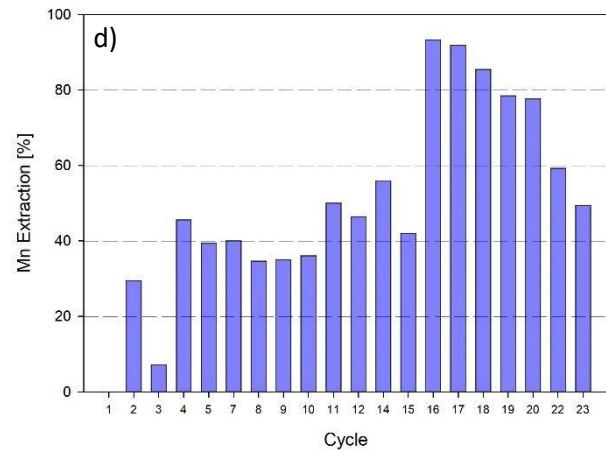
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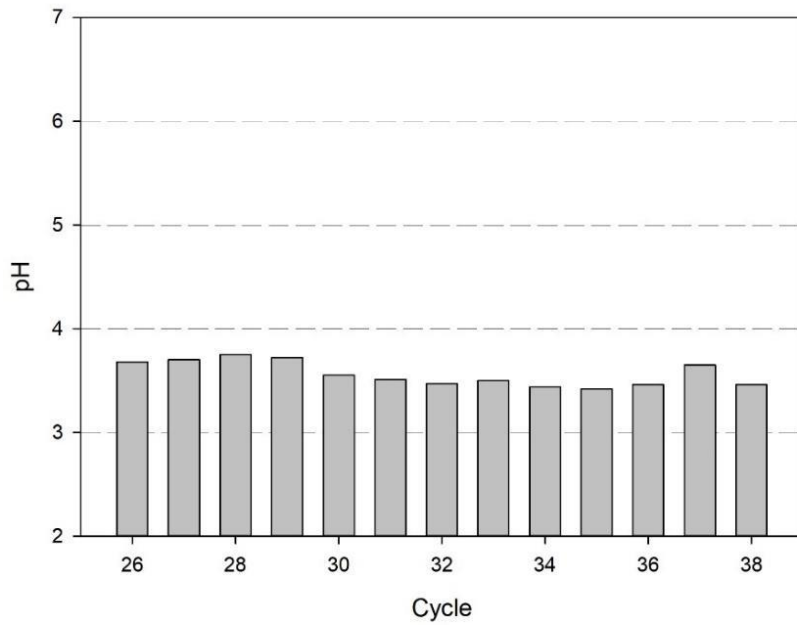
206

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

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

209 In general, it can be observed that the lower the pH, the higher the metals concentration in the liquid  
210 phase, but there was an upward trend in terms of extraction for some metals from around cycle 5,  
211 even if the pH showed slight variations. Aluminium shows an increasing trend, with an average  
212 extraction of  $51.3 \pm 2.9$  %. Magnesium and manganese also increased over time ( $58 \pm 4.5$  % for Mg and  
213  $68 \pm 18.4$  % for Mn). As expected for highly oxidated materials, low extraction efficiencies of  $0.03 \pm$   
214  $0.01$ % were observed for iron under the conditions reached during the experiment (i.e., pH >3.5;  
215 approx. 20 g/L acetic acid concentration; 0.1 V redox potential; temperature 22 °C), in agreement  
216 with the known Pourbaix diagram combined with the speciation diagrams in the presence of acetic  
217 acid (Taylor et al., 1998). Nevertheless, due to the high concentration in the untreated red mud, the  
218 final leachate contained a significant iron concentration (i.e., around 2 ppm), which is potentially  
219 suitable for recovery. Finally, Ti showed the same growing trend, though the highest extractions  
220 achieved were still lower than 1%. The averages were measured considering the cycles from the 15<sup>th</sup>,  
221 when the metals extractions results started to stabilize.

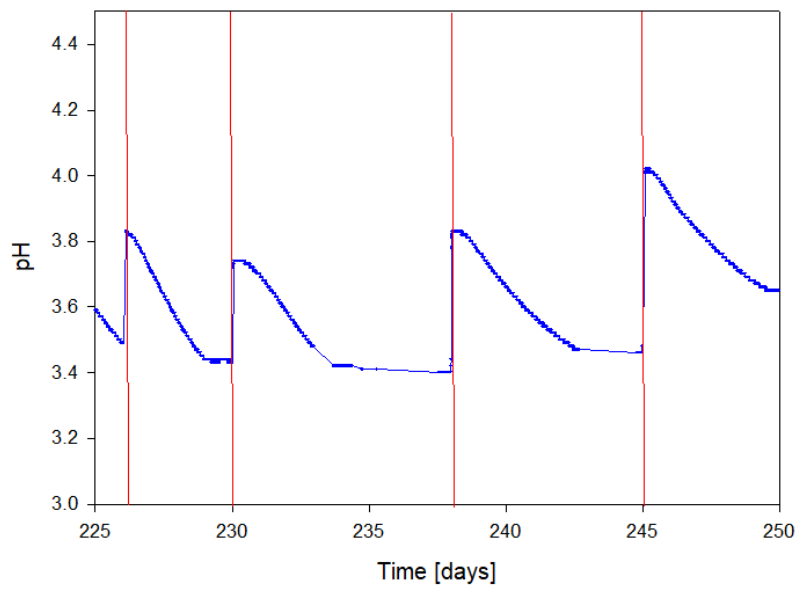
222 Process performance at 1.5% S/L. The applied S/L ratio was reduced from 2 to 1.5, to check if lower  
223 amounts of RM would have an effect on process performance. In particular, the possibility to reduce  
224 the potential inhibiting effects and extend the duration of working cycles at low pH conditions was  
225 investigated, supposing that longer exposures at low pH would enhance metals extraction. Figure 4  
226 shows the minimum pH values for each cycle over time at 1.5% S/L, Figure 5 shows some  
227 representative cycles under stable conditions at 1.5%. Table 4 reports the kinetics of pH drop and the  
228 pH plateaux values.



229

230

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



231

232

Figure 5. pH trends of some representative cycles at 1.5% S/L

233

234

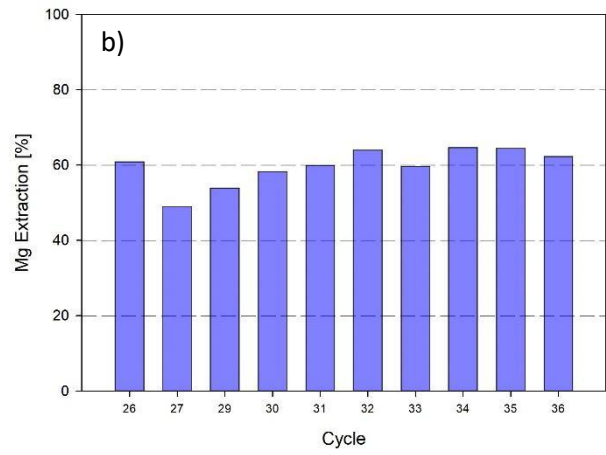
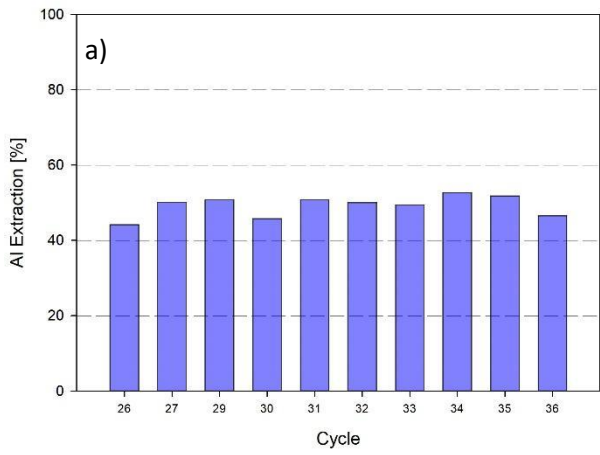
*Table 4. Process kinetics and pH plateaux at 1.5% S/L*

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

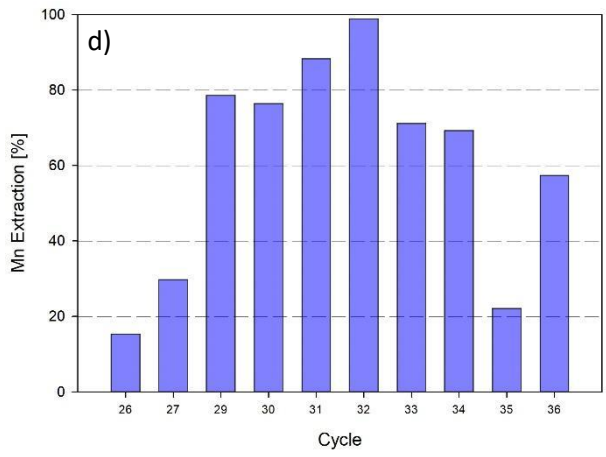
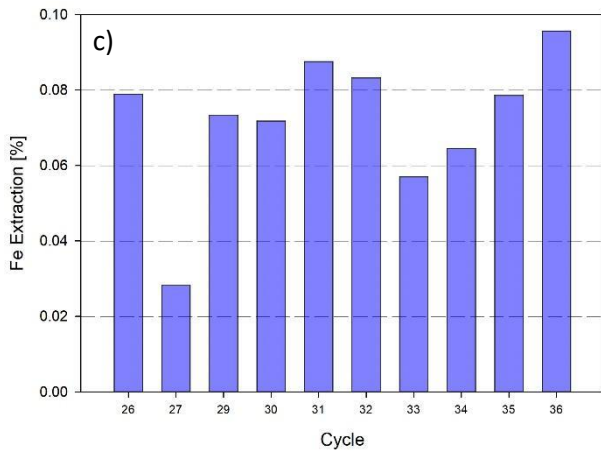
235

236 As expected, longer cycles were achieved, probably connected to a lower amount of RM fed into the  
 237 reactor. However, the lower RM concentration may correspondingly reduce the amount of biomass  
 238 concentration entering in the reactor.

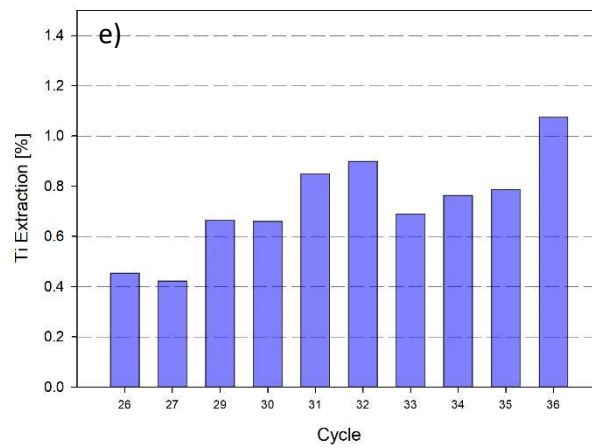
239 Figure 6 shows the trend of metal extraction efficiencies. The aluminium extraction was around  
 240  $49 \pm 2.6$  %, and remained constant during the test at S/L 1.5%. A very low iron extraction was obtained  
 241 ( $<0.1$ %). A very fluctuating extraction efficiency was observed for Mn ( $60 \pm 27$  %). Mg extraction  
 242 efficiency reached an average of  $59 \pm 4$  %. Finally, the extraction of titanium increased, as well, but  
 243 remained lower than 1%. Given the relatively stable operation of the SBR at S/L 1.5%, all the cycles  
 244 have been considered to calculate the average extraction.



245



246



247

248 *Figure 6 . Metals extraction percentages at 1.5% S/L: Al (a), Mg (b), Fe (c), Mn (d), Ti (e)*

249



250 The daily extraction capacity was also calculated. Table 5 summarizes the values obtained for each  
 251 metal, using the results from cycle 18 (2% S/L) and 31 (1.5% S/L), as examples of the best results  
 252 obtained.

253 *Table 5. 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

254

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

257 The results for Ti and Mn at 1.5% are quite similar to those achieved at 2% S/L, whereas Fe is higher  
 258 because of the higher extraction at 1.5%. Al showed a lower value at 1.5%: since less RM was fed,  
 259 longer cycles were obtained with a consequent smaller daily extraction capacity. This behaviour is  
 260 less evident with the other metals. At 1.5% S/L some REEs were measured in the liquid phase at pH  
 261 3.5, and the extraction percentages are reported in Table 6.

262 *Table 6. REEs extractions from the RM using 1.5% S/L*

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

263 Acid production. From the HPLC analysis it was found that acetic acid was the one present at the  
264 highest concentrations (20 g/l). This is an average value among the cycles at 2% S/L shown in this  
265 study. Because of this very high concentration in the leached solution, it was not possible to detect  
266 the presence of other acids, which may, however, be present (Cozzolino *et al.*, 2023).

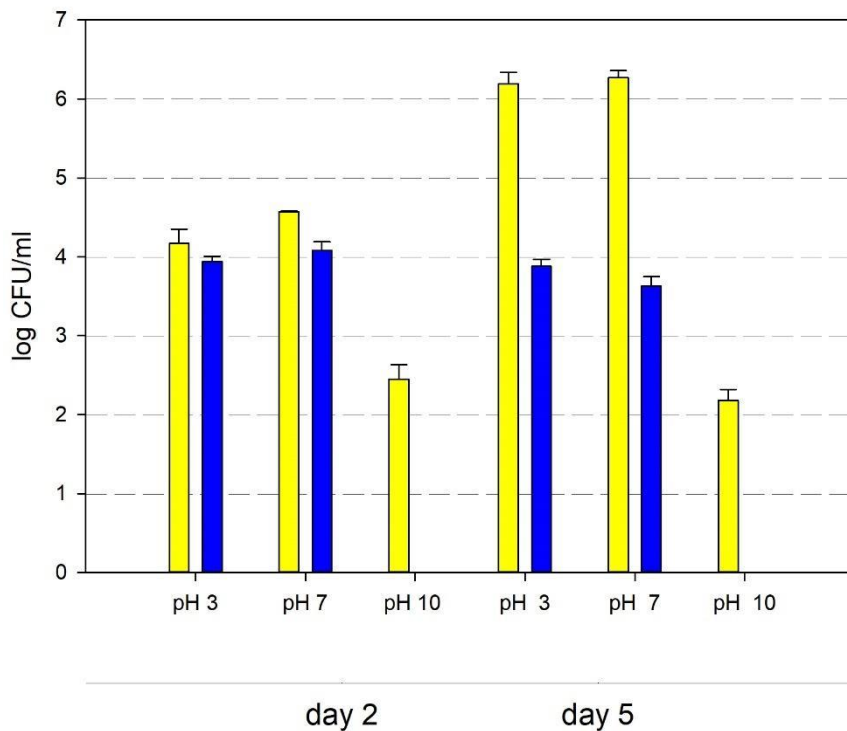
267

268 Mineralogical analysis results. Figure S2 shows the XRD analysis for untreated and treated RM  
269 (cycle 18). Untreated RM composition was based on hematite ( $\text{Fe}_2\text{O}_3$ ), calcite ( $\text{CaCO}_3$ ), sodalite  
270 ( $\text{KNa}_3\text{Al}_3\text{Cl}(\text{SiO}_4)_3$ ), boehmite ( $\text{AlO}(\text{OH})$ ), gibbsite ( $\text{Al}(\text{OH})_3$ ), rutile ( $\text{TiO}_2$ ). After bioleaching, there  
271 was no evidence of sodalite, gibbsite, boehmite and calcite, indicating the transfer of metals  
272 associated with these components into the liquid phase and/or a reprecipitation of amorphous phases.  
273 One component clearly present in the residue after the treatment was hematite, in agreement with the  
274 low concentration of Fe in the liquid phase Weddellite ( $\text{Ca}(\text{C}_2\text{O}_4)\cdot 2(\text{H}_2\text{O})$ ) was formed, probably as  
275 the result of  $\text{Ca}^{2+}$  precipitation due to the due reaction with oxalic acid (Qu *et al.*, 2019, Cozzolino *et*  
276 *al.*, 2023).

277

### 278 **3.3 Selective enumeration of microorganisms**

279 The viable titres of heterotrophic alkaliphiles, neutrophiles, and acidophiles are shown in Figure 7 .  
280 At the beginning of the cycle, the viable titres of neutrophilic and acidophilic microorganisms were  
281 comparable and in the order of  $10^4$  CFU/ml for both bacteria and moulds. Moreover, a two orders of  
282 magnitude lower viable titre was found for alkaliphilic bacteria. At the end of the cycle, the titre of  
283 alkaliphilic bacteria was almost stable. On the contrary, an enrichment in acidophilic and neutrophilic  
284 bacteria was observed, as highlighted by a two order of magnitude increase in the viable titres. The  
285 alkaliphilic moulds were not detected at all, both at the beginning and at the end of the analysed  
286 cycle.



287

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

#### 290 **4 Discussion**

291 At 2% S/L, a pH below 4 (3.9) was reached on cycle #5 for the first time. This value is quite low  
 292 compared to the minimum values obtained in batch tests performed with the same operational  
 293 conditions, i.e., 5.9 (Cozzolino *et al.*, 2023). Such behaviour can be linked to the progressively  
 294 enhanced biological activity, compared to the initial one. In line with the pH values, also the  
 295 extractions observed in the first SBR cycles were similar to those achieved with batch tests conducted  
 296 at the same operating conditions and LM, then the extracted quantity progressively increased. For  
 297 instance, Al in SBR reached an extraction 5-6 times higher than the batch tests conducted at the same  
 298 S/L and temperature (51% vs 10%). Also, Fe and Ti extraction efficiencies were a few  
 299 times higher than those achieved in batch tests, though still lower than 1%. Mg and Mn extraction  
 300 efficiencies in SBR and batch tests were 58% and 68% vs 18% and 9%, respectively. Such an increase

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

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

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

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

321 During the experimentation, acetic, oxalic, lactic, and citric acids were measured in the samples. A  
322 high concentration of acetic acid was expected because of the presence of ethanol, which is converted  
323 into acetic acid through acetic fermentation. The leaching medium employed has a stimulating effect  
324 on the growth of heterotrophic biomass. When heterotrophic biomass is involved in a leaching  
325 process, acidolysis is primarily responsible for the release of metals (Qu *et al.*, 2013b).

326

327 Considering REEs, Ilyas et al. (2021) correlated the extraction with different mixtures of organic  
328 acids produced by fungi and pointed out that REEs solubilization from red mud can be due to either  
329 an enzymatic reduction of highly oxidized metal compounds or the production of organic acids  
330 (acidolysis), the latter appearing to be the main mechanism of REEs dissolution. According to our  
331 results, the main role in REEs dissolution was likely played by acetic acid, as its concentration was  
332 very high compared to other acids. Compared to Qu *et al.* (2019) and Qu and Lian (2013), our study  
333 showed extraction efficiencies higher for Nd (65.2% vs almost 25% and 30%, respectively), similar  
334 for Ce (19.5% vs 22% and 25%, respectively), and lower for Y (30.2% vs 60% and 80%). Comparable  
335 Nd extractions were obtained by Abhilash *et al.* (2021), who treated RM from India by using  
336 *Gluconobacter oxydans* with higher RM concentrations. Qu *et al.* (2019) reported a Sc extraction of  
337 50%, and Qu and Lian (2013) more than 70%, both higher than our results (34.4%). Campo Pedram  
338 *et al.* (2020) achieved similar results in Sc extraction, in which the ability of the mould *Aspergillus*  
339 *niger* in bioleaching was tested. Lanthanum extraction was similar to the study performed by Qu and  
340 Lian (2013). La and Sc extraction efficiencies in our study were comparable to those obtained by Qu  
341 *et al.*, 2015 using a continuous set-up (i.e., 27% for La, and 30% for Sc). The difference in extraction  
342 results can be linked to the wide spectrum of microorganisms used in the literature and connected to  
343 the metabolites produced. Moreover, the mineralogical phases, in which these minor components are  
344 present, can affect the results.

## 345 **5 Conclusions**

346 A sequencing batch reactor was started-up to investigate the possibility of achieving bioleaching of  
347 RM in a SBR in the long-term operation, without any preliminary biomass selection/enrichment. An  
348 operating strategy based on pH evolution over time was adopted to set optimal cycle duration. All the  
349 elements of interest were released, and the highest extraction efficiency was achieved for Al, Mn and  
350 Mg. Some of them were better released with a lower S/L. Promising results were obtained for minor

351 elements with a higher economic interest (REEs), as well. The metals extraction was higher if  
352 compared to batch tests conducted at the same operational conditions, demonstrating the biological  
353 activity enhancement and the possibility of not using an external inoculum. After the bioleaching  
354 treatment, the reactor produces a slurry containing a liquid phase with dissolved metal ions and a  
355 residual RM. In a hypothetical scale-up of the  
356 treatment, it would be necessary to separate the liquid phase to recover all the leached metals. To  
357 ensure proper disposal or potential reuse, a physico-chemical characterization is required to assess  
358 the suitability of the treated RM. Further experimentation will be focused on increasing the S/L ratio  
359 in order to treat more waste and recover a higher metals amount at the same time. Moreover, the  
360 possibility of replacing the synthetic organic leaching medium with organic waste as the carbon and  
361 energy source worths of being investigated, in the perspective of further reducing the operating costs.

362

363 **Supplementary Materials:** The following supporting information can be downloaded at [xxx](#), Figure  
364 S1: Schematic representation of the semi-continuous reactor; Figure S2: XRD analysis of raw RM  
365 and bioleached one at 2% S/L.

366

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374 Tamburini, Jesús Angel Muñoz, Angela Serpe and Stefano Milia; *supervision*, Alessandra Carucci,  
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383

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386

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