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

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ABSTRACT

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

KEYWORDS: biohydrometallurgy, circular economy, metals, red mud, rare earth elements, secondary raw materials, sequencing batch reactor

1. Introduction

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

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

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

A way to recover valuable substances from RM is to leach metals into solution via chemical reagents. A possible option could be biohydrometallurgy, which exploits the metabolism of microorganisms to achieve the leaching of metals from solid wastes, ores or minerals (Jain and Sharma, 2004; Sethurajan *et al.*, 2018). This eco-friendly approach offers advantages over known chemical methods, including lower energy consumption, reduced use of chemicals and generation of hazardous fluids, and enhanced cost-effectiveness. Bioleaching, a key process in biohydrometallurgy, can be operated by autotrophic or heterotrophic microorganisms. Autotrophic bioleaching employs bio-oxidation/reduction cycles with inorganic acids, while heterotrophic leaching relies on the microbial production of organic metabolites and the formation of soluble chelates to mobilize elements (Pedram *et al.*, 2020). One option to leach metals contained in non-sulfidic ores, such as RM, may be to use metabolites produced by heterotrophic bacteria and fungi sustained by a suitable organic substrate (Jain and Sharma, 2004).

Up to now, most of the studies concerning the bioleaching of valuable elements from RM were conducted in batch mode with the addition of an external and/or preliminarily enriched inoculum (Ghorbani *et al.*, 2008; Qu *et al.*, 2019; Vakilchap *et al.*, 2016). This study aims to explore possible strategies for process optimization, shifting the biomass enrichment from a batch- to a semi-continuous scale. Furthermore, the possibility of achieving bioleaching of RM in SBR without any preliminary biomass selection and enrichment is investigated, thus reducing the operational costs due to an external inoculum.

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

2. Materials and Methods

2.1 Red Mud characterization

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

2.2 Bioleaching experiments

The experiments were carried out in a 5-litre sequencing batch reactor (SBR) equipped with an external water jacket for temperature control, maintaining the process at 22 ± 2 °C. To preserve the autochthonous biomass possibly colonizing RM, the material was not de-hydrated before the SBR start-up. A synthetic leaching medium (LM) consisting of 30 g/l of ethanol 96%, 10 g/l of glucose, and 10 g/l of yeast extract (YE), according to Qu *et al.* (2019), was used. During the experimentation, the concentration of YE was reduced due to issues related to foam formation. Different solid to liquid ratios (S/L) were used: 2% and 1.5%, referred to the dry mass. More specifically, the 2% ratio was adopted on the basis of our previous study which investigated the bioleaching of metals from red mud in batch conditions using non-enriched biomass (Cozzolino *et al.*, 2022). The S/L ratio was reduced at 1.5% in the second part of the experiment based on the hypothesis that a lower S/L ratio may reduce the potential inhibiting effect of RM on biomass, without significantly reducing the amount of red mud treated per cycle. A mechanical stirrer was used to provide mixing (rotating speed, 100-120 rpm). To avoid oxygen limitation, the reactor was aerated, maintaining the dissolved oxygen concentration above 1 ppm. The air was flushed through porous stones inside the reactor, and the flowrate was kept in the range 1-2 l/min with a flowmeter. A reactor scheme is shown in supplementary materials (Figure S1). In a typical working cycle, pH increased soon after the feeding, then rapidly decreased due to biological activity and reached a plateau. Each new feeding was added at the end of the plateau, when signals of pH rise were detected. According to the observed pH trend over time, 2 litres of fresh LM and RM with the desired S/L ratio were periodically replaced in the reactor to start a new working cycle. Samples were taken at the beginning, during and at the end of each working cycle. The experimentation lasted 170 and 58 days at 2% and 1.5% S/L, respectively. A schedule of the experimental campaign and main operating conditions are resumed in Table 1.

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

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

2.3 Analytical methods

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

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

The RM water content was determined after drying the sample at 105 °C. The ANC was assessed by mixing several subsamples of dry RM with distilled water (10:1 *v/w*) containing pre-selected amounts of acid (HNO_3 65%) and stirring the suspension for 48 h (UNI CEN/TS 15364). The ANC values were determined considering a target pH of 4.5 ($ANC_{4.5}$).

During bioleaching tests, 15 ml samples were periodically taken from the reactor and centrifuged at 5000 rpm for 15 minutes to remove the solid part. The supernatants were used to evaluate pH, EC, metals and organic acids concentration. For metals analysis, part of the supernatant was filtered at 0.45 µm, acidified (1% *v:v* of nitric acid 65%), diluted with grade-I water, and then analysed with ICP-OES (Varian 710-ES; Perkin Elmer Optima 2100 DV) (Cozzolino *et al.*, 2023). The remaining part of the samples was filtered at 0.45 µm, diluted with grade-I water and used to evaluate organic acid concentrations using a LC4000 HPLC (Jasco) equipped with a Water – Fast fruit juice analytical column (7.8x150 mm, 50 angstrom, 7 µm) and run at T=60 °C, H3PO4 0.01N eluent solution, isocratic mode, flow 0.8 ml/min.

2.4 Microbiological analysis

Samples of the slurry were axenically collected two and five days after the beginning of the 18th cycle and used for the enumeration of cultivable aerobic microorganisms. The viable titres were determined as colony forming units (CFU) by plating serial 10-fold dilutions of each sample onto three different culture media. The Horikoshi Medium (yeast extract 1 g/l, K_2HPO_4 0.2 g/l, $MgSO_4$ $7H_2O$ 0.2 g/l, Na_2CO_3 10 g/l, glucose 10 g/l, agar 17 g/l, pH 10.0) was used for enumeration of alkalophilic microorganisms (Horikoshi, 1999). The medium was modified by omitting the addition of sodium carbonate and adjusting the pH at 7.0 and 3.0 for enumeration of neutrophilic and acidophilic microorganisms, respectively. After five days incubation at 25 °C bacteria and moulds were separately counted.

2.5 Calculations

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

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

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

$$Daily\ extraction\ capacity\ \left[\frac{mg}{day * l}\right] = \frac{Metal\ concentration\ \left[\frac{mg}{l}\right] * V_O\ [l]}{cycle\ duration\ [day] * V_R\ [l]} \qquad (2)$$

Where “metal concentration” is the concentration for each metal used to evaluate the highest extraction for each S/L (2%, 1.5%), the “cycle duration” is the number of days of the considered cycle, Vo is the volume of slurry discharged at the end of the cycle and V_R is the reactor volume.

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

3 Results

3.1 Red Mud characterization

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

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

Fe_2O_3 [%]	43.55 ± 7.26	**TiO_2 [%]**	6.98 ± 1.70	**Nd [mg/kg]**	190
Al_2O_3 [%]	20.7 ± 3.78	**CaO [%]**	4.08 ± 0.1	**Ce [mg/kg]**	651
SiO_2 [%]	9.7 ± 2.35	**Na_2O [%]**	6.68 ± 0.02	**Y [mg/kg]**	199
MgO [%]	0.31 ± 0.02	**K_2O [%]**	0.1 ± 0.01	**Sc [mg/kg]**	97
MnO [%]	0.103 ± 0.03			**La [mg/kg]**	280

3.2 Bioleaching experimentation

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

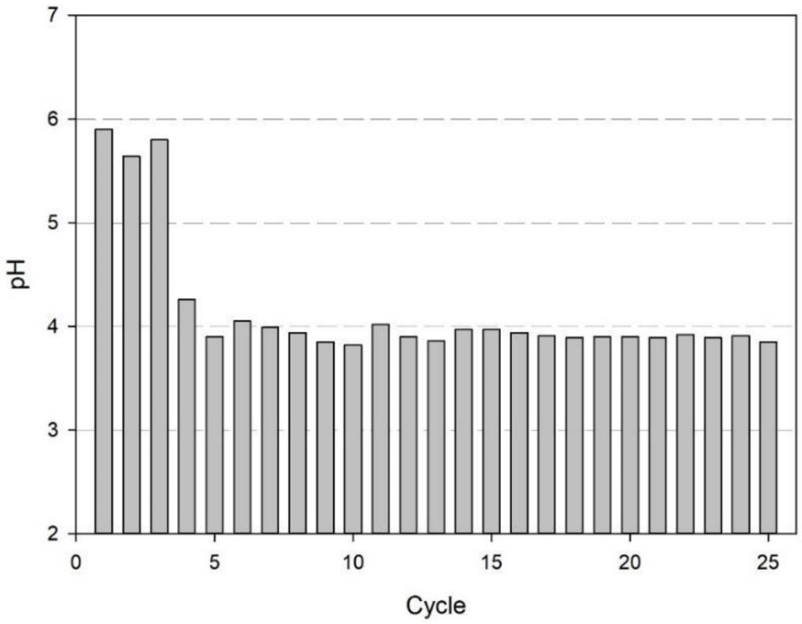

Figure 1. Lowest pH values for each cycle over time at 2% S/L

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

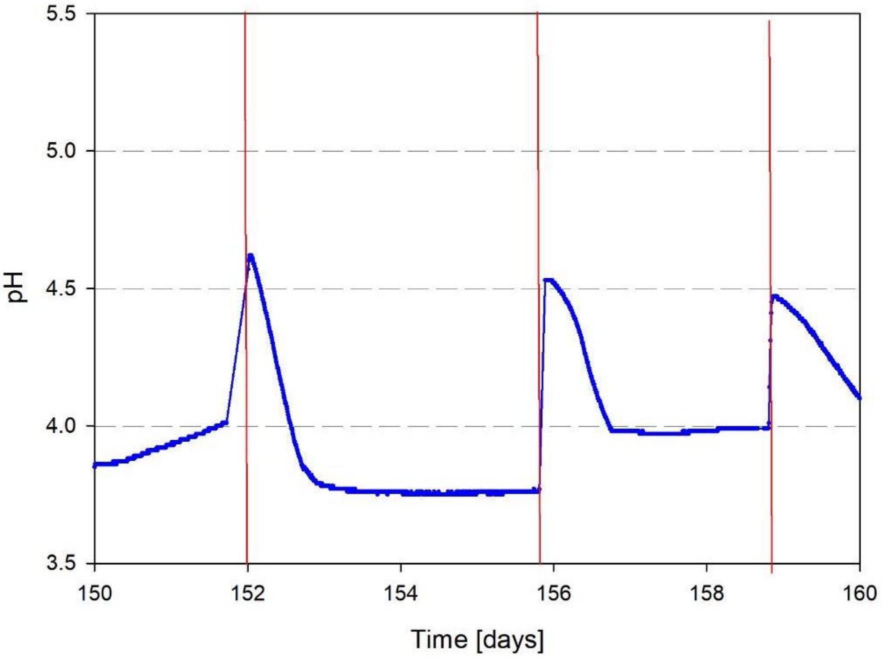

Figure 2. pH trends of some representative cycles at 2% S/L

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

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

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

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

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

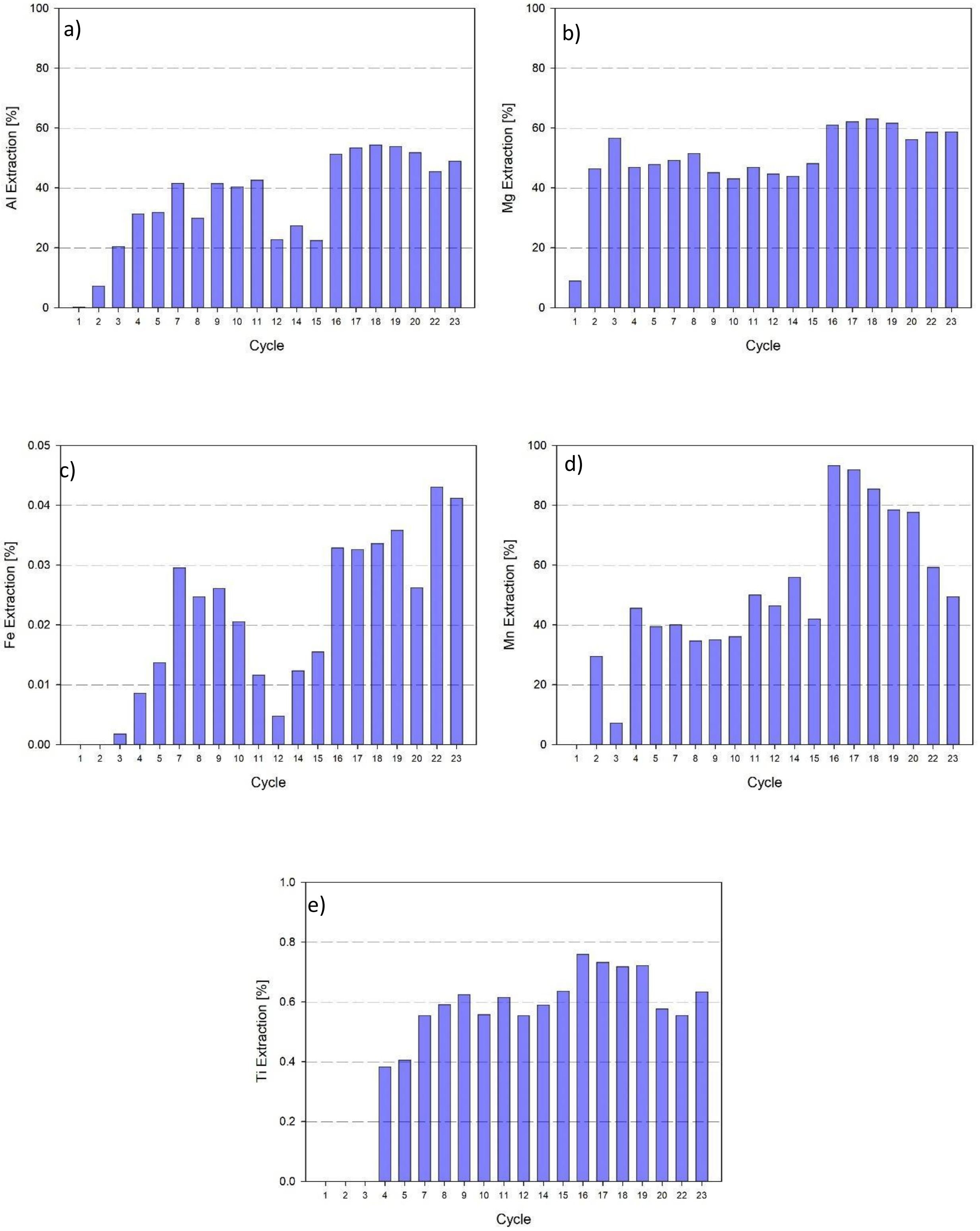

Figure 3 Metals extraction percentages during the first 23 cycles at 2% S/L: Al (a), Mg (b), Fe (c), Mn (d), Ti (e)

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

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

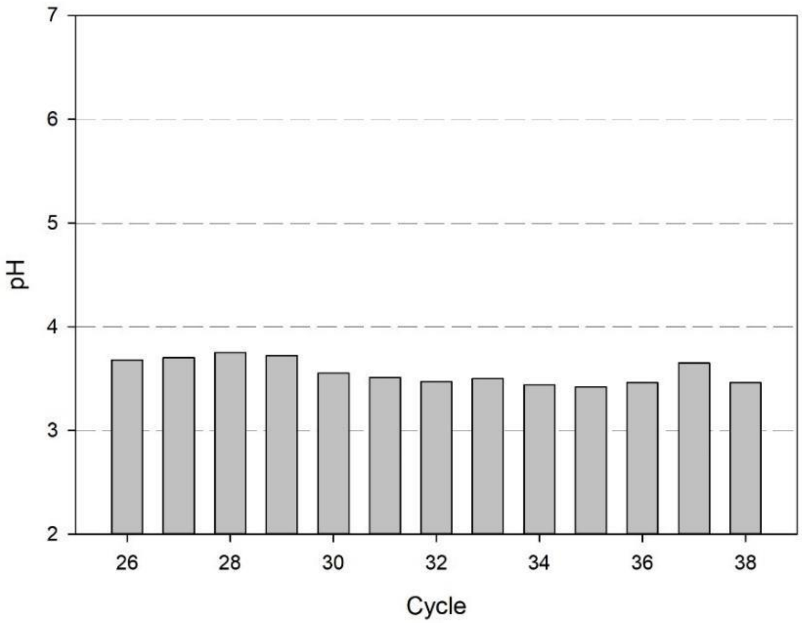

Figure 4. Lowest pH values for each cycle over time at 1.5% S/L

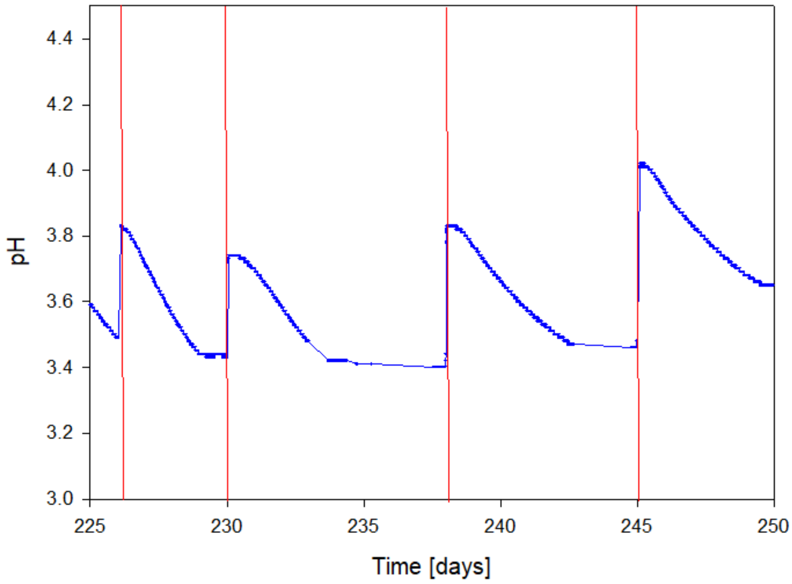

Figure 5. pH trends of some representative cycles at 1.5% S/L

Table 4. 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

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

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

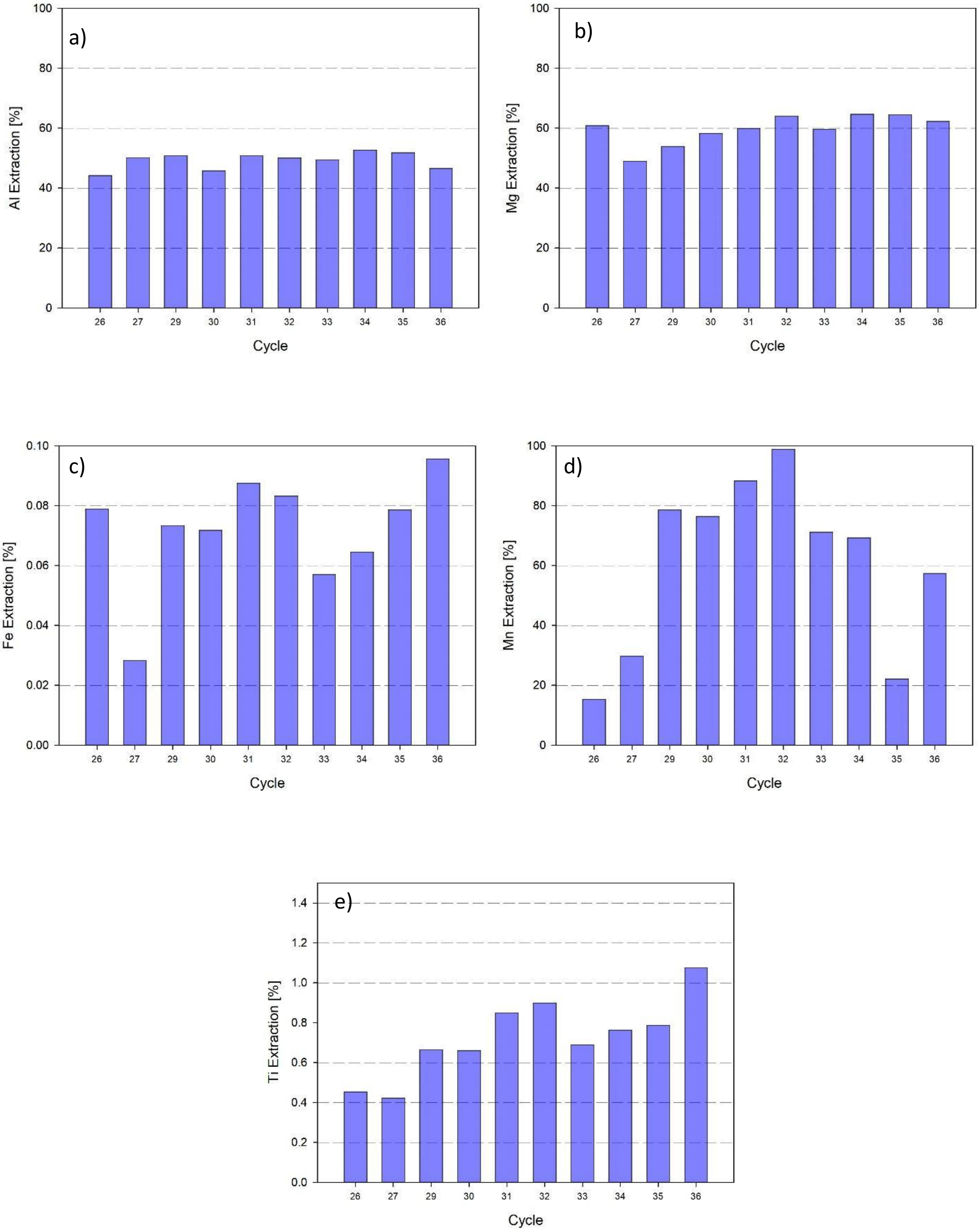

Figure 6 . Metals extraction percentages at 1.5% S/L: Al (a), Mg (b), Fe (c), Mn (d), Ti (e)

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

*Table 5. Estimated daily extraction capacity [mg/(day*l)] at 2% and 1.5% S/L*

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

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

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

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

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

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

Mineralogical analysis results. Figure S2 shows the XRD analysis for untreated and treated RM (cycle 18). Untreated RM composition was based on hematite (Fe_2O_3), calcite ($CaCO_3$), sodalite ($KNa_3Al_3Cl(SiO_4)_3$), boehmite (AlO(OH), gibbsite ($Al(OH)_{3,}$ rutile (TiO_2). After bioleaching, there was no evidence of sodalite, gibbsite, boehmite and calcite, indicating the transfer of metals associated with these components into the liquid phase and/or a reprecipitation of amorphous phases. One component clearly present in the residue after the treatment was hematite, in agreement with the low concentration of Fe in the liquid phase Weddellite ($Ca(C_2O_4)$-$2(H_2O)$) was formed, probably as the result of Ca_{2+} precipitation due to the due reaction with oxalic acid (Qu *et al.*, 2019, Cozzolino et al., 2023).

3.3 Selective enumeration of microorganisms

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

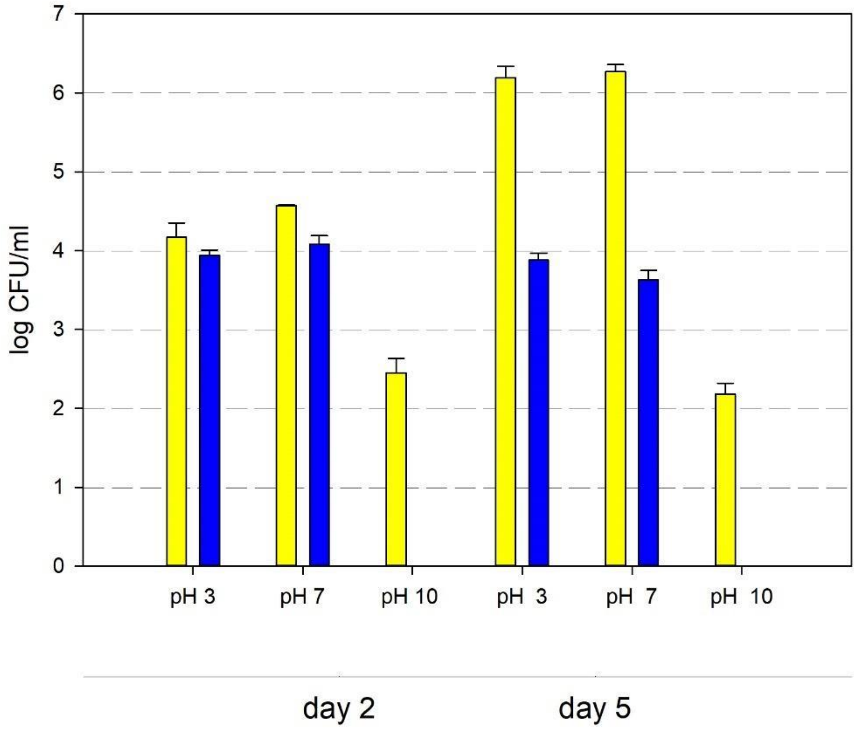

Figure 7. Viable titres (log-transformed) of alkaliphilic (pH 10), neutrophilic (pH 7), and acidophilic (pH 3) microorganisms. Yellow: bacteria. Blue: moulds.

4 Discussion

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

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

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

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

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

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

5 Conclusions

A sequencing batch reactor was started-up to investigate the possibility of achieving bioleaching of RM in a SBR in the long-term operation, without any preliminary biomass selection/enrichment. An operating strategy based on pH evolution over time was adopted to set optimal cycle duration. All the elements of interest were released, and the highest extraction efficiency was achieved for Al, Mn and Mg. Some of them were better released with a lower S/L. Promising results were obtained for minor elements with a higher economic interest (REEs), as well. The metals extraction was higher if compared to batch tests conducted at the same operational conditions, demonstrating the biological activity enhancement and the possibility of not using an external inoculum. After the bioleaching treatment, the reactor produces a slurry containing a liquid phase with dissolved metal ions and a residual RM. In a hypothetical scale-up of the treatment, it would be necessary to separate the liquid phase to recover all the leached metals. To ensure proper disposal or potential reuse, a physico-chemical characterization is required to assess the suitability of the treated RM. Further experimentation will be focused on increasing the S/L ratio in order to treat more waste and recover a higher metals amount at the same time. Moreover, the possibility of replacing the synthetic organic leaching medium with organic waste as the carbon and energy source worths of being investigated, in the perspective of further reducing the operating costs.

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

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References

Abhilash, Hedrich, S., Schippers, A., 2021. Distribution of Scandium in Red Mud and Extraction Using *Gluconobacter oxydans*. Hydrometallurgy 202, 105621. doi: 10.1016/j.hydromet.2021.105621.

Charalampides, G., Vatalis, K.I., Apostoplos, B., Ploutarch-Nikolas, B., 2015. Rare Earth Elements: Industrial Applications and Economic Dependency of Europe. Procedia Econ.Financ. 24(15), 126–135. doi: 10.1016/s2212-5671(15)00630-9.

Cozzolino, A., Cappai, G., Cara, S., Milia, S., Ardu, R., Tamburini, E., Carucci, A., 2023. Bioleaching of Valuable Elements from Red Mud: A Study on the Potential of Non-Enriched Biomass. Minerals, 13(7), 856. doi:10.3390/min13070856.

EIT RawMaterials Call for KAVA Projects SeedBook User Guide; European Union: Brussels, Belgium, 2020; pp. 1–2.

European Commission. 2023. *Study on the Critical Raw Materials for the EU*.

Ghorbani, Y., Oliazadeh, M., Shahverdian, A., 2008. Aluminum Solubilization from Red Mud by Some Indigenous Fungi in Iran. J. App. Biosci. 7, 207–213.

Giese, E.C., 2020. Biosorption as Green Technology for the Recovery and Separation of Rare Earth Elements. World J. Microbiol. Biotechnol. 36(4), 1–11. doi: 10.1007/s11274-020-02821-6.

Hind, A.R., Bhargava, S.K., Grocott, S.C., 1999. The Surface Chemistry of Bayer Process Solids: A Review. Colloids Surf. A: Physicochem. Eng. 146(1–3), 359–374. doi: 10.1016/S0927-7757(98)00798-5.

Horikoshi, K., 1999. Alkaliphiles: Some Applications of Their Products for Biotechnology. *Microbiol. Mol. Biol.* 63(4), 735–750. doi:10.1128/mmbr.63.4.735-750.1999.+

Ilyas, S., Kim, H., Srivastava, R.R., 2021. Feasibility of the Bio-Mobilization of Rare Earth Elements from Bauxite Residual Red Mud. Environ. Sci. Proc. 6(1), 5. doi:10.3390/iecms2021-09334.

Nalini Jain, Sharma, D.K., 2004. Biohydrometallurgy for Nonsulfidic Minerals - A Review.

Geomicrobiol. J. 21(3), 135–44. doi: 10.1080/01490450490275271.

Pedram, H., Hosseini, M.R., Bahrami, A., 2020. Utilization of *A. niger* Strains Isolated from Pistachio Husk and Grape Skin in the Bioleaching of Valuable Elements from Red Mud. Hydrometallurgy 198, 105495. doi: 10.1016/j.hydromet.2020.105495.

Power, G., Gräfe, M., Klauber, C., 2011. Bauxite Residue Issues: I. Current Management, Disposal and Storage Practices. Hydrometallurgy 108(1–2), 33–45. doi: 10.1016/j.hydromet.2011.02.006.

Qu, Y., Lian, B., 2013a. “Bioleaching of Rare Earth and Radioactive Elements from Red Mud Using *Penicillium tricolor* RM-10.” Bioresour. Technol. 136, 16–23. doi: 10.1016/j.biortech.2013.03.070.

Qu, Y., Lian, B., Mo, B., Liu, C., 2013b. Bioleaching of Heavy Metals from Red Mud Using *Aspergillus niger*. Hydrometallurgy 136, 71–77. doi: 10.1016/j.hydromet.2013.03.006.

Qu, Y., Li, H., Tian, W., Wang, X., Wang, X., Jia, X., Shi, B., Song, G., and Tang, Y., 2015. Leaching of Valuable Metals from Red Mud via Batch and Continuous Processes by Using Fungi. Miner. Eng. 81, 1–4. doi: 10.1016/j.mineng.2015.07.022.

Qu, Y., Li, H., Wang, X., Tian, W., Shi, B., Yao, M., and Zhang, Y., 2019. Bioleaching of Major, Rare Earth, and Radioactive Elements from Red Mud by Using Indigenous Chemoheterotrophic Bacterium *Acetobacter sp*. Minerals 9(2), 67. doi: 10.3390/min9020067.

Rasoulnia, P., Barthen, R., Lakaniemi, A.M., 2021. A Critical Review of Bioleaching of Rare Earth Elements: The Mechanisms and Effect of Process Parameters. Crit. Rev. Environ. Sci. Technol. 51(4), 378–427. doi: 10.1080/10643389.2020.1727718.

Sethurajan, M., van Hullebusch, E.D., Nancharaiah, Y.V., 2018. Biotechnology in the Management and Resource Recovery from Metal Bearing Solid Wastes: Recent Advances. J. Environ. Manage. 211, 138–53. doi: 10.1016/j.jenvman.2018.01.035.

Taylor, K.C., Nasr-El-Din, H.A., Al-Alawi, M.J., 1998. A systematic study of iron control chemicals used during well stimulation. *SPE Formation Damage Control Conference*, Lafayette, Louisiana, SPE-39419-MS, doi:10.2118/39419-MS.

Vakilchap, F., Mousavi, S.M., Shojaosadati, S.A., 2016. Role of *Aspergillus niger* in Recovery Enhancement of Valuable Metals from Produced Red Mud in Bayer Process. Bioresour. Technol. 218, 991–98. doi: 10.1016/j.biortech.2016.07.059.

Vidal, O., Weihed, P., Hagelüken, C., Bol, D., Christmann, P., Arndt, N., 2013. ERA-MIN Research Agenda. 2013, p. 125. Available online: https://hal.univ-grenoble-alpes.fr/insu-00917653/

Zhang, D.-r., Chen, H.-r., Nie, Z.-y., Xia, J.-l., Li, E.-p., Fan, X.-l., 2020. Extraction of Al and Rare Earths (Ce, Gd, Sc, Y) from Red Mud by Aerobic and Anaerobic Bi-Stage Bioleaching. J. Chem. Eng. 401, 125914. doi: 10.1016/j.cej.2020.125914.