

Article

Bioleaching of Valuable Elements from Red Mud: A Study on the Potential of Non-Enriched Biomass

Anna Cozzolino ¹, Giovanna Cappai ^{1,2,*} , Stefano Cara ² , Stefano Milia ^{2,*} , Riccardo Ardu ^{1,3}, Elena Tamburini ³ 
and Alessandra Carucci ^{1,2}

¹ Department of Civil-Environmental Engineering and Architecture (DICAAR), University of Cagliari, Via Marengo 2, 09123 Cagliari, Italy; anna.cozzolino@unica.it (A.C.); riccardo.ardu@unica.it (R.A.); carucci@unica.it (A.C.)

² National Research Council of Italy—Institute of Environmental Geology and Geoengineering (CNR-IGAG), Via Marengo 2, 09123 Cagliari, Italy; stefano.cara@cnr.it

³ Department of Biomedical Sciences, University of Cagliari, 09042 Monserrato, Italy; etamburini@unica.it

* Correspondence: gcappai@unica.it (G.C.); stefano.milia@cnr.it (S.M.)

Abstract: Red mud (RM) is the main residue produced by the alkaline extraction of alumina (Al_2O_3) from bauxite, and it contains valuable metals such as iron (Fe), aluminium (Al), titanium (Ti), magnesium (Mg), manganese (Mn), rare earth elements, etc. This research aimed to investigate the biologically induced leaching of some valuable elements from raw RM without preliminary biomass enrichment and inoculum, simultaneously reducing RM polluting potential and extracting metals for their subsequent recovery within a circular economy-based approach. In addition to the missing inoculum, such an approach is challenging since high RM alkalinity and pH, as well as the absence of any sulphides, constrain the use of the most common biohydrometallurgical techniques. Red Muds from two European locations were tested (RM-I and RM-II, respectively). Bioleaching tests were performed at different temperatures ($T = 22^\circ\text{C}$ and 28°C ; and also 15°C for RM-II) and solid-to-liquid ratios ($S/L = 2\%$, 5%). A sudden drop in pH from alkaline to constant neutral/acidic values was observed in almost all tests, and such results were attributed to biological activity since abiotic tests did not show any pH decrease. The best results in terms of extraction were achieved with RM-II, in particular for Al, Mg and Mn (17%, 42% and 47%, respectively). At 2% S/L, the highest temperature allowed for a better metal release, while at 5% S/L, the highest extraction of Al, Mg and Ti was observed at 22°C . As expected, iron was less available to leach at the achieved pH values, as it was mostly present as hematite in both RMs. Finally, the first microbiological characterisation of the autochthonous biomass selected during the bioleaching treatment of RM was provided.

Keywords: 16S rRNA gene; biohydrometallurgy; circular economy; metals; red mud; secondary raw materials



Citation: Cozzolino, A.; Cappai, G.; Cara, S.; Milia, S.; Ardu, R.; Tamburini, E.; Carucci, A. Bioleaching of Valuable Elements from Red Mud: A Study on the Potential of Non-Enriched Biomass. *Minerals* **2023**, *13*, 856. <https://doi.org/10.3390/min13070856>

Academic Editor: María Ángeles Martín-Lara

Received: 3 May 2023

Revised: 13 June 2023

Accepted: 20 June 2023

Published: 24 June 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

During the last decades, the demand and consumption of raw materials have grown dramatically. Metallurgical industries produce several types of waste which still contain large amounts of metals. Such kind of waste can be considered as a new source for the extraction of secondary and critical raw materials, in agreement with United Nations' and European Union's policies promoting the shift from a linear to a circular economy, the sustainable development and the transition toward a “zero waste” society [1,2].

Aluminium is one of the most important light metals, commonly present in nature as oxide (i.e., alumina, Al_2O_3) in a mineral known as bauxite [3]. The alkaline extraction of alumina from bauxite is known as the Bayer process, which is based on caustic soda (NaOH) dosage to chemically dissolve the bauxite compounds and extract the aluminium oxide [4]. The residue produced from this process is called red mud (RM). Nowadays, about 95% of alumina is extracted from bauxite ores, and 90% of it is extracted using

the Bayer process [5]. The RM contains the substances originally present in the bauxite (e.g., Al, Fe, Ti, rare earth elements, etc.) [6], the minerals formed and the elements added during the Bayer process. Due to its composition and the presence of valuable elements, including critical raw materials, RM is defined as a “polymetallic raw material” or an “artificial ore” [3,7]. The production of one ton of alumina results in almost two metric tons of RM produced [8]. Worldwide, in 2019, the volume of RM stockpiled in storage areas was estimated at over 3.5 billion tons [3], and this number seems to increase at a rate of 160 million tons every year [9]. RM is largely studied because of its potentially dangerous impact on the environment [5]. The failure of dams has already caused the release of metals into the environment. In 2010, a red mud lake released one million cubic meters of RM slurry in Ajka (Hungary), causing the death of several people [10].

A way to recover valuable substances in RM is to leach metals into the solution. Chemical extraction using different acids, such as sulfuric, nitric and hydrochloric acids, has been largely used and studied [3]. An alternative treatment could be biohydrometallurgy, which exploits the metabolism of microorganisms to achieve the leaching of metals from solid wastes, ores or minerals [11,12].

The biohydrometallurgical process can be defined as relatively inexpensive and environmentally friendly since it requires a low energy supply and very low or no chemical dosage [8,11,13]. Bioleaching is applied to low grades ores and rocks, but also to mining and industrial wastes containing metals and to sewage sludge [11]. The most studied topic in the bioleaching field is sulphide bioleaching mediated by autotrophic chemolithotrophic bacteria, such as *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* [13]. However, using these bacteria may not be the ideal solution for metals recovery from RM due to the absence of sulphur in bauxite residues and their high alkalinity.

Microbial extraction of metals from non-sulphidic ores has been less studied. Non-sulphidic ores can be leached using metabolites produced by heterotrophic bacteria and fungi sustained by a suitable organic substrate [11]. Fungi were widely studied [5,6,8] to extract metals from RM (such as *Aspergillus niger* and *Penicillium*), whilst less attention has been paid to bacteria so far [3] due to the alkaline environment provided by RM: in previous studies, selected biomass such as *Acetobacter* sp., *Aspergillus niger*, *Penicillium*, etc., was enriched and inoculated into sterilised RM to investigate the bioleaching of metals [3,5–8].

Based on the hypothesis that biomass can adapt and survive in such an alkaline environment, this research was aimed at evaluating the biological activity and bioleaching potential of autochthonous biomass present in untreated RM without the preliminary selection and addition of an external microbial inoculum, with the perspective of a simpler and more cost-effective process application in engineered systems. Two different RMs, coming from two sites in Europe (RM-I and RM-II, respectively), were tested under different temperatures and solid-to-liquid ratios (S/L). For RM-I, the fate of Fe, Al and Ti was specifically investigated due to their well-recognised economic importance. For RM-II, the fate of Mg and Mn was also studied. Results were promising and paved the ground for applying bioleaching in engineered systems without preliminary biomass selection and enrichment.

2. Materials and Methods

2.1. Red Mud Characterisation

RM-I and RM-II were characterised in terms of pH, electric conductivity (EC), acid neutralisation capacity (ANC), metals content and mineral composition. RM samples were previously mixed and ground to 2 mm. Both samples were collected from plants' storage sites, where they had been exposed to the atmosphere. More specifically, the last displacement of RM-II in the storage site is less recent than RM-I, and there's approximately a 35-year difference in RM exposure to weathering agents.

2.2. Bioleaching Experiments

Batch experiments were carried out in 500 mL glass bottles. To preserve the autochthonous microbial biomass possibly colonising RMs, the material was not dehydrated nor sterilised before the tests. A synthetic leaching medium (LM, 400 mL) consisting of 30 g/L of ethanol 96%, 10 g/L of glucose, and 10 g/L of yeast extract, according to Qu et al. [3], was added to each bottle. The concentrations of calcium (Ca^{2+}), sodium (Na^+) and potassium (K^+) in the LM were 20 mg/L, 32 mg/L and 632 mg/L, respectively, due to the presence of the yeast extract. The LM was sterilised with an autoclave (ASAL 760) at 121 °C for 15 min. Different S/L ratios were used: 2% and 5%, referred to the RM dry mass. Each S/L was tested at 28 °C (i.e., the usual temperature for the incubation of mesophilic microorganisms) and 22 °C (i.e., room temperature). RM-II was also tested at 15 °C (i.e., a relatively low temperature corresponding to approximately 50% of the optimal one). The temperature was controlled at 28 °C using a Digital Thermostatic Unit (TRM 730), and at 15 °C using a chiller (TECO, TK500). Tests were carried out in duplicate. Each test lasted about 30 days, during which the slurry was stirred at 120 rpm. Oxygen diffusion from the bottles' headspace was provided by mechanical stirring. The oxygen concentration was periodically checked using a portable meter, and 3 h aeration (once a week) was sufficient to keep oxygen concentration above 1 ppm. Table 1 shows a summary of all the tests conducted during this study. The abiotic control tests were performed under the same S/L ratio as the batch experiments, with the addition of HgCl_2 (0.3 g/L). The experimental setup is shown in Figure S1 (Supplementary Materials).

Table 1. Tests performed during the experimentation.

| Test Code | Red Mud | Temperature [°C] | S/L [%] | Duration [Days] | Metals Extracted |
|----------------|---------|------------------|---------|-----------------|--------------------|
| RM-I_2%_22 °C | RM-I | 22 | 2 | 31 | Al, Ti, Fe |
| RM-I_2%_28 °C | RM-I | 28 | 2 | 31 | Al, Ti, Fe |
| RM-I_5%_22 °C | RM-I | 22 | 5 | 31 | Al, Ti, Fe |
| RM-I_5%_28 °C | RM-I | 28 | 5 | 31 | Al, Ti, Fe |
| RM-II_2%_15 °C | RM-II | 15 | 2 | 36 | Al, Ti, Fe, Mn, Mg |
| RM-II_2%_22 °C | RM-II | 22 | 2 | 36 | Al, Ti, Fe, Mn, Mg |
| RM-II_2%_28 °C | RM-II | 28 | 2 | 36 | Al, Ti, Fe, Mn, Mg |
| RM-II_5%_15 °C | RM-II | 15 | 5 | 37 | Al, Ti, Fe, Mn, Mg |
| RM-II_5%_22 °C | RM-II | 22 | 5 | 37 | Al, Ti, Fe, Mn, Mg |
| RM-II_5%_28 °C | RM-II | 28 | 5 | 37 | Al, Ti, Fe, Mn, Mg |

2.3. Analytical Methods

The pH and EC of untreated RMs were determined after mixing and stirring the RM suspension in distilled water ($v/w = 5 \text{ L/kg}$) for 16 h [10], using a benchtop meter (HI5522, Hanna Instruments, Woonsocket, RI, USA). RM chemical composition was analysed by ICP-OES (Optima 7000, PerkinElmer, Waltham, MA, USA) after total digestion according to EPA method 3052. 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 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 water content of the two RMs was determined after having dried 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 ($\text{ANC}_{4.5}$). The ANC data were integrated with the total limestone determination. Hydrochloric acid was used to treat the samples, and the CO_2 produced was proportional to the limestone contained in the samples. The volumetric gas determination was carried out with a Dietrich-Frühling calcimeter.

During bioleaching tests, 15 mL samples were taken twice per week from the bottles and centrifuged at 5000 rpm for 15 min 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) for the quantification of Al (detection limit 0.03 mg/L), Fe (d.l. 0.005 mg/L), Ti (d.l. 0.01 mg/L), Mn (d.l. 0.002 mg/L) and Mg (0.0005 mg/L). The remaining part of the samples was filtered at 0.45 µm, diluted with grade-I water and used to evaluate organic acid concentrations through HPLC (LC 4000, Jasco, Oklahoma City, OK, USA).

Scanning Electron Microscope (SEM) images of RM after biological treatment were captured using an FEI Quanta 200 SEM microscope. The analyses were performed in low vacuum mode (i.e., residual pressure in the experimental chamber in the range of 0.3–0.9 Torr) to minimise electrostatic charge effects, or high vacuum mode (pressure below 10^{-4} Torr). Images were collected in either secondary electrons or backscattered electrons.

2.4. Calculations

Metals extraction efficiency was calculated according to Equation (1) [14]:

$$\text{Extraction [\%]} = \frac{\text{Metal in leachate [mg]}}{\text{Metal in untreated RM [mg]}} \cdot 100 \quad (1)$$

The kinetics of the bioleaching process were calculated as the slope of the steepest part of pH profile over time and R^2 describes the linear correlation of this slope.

2.5. Microbial Characterisation

Samples of slurry from RM-II_2%_22 °C were periodically collected during the batch tests and used for the enumeration of cultivable aerobic microorganisms and characterisation of the bacterial communities.

The viable titres of heterotrophic neutrophilic and alkaliphilic microorganisms were determined as colony forming units (CFU) by plating serial 10-fold dilutions onto NHM medium (yeast extract 1 g/L, K_2HPO_4 0.2 g/L, $MgSO_4 \cdot 7H_2O$ 0.2 g/L, glucose 10 g/L, agar 17 g/L, pH 7.0) and Alkaliphilic Horikoshi Medium (yeast extract 1 g/L, K_2HPO_4 0.2 g/L, $MgSO_4 \cdot 7H_2O$ 0.2 g/L, Na_2CO_3 10 g/L, glucose 10 g/L, agar 17 g/L, pH 10.0), respectively [15]. Cultures were incubated at 22 °C for 5 days.

The diversity and composition of the bacterial community during the treatment were determined by Next Generation Sequencing (NGS) of the 16S rRNA gene. At each kinetic time, an aliquot of slurry (1 mL) was centrifuged at 11,000 rpm, washed with SET buffer (Sucrose 20%, EDTA 50 mM, Tris-HCl pH 8.0 50 mM), and stored at -20 °C until further processing. Total DNA was extracted by the DNeasy PowerSoil Pro Kit (QIAGEN) following manufacturer's instructions. DNA was subsequently purified using the DNeasy PowerClean Cleanup Kit (QIAGEN). The DNA quality and concentration were determined on agarose gel using a DNA quantitation standard. DNA samples were submitted to Bio-Fab Research Srl (Rome, Italy) for sequencing of the V3–V4 region of the bacterial 16S rRNA gene on an Illumina Miseq platform (Illumina, San Diego, CA, USA) using 2×300 bp paired-end reads. The primer pair was used S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 [16]. For data processing, raw sequences were demultiplexed by the sequencing facility. Reads were trimmed to remove primer sequences using the CutAdapt version 3.5. Sequences were imported into Quantitative Insights into Microbial Ecology (QIIME 2) version 2020–11 [17]. Using the DADA2 pipeline [18], reads with ambiguous and poor-quality bases were discarded, good-quality reads dereplicated and denoised, and the paired reads merged. Chimeras and singletons were identified and removed from the dataset. DADA2 was used to produce amplicon sequence variants (ASVs), thus obtaining a filtered ASV-abundance table. For each ASV, taxonomy assignment to the lowest taxonomic level (i.e., from genus to phylum) was performed against the Silva database release 132 [19].

The indices of diversity (richness as number of observed ASV, Shannon index with an e log base) and evenness (Pielou's index) were calculated by using the vegan R package [20].

3. Results

3.1. Red Mud Characterisation

The pH, EC and ANC_{4.5} values assessed for RM-I and RM-II were 10.5 and 11.6, 2.08 and 2.46 mS/cm and 3.4 and 5.2 mmolH⁺/g, respectively. The pH was in the alkaline range for both RMs, as expected due to the chemicals involved in the Bayer process, and within the range of 9.7–12.9 reported in the literature for this kind of residues [21]. The ANC_{4.5} values of both RMs were between the reported range (1.4–28.4 mS/cm) [21]. The ANC curves determined with RM-I and RM-II were very similar, except for the behaviour around pH 6.5, where a higher buffering capacity was shown by RM-II (Figure 1). Since this is the typical buffering pH range of carbonates, a higher content of these compounds in RM-II was likely the cause of such behaviour. The total limestone analysis resulted in 48 g CaCO₃/kg for RM-I versus 75 g CaCO₃/kg for RM-II, thus confirming the hypothesis. Such difference between the samples can be attributed to the different exposure of RMs to weathering agents with RM-I being more recent than RM-II.

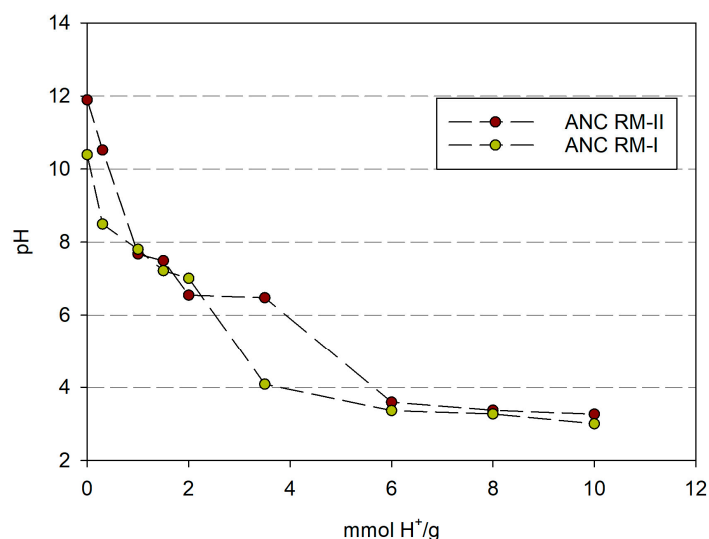


Figure 1. ANC trends observed in RM-I and RM-II samples.

The water content of RM-I and -II was about 30% and 15%, respectively. Table 2 shows the concentrations of the most relevant chemical elements in the two RMs, expressed as a weight percentage relative to dry mass. The data represent the average values \pm the standard deviation (three replicates were performed). In addition to the elements originally present in bauxite, RM composition was influenced by the reagents used to extract alumina, such as NaOH.

Table 2. Main elements contained in red muds (weight percentage).

| | RM-I | RM-II |
|------------------------------------|------------------|------------------|
| Fe ₂ O ₃ [%] | 27.7 \pm 1.16 | 43.55 \pm 7.26 |
| Al ₂ O ₃ [%] | 6.04 \pm 0.38 | 20.7 \pm 3.78 |
| SiO ₂ [%] | 5.99 \pm 2.18 | 9.7 \pm 2.35 |
| MgO [%] | 0.05 \pm 0.02 | 0.31 \pm 0.02 |
| MnO [%] | 0.02 \pm 0.02 | 0.103 \pm 0.03 |
| TiO ₂ [%] | 5.76 \pm 0.11 | 6.98 \pm 1.70 |
| CaO [%] | 2.91 \pm 0.06 | 4.08 \pm 0.1 |
| Na ₂ O [%] | 6.29 \pm 0.013 | 6.68 \pm 0.02 |
| K ₂ O [%] | 0.05 \pm 0.01 | 0.1 \pm 0.01 |

3.2. Bioleaching Experimentations

3.2.1. RM-I Bioleaching Results

Process kinetics and extraction efficiencies. Two different temperatures (i.e., 22 °C and 28 °C) and S/L (i.e., 2% and 5%) were tested for RM-I. In all the experiments, pH decreased very quickly (days 0–7), then fluctuated until the end of the tests (Figure 2a,b). The lowest pH was achieved with the highest temperature (28 °C) and the highest RM content (5% S/L). The decrease in pH can be reasonably attributed to microbial activity since the abiotic controls did not show significant variations in pH, which remained high during the tests.

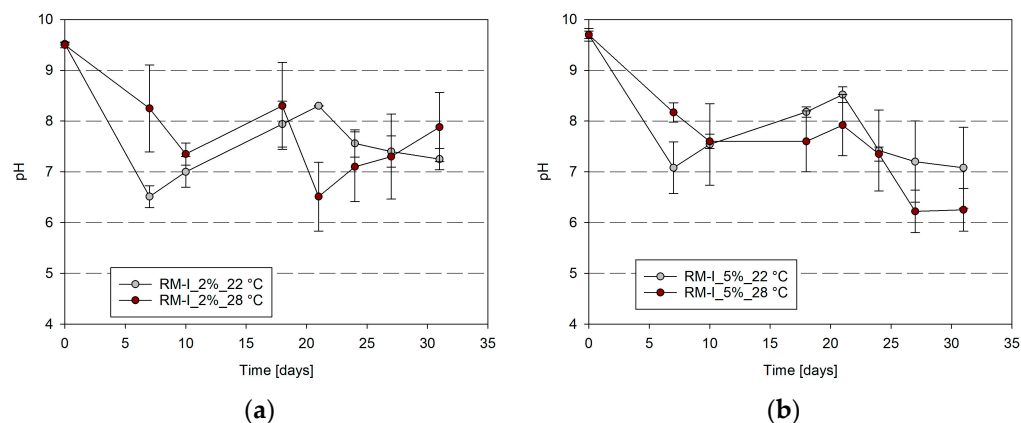


Figure 2. pH trends (mean values \pm standard deviations) of RM-I tests at different temperatures and S/L ratios: (a) tests at 2% S/L, (b) tests at 5% S/L.

Table 3 shows the initial and the lowest pH values reached and the process kinetics for each batch test.

Table 3. Initial and lowest pH values measured in batch tests, process kinetics with the corresponding R^2 for RM-I (mean values \pm standard deviations).

| TEST CODE | Initial pH | Lowest pH | Kinetics [pH Units/Day] | R^2 |
|---------------|------------------|-----------------|-------------------------|-------|
| RM-I_2%_22 °C | 9.52 \pm 0.02 | 7.25 \pm 0.21 | 0.28 | 0.81 |
| RM-I_2%_28 °C | 9.50 \pm 0.057 | 6.51 \pm 0.68 | 0.21 | 0.97 |
| AB-I_2% | 9.2 | 8.7 | - | - |
| RM-I_5%_22 °C | 9.70 \pm 0.073 | 7.08 \pm 0.8 | 0.24 | 0.8 |
| RM-I_5%_28 °C | 9.70 \pm 0.12 | 6.25 \pm 0.42 | 0.21 | 0.9 |
| AB-I_5% | 9.1 | 8.8 | - | - |

For both the S/L tested, the lowest pH was achieved at the highest temperature (28 °C), thus confirming the temperatures indicated by Qu et al. [3] as optimal for mesophilic growth (28–30 °C).

The extraction of aluminium, iron and titanium obtained during tests on RM-I is reported in Figure 3. As expected, the highest extraction of target metals was observed in test RM-I_5%_28 °C, where the lowest pH was achieved.

Coherently, although the observed extraction rates were significantly lower than in previous studies with selected and enriched biomass [3,5] (see Paragraph 4), the results suggested that raw RM can sustain bioleaching despite the extremely alkaline environment. In this sense, abiotic tests used as control showed negligible pH decrease and metals extraction.

Mineralogical assessment of RM-I samples. The results of XRD analyses on RM-I before and after bioleaching (RM-I_5%_28 °C, as the best result) are shown in Figure 4. The main mineralogical components of RM-I before bioleaching were boehmite (AlO(OH)), hematite (Fe₂O₃), calcite (CaCO₃), sodalite (KNa₃Al₃Cl(SiO₄)₃), rutile (TiO₂), compatible with its

chemical composition for major elements (Table 2). After treatment, the main mineralogical components were the same, except for the formation of weddellite ($\text{Ca}(\text{C}_2\text{O}_4)\cdot 2(\text{H}_2\text{O})$).

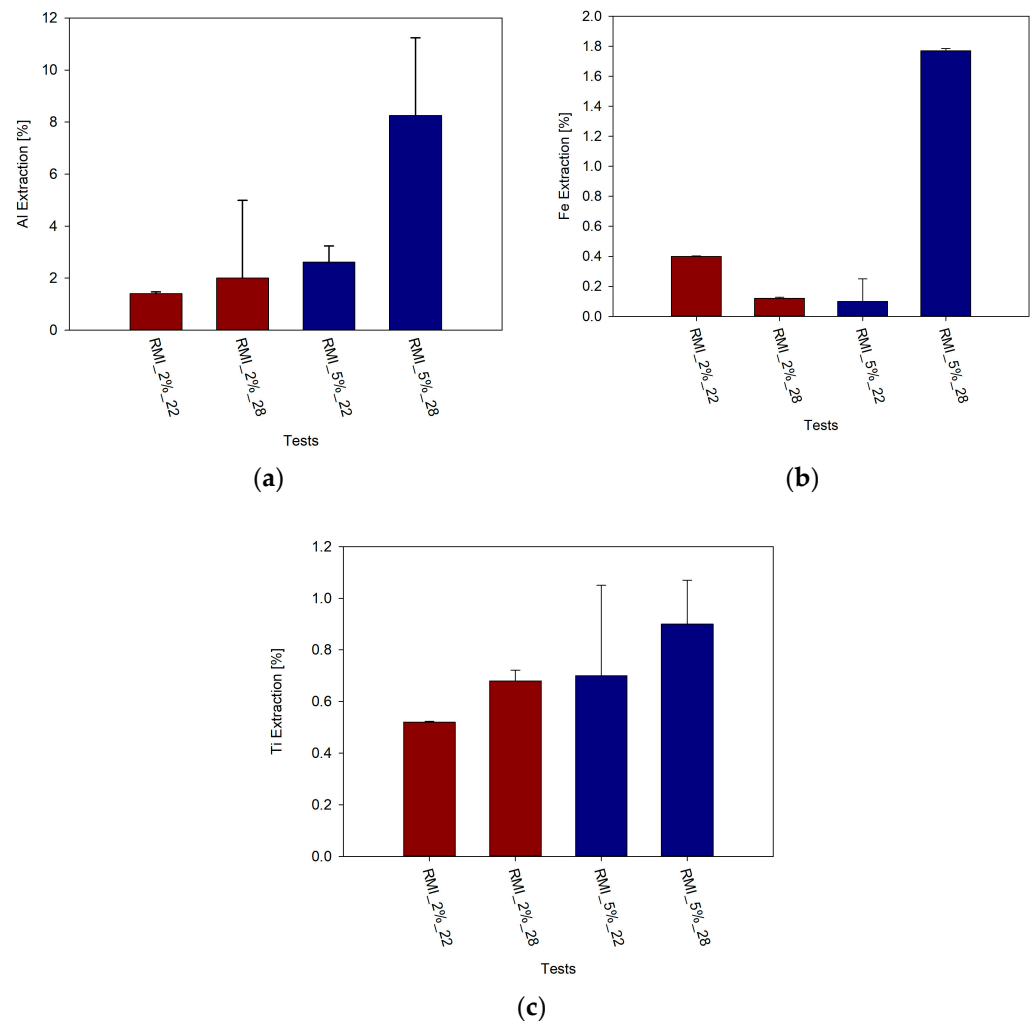


Figure 3. Aluminium (a), iron (b) and titanium (c) extraction efficiencies observed for RM-I at different S/L ratios and temperatures.

3.2.2. RM-II Bioleaching Results

Process kinetics and extraction efficiencies. Three different temperatures were tested at S/L 2% and 5% with RM-II: 15 °C (RM-II_2%_15 °C and RM-II_5%_15 °C), 22 °C (RM-II_2%_22 °C and RM-II_5%_22 °C) and 28 °C (RM-II_2%_28 °C and RM-II_5%_28 °C).

Figure 5 shows the pH trends of the tests, the errors bars indicate the standard deviation evaluated from the two duplicates. Figure 5a shows the pH trends of the three experiments at S/L 2%. In all the tests, pH values decreased very quickly and then remained stable until the end. This decrease was attributed to the biological activity since negligible pH variation was assessed in the abiotic controls, as observed also for the tests with RM-I. As shown in Figure 5a, test RM-II_2%_28 °C showed the fastest pH decrease and the lowest pH achieved (i.e., 5.4 within the first 7 days), compared with the other trials. In test RM-II_2%_22 °C the pH dropped to 6, while in test RM-II_2%_15 °C the lowest pH achieved was 6.5 (day 5) and then it remained stable around a neutral value.

Tests at 5% S/L ratio showed less differences among pH plateau values with decreasing temperatures compared to tests at 2% S/L (Figure 5b).

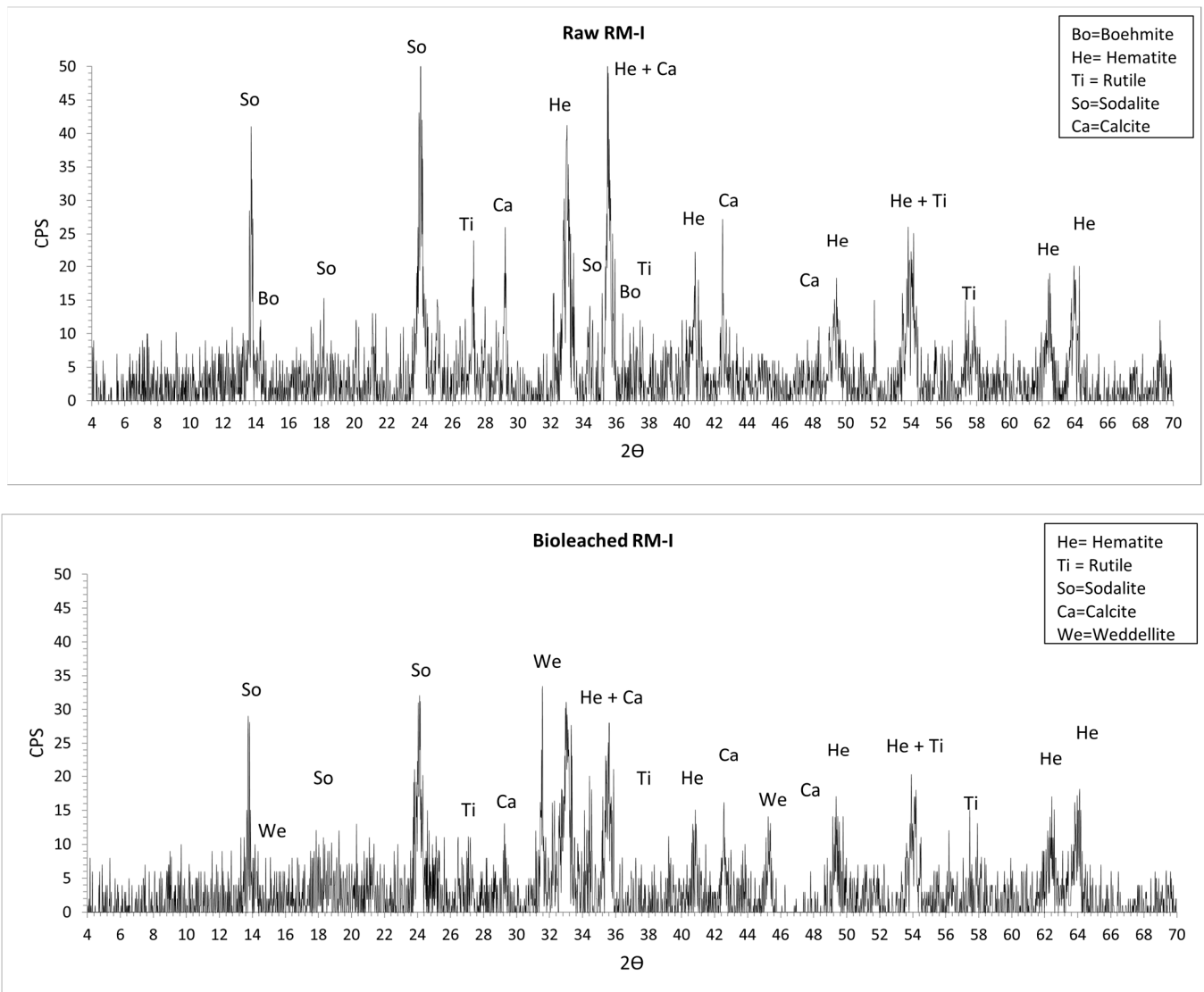


Figure 4. XRD analyses of RM-I before (top) and after (bottom) bioleaching.

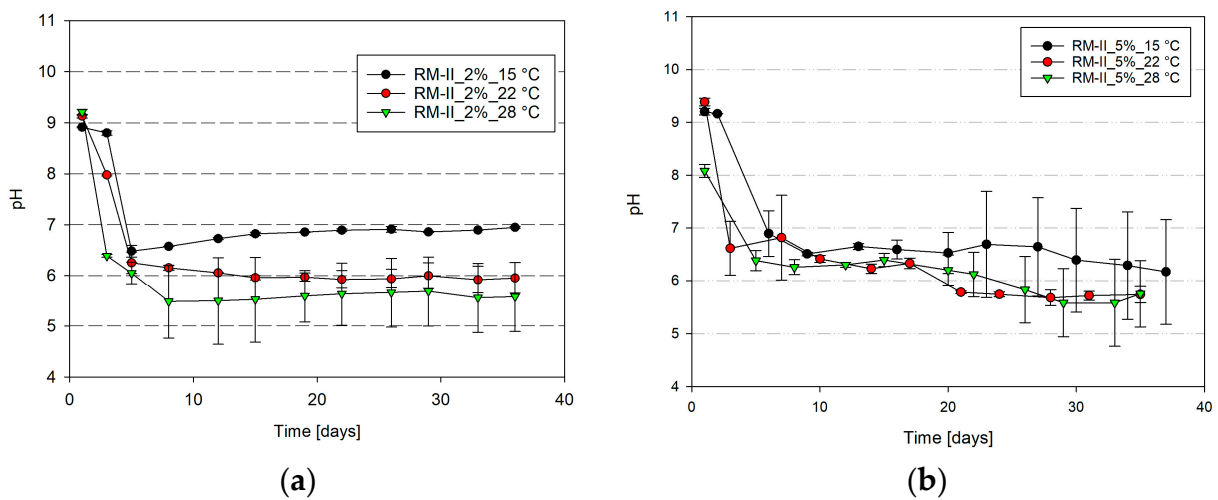


Figure 5. pH trends of RM-II tests at different temperatures: (a) 2% S/L; (b) 5% S/L.

In particular, the pH trends observed in RM-II_5%_22 °C and RM-II_5%_28 °C tests were very similar, while RM-II_5%_15 °C was similar to RM-I_2%_15 °C. The pH trends from RM-II batch tests have been fitted with a pseudo-first order (PFO) model. The model fitted well ($R^2 > 0.95$) for RM-II_2%_22 °C and RM-II_2%_28 °C, while it was not that accurate for the other data sets ($R^2 < 0.93$). Figure S2 shows the plots of the model-fitting. The Equations (S1) and (S2) are the model equation and the error, respectively. Table 4 shows the initial and the lowest pH values reached and the process kinetics for each batch test.

Table 4. Initial and lowest pH values measured in batch tests, process kinetics with the corresponding R^2 for RM-II (mean values \pm standard deviations).

| TEST CODE | Initial pH | Lowest pH | Kinetics [pH Units/Day] | R^2 |
|----------------|-----------------|-----------------|----------------------------|-------|
| RM-II_2%_15 °C | 8.9 \pm 0.009 | 6.5 \pm 0.12 | 0.48 | 0.8 |
| RM-II_2%_22 °C | 9.13 \pm 0.04 | 5.9 \pm 0.04 | 0.61 | 0.98 |
| RM-II_2%_28 °C | 9.2 \pm 0.06 | 5.4 \pm 0.86 | 0.46 | 0.82 |
| AB-II_2% | 9.1 | 8.7 | - | - |
| RM-II_5%_15 °C | 9.2 \pm 0.06 | 6.2 \pm 0.99 | 0.37 | 0.78 |
| RM-II_5%_22 °C | 9.4 \pm 0.07 | 5.6 \pm 0.147 | 0.34 | 0.7 |
| RM-II_5%_28 °C | 8.2 \pm 0.122 | 5.5 \pm 0.65 | 0.3 | 0.7 |
| AB-II_5% | 9.1 | 8.8 | - | - |

Target metals were magnesium, iron, aluminium, titanium and manganese. The maximum metal extraction efficiencies observed after two weeks of batch operation are shown in Figure 6. Aluminium showed the worst results at 15 °C for both the S/L ratios. At 2% S/L, the Al extraction increased with the temperature (maximum value of 17.3% obtained in RM-II_2%_28 °C), while at 5% S/L the highest value was obtained at room temperature (i.e., 10.5%). As for iron, although the best results were obtained for RM-II_2%_28 °C, the extraction efficiencies were always very low (<1%) due to the mineralogical phase in which iron was present in RM (i.e., hematite). Hence, the process would need a lower pH (i.e., below 3) to maximise Fe extraction. As for Titanium, a low extraction was achieved as well. A slight increase with temperature was observed at 2% S/L, even if the best results were obtained for RM-II_5%_22 °C (0.32%). The extraction of magnesium and manganese was higher than the other metals. At 2% S/L, the highest magnesium extraction was achieved at 28 °C (i.e., 30.8%); at 5% S/L, the highest Mg extraction was observed at 22 °C (i.e., 42.4%). At 5% S/L, Mn had a peculiar behaviour, since the best extraction efficiency was achieved at 15 °C (i.e., 47.4%). It should be also noticed that the concentrations of metals assessed in the liquid phase at the end of the tests were lower than those reported in Figure 6 and, consequently, the extraction of metals at the end of the batch trials was lower, showing a decrease of around 25%–30%.

Mineralogical assessment of RM-II samples. XRD analysis showed a mineralogical composition of the raw (untreated) RM-II sample mainly consisting of hematite (Fe_2O_3), calcite (CaCO_3), sodalite ($\text{KNa}_3\text{Al}_3\text{Cl}(\text{SiO}_4)_3$), boehmite ($\text{AlO}(\text{OH})$), gibbsite ($\text{Al}(\text{OH})_3$) and rutile (TiO_2) (Figure 7).

Figure 8 shows the XRD analysis results after treatment at both S/L, at 28 °C, as best results obtained. The XRD analysis after the bioleaching process showed lower peaks, indicating the possible dissolution of minerals. Figure S3 shows the XRD results after the treatment at both S/L ratios and 22 °C.

A variation in the structure of RM has been highlighted by SEM analyses before and after the treatment, with a more amorphous structure characterising the raw RM (Figure S4 in Supplementary Materials). The treated RM was characterised by smaller size particles, probably due to the acidic metabolite corrosion, as reported by Qu et al. [3]. The RM erosion and structure modification can be attributed not only to the compounds produced by the biomass but also to the microorganisms' physical action [3,7].

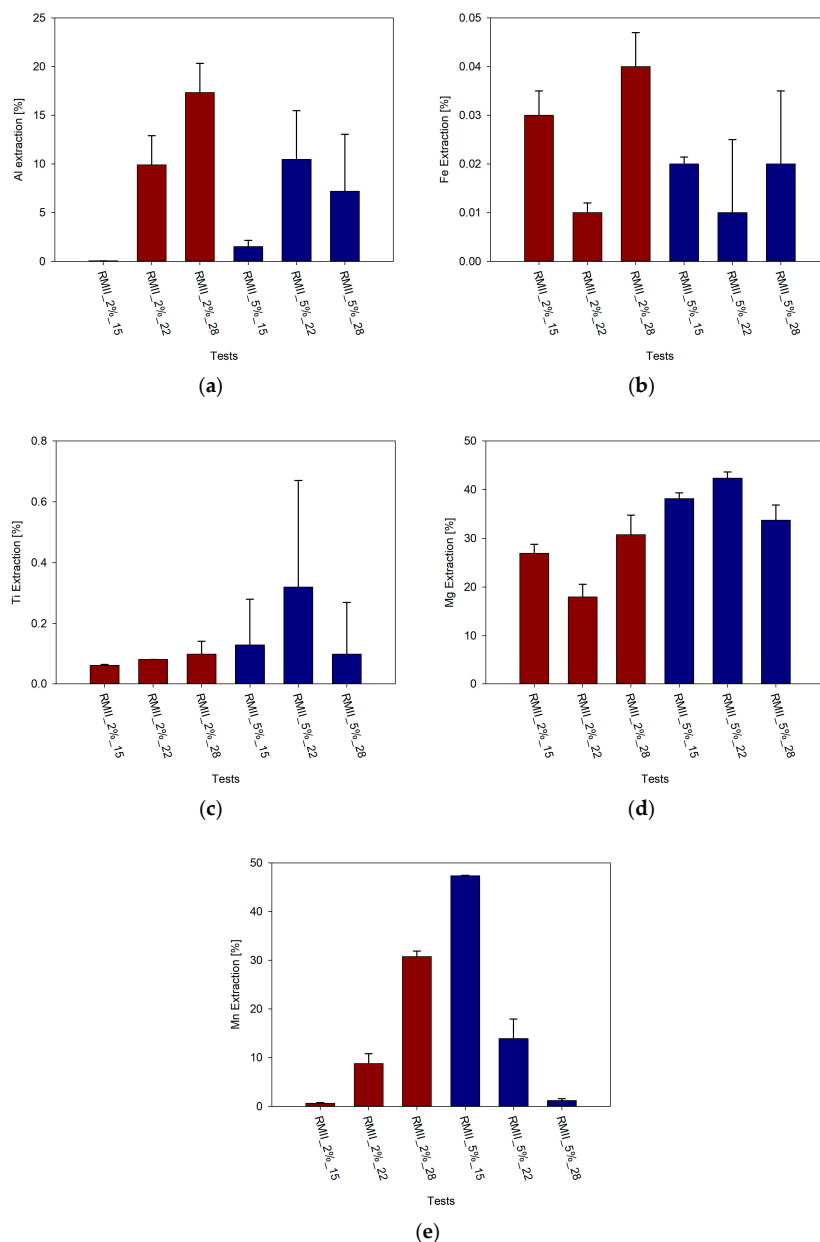


Figure 6. Aluminium (a), iron (b), titanium (c), magnesium (d) and manganese (e) extraction for RM-II at different S/L ratios and temperatures.

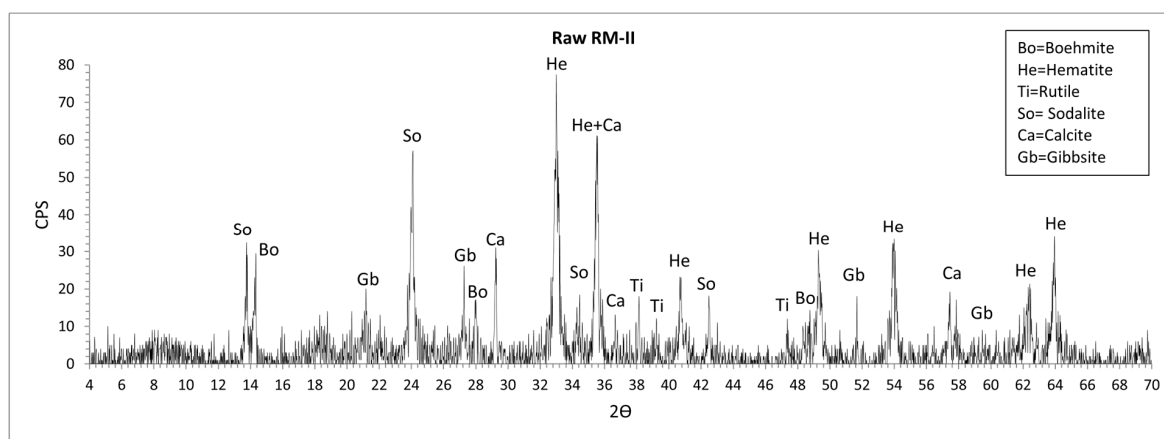


Figure 7. XRD pattern of untreated RM-II sample.

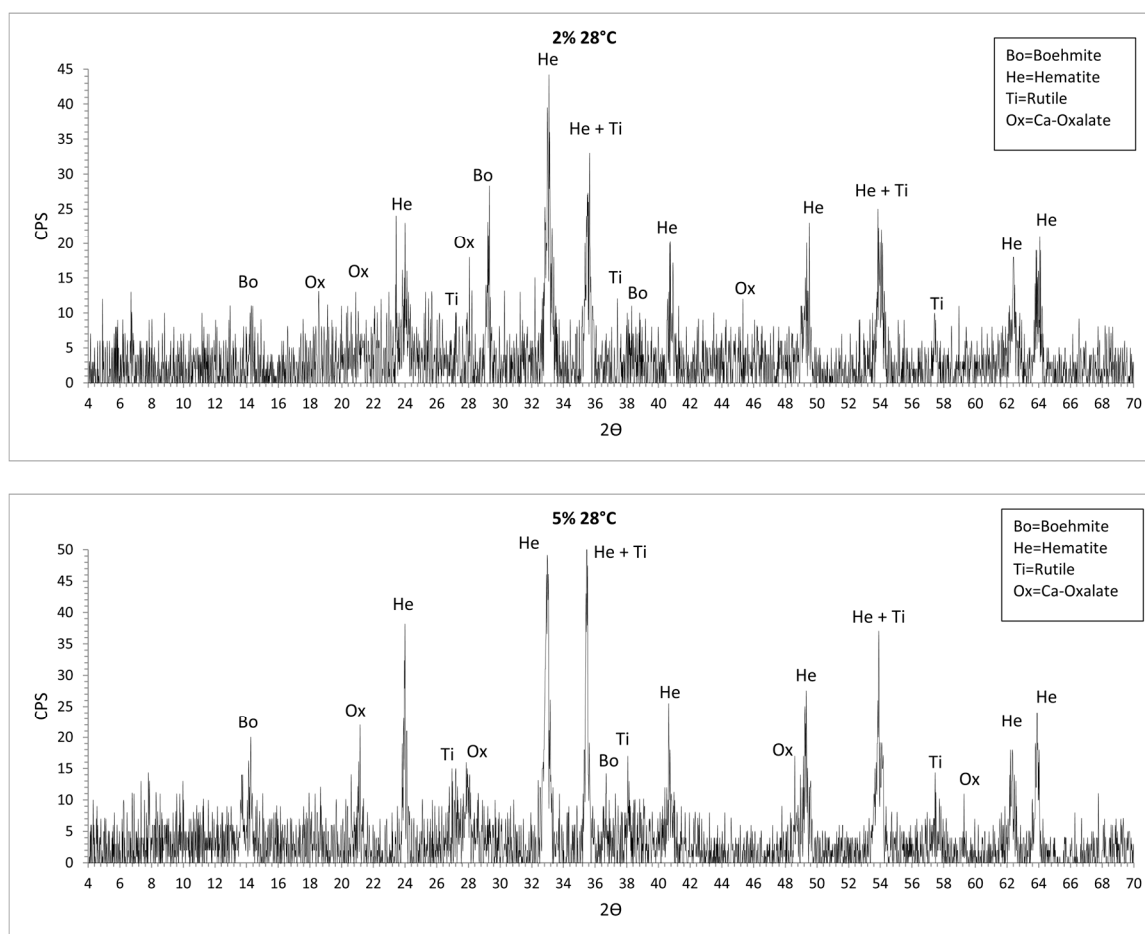


Figure 8. XRD patterns of treated RM-II samples at different S/L ratios and 28 °C.

Organic acids production. The leaching medium used in our study enhances the growth of heterotrophic biomass. When heterotrophic biomass is involved in a leaching process, the organic acids produced are responsible for acidolysis, the most important mechanism in metals release [8]. The acids break down the metal bonds on the mineral surface and promote the solubility of the metals through complexation [6,7,21]. Figure 9 shows the production of acetic, oxalic, lactic and citric acids at the end of the tests with RM-II. These are probably not the only acids produced during the experimentation, considering the mass balance between the carbon initially present in the LM and in the organic acids, even taking into account that part of the carbon was probably not completely used and that another part was converted to CO₂ and into new biomass' cells.

At both S/L ratios, the organic acid production increased with temperature, confirming that increasing the temperature resulted in increased biological activity. At 5% S/L, citric acid was less present than at 2% S/L.

Observing all the tests results, it can be said that 5% S/L tests led to a higher total concentration of acids: this might be due to a higher amount of biomass in the system.

Characterisation of autochthonous microbial biomass. The viable titres of heterotrophic alkaliphilic and neutrophilic microorganisms were monitored during the initial growing phase of the biomass in the test with RM-II with 2% S/L at 22 °C (Figure 10). At the start-up of the test, the two groups had a comparable titre of 10² CFU/mL. During the treatment, the pH decrease was paralleled by a sudden increase in the titre of neutrophilic microorganisms, while a slow decrease in alkaliphilic microorganisms was found.

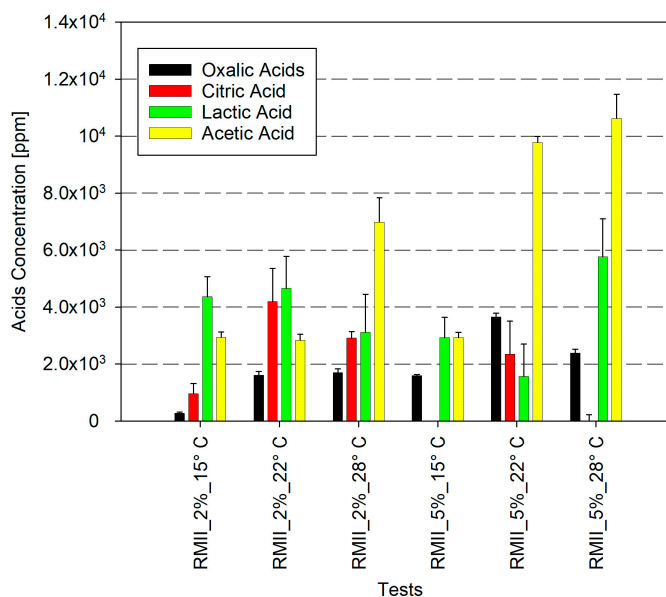


Figure 9. Acids production during tests with RM-II (average values ± standard deviations).

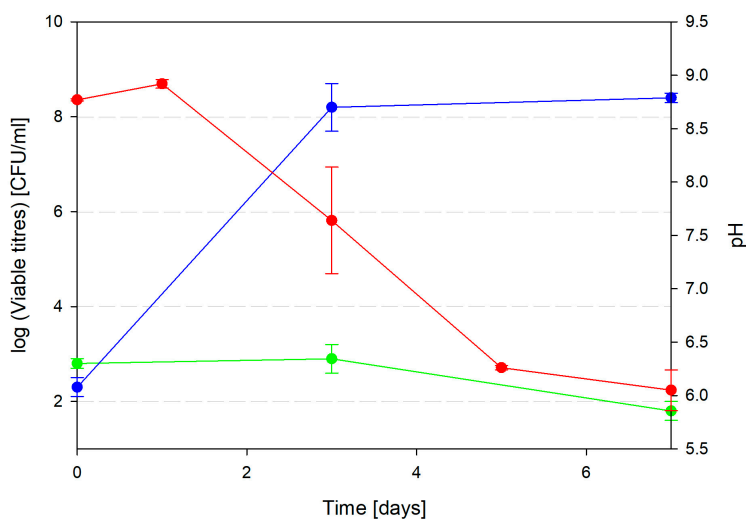


Figure 10. Log-transformed viable titres (mean of two replicates ± standard deviation) of heterotrophic alkaliphilic (green) and neutrophilic (blue) microorganisms as well as the pH (red) during the bioleaching test RM-II_2%_22 °C.

The total bacterial community in the biomass underwent a rapid reduction in diversity, decreasing the richness from 281 ASVs (amplicon sequence variants) at the beginning of the treatment to less than 12 detected ASVs at the end of the process (Figure 11). Moreover, a reduction in the evenness was also observed, as highlighted by the decrease in the Pielou’s index. At phylum level, Proteobacteria (31%), Firmicutes (20%), Bacteroidota (15%) and Actinobacteriota (9%) were the more abundant taxa in the community at the beginning of the treatment (3rd day). Then, Proteobacteria increased up to 70% at day 27 after the start-up. During the process, Firmicutes were almost constant with an average relative abundance (RA) of 25% ± 4%, while Bacteroidota and Actinobacteriota decreased to less than 10% and 6%, respectively.

At genus level, the bacterial community was dominated by *Bacillus* and *Acinetobacter*. More specifically, the RAs of the two genera were 3% three days after the start-up. The RAs of these two dominant taxa increased up to 19.1% and 24.3%, respectively, at 5 days after the beginning of the treatment and then remained almost constant until the end of the

process. The third genus in the community was *Bacteroides*, which increased from a RA of 2.7% 3 days after the beginning of the process up to an almost constant RA of $4.9\% \pm 0.6\%$.

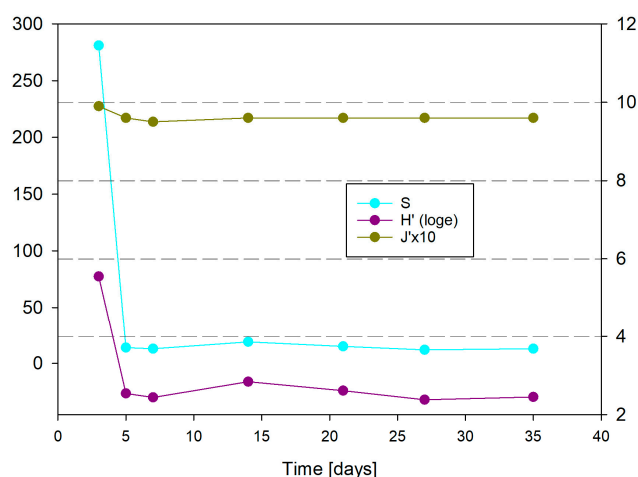


Figure 11. Indices of diversity and evenness of the bacterial community during the bioleaching test RM-II_2%_22 °C. Note: Richness as number of observed ASVs, H': Shannon index with an e log base, and evenness (J': Pielou's).

4. Discussion

Red mud characterisation. RM is a very heterogeneous material, whose composition is strictly connected to the origin site of the bauxite. Consequently, the elements' content can vary within a wide range, which includes RM-I and RM-II compositions. Table 5 shows the minimum and maximum content of some RMs' major elements as reported in the literature, expressed as oxides [21,22].

Table 5. Average reported composition (min and max) [%] of RMs, and average composition of RM-I and RM-II.

| Constituent | Reported Range | RM-I | RM-II |
|--------------------------------|----------------|------|-------|
| Fe ₂ O ₃ | 6.8–71.9 | 27.7 | 43.6 |
| Al ₂ O ₃ | 2.1–33.1 | 6.1 | 20.7 |
| TiO ₂ | 2.5–22.6 | 5.8 | 8.2 |
| Na ₂ O | 0.1–12.4 | 6.3 | 6.7 |
| SiO ₂ | 0.6–23.8 | 6.0 | 9.7 |
| CaO | 0.6–47.2 | 2.9 | 4.08 |

Biological activity. The same recipe adopted by Qu et al. [3] was used to prepare the leaching medium. In that study, *Acetobacter* was selected as inoculum for RM bioleaching. For this type of microorganisms, ethanol is used to produce acetic acid (acetic fermentation). In fact, acetic acid is the result of glucose oxidation and ethanol acetic fermentation, both occurring under aerobic conditions. In our study, since the initial culture was mixed and no preliminary selection and enrichment were carried out, glucose and yeast extract were used as general carbon and nutrients source, respectively. A more specific ingredient is ethanol, evaluated as a potential carbon source for microorganisms autochthonous of the RMs. In fact, from experiments conducted without it, after a fast decrease until 6.3 during the first 3 days of treatment, pH started to increase, reaching 8.5 at day 20 (data not shown). This may indicate that this element has a specific role during the bioleaching treatment.

pH trends and metals extraction. The pH plateau values observed in the present study during all the treatments are higher in comparison to others reported in the literature [5,8,10], in which a strictly acid environment was achieved using selected cultures. For example, in the *Acetobacter* study [3], during the one-step process (microorganisms and waste/ores are put together in contact with the LM), a pH of 4 was achieved after 10 days of treatment

at the same S/L ratios used in our research, showing an extraction of 55%, 35% and 45% for Al, Fe and Ti, respectively. Vakilchap et al. [5] obtained pH 4 and an Al extraction of around 70% using *Aspergillus niger*.

However, a direct comparison between the results achieved in our study and those previously reported in the literature may be misleading since the latter were achieved using sterilised RM inoculated with pre-selected and enriched biomass, which could operate at its maximum capacity under ideal conditions. In our study, no pre-selected/enriched inoculum was added to the untreated RM; therefore, one reason for such a difference in pH drop can be reasonably ascribed to the lower concentration of specialised biomass, as well as to the higher ANC of RM-I and -II, compared to other studies [3,10].

A pseudo-first-order model was applied to the experimental data to see if bioleaching behaviour could be predicted. As shown in Figure S2, the model did not fit the entire dataset obtained during the experimentation. Expanding the database and/or testing different and more complex models may be the key to better-predicting process behaviour. In previous studies, lower extraction efficiencies were obtained when using higher amounts of RM because of the too-harsh environment: for instance, Kiskira et al. [23] tested an S/L ratio from 1% to 20% for the extraction of scandium (Sc), and Qu et al. [3] tested 2%, 5% and 10% S/L, achieving the best results with the lower ratio. In our study, at a given temperature, the S/L ratio of 5% showed better results, likely due to the higher initial content of biomass in the system, compared to 2% S/L.

In general, our results showed that at 2% S/L metals extraction increased with the temperature, while this trend was not confirmed at 5% S/L. As for the effect of S/L ratio, a clear trend for the different metals cannot be observed: in a few cases, we obtained worst results in tests at 5% than at 2% even with a lower pH. These results may be the consequence of the two opposite effects of using increased RM quantities in the process: higher S/L could result in an increased quantity of biomass at the beginning of the tests, but at the same time, a higher quantity of RM could have led to potentially higher toxicity for the biomass [24].

Qu et al. [8] have reported the chemical pathways from the oxalic and citric acids to their metals' complexes. In the literature, the presence of other acids has been reported, for instance, gluconic acid [5], malic acid [10], and succinic, fumaric and tartaric acids [3]. During the heterotrophic bioleaching, the second most important part of the process, after acidolysis, is the complexolysis, i.e., the formation of complexes from microorganisms' metabolites (ligands) and the metals cations. These complexes can affect the metals' solubilisation, in general, increasing their solubility. The acids production obtained in the present study was compared with results obtained by Qu et al. [3] since the same LM was used, even if they performed the process under axenic conditions with an inoculum of *Acetobacter*. The trends of the acids production are comparable, increasing with the S/L ratios. Moreover, the acetic acid production is similar to the one observed in our study (slightly higher for RM-II_5%_22° and RM-II_5%_28°), whilst lower quantities of lactic and oxalic acids were produced [3].

Metal extraction efficiencies reported in our study are the maximum ones obtained after 10–14 days of batch operation. Indeed, it was reported that oxalic acid might lead to the formation of metals (rare earth elements) oxalates and reduce metals' solubility with their consequent precipitation [25,26]. This can explain the decreased metals solubilisation observed in our study, as previously highlighted.

Mineralogical analysis. From XRD analysis on RM-I, weddellite peak intensity significantly increased after the bioleaching process, probably as the result of Ca²⁺ precipitation due to the reaction with oxalic acid, as previously reported [3]. The presence of an oxalate (C₂CaO₄) in treated RM-II samples, reasonably formed at the expense of calcite which disappeared after bioleaching, is another example. The RM-I showed a similar composition after bioleaching, but with a clear decrease in almost all peak intensities, consistently with the release of metals in the liquid phase. Moreover, the mineralogical composition is compatible with its chemical composition for major elements (Table 2). Despite the higher content of

iron than aluminium in RM, a greater Al extraction efficiency was achieved, likely due to RMs mineralogical composition, e.g., to the more insoluble iron components contained in RM-I such as hematite, which requires a more acidic environment to be successfully leached. Comparison of XRD analysis of untreated and treated RM-II at 22 °C and 28 °C showed a similar composition (Figure 8), but again with a clear decrease in almost all peak intensities, consistent with the decrease in concentrations following bioleaching treatment.

Microbial communities. The microbial community underwent a rapid change during the process, with a sudden increase in the abundance of heterotrophic neutrophilic microorganisms and a marked reduction in bacterial diversity. The autochthonous community was dominated by *Bacillus* and *Acinetobacter*, bacteria previously characterised for their alkaliphilic properties but also for their ability to produce acids by a heterotrophic metabolism. Moreover, the two genera have been recently identified in the community of alkaline drainage water at a brown mud disposal site [27].

5. Conclusions

In this study, the possibility of exploiting the bioleaching of valuable metals from RM without preliminary biomass selection and enrichment was successfully assessed. Despite the alkaline environment and the absence of any external inoculum, pH dropped from alkaline to neutral values in all the conditions tested, and all the elements of interest were released in the liquid phase. Indeed, the chances of achieving high metal extraction efficiencies in batch mode using autochthonous biomass not previously enriched were negligible. The highest extraction efficiency was achieved in RM-II tests for Al, Mg and Mn (17%, 42% and 47%, respectively). As for RM-I, high S/L ratio and temperature were proved to enhance pH drop and consequent metals release in the liquid phase. As for RM-II, contradictory results were observed depending on the target metal. Moreover, the first characterisation of the autochthonous microbial community during the bioleaching process of RM was performed. Results are promising, and further investigation is needed to foster pH decrease and strengthen acidic conditions; thus, maximising metals release. In this sense, the switch from batch to semi-continuous operation may help in naturally enriching the biomass and increase process performance consequently. The slurry obtained after batch bioleaching treatment contains dissolved metal ions and residual RM. The subsequent steps are: scaling up the treatment and improving metal extraction. To recover metals, a solid/liquid separation is necessary. Various methods can be employed to recover metals from the liquid phase, depending on the target metal and its concentration, namely chemical precipitation, flotation, ion exchange, adsorption, solvent extraction, etc. The residual RM can be reused after undergoing a physico-chemical characterisation to ensure compliance with national guidelines and assess its suitability for other applications.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/min13070856/s1>, Figure S1: Experimental setup for bioleaching tests carried out at low (a), room (b), and high temperature (c); Figure S2: Pseudo-first order model fitting for RM-II_2%_15 °C (a) ($R^2 = 0.72$), RM-II_2%_22 °C (b) ($R^2 = 0.96$), RM-II_2%_28 °C (c) ($R^2 = 0.98$), RM-II_5%_15 °C (d) ($R^2 = 0.93$), RM-II_5%_22 °C (e) ($R^2 = 0.85$), RM-II_5%_28 °C (a) ($R^2 = 0.87$); Figure S3: XRD patterns of treated RM-II samples at different S/L ratios and 22 °C; Figure S4: SEM images of raw RM-II (a) and RM after trials RMII_5%_28 °C (b), RMII_2%_28 °C (c,d), RMII_2%_22 °C (e,f), RMII_5%_22 °C (g,h).

Author Contributions: Conceptualization, A.C. (Alessandra Carucci), G.C., A.C. (Anna Cozzolino) and S.M.; methodology, A.C. (Alessandra Carucci), G.C., A.C. (Anna Cozzolino), E.T., S.C. and S.M.; validation, A.C. (Alessandra Carucci), G.C., S.C. and S.M.; investigation, A.C. (Anna Cozzolino) and R.A.; resources, A.C. (Alessandra Carucci), E.T.; data curation, A.C. (Anna Cozzolino), G.C., E.T. and S.M.; writing—original draft preparation, A.C. (Anna Cozzolino); writing—review and editing, A.C. (Alessandra Carucci), G.C., S.C., E.T. and S.M.; supervision, A.C. (Alessandra Carucci), G.C. and S.M.; funding acquisition, A.C. (Alessandra Carucci). All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available in this article and its Supplementary Materials.

Acknowledgments: Anna Cozzolino gratefully acknowledges the Sardinia Regional Government for the financial support of her PhD scholarship (P.O.R. Sardegna F.S.E. Operational Programme of the Autonomous Region of Sardinia, European Social Fund 2014-2020—Axis III Education and training, Thematic goal 10, Investment Priority 10ii, Specific goal 10.5). The Authors wish to thank Orietta Masala (CNR-IGAG) and Martina Piredda for their valuable help with ICP-OES analyses. The authors acknowledge the CeSar (Centro Servizi d’Ateneo per la Ricerca) of the University of Cagliari, Italy, for the SEM analysis.

Conflicts of Interest: The authors declare that they have no known competing financial interest or personal relationship that could have appeared to influence the work reported in this paper.

References

1. EIT. *EIT RawMaterials Call for KAVA Projects SeedBook User Guide*; European Union: Brussels, Belgium, 2020; pp. 1–2.
2. Vidal, O.; Weihed, P.; Hagelüken, C.; Bol, D.; Christmann, P.; Arndt, N. ERA-MIN Research Agenda. 2013, p. 125. Available online: <https://hal.univ-grenoble-alpes.fr/insu-00917653/> (accessed on 19 June 2023).
3. Qu, Y.; Li, H.; Wang, X.; Tian, W.; Shi, B.; Yao, M.; Zhang, Y. Bioleaching of Major, Rare Earth, and Radioactive Elements from Red Mud by Using Indigenous Chemoheterotrophic Bacterium *Acetobacter* sp. *Minerals* **2019**, *9*, 67. [[CrossRef](#)]
4. Hind, A.R.; Bhargava, S.K.; Grocott, S.C. The Surface Chemistry of Bayer Process Solids: A Review. *Colloids Surf. A Physicochem. Eng. Asp.* **1999**, *146*, 359–374. [[CrossRef](#)]
5. Vakilchah, F.; Mousavi, S.M.; Shojaosadati, S.A. Bioresource Technology Role of *Aspergillus Niger* in Recovery Enhancement of Valuable Metals from Produced Red Mud in Bayer Process. *Bioresour. Technol.* **2016**, *218*, 991–998. [[CrossRef](#)] [[PubMed](#)]
6. Ghorbani, Y.; Oliazadeh, M. Shahverdiyan, Aluminum Solubilization from Red Mud by Some indigenous Fungi in Iran. *J. Appl. Biosci.* **2008**, *7*, 207–213.
7. Qu, Y.; Li, H.; Tian, W.; Wang, X.; Wang, X.; Jia, X.; Shi, B.; Song, G.; Tang, Y. Leaching of Valuable Metals from Red Mud via Batch and Continuous Processes by Using Fungi. *Miner. Eng.* **2015**, *81*, 1–4. [[CrossRef](#)]
8. Qu, Y.; Lian, B.; Mo, B.; Liu, C. Bioleaching of Heavy Metals from Red Mud Using *Aspergillus Niger*. *Hydrometallurgy* **2013**, *136*, 71–77. [[CrossRef](#)]
9. Zeng, H.; Lyu, F.; Sun, W.; Zhang, H.; Wang, L.; Wang, Y. Progress on the Industrial Applications of Red Mud with a Focus on China. *Minerals* **2020**, *10*, 773. [[CrossRef](#)]
10. Qu, Y.; Lian, B. Bioleaching of Rare Earth and Radioactive Elements from Red Mud Using *Penicillium Tricolor* RM-10. *Bioresour. Technol.* **2013**, *136*, 16–23. [[CrossRef](#)]
11. Jain, N.; Sharma, D.K. Biohydrometallurgy for Nonsulfidic Minerals—A Review. *Geomicrobiol. J.* **2004**, *21*, 135–144. [[CrossRef](#)]
12. Sethurajan, M.; van Hullebusch, E.D.; Nancharaiyah, Y.V. Biotechnology in the Management and Resource Recovery from Metal Bearing Solid Wastes: Recent Advances. *J. Environ. Manag.* **2018**, *211*, 138–153. [[CrossRef](#)]
13. Lee, J.C.; Pandey, B.D. Bio-Processing of Solid Wastes [and Secondary Resources for Metal Extraction—A Review. *Waste Manag.* **2012**, *32*, 3–18. [[CrossRef](#)]
14. Zhou, G.; Zhang, H.; Yang, W.; Wu, Z.; Liu, W.; Yang, C. Bioleaching Assisted Foam Fractionation for Recovery of Gold from the Printed Circuit Boards of Discarded Cellphone. *Waste Manag.* **2020**, *101*, 200–209. [[CrossRef](#)]
15. Horikoshi, K. Alkaliphiles: Some Applications of Their Products for Biotechnology. *Microbiol. Mol. Biol. Rev.* **1999**, *63*, 735–750. [[CrossRef](#)]
16. Klindworth, A.; Pruesse, E.; Schweer, T.; Peplies, J.; Quast, C.; Horn, M.; Glöckner, F.O. Evaluation of General 16S Ribosomal RNA Gene PCR Primers for Classical and Next-Generation Sequencing-Based Diversity Studies. *Nucleic Acids Res.* **2013**, *41*, e1. [[CrossRef](#)] [[PubMed](#)]
17. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, Interactive, Scalable and Extensible Microbiome Data Science Using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [[CrossRef](#)] [[PubMed](#)]
18. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.H. DADA2: High-Resolution Sample Inference from Illumina Amplicon Data. *Nat. Methods* **2016**, *13*, 581–583. [[CrossRef](#)] [[PubMed](#)]
19. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools. *Nucleic Acids Res.* **2013**, *41*, D590–D596. [[CrossRef](#)] [[PubMed](#)]
20. Oksanen, A.J.; Blanchet, F.G.; Kindt, R.; Legendre, P.; Minchin, P.R.; Hara, R.B.O.; Simpson, G.L.; Solymos, P.; Stevens, M.H.H. Community Ecology Package. *Ecol. Packag.* **2019**, *3*, 263.
21. Gräfe, M.; Power, G.; Klauber, C. Bauxite Residue Issues: III. *Alkalinity Assoc. Chem. Hydrometall.* **2011**, *108*, 60–79. [[CrossRef](#)]
22. Liu, Z.; Li, H. Metallurgical Process for Valuable Elements Recovery from Red Mud—A Review. *Hydrometallurgy* **2015**, *155*, 29–43. [[CrossRef](#)]

23. Kiskira, K.; Lympelopoulou, T.; Tsakanika, L.A.; Pavlopoulos, C.; Papadopoulou, K.; Ochsenkühn, K.M.; Lyberatos, G.; Ochsenkühn-Petropoulou, M. Study of Microbial Cultures for the Bioleaching of Scandium from Alumina Industry By-Productst. *Metals* **2021**, *11*, 951. [[CrossRef](#)]
24. Gadd, G.M. Metals and Microorganisms: A Problem of Definition. *FEMS Microbiol. Lett.* **1992**, *100*, 197–203. [[CrossRef](#)] [[PubMed](#)]
25. Kang, X.; Csetenyi, L. Monazite Transformation into Ce- and La-Containing Oxalates by *Aspergillus Niger*. *Environ. Microbiol.* **2020**, *22*, 1635–1648. [[CrossRef](#)]
26. Castro, L.; Blázquez, M.L.; González, F.; Muñoz, J.A. Bioleaching of Phosphate Minerals Using Earth Elements. *Metals* **2020**, *10*, 978. [[CrossRef](#)]
27. Kisková, J.; Stramová, Z.; Javorský, P.; Sedláková-Kaduková, J.; Pristaš, P. Analysis of the Bacterial Community from High Alkaline (PH > 13) Drainage Water at a Brown Mud Disposal Site near Žiar Nad Hronom (Banská Bystrica Region, Slovakia) Using 454 Pyrosequencing. *Folia Microbiol.* **2019**, *64*, 83–90. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.