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Analysis of element yield, bacterial community structure and the impact of carbon sources for bioleaching rare earth elements from high grade monazite

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ABSTRACT

Rare earth element (REE) recovery from waste streams, mine tailings or recyclable components using bioleaching is gaining traction due to the shortage and security of REE supply as well as the environmental problems that occur from processing and refining. Four heterotrophic microbial species with known phosphate solubilizing capabilities were evaluated for their ability to leach REE from a high-grade monazite when provided with either galactose, fructose or maltose. Supplying fructose resulted in the greatest amount of REE leached from the ore due to the largest amount of organic acid produced. Gluconic acid was the dominant organic acid identified produced by the cultures, followed by acetic acid. The monazite proved difficult to leach with the different carbon sources, with preferential release of Ce over La, Nd and Pr.

1. Introduction

The utilization of rare earth elements (REE) in technological driven industries including medical, electronic and defense has seen a rapid increase in their value as well as stimulating research into extraction and recovery processes. The supply of REE currently meeting demand is being met, and dominated by China, who have exhaustive extraction and commercialization practices. The monopoly on this industry creates a worldwide supply risk and to combat this bottleneck, secondary suppliers including the USA, South Africa and Australia have entered the market to diversify the supply of these essential elements. REE scattered throughout the globe are most commonly found in oxidic form as rare earth oxides (REO), phosphates, carbonates and silicates [1]. Profiting from the extraction of REE occurs by mining of only a few of these oxides including bastnasite ($(Ce,La)(CO_3)F$), monazite ($(Ce,La,Nd,Th)PO_4$) and xenotime (YPO_4) [2].

Australia is host to approximately 36 REO mines including Mount Weld (the largest), Nolans, Olympic Dam and Avonbank to name a few, with REO production in 2023 reaching 18 thousand tonnes, ranking 3rd in the worlds' supply behind China and the United States [3]. The Mount Weld mine (operated by Lynas corporation) holds claim to the having the highest grade REE mine in the world [4] but these extensive reserves are bound within the deeply weathered monazite component. Recovery of REE from these sources involves orthodox extraction and separation procedures that require the input of hazardous chemicals (ion exchange, solvent extraction) resulting in the generation of dangerous wastes, often including radionucleotides such as thorium. A viable alternative to this highly energy intensive extraction processes is bioleaching.

Bioleaching of REO, in particular monazite ores has been demonstrated by several groups [5–7] relying on the principle of phosphate solubilization to liberate the REE from the ore matrix. The application of phosphate solubilizing microorganisms (PSM) has seen success, but the levels of REE released and recoverable from the ore can differ widely. In 2018 we reported higher rates of REE leaching with fungal species than bacteria [5] as have others [8,9]. With pulp densities of up to 10%, recoveries of REE from red mud saw 61% of Yttrium recovered [10], whereas with a 1% pulp density, *Acidithiobacillus ferrooxidans* leaching permitted maximum recovery of 37% lanthanum after 14 days in batch culture [11]. Recycling of REE containing wastes such as fluid catalytic cracking catalyst by application of *Gluconobacter oxydans* in a batch bioleaching system allowed for the salvage of 49% REE present [12].

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For successful bioleaching of phosphate ores using heterotrophic organisms, a source of organic carbon must be supplied to the system, as the resulting organic acids generated via metabolism have been suggested to be primary mechanism responsible for the release of cations from the phosphate structure. Other suggested processes have included the production of phosphatases that are known to be active in phosphate limited soils [13] as well as production of siderophores that are effective in solubilizing P from inorganic minerals including $FePO_4$ and phosphate rock [14]. Siderophore release from PSM not only enables the iron chelation from Fe–P complexes [15], freeing up the phosphate, but has an additional defensive property in soil environments preventing pathogenic predation for some organisms [16].

Some research has been done on the impact of different sugars on leaching efficiencies and the mechanisms previously listed [9,17,18] in an attempt to lower costs of leaching as well as understand how secondary metabolites affect the growth of organisms present in the leaching environment. This study evaluated the ability of PSM to leach REE from monazite ore sourced from the Mt Weld Mine in Western Australia. The ore from this site is of a suitable grade by which traditional processes of chemical and electrical methods of recovery are usually employed. Its suitability to bioleaching as an alternative extraction method was to be determined.

2. Methods

2.1. Monazite high grade ore composition

A weathered lateritic monazite ore from the Mt Weld mine in Western Australia was donated for this research by Lynas Corporation. The ore was ground initially by a rod mill, then pulverized in a ring mill and finally sieved to 1–35 μm in size. The ore sample was gamma irradiated at 50 kGy for 11 h (ChemCentre, Western Australia). The mineralogical and chemical data of the ore was obtained by X-ray diffraction (XRD), inductively coupled plasma mass spectrometry (ICP-MS) (performed by Bureau Veritas). For XRD-phase identification, the high-grade monazite ore was micronized and front-loaded into a specimen holder, with diffraction data collected using a Bruker D8 Discover diffractometer with Ni-filtered Cu Kα radiation (40 kV, 40 mA) over the range 7–120° 2θ, with a step size of 0.015°. Phase identification was performed using a Bruker EVA 5.2 with the COD database. Elemental composition can be found in Supplementary Table 1.

2.2. Microorganisms and media

Klebsiella aerogenes was obtained from the American Tissue Culture collection (ATCC 13048), *Burkholderia* T48 (Genbank: FJ687970) was isolated from soil collected from the rhizosphere of a natural stand of *Acacia stenophylla* in the Murray–Darling Basin in south-eastern Australia [19], *Pseudomonas putida* (DSMZ 1693) and *G. oxydans* (DSMZ 2343) were obtained from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures.

National Botanical Research Institute's Phosphate medium (NBRIP: 5 g L^{-1} $MgCl_2(H_2O)_6$, 0.25 g L^{-1} $MgSO_4(H_2O)_7$, 0.2 g L^{-1} KCl, 0.1 g L^{-1} NH_4SO_4, 2 g L^{-1} KH_2PO_4, 30 g L^{-1} glucose, pH 6.8 ± 0.4) was used as the growth medium for all organisms. Organisms were maintained at 30 °C in liquid culture prior to use.

2.3. DNA extraction for phylogeny determination and diversity profiling

Genomic DNA was extracted using a FastDNA™ Spin Kit for soil (MP Bio, CA, USA). Diversity profiling of extracted community DNA following experiments was carried out by the Australian Genomics Research Facility using universal primers 341F and 806R specific for the 16S rRNA gene of bacteria and archaea [20]. Sequence analysis was conducted as described in [21]. Quality filtering and removal of operational taxonomic units (OTU) with relative abundance below 0.1% was performed.

2.4. Bioleaching experiments

Bioleaching experiments examining the impact of different carbon sources on leaching capabilities of the different microorganisms were performed on monazite. All leaching experiments were conducted in triplicate, in 500 mL Erlenmeyer flasks containing 0.5% ore (w/v) supplemented with 1% maltose (Merck), 1% fructose (Merck) or 1% galactose (Merck) in modified NBRIP media with no soluble phosphate source, pH 7.0. No flask contained glucose in the experiments. From stock, isolates were harvested at exponential growth phase, centrifuged, and washed to remove traces of NBRIP containing phosphate and glucose. Cells were resuspended in NBRIP media with no soluble phosphate to a final density of 10^7 cells ml^{-1}. Flasks were incubated aerobically at 30 °C and 130 rpm for 7 days. Four mL was withdrawn for analysis at time 0, 1, 3, 7 days, removing no more than 10% of the overall total volume.

Measurements were performed on both, cells and supernatant, and included pH, acid phosphatase activity, REE concentration in the leachate, organic acids and species diversity (after 7 days only). Supernatants were separated from ore and cells by centrifugation (13,000 rpm, 20 min) and filter sterilized (0.22 μm pore size).

2.5. Analytical methods

2.5.1. Acid phosphatase activity and pH

Leachate pH was measured (Ionode IJ series pH probe) on all samples taken. Cellular and extracellular acid phosphatase (AP) activity was assayed by the rate of ρ-nitrophenol phosphate (ρNPP) conversion to ρNP following a modified protocol [22]. A 300 μL sample of culture was centrifuged at 13,000 rpm for 10 min, the supernatant, collected and filtered (0.20 μm PES, Sartorius). The cell pellet was re-suspended and washed with 300 μL of Tris-Cl pH 7. Triplicate tests were performed on 100 μL of cells, placed in a 96-well plate and centrifuged at 3000 rpm for 20 min. Wash buffer was discarded. Triplicate tests were also performed on 100 μL of supernatant per sample. To each sample, for both cells and supernatant, 100 μL of buffer containing 0.1 M sodium acetate (pH 5.2), 0.1% Triton X-100 and 5 mM ρ-nitrophenol phosphate (Sigma–Aldrich) was added, the plates returned to 37 °C for 1 h and the reaction stopped with the addition of 10 μL 1 N NaOH. Colour development was assayed at 405 nm using a microplate reader (EnSpire Multimode plate reader, PerkinElmer). Enzyme activity is expressed as mmol ρNP ml^{-1} min^{-1}.

2.5.2. Organic acid analysis

Using HPLC, organic acids (OA) were separated and analysed from culture extracts at each interval, based on a previously published protocol [5]. In brief, filter sterilised (0.2 μm) culture extracts were centrifuged (13,000 rpm for 10 min at room temperature) and the supernatant diluted in H_3PO_4-acidified MQ water (pH = 2.00). Samples were run on reverse-phase HPLC using a Luna (Phenomenex) 5u C18(2) column (150 × 4.6 mm; 5 mm particle size) fitted with a C18(2) guard column, using a 5:95 acetonitrile: 5 mM ammonium phosphate buffer ($(NH_4)_2HPO_4$); pH = 2.00 using H_3PO_4) as the mobile phase. All samples were run in 50 μL-injections under isocratic elution with 1.0 mL/min-1 flow rate at 50 °C for 15 min. The instrument used was a Thermo Scientific UltiMate ™ 3000 LC System, coupled with a Thermo Scientific™ Dionex™

UltiMate™ Diode Array Detector (DAD) 3000 set to 210 nm wavelength for detection.

Data acquisition and analysis was conducted on HPLC traces acquired using Thermo Scientific™ Dionex™ Chromeleon ™ 7 Chromatography Data System software (Version 7.2 SR4). Sample peaks resolved were matched to elution times of pure OA standards for: D-gluconic, oxalic, DL-malic, formic (Scharlab; Barcelona, Spain), Δ-lactic, α-ketoglutaric, citric, acetic (VWR; Radnor, US) and succinic (VWR; Radnor, US). Unless otherwise indicated, all OA were sourced from Sigma-Aldritch (St. Louis, US). Peak area was assessed via manual integration, with data exported to Microsoft Excel. Using standard curves generated from separated OA of known concentration, OA were quantified for all samples via peak area interpolation and expressed in mmol L^{-1}. Although Δ-lactic and citric acid were detected, these standards had an unresolvable co-elution, thus are not reported here. Pyruvic and butyric acids were also assessed but were undetectable in all samples.

2.5.3. REE concentrations

Measuring the concentration of total REE in the leachate was performed colorimetrically following the protocol by Hoggendoorn et al. [23] with a wavelength of 650 nm for qualitative assessment. Further analysis for quantitative results of individual elements La, Ce, Nd and Pr for specific strains were measured using ICP-MS. The filtered sample aliquots were heated to 85 °C and pre-treated with hydrogen peroxide (H_2O_2), prior to aqua regia digestion (1:3 HCl:HNO_3), based off methods by Cao et al. [24] and Gonzalez, van Lier & de Kreuk [25]. The instrument (iCAP Q, Thermo Scientific) was operated in accordance with manufacturer instructions.

3. Results

3.1. Impact of changing sugars on acid phosphatase production

The levels of acid phosphatase (AP) in both the cellular fraction and supernatant under different sugars with 0.5% sterile monazite were measured at 1,3 and 7 days post inoculation. AP activities were significantly greater ($P < 0.05$) when provided with fructose for *Burkholderia* T48 than any other species and peaked at day 3 (Fig. 1A) in both cells (16.96 ± 1.25 mM pNP) and supernatant (14.9 ± 1.78 mM pNP), with a detectable increase for *K. aerogenes* cellular fraction at day 3 with fructose (4.70 ± 1.44 mM pNP) holding until day 7 (4.85 ± 0.71 mM pNP) (Fig. 1B). Changes in the levels of AP produced by *G. oxydans* and *P. putida* were negligible when the carbon source changed with little difference between recorded between cellular and supernatant fractions (Fig. 1C and D), and at concentrations no greater than those recorded in the abiotic controls.

3.2. REE release and pH

Initial qualitative colorimetric assessment of monazite solubilization and REE release demonstrated that total REE yield from *G. oxydans* was minimal (Fig. 2). *G. oxydans* with fructose failed to solubilize monazite with the final pH at day 7 staying high at 5.56 with a total of 1.87 ± 0.73 mM REE detected and with maltose the pH reached 5.86 with 1.58 ± 0.34, performing no better than what was recorded with the non-inoculated control (Fig. 2B). *G. oxydans* produced the greatest amount of soluble REE (9.17 ± 0.34 mM) when galactose was provided. *P. putida*, on the other hand, produced the greatest concentration of REE in solution when grown in the presence of fructose with a final concentration of 9.37 ± 0.1 mM REE in solution by day 7, halving when provided with galactose (4.99 ± 0.1), and decreasing to 1.51 ± 0.28 mM REE when given maltose. *K. aerogenes* had the greatest ability to solubilize the monazite with 24.03 ± 0.1 mM REE in solution from fructose, 16.33 ± 1.4 mM REE with galactose and 11.99 ± 0.86 mM REE from maltose. *Burkholderia* T48 with fructose had a final concentration of 7.47 ± 2.75 mM REE in solution by day 7, with galactose (5.06 ± 0.59), and 1.96 ± 0.34 mM REE when given maltose. Based on the amount of total REE released into solution, AP concentrations and changes in pH, further analysis by ICP-MS was performed to determine the proportion of individual REE released by the two better performing species, *Burkholderia* T48 and *K. aerogenes* (Fig. 3). No further REE or organic acid data was collected for either *P. putida* or *G. oxydans*. ICP-MS revealed that Ce was preferentially leached by both *Burkholderia T48* and *K. aerogenes,* followed by La and Nd at similar concentrations when supplemented with either fructose or galactose. Total soluble REE were at the lowest concentrations when maltose was the carbon source, with *K. aerogenes* releasing more than *Burkholderia* T48 . Non-inoculated controls saw negligible amounts of REE solubilized with the greatest amount detected with fructose after 1 day (35 μg L^{-1}). REE release was faster with *K. aerogenes.*

3.3. Organic acid production

A number of peaks were detected in HPLC chromatograms with the most abundant compounds being identified and quantified. The microorganisms produced micromolar amounts of organic acids over three days. Assessment was ceased after day 3 as the pH had reached its lowest value at this time for *Burkholderia* T48 and *K. aerogenes.* Previous research had determined >90% of sugars had been consumed by day 3 when given glucose [5]. Fig. 4 shows the maximum observed concentrations for all identified organic acids during bioleaching experiments with each sugar. D-gluconic, oxalic, formic, acetic, α-ketoglutaric, DL-malic and succinic acids were detected in varying concentrations depending on the carbon source provided. *K. aerogenes* provided with fructose produced the greatest amount (0.42 mmol L^{-1}) and number of organic acid species, however identification and analysis of D-gluconic acid was problematic. In fructose-fed experiments, the D-gluconic peak was detected in both non-inoculated and inoculated media alike, suggesting the presence of an interfering compound with identical retention time (Fig. S1). Taking this point into consideration, the amount of OA produced by *K. aerogenes* other than D-gluconic acid was similar between the sugars at day 1, but on fructose fed-cultures there was approximately a 3-fold increase in all other OA by day 3. Detection of DL-malic, succinic and α-ketoglutaric acids were notable in fructose-fed *K. aerogenes* cultures at this timepoint, compared with other groups. Moreover, a large amount of acetic acid (0.178 mmol L^{-1}) was generated in these cultures, equal to that of the theoretical amount of D-gluconic acid (0.175 mmol L^{-1}). Less was detected when *K. aerogenes* was given galactose or maltose. *Burkholderia* T48 generated similar amounts of formic and DL-malic acid to *K. aerogenes* after 3 days when provided with galactose. Both species produced the lowest amount and diversity of OA when provided with maltose.

The amount of REE released by *Burkholderia* T48 and *K. aerogenes* was examined against the overall concentration of OA recorded and a correlation calculated of $r = 0.72$ (Supplementary Fig. 3). This highlights a weak relationship between the amount of OA produced and REE in solution. While there was a correlation seen between the amount of total organic acids produced and the amount of rare earths in solutions, no relationship was observed between the amount of AP produced (cellular or supernatant) with the amount of REE released (Supplementary Fig. 3).

3.4. Microbial diversity

Despite gamma irradiation of the monazite, microscopic analysis detected microbial activity in the non-inoculated controls.

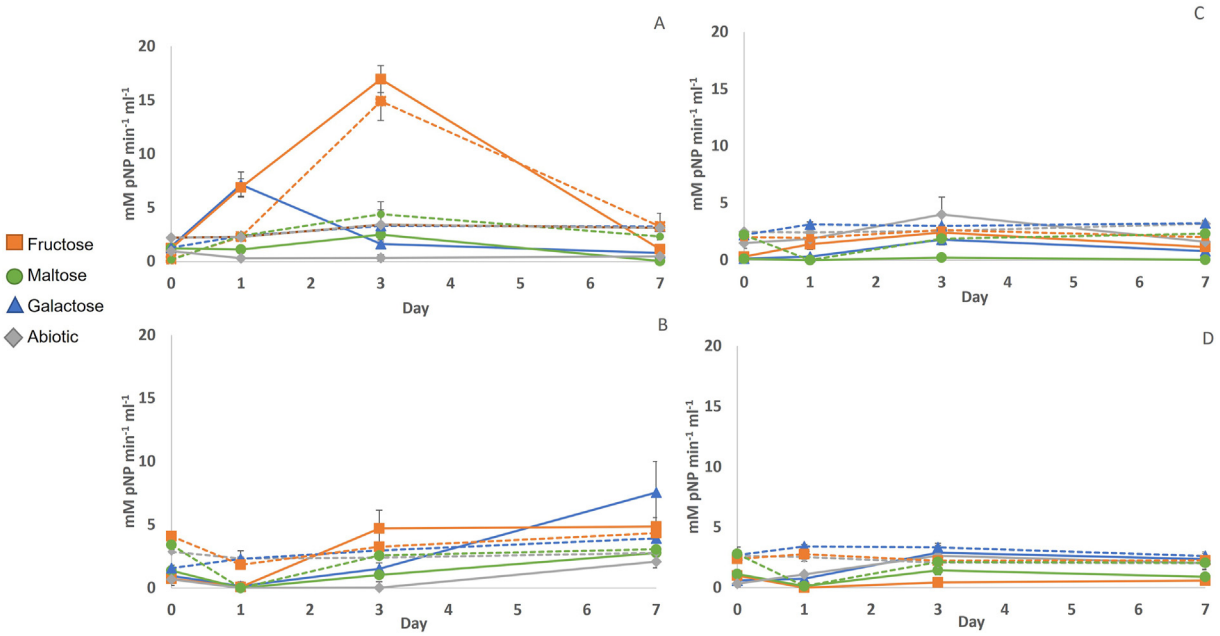

Fig. 1. Acid phosphatase production by *Burkholderia* T48 (A), *K. aerogenes* (B), *P. putida* (C) and *G. oxydans* (D). Solid lines represent the cellular fraction and dotted lines are the supernatant. Data are averages of three experiments each with three biological replicates. Non-inoculated results are the average of the three sugars.

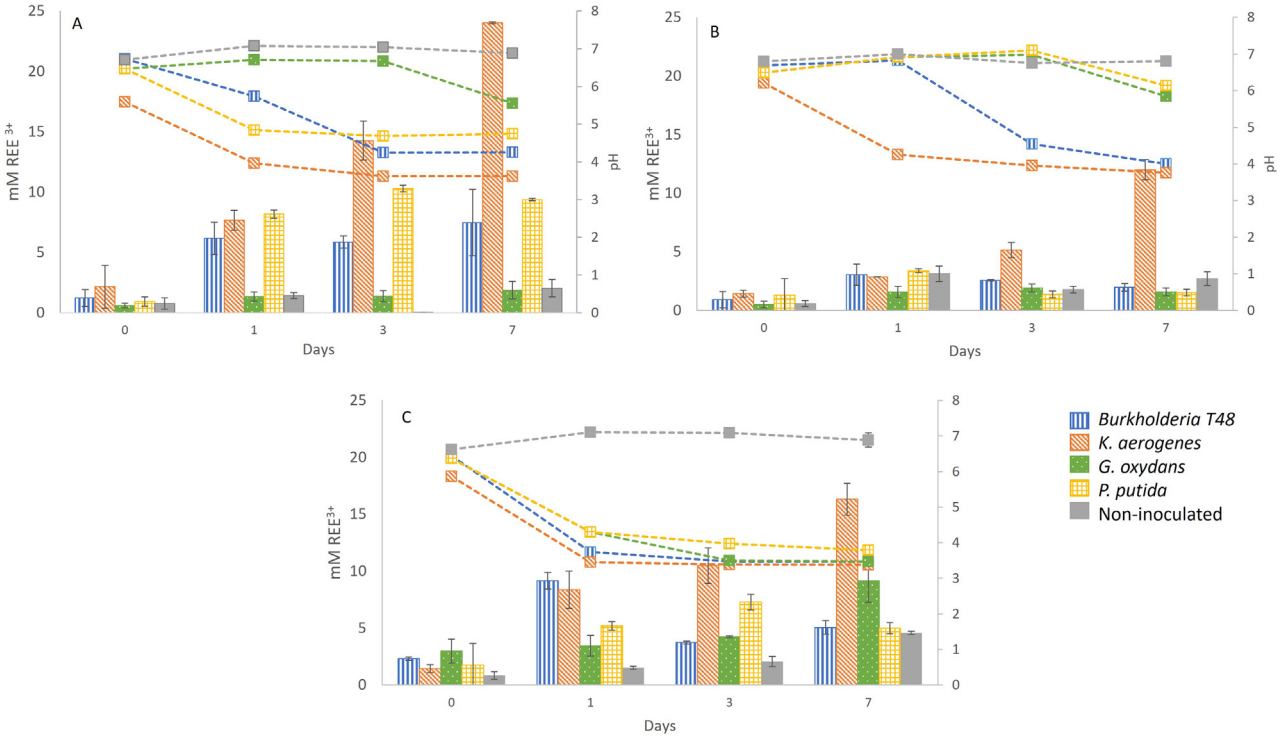

Fig. 2. Total concentration of REE released into solution by *Burkholderia* T48, *K. aerogenes*, *P. putida* and *G. oxydans* when provided with fructose (A), maltose (B) and galactose (C). Corresponding changes in pH for each organism per sugar are recorded on the secondary axis as dotted lines.

Therefore, microbial diversity analysis was performed on all samples at day 7. At day 7, *Burkholderia* T48, *K. aerogenes* and *P. putida*, still constituted >99% of the microbial population. In cultures initially inoculated with *Burkholderia* T48 this remained the only organism detected after 7 days growth in the presence of any of the sugars (Supplementary Table 2). Two additional families, *Nocardiaceae* and *Pseudonocardiaceae,* were present in very low proportions (less than 0.1%) in the *K. aerogenes* cultures after 7 days on all sugars, however these families were not present in our extraction blanks (data not shown). This was also seen for *P. putida* grown in the presence of fructose and maltose, however in the presence of galactose, Nitrobacteraceae *(Bradyrhizobium)* was

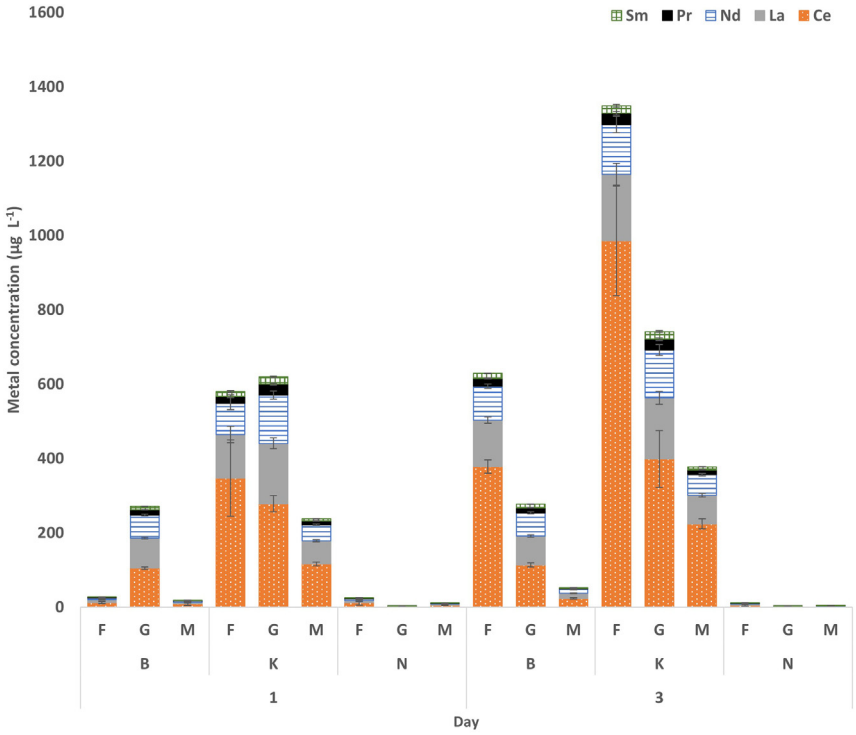

Fig. 3. Proportions of REE in bioleaching supernatant after recorded pH changes from day 1 and day 3. Concentrations are of total detected light REE determined by ICP-MS. Error bars indicate standard errors around the means. Bioleaching samples are from growth either on fructose (F), galactose (G) and maltose (M) inoculated with either *K. aerogenes* (K) *or Burkholderia* T48 (B). Non-inoculated controls (N) are an average of three replicates.

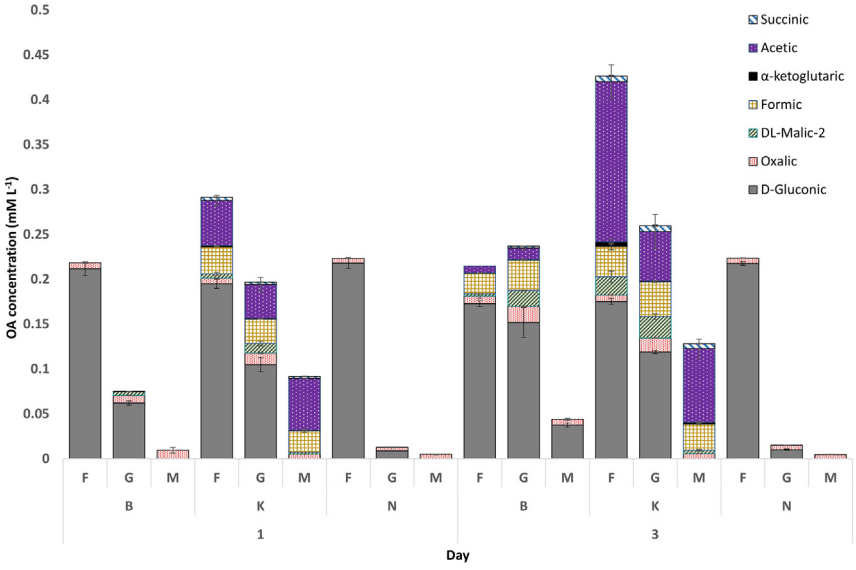

Fig. 4. Leachate concentrations of organic acids following inoculation of gamma irradiated monazite with *Burkholderia* T48 (B) and *K. aerogenes* (K) supplemented with fructose (F), maltose (M) and galactose (G). Non-inoculated (N) controls.

detected at 0.1% of the total population. In contrast, the population of *G. oxydans* after 7 days substantially decreased. Significant proportions of *Sphingomonadaceae* and *Enterobacteriaceae* were present, with specific species identified including *Burkholderia* and *Pseudomonas*. These species were also detected in the non-inoculated controls; however, many others were also present in higher numbers including *Afipia*, *Bradyrhizobium*, *Actinomyces, Micrococcus*, *Ralstonia* and *Bacillus.* Interestingly non-inoculated cultures provided with maltose had the greatest species diversity compared with the fructose and galatose. *Burkholderia* and *Pseudomonas* species dominated the non-inoculated controls cultures incubated in the presence of fructose and galactose, with strain identification different to those being maintained for inoculation. No carbon controls routinely had few species including *Burkholderia, Pseudomonas, Bacilli, Bradyrhizobium and Sphignomonas,* however reproducibility was difficult due to low cell numbers.

4. Discussion

The use of microbially produced small organic acids to solubilize REE containing ores has been the subject of increasing interest over recent years. However, optimization of microbially driven monazite solubilization for REE recovery is in its infancy and knowledge of the impact of differing carbon sources on the production of these organic acids has not been fully evaluated. The experiments conducted here demonstrated that while all species maintained growth on the differing carbon sources, they produced differing suites of organic acids, which impacted their ability to solubilize the monazite.

Initial experiments conducted with four microbial species demonstrated that not all organisms solubilize inorganic phosphate equally with evaluation of successful leaching determined by the concentration of REE in solution. *G. oxydans* and *P. putida* were discarded from further studies after initial analysis of REE in solution and concentration of AP produced were low on all carbon sources examined. This does not automatically preclude their inclusion from future leaching studies but highlights that the conditions established here were less than optimal as both *Pseudomonas* sp. and *G. oxydans* have been used successfully for bioleaching a variety of minerals [26,27].

Bacterial species that produce acid phosphatases (AP) are often associated with plant growth promotion whereby these enzymes enable phosphate solubilization resulting in stabilization of the microcosm and nutrient exchange pathways to be established [13]. As *Burkholderia* species are commonly found in the rhizosphere [28] and aid in preventing P limitation in agricultural soils, the production of this enzyme in high concentrations by *Burkholderia* T48 up to day 3 supplemented with fructose was not unexpected. The production of phosphatases are strictly regulated by phosphate levels [29] (however phosphate-insensitive phosphates do exist [30]), and while the levels of available phosphate in all cultures were the same at day 0, the lack of soluble phosphate did not trigger a release of AP from the other three species regardless of the carbon source provided. With galactose and maltose, AP production was minimal with *Bukholderia* T48 indicating a separate role or response to fructose.

Fructose in the environment is known to have an effect on some PSM, as fungal secretion of fructose by *Rhizophagus irregularis* has been documented to increase the extracellular secretion of phosphatase by *Rahnella aquatilis,* thus triggering P mineralization [31]. Therefore, as all strains recorded low levels of AP when provided with either galactose or maltose, the presence of fructose may play a role as a signal molecule with some organisms, triggering bacteria-mediated phosphorus mineralization with AP regulation. As these effects are likely to be occurring at the cell surface, attachment and biofilm formation to the phosphate of the monazite in the presence of different carbon sources requires further examination to support this theory.

The lack of a clear correlating relationship between production of an AP and REE release indicates that this enzyme is not a major driver of monazite solubilization, although it is likely that the drop in pH below 5.0 after days 1–2 rendered the enzyme less effective by day 3 (*Burkholderia* T48 pH 4.2 with fructose at day 3) as optimal pH has been documented to be between 5 and 6 [13,28]. *Burholderia* T48 produced more AP but solubilized less monazite than *K. aerogenes* which produced more OA and solubilized more monazite.

Previous research conducted on sterile and non-sterile monazite from Mt Weld with glucose provided as the carbon source [5] demonstrated that gluconic acid was a large contributor to the overall organic acid profile of each species and generated a far greater amount of total organic acids than seen with the organisms tested here on fructose, galactose or maltose. The most dominant OA across experiments was D-gluconic acid when galactose and maltose were provided. However, the D-gluconic peak was also detected in the non-inoculated samples supplemented with fructose, suggesting the presence of an interfering compound with identical retention. As both are 6-carbon molecules, D-gluconic acid and fructose may be structurally similar enough to confer properties (i.e. hydrophobicity) that result in the sharing of an elution time under the HPLC conditions chosen. Conversely, Amaniampong et al. [32] demonstrated the oxidation of D-glucose to glucuronic acid through free radical interaction in water in the absence of chemical catalysts. In line with this, fructose may be readily oxidized into D-gluconic acid in our media, more so in the presence of pro-oxidant metals from the ore. This has not yet been clarified however, so it is difficult to confirm D-gluconic acid as the dominant acid in all fructose leaching experiments.

On day 3, fructose-fed *K. aerogenes* cultures exhibited the highest yield of lanthanides (>1 mg L^{-1}) in the leachate accompanied by the highest OA levels (>0.4 mM L^{-1}), with the greatest spectrum of OA-species produced. Whilst D-gluconic was the most abundant OA in many groups, this did not uniformly correspond with lanthanide abundance. In fact, D-gluconic levels detected in fructose-fed *K. aerogenes* cultures decreased from day 1 to day 3, whilst total OA and REE more than doubled over time. The OA with marked increases in this group were DL-malic, α-ketoglutaric, acetic and succinic acids, suggesting that these OA may play a more substantial role in mineral interactions and lanthanide bioleaching, independent of phosphatase activity. The structure of OA species secreted underlyingly determines REE-interactions.

The increased REE in culture extracts in association with particular OA species may be partly explained by hydrophobicity. Oxalic acid (very hydrophilic) was detectable in all experimental groups and timepoints without much effect on lanthanides in culture extracts, apparently inconsequential to the amount of REE solubilized. Acetic acid and the di-carboxylic acids (DL-malic, α-ketoglutaric, and succinic acids) were the most hydrophobic of the OA detected, with longer column retention times, with the greatest effects on lanthanide bioleaching seen in their presence. As cationic adsorption tends to strengthen with organic hydrophobicity [33–35], the exposure of ore to these OA could result in increased interactions with lanthanides in solution, OA-adsorption to the mineral surface, and/or with other cations and thereafter secondary release of REE into solution. This is in part supported by Lombardo et al. [36], reporting that silica functionalized with succinic-acid had a very high partition coefficient ($K_d = 250.0$) and accordingly high capacity to adsorb and chelate lanthanides in solution, and at a comparable pH to these experiments (pH = 4.50).

Wang & Golden [37] demonstrated that acetate ions bind cerium ions, deter redox cycling, and keep it in solution for cerium-film formation processes. This is largely attributed to monodentate and bidentate REE-coupling with acetic acid facilitated by its carboxyl group [38]. Carboxyl-metal binding has also been characterized in dicarboxylic acids [39], with both the number and strength of electrostatic interactions suggested to increase relative to the number of carboxyl functional groups [40–42]. In this study, the constitutive increases in the more hydrophobic dicarboxylic acids may reflect more functional sites for stronger interactions with lanthanides, improved OA-REE chelation, and thereafter solubilization as a complex. The increased lanthanide bioleaching measured in association with acetic acid likely represents weaker and lesser ratio of lanthanide interactions per molecule of acetic acid but compensated for by sheer numbers. Collectively, this suggests particular OA may be more integral to REE-leaching and OA-sequestering of REE, independent of phosphatase activity.

D-gluconic acid production is a result of glucose dehydrogenase (GDH) activity which is affected by phosphate regulation. Phosphate starvation is known to induce GDH activity thereby increasing OA production, with a resultant decrease in pH, releasing phosphate [43]. Experiments here demonstrate that gluconic acid was produced from all of the carbon sources, albeit at low concentrations (<0.5 mM L^{-1}). If phosphate needs were being met through solubilization of the ore, there should have been a higher concentration of REE in solution, however this was not seen. It is possible that phosphate starvation was occurring but without the expected increase in gluconic acid produced as a result of triggering of GDH activity. Maintenance of cellular phosphate levels are often tightly controlled in bacteria [44] as excessive intracellular phosphate is toxic [45] so a counternarrative may be that phosphate starvation is more likely to induce lipid accumulation and activate pathways for fatty acid biosynthesis [46]. This would lower the total amount of OA generated in these experiments. This highlights that changing sugars significantly impacts OA production. Hypothetically it appears that the lack of phosphate (from monazite solubilization) could trigger a preference for energy storage rather than growth.

For the microorganisms tested to access energy for cellular requirements, all carbon sources provided here needed to be converted to glucose necessitating the presence of specific genes. The ability to utilise maltose requires a complex set of genes, with all species examined possessing the maltose systems responsible for use of maltodextrins according to current literature [47–50]. The lack of REE in the leachate following maltose utilization could be attributed to the low levels of AP produced, as well as a difference in the suite of OA produced, however as the amount of AP produced did not correlate to the amount of REE in solution, it is more likely that the lower OA produced are responsible. As all cultures were fully aerated (shaking flasks), oxygen limitation is not to blame for the lack of success with maltose as has been demonstrated with yeast [51] and *Alicyclobacillus acidocaldarius* [52].

Uptake of maltose has been demonstrated to be slower by up to half the rate in comparison to other sugars, especially glucose, in other bacterial species [53] with further utilization of maltose affected by lack of available phosphate. Additionally, during periods of phosphate stress, maltose can be converted to glycogen for storage (if glucose is absent) [54,55]. Therefore, the very low levels of OA produced by *Burkholderia* T48 and *K. aerogenes* in the presence of maltose maybe attributed to the slow uptake of maltose combined with the conversion of maltose to glycogen during phosphate starvation. It will be of interest to repeat these experiments with a longer time frame to determine if the slow update of maltose could result in an extended leaching pattern and subsequent release of REE.

During periods of phosphate limitation (starvation) high cytoplasmic trehalose content has been detected in bacteria [55], implying the bioconversion of carbon sources to an osmoprotectant could be necessary to manage the phosphate-dependent regulation of central and storage pool metabolism (glycogen, polyphosphate) pathways. However, most less-preferred sugars are taken up by cells with the assistance of the ATP-binding cassette (ABC) transport systems, which are the largest group of carbohydrate-transport systems found in bacteria [56]. The additional enzymes and energy input required for the conversion of these alternate sugars and activation of multiple pathways for their incorporation into other substances could be responsible for depleting available phosphate stores. Future metabolite analysis is required to unravel this issue.

Galactose metabolism in microorganisms is often examined in reference to lactic acid bacteria [57] for exploitation in the food industry with insufficient research conducted in bioleaching systems [58] to thoroughly understand its impact on numerous leaching kinetics. Utilization of fructose or galactose by *Enterobacter aerogenes* (now *K. aerogenes* as is being used in this paper) has previously demonstrated that after 20 h, more formate was produced on fructose than galactose [59], whereas our results demonstrate very little difference in the total amount produced on either sugar. In this instance, phosphate limitation is likely impacting the types of OA being produced due to the P requirements of the various metabolic cycles. With the total overall concentration of OA on galactose less than that of fructose by the end of the experiment, both *K. aerogenes* and *Burkholderia* T48 may have been converting more of the available galactose into forming an extra polymeric substance on the monazite to remain in contact with the surface, thus accessing available phosphate. Galactose metabolism plays a crucial role in biofilm formation in stressful conditions and in the case of species of *Burkholderia*, D-galactose is incorporated into branched acetylated heptasaccharide repeating units [60]. This phenomenon has previously been seen in sulfide bioleaching systems with *At. ferrooxidans* [61] as well as in *Bacillus subtilis* [62].

Despite two rounds of gamma irradiation of the monazite ore, the non-inoculated controls had considerable microbial diversity after 7 days however, the reads were low in number in comparison to the inoculant strains. This diversity was not detected in the extraction blanks performed (data not shown). The monazite ore is extremely friable, with small particle size and is easily compacted. It is likely that some organisms were occupying cracks and pits in the ore and were unable to be destroyed. Chemical cleaning and autoclaving of the ore was excluded as both methods will result in surface changes to the ore and overall composition; impacting the ability to assess each species leaching capabilities. Regardless of the carbon source provided, *Burkholderia* T48, *K. aerogenes and P. putida* remained the dominant bacterial species in monazite cultures while cultures inoculated with *G. oxydans* had a varying microbial diversity by day 7. *Burkholderia* sp are generally acknowledged as having significant biocontrol properties [63] as they produce a number of bioactive materials including volatile organic compounds, polyenes, polyynes, siderophores, macrolides, bacteriocins and quinolones [64], so therefore it is not surprising that any microorganisms left on the ore could not proliferate to make a significant contribution if secretion of any of these compounds were present in the media. Similarly, *K. aerogenes* has a probiotic role in the gastrointestinal tract producing substances that deter possible pathogens [65] and *Pseudomonas* species produce up to 795 different bioactive compounds [66]. These species ability to dominate regardless of the carbon source reflect their greater biocontrol properties preventing any of the indigenous microorganisms from establishing to significant levels of detection.

G. oxydans inability to remain as the dominant species in the leaching cultures was unexpected as it does secrete acetic acid and is known to oxidize a variety of carbohydrate sources [67]. Analysis of those organisms that began to proliferate included *Bradyrhizobium* when provided with fructose, several Enterobacteriaceae when given galactose and numerous genera including Bacilli, *Burkholderia*, and *Pseudomonas* when maltose was supplied; all of which are frequently identified as soil inhabitants and found in the uninoculated controls. As the OA produced by *G. oxydans* was not examined due to the poor leaching rates initially detected, a cursory analysis in the future could possibly help to explain the loss of culture prevalence with inspection of the various bioactive compounds in the other three cultures as well as examination of the different OA produced.

5. Conclusions and future directions

Microbial driven solubilization of monazite leading to the liberation of REE into solution resulted from organic acid release,

and was driven by the carbon source provided, with fructose demonstrating the greatest leaching capabilities. The survival of indigenous microbes on the ore after irradiation demonstrates native populations can withstand extreme conditions. Follow up studies will evaluate the changes in leaching actions when purposely non-sterilized ore is used with varying carbon sources as well as investigations into the role of AP in the bioleaching process. The goal will be to understand how the native population, monazite breakdown and phosphate regulation are impacted and if this enables an economically viable recovery process for REE to be established.

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.resmic.2023.104133.

References

[1] Yang X, Makkonen HT, Pakkanen L. Rare earth occurrences in streams of processing a phosphate ore. Minerals 2019;9:262.
[2] Dostal J. Rare earth element deposits of alkaline igneous rocks. Resources 2017;6:34.
[3] USGS. Mineral commodity summaries 2023. In: USG survey; 2023. p. 210.
[4] Lynas Corporation Ltd. The rare earths market. NASDAQ Virtual Investor Conference 2018:1–19.
[5] Corbett MK, Eksteen JJ, Niu XZ, Watkin ELJ. Syntrophic effect of indigenous and inoculated microorganisms in the leaching of rare earth elements from Western Australian monazite. Res Microbiol 2018;169:558–68.
[6] Castro L, Blázquez ML, González F, Muñoz J. Biohydrometallurgy for rare earth elements recovery from industrial wastes. Molecules 2021;26.
[7] Barnett MJ, Palumbo-Roe B, Gregory SP. Comparison of heterotrophic bioleaching and ammonium sulfate ion exchange leaching of rare earth elements from a madagascan ion-adsorption clay. Minerals 2018;8:236.
[8] Hassanien WAG, Desouky OAN, Hussien SSE. Bioleaching of some rare earth elements from Egyptian monazite using *Aspergillus ficuum* and *Pseudomonas aeruginosa*. Walailak J Sci Technol 2014;11:809–23.
[9] Brisson VL, Zhuang WQ, Alvarez-Cohen L. Bioleaching of rare earth elements from monazite sand. Biotechnol and bioengin 2016;113:339–48.
[10] Qu Y, Li H, Wang X, Tian W, Shi B, Yao M, et al. Bioleaching of major, rare earth, and radioactive elements from red mud by using indigenous chemoheterotrophic bacterium acetobacter sp. Minerals 2019;9:67.
[11] Tian Y, Hu X, Song X, Yang AJ. Bioleaching of rare-earth elements from phosphate rock using *Acidithiobacillus ferrooxidans*. Lett Appl Microbiol 2022;75:1111–21.
[12] Reed DW, Fujita Y, Daubaras DL, Jiao Y, Thompson VS. Bioleaching of rare earth elements from waste phosphors and cracking catalysts. Hydromet 2016;166.
[13] Behera BC, Yadav H, Singh SK, Mishra RR, Sethi BK, Dutta SK, et al. Phosphate solubilization and acid phosphatase activity of *Serratia* sp. isolated from mangrove soil of Mahanadi river delta, Odisha, India. J Genet Eng Biotechnol 2017;15:169–78.
[14] Ghosh P, Rathinasabapathi B, Ma LQ. Phosphorus solubilization and plant growth enhancement by arsenic-resistant bacteria. Chemosphere 2015;134:1–6.
[15] Collavino MM, Sansberro PA, Mroginski LA, Aguilar OM. Comparison of in vitro solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote *Phaseolus vulgaris* growth. Biol Fertil Soils 2010;46:727–38.
[16] Figueiredo ART, Özkaya Ö, Kümmerli R, Kramer J. Siderophores drive invasion dynamics in bacterial communities through their dual role as public good versus public bad. Ecol Lett 2022;25:138–50.
[17] Sjöberg V, Grandin A, Karlsson L, Karlsson S. Bioleaching of shale – impact of carbon source. In: Merkel B, Schipek M, editors. The new uranium mining boom: challenge and lessons learned. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012. p. 449–54.
[18] Naseri T, Mousavi SM, Kuchta K. Environmentally sustainable and cost-effective recycling of Mn-rich Li-ion cells waste: effect of carbon sources on the leaching efficiency of metals using fungal metabolites. Waste Manag 2023;157:47–59.
[19] Hoque MS, Broadhurst LM, Thrall PH. Genetic characterization of root-nodule bacteria associated with *Acacia salicina* and *A. stenophylla* (Mimosaceae) across south-eastern Australia. Int J Syst Evol Microbiol 2011:299–309.
[20] Muyzer G, de Waal EC, Uitterlinden AG. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 1993;59:695–700.
[21] Khaleque HN, Shafique R, Kaksonen AH, Boxall NJ, Watkin ELJ. Quantitative proteomics using SWATH-MS identifies mechanisms of chloride tolerance in the halophilic acidophile *Acidihalobacter prosperus* DSM 14174. Res Microbiol 2018;169:638–48.
[22] Yang T-T, Sinai P, Kain SR. An acid phosphatase assay for quantifying the growth of adherent and nonadherent cells. Anal Biochem 1996;241:103–8.
[23] Hogendoorn C, Roszczenko-Jasińska P, Martinez-Gomez NC, de Graaff J, Grassl P, Pol A, et al. Facile arsenazo III-based assay for monitoring rare earth element depletion from cultivation media for methanotrophic and methylotrophic bacteria. Appl Environ Microbiol 2018;84.
[24] Cao X, Xu L, Decho AW, Lead J. A method for cultivation, fractionation, and metal determination to evaluate metal removal by the combination of NPs and bacteria. MethodsX 2021;8:101550.
[25] Gonzalez A, van Lier JB, de Kreuk MK. Effects of mild thermal pre-treatment combined with H_2O_2 addition on waste activated sludge digestibility. Waste Manag 2022;141:163–72.
[26] Hosseini Nasab M, Noaparast M, Abdollahi H, Amoozegar MA. Indirect bioleaching of Co and Ni from iron rich laterite ore, using metabolic carboxylic acids generated by *P. putida, P. koreensis, P. bilaji* and *A. niger*. Hydromet 2020;193:105309.
[27] Jin H, Reed DW, Thompson VS, Fujita Y, Jiao Y, Crain-Zamora M, et al. Sustainable bioleaching of rare earth elements from industrial waste materials using agricultural wastes. ACS Sustain Chem Eng 2019;7:15311–9.
[28] Rombola TH, Pedrinho EA, de Macedo Lemos EG, Goncalves AM, dos Santos LF, Pizauro Jr JM. Identification and enzymatic characterization of acid phosphatase from *Burkholderia gladioli*. BMC Res Notes 2014;7:221.
[29] Margalef O, Sardans J, Fernandez-Martinez M, Molowny-Horas R, Janssens IA, Ciais P, et al. Global patterns of phosphatase activity in natural soils. Sci Rep 2017;7:1337.
[30] Lidbury I, Scanlan DJ, Murphy ARJ, Christie-Oleza JA, Aguilo-Ferretjans MM, Hitchcock A, et al. A widely distributed phosphate-insensitive phosphatase presents a route for rapid organophosphorus remineralization in the biosphere. Proc Natl Acad Sci U S A 2022:119.
[31] Zhang L, Feng G, Declerck S. Signal beyond nutrient, fructose, exuded by an arbuscular mycorrhizal fungus triggers phytate mineralization by a phosphate solubilizing bacterium. Isme j 2018;12:2339–51.
[32] Amaniampong PN, Karam A, Trinh QT, Xu K, Hirao H, Jerome F, et al. Selective and catalyst-free oxidation of D-glucose to D-glucuronic acid induced by high-frequency ultrasound. Sci Rep 2017;7:40650.
[33] Bohm L, Grancic P, Scholtzova E, Heyde BJ, During RA, Siemens J, et al. Adsorption of the hydrophobic organic pollutant hexachlorobenzene to phyllosilicate minerals. Environ Sci Pollut Res Int 2023;30:36824–37.
[34] Maghsoodi M, Jacquin C, Teychene B, Lesage G, Snow SD. Delineating the effects of molecular and colloidal interactions of dissolved organic matter on titania photocatalysis. Langmuir 2023;39:3752–61.
[35] Polubesova T, Sherman-Nakache M, Chefetz B. Binding of pyrene to hydrophobic fractions of dissolved organic matter: effect of polyvalent metal complexation. Environ Sci Technol 2007;41:5389–94.
[36] Lombardo MV, Mirenda M, Bordoni AV, Wolosiuk A, Regazzoni AE. Chemisorption of lanthanide ions on succinate-functionalized mesoporous silica: an in situ characterization by fluorescence. J Colloid Interface Sci 2017;507:139–44.
[37] Wang AQ, Golden TD. Electrodeposition of oriented cerium oxide films. Int J Electrochem 2013;2013:482187.
[38] Nakajima K, Shimizu A, Takekiyo T, Yoshimura Y, Koizumi T. Effect of pressure on the coordination structure of acetate-rare earth complex in water. J Phys Conf 2010;215:12072.
[39] Zabiszak M, Frymark J, Nowak M, Grajewski J, Stachowiak K, Kaczmarek MT, et al. Influence of d-electron divalent metal ions in complex formation with L-tartaric and L-malic acids. Molecules 2021;26.
[40] Kondoh A, Oi T. Interaction of alkaline earth metal ions with carboxylic acids in aqueous solutions studied by 13C NMR spectroscopy 1998;53:77–91.
[41] Mihalache M, Oprea O, Guran C, Holban AM. Synthesis, characterization, and biological activity of some complex combinations of nickel with α-ketoglutaric acid and 1-(o-tolyl)biguanide. Compt Rendus Chem 2018;21:32–40.
[42] Büker L, Dickbreder R, Böttcher R, Sadowski S, Bund A. Investigation of the reaction kinetics of chromium(III) ions with carboxylic acids in aqueous solutions and the associated effects on chromium deposition. J Electrochem Soc 2020;167:162509.
[43] Gyaneshwar P, Parekh LJ, Archana G, Poole PS, Collins MD, Hutson RA, et al. Involvement of a phosphate starvation inducible glucose dehydrogenase in

soil phosphate solubilization by *Enterobacter asburiae*. FEMS Microbiol Lett 1999;171:223–9.
[44] Smirnov A, Esnault C, Prigent M, Holland IB, M-J V. Phosphate homeostasis in conditions of phosphate proficiency and limitation in the wild type and the phoP mutant of *Streptomyces lividans*. PLoS ONE 2015;10.
[45] Bruna RE, Kendra CG, Groisman EA, Pontes MH. Limitation of phosphate assimilation maintains cytoplasmic magnesium homeostasis. Proc Natl Acad Sci U S A 2021:118.
[46] Yang F, Xiang W, Li T, Long L. Transcriptome analysis for phosphorus starvation-induced lipid accumulation in *Scenedesmus sp*. Sci Rep 2018;8:16420.
[47] Belda E, van Heck RGA, José Lopez-Sanchez M, Cruveiller S, Barbe V, Fraser C, et al. The revisited genome of *Pseudomonas putida* KT2440 enlightens its value as a robust metabolic chassis. Environ Microbiol 2016;18:3403–24.
[48] Szczerba H, Komoń-Janczara E, Dudziak K, Waśko A, Targoński Z. A novel biocatalyst, *Enterobacter aerogenes* LU2, for efficient production of succinic acid using whey permeate as a cost-effective carbon source. Biotechnol Biofuels 2020;13:96.
[49] Sievers M, Swings J. *Gluconobacter*. Bergey's Manual of Systematics of Archaea and Bacteria, pp. 1-9.
[50] Woods DE, Sokol PA. The genus *Burkholderia*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E, editors. The prokaryotes: volume 5: proteobacteria: alpha and beta subclasses. New York, NY: Springer New York; 2006. p. 848–60.
[51] Weusthuis RA, Visser W, Pronk JT, Scheffers WA, van Dijken JP. Effects of oxygen limitation on sugar metabolism in yeasts: a continuous-culture study of the Kluyver effect. Microbiol 1994;140:703–15.
[52] Hülsmann A, Lurz R, Scheffel F, Schneider E. Maltose and maltodextrin transport in the thermoacidophilic gram-positive bacterium *Alicyclobacillus acidocaldarius* is mediated by a high-affinity transport system that includes a maltose binding protein tolerant to low pH. J Bacteriol 2000;182:6292–301.
[53] Liao C-H, Yao L, Xu Y, Liu W-B, Zhou Y, Ye B-C. Nitrogen regulator GlnR controls uptake and utilization of non-phosphotransferase-system carbon sources in actinomycetes. Proc Natl Acad Sci USA 2015;112:15630–5.
[54] Park J-T, J-H S, Tran PL, Hong I-H, Yong H-U, Oktavina EF, et al. Role of maltose enzymes in glycogen synthesis by *Escherichia coli*. J Bacteriol 2011;193:2517–26.
[55] Woo HM, S N, Seibold GM, Willbold S, Eikmanns BJ, Bott M. Link between phosphate starvation and glycogen metabolism in *Corynebacterium glutamicum*, revealed by metabolomics. Appl Environ Microbiol 2010;76:6910–9.
[56] Ames GF-L. Bacterial periplasmic transport systems: structure, mechanism, and evolution. Annu Rev Biochem 1986;55:397–425.
[57] Iskandar CF, Cailliez-Grimal C, Borges F, Revol-Junelles A-M. Review of lactose and galactose metabolism in Lactic Acid Bacteria dedicated to expert genomic annotation. Trends Food Sci Technol 2019;88:121–32.
[58] Saavedra A, Aguirre P, Gentina J. Biooxidation of iron by *Acidithiobacillus ferrooxidans* in the presence of D-galactose: understanding its influence on the production of EPS and cell tolerance to high concentrations of iron. Front Microbiol 2020;11:759.
[59] Wu Y, Chu W, Yang J, Xu Y, Shen Q, Yang H, et al. Metabolic engineering of *Enterobacter aerogenes* for improved 2,3-butanediol production by manipulating NADH levels and overexpressing the small RNA RyhB. Front Microbiol 2021;12:754306.
[60] Ferreira AS, Silva IN, Oliveira VH, Cunha R, Moreira LM. Insights into the role of extracellular polysaccharides in *Burkholderia* adaptation to different environments. Front Cell Infect Microbiol 2011;1:16.
[61] Moncayo EA, Debut A, Vizuete K, Jumbo-Flores D, Aguirre P. Sticky bacteria: combined effect of galactose and high ferric iron concentration on extracellular polymeric substances production and the attachment of *Acidithiobacillus ferrooxidans* on a polymetallic sulfide ore surface. Front Microbiol 2022;13.
[62] Chai Y, Beauregard PB, Vlamakis H, Losick R, Kolter R. Galactose metabolism plays a crucial role in biofilm formation by *Bacillus subtilis*. mBio 2012;3:e00184-12.
[63] Depoorter E, Coenye T, Vandamme P. Biosynthesis of ditropolonyl sulfide, an antibacterial compound produced by *Burkholderia cepacia* complex strain R-12632. Appl Environ Microbiol 2021;87:e0116921.
[64] Rodríguez-Cisneros M, Morales-Ruíz LM, Salazar-Gómez A, Rojas-Rojas FU, Estrada-de los Santos P. Compilation of the antimicrobial compounds produced by *Burkholderia sensu stricto*. Molecules 2023;28(4):1646.
[65] Huber TW. Enterobacter. In: Robinson RK, editor. Encyclopedia of food microbiology. Oxford: Elsevier; 1999. p. 598–603.
[66] Simionato AS, Cano BG, Navarro MOP, Tavares ER, Ribeiro RA, Hungria M, et al. Whole-Genome sequence of bioactive compound-producing Pseudomonas aeruginosa strain LV. Microbiol Res Announce 2021;10:e01120-20.
[67] Prust C, Hoffmeister M, Liesegang H, Wiezer A, Fricke WF, Ehrenreich A, et al. Complete genome sequence of the acetic acid bacterium *Gluconobacter oxydans*. Nat Biotechnol 2005;23:195–200.