

Interactions of phosphate solubilising microorganisms with natural rare-earth phosphate minerals: a study utilizing Western Australian monazite

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Abstract Many microbial species are capable of solubilising insoluble forms of phosphate and are used in agriculture to improve plant growth. In this study, we apply the use of known phosphate solubilising microbes (PSM) to the release of rare-earth elements (REE) from the rare-earth phosphate mineral, monazite. Two sources of monazite were used, a weathered monazite and mineral sand monazite, both from Western Australia. When incubated with PSM, the REE were preferentially released into the leachate. *Penicillium* sp. released a total concentration of 12.32 mg L⁻¹ rare-earth elements (Ce, La, Nd, and Pr) from the weathered monazite after 192 h with little release of thorium and iron into solution. However, cultivation on the mineral sands monazite resulted in the preferential release of Fe and Th. Analysis of the leachate detected the production of numerous low-molecular weight organic acids. Gluconic acid was produced by all microorganisms; however, other organic acids produced differed between microbes and the monazite source provided. Abiotic leaching with equivalent combinations of organic acids resulted in the lower release of REE implying that other microbial processes are playing a role in solubilisation of the monazite ore. This study demonstrates that microbial solubilisation of monazite is promising; however, the extent of the

reaction is highly dependent on the monazite matrix structure and elemental composition.

Keywords Monazite · Rare-earth elements · Phosphate solubilising microorganisms · Bioleaching · Recovery rates

Introduction

Nested within the periodic table are 15 elements with atomic numbers ranging from 57 to 71 commonly referred to as the ‘Lanthanides’ [1]. Demonstrating similar physicochemical properties to these Lanthanides is Scandium (21) and Yttrium (39), and together, these 17 elements, commonly found in the same mineral assemblages [2], are convened together to form what are called the rare-earth elements (REE). REEs, although moderately abundant in the earth’s crust, rarely occur in concentrated forms, making them economically challenging to recover with complex, time intensive, conventional mining approaches. Their distinctive electronic, optical, and magnetic properties have enabled them to be fundamental components of many imperative technologies, including mobile phones, superconductors, hybrid vehicles, and manufacturing industries [2]. The importance of REEs in a technologically dependent society cannot be understated as their unique characteristics do not permit replacement by any other metals or synthetic substitutes. As consumer demand for these products escalates, REE requirements throughout the world are expected to increase, placing stress on uncertain supply routes, and already heavily burdened markets [3]. Despite more than 200 known REE-bearing ores [2], only three are considered to be primary REE mineral ores feasibly allowing for the extraction of REEs: bastnasite, monazite, and xenotime [4]. Acquisition of REEs obtained via the mining

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of bastnasite (carbonate rich-REEs) and monazite (thorium–phosphate mineral) [5] is predominately performed in the USA and China, contributing to resource scarcity and restricting commercial exploitation. Separation of the REEs from the ore matrix is expensive and complex and involves different processes including but not restricted to: grinding, sifting, and gravity concentration [6], low intensity magnetic separation [7], and flotation [8] followed by chemical treatment with either acidic or alkaline reagents, under high temperatures and varying lengths of time [9]. As rare-earth ores may contain concentrations of thorium and uranium up to 10% of the total ore matrix [10], potential radioactive waste products created during leaching lead to complicated disposal protocols or contamination of the REE concentrate. As phosphate precipitation is common in many natural systems [11], REEs can be found bound within igneous phosphate rock, placer deposits, and mineral sands [12].

Phosphorus (P), a vital element for all living organisms, is necessary for sustaining levels of key cellular reactions, carbon, and amino acid metabolic processes, production of ATP, enzyme regulation, and energy transfer [13]. Microorganisms that have the ability to obtain phosphorus from insoluble sources, known as phosphate solubilising microorganisms (PSMs), can extricate insoluble P into a soluble form by acidification, chelation, and exchange reactions [14]. Commercial applications of this naturally occurring phenomena are currently exploited by the agricultural industry [15] sidestepping the expensive addition of phosphate to soils. However, this technique is currently underutilised for the recovery of minerals embedded in phosphorus rock. Microbial solubilisation of phosphate from REE phosphate ores has been reported [16, 17] with microbial colonization of the mineral surface suggesting biosolubilisation, attributed to electron transfer via the secretion of low molecular weight organic acids, such as gluconic acid, citric acid, oxalic acid, formic acid, butyric acid, and malic acid [18]. Research conducted by Qu and Lian [19], Brisson et al. [20], and Shin [21] demonstrates that bioleaching of monazite ores by PSM belonging to the genera *Pseudomonas*, *Enterobacter*, *Serratia*, *Pantoea*, *Bacillus*, and *Aspergillus* provided with an organic carbon source for growth is possible; however, recovery rates of REEs are variable. The application of microbial leaching is a proven technology for the recovery of copper, nickel, zinc, and cobalt from low-grade ores [22]. It is a well-established industrial technique that can decrease extraction expenditures [23], as well as decrease the generation of damaging environmental waste products that arise when traditionally mining methodologies are engaged. The identification and characterisation of phosphate solubilising microbes capable of phosphate mobilization and REE release have the potential to reduce recovery costs, decrease environmental

impacts, and secure REE for future advanced technological applications.

For the application of PSMs to industrial bioprocessing of rock phosphate containing rare-earth elements, initial experiments conducted here focussed on examining PSMs previously identified in the literature as having phosphate solubilising capabilities and examining their ability to solubilise the phosphate present in insoluble tricalcium phosphate [Ca_3PO_4 , (TCP)] or a monazite source. In an effort to establish whether microbial mobilization of inorganic P would allow continued microbial sustenance and release REE from the ore, we report on the biogeochemical changes observed, including changes in pH, organic acid production, soluble P levels, and REE concentrations detected in the leachate.

Methods

Microbes used in this study

The bacterial and fungal isolates used in this study are listed in Table 1.

DNA extraction, amplification, sequencing and identification

Bacteria

Pure isolates (Table 1) were grown overnight in 2 mL LB broth with rotation at 160 rpm at either 30 or 37 °C depending on species tested. Cells were centrifuged at 12,000×g for 20 min, DNA extracted using the MoBio DNA extraction kit (MO BIO Laboratories, Inc) following manufacturer's instructions. The 16S rRNA gene region was amplified by PCR using Bioline MyTaq with 27F, 800F, 802R, and 1492R universal bacterial primers [24].

Fungi

Fungal isolates (Table 1) were inoculated onto Sabouraud agar (SAB) and incubated for 5 days at 30 °C. DNA was extracted from a 5 mm diameter section of fungi using the Lysing matrix A tube (MP Biomedicals) with 500 µL of lysis buffer [25] in a ribolyser (MP Biomedicals) for 30 s at a speed of 4.0. RNase A (20 µL, Sigma–Aldrich)(1 mg/ml) was added and incubated at 37 °C for 5 min. NaCl (165 µL of 5 M) was added, mixed by inversion, and centrifuged at 21,920×g for 20 min at 4 °C. The supernatant was mixed with 800 µL of Phenol:Chloroform:Isopropanol (25:24:1, Sigma–Aldrich) and mixed by inversion. The sample was centrifuged for 5 min at 21,920×g and the top layer

Table 1 Microbial isolates used in this study

Species and strain	References	NCBI Genbank accession/culture collection	Growth temperature °C
Bacteria			
<i>Azospirillum brasilense</i> DSMZ 1843	Rodriguez et al. [69]	NR_117478.1	37
<i>Bacillus megaterium</i> DSMZ 2894	Feng et al. [17]	KP036928.1	37
<i>Paenibacillus polymyxa</i>		KT783525	37
<i>Bacillus subtilis</i>		KJ87280.1	37
<i>Burkholderia glathei</i>	Kim et al. [67]	DSMZ 50014	30
<i>Enterobacter aerogenes</i> ATCC 13,048	Chung et al. [60]	KF516237.1	37
<i>Klebsiella oxytoca</i>	Walpolo et al. [59]	AB004754	37
<i>Klebsiella pneumoniae</i>	Chung et al. [60]	AY17656	37
<i>Pantoea agglomerans</i> DSMZ 8570	Feng et al. [17]	AM421978.1	30
<i>Pseudomonas aeruginosa</i>		DSMZ 50071	30
<i>Pseudomonas putida</i> DSMZ 1693	Malboobi et al. [68]	FM163468.1	30
<i>Serratia marcescens</i>	Ben Farhat et al. [71]	AB909432.1	37
Fungi			
<i>Aspergillus niger</i> DSMZ 821	Schneider et al. [70]	ATCC 9142	30
<i>Aspergillus tubigenensis</i>		KT033072.1	30
<i>Penicillium</i> sp		JN7982529.1	30

removed and the process repeated. The supernatant was transferred to a new tube, and an equal amount of isopropanol added and incubated at -20°C for 2 h. Tubes were centrifuged at $21,920\times g$ for 20 min at 4°C . The supernatant was discarded, the pellet washed twice in 70% ethanol, centrifuged, and then air-dried and resuspended in 40 μL of TE buffer. Fungal ITS regions were amplified by PCR using Bioline MyTaq with the ITS4 and ITS5 universal fungal primers [26]. All PCR products were purified by Invitrogen PCR clean up kit. Sequencing was performed by the State Agricultural Biotechnology Centre, Murdoch, W.A. Sequences were compared to those in the NCBI database and isolate identity confirmed.

Bioleaching

Preparation of inocula

Bacterial species were grown in LB broth with rotation at 160 rpm, at either 30 or 37°C depending on species tested, to exponential phase and harvested by centrifugation at $12,000\times g$ for 10 min. Cells were resuspended in sterile Tris–HCl buffer (pH 7), centrifuged, and washed twice more to remove any traces of phosphate. Cells were resuspended to a density of 1×10^8 CFUs/mL in modified Pivovskaya (PVK) media [27] with 3% w/v glucose and pH 7. Inoculating PVK media with 5 mm plugs taken from SAB plates grew fungal isolates.

Monazite sample preparation and composition analysis

Two monazite samples from Western Australia were obtained for leaching analysis. A monazite concentrate sourced from the Busselton Mineral Sands deposit, Western Australia (Cable Sands Pty Ltd) referred to as CSM, and a weathered lateritic monazite ore from the Mt Weld mine (Lynas Corporation) referred to as MWM. CSM particulate size was 90–200 μm , and the monazite concentrate was diluted 1:10 with Silica flour to obtain a safe Th/U working concentration. MWM was ground initially by a rod mill, then pulverized in a ring mill and finally sieved to 1–35 μm in size. Both ores were subsequently gamma irradiated at 50 kGy for 11 h (ChemCentre, Western Australia) to ensure sterility. The mineralogical data of both ores were obtained by X-ray diffraction and elemental composition determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (performed by CSIRO Minerals, Waterford, WA). Scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX) was performed on a Zeiss Evo 40XVP SEM with an Oxford Instruments SiLi X-ray detector and Inca operating software (John de Laeter Centre, Curtin University, WA).

Bioleaching experiments

All leaching experiments were conducted in triplicate and included an abiotic control in modified PVK media. The initial pH was adjusted to approximately 7 with 1 N NaOH and the flasks weighed prior to and after autoclaving. Each

flask was inoculated to a concentration of 10^8 cells mL^{-1} and incubated for 192 h at 130 rpm at either 30 or 37 °C depending on species tested.

Tricalcium phosphate

TCP (1% w/v) was added to modified PVK media prior to autoclaving. Soluble phosphate concentration was determined after autoclaving by following Murphy and Riley [28] colourimetric method.

Monazite ore

MWM (0.5% w/v) or 5% CSM w/v was added to flasks containing modified PVK media after autoclaving. Soluble phosphate was determined via colourimetric analysis to assess the amount of P released upon ore addition to liquid. The weight of the ore was determined at 192 h (experimental end) for all bacterial species.

Abiotic leaching with organic acids

MWM (0.5% w/v) was added to modified PVK media, without glucose, after autoclaving, and the following combinations of organic acids added: gluconic acid (0.24 mM), acetic acid (2.72 mM), citric acid (0.122 mM) to mimic those produced by *Penicillium sp* and *Aspergillus sp.*, gluconic acid (0.007 mM), formic acid (0.007 mM), citric acid (0.03 mM) and malic acid (0.1 mM) to mimic those produced by *Enterobacter*, and gluconic acid (0.007 mM), formic acid (0.0042 mM), citric acid (0.226 mM), malic acid (0.17 mM), and acetic acid (0.13 mM) to mimic those produced by *Pseudomonas* and *Pantoea*.

Analysis

Samples were taken at 0, 24, 144, and 192 h, and pH determined (Ionode IJ series pH probe). Samples were then filtered [0.20 μm (Satorius)] and assayed for soluble PO_4^{-3} by colourimetric method analysis. Evaporation rates were determined by weighing each flask after sampling. The concentrations of P were corrected for decreases in fluid volume due to evaporation and the sampling process. Separation and identification of organic acids produced were determined by a high-performance liquid chromatography (HPLC) instrument (Agilent 1200) coupled with a diode array detector (DAD, Agilent). Injection volume was set as 50 μL for the samples. Compound separation was achieved with a C18 reverse phase column (Agilent, 5 μm , 4.6 \times 250 mm). The isocratic elution flow rate was 1.0 mL min^{-1} . The mobile phase consists of 70% methanol and 30% phosphate buffer (pH 2.0). A detection wavelength of 220 nm was used. Identification and concentration of

organic acids were determined by comparing the retention times and peak areas of chromatograms of the samples with standards. Organic acid identity was further confirmed by High-Resolution Mass Spectrometry. Organic standards included gluconic, malic, formic, butyric, citric, acetic, lactic, oxalic, and pyruvic. REEs (Ce, La, Nd, and Pr), and Th and U in solution were analysed by ICP-EOS (CSIRO Minerals, Waterford).

pH calculations

The concentration of protons generated by the organic acid concentrations as determined by HPLC at 192 h was calculated assuming a less than 10% dissociation for weak acid functional groups. Malic acid (diprotic) and citric acid (triprotic) were treated as individual monoprotic acids for simplicity [29]. Individual pKa values for each acid were employed:

$$[H^+] = \sum_{i=1}^n \sqrt{\text{pKai}[\text{organic acid}]}$$

Due to these simplifications, it is likely that $[H^+]$ concentrations have been overestimated.

Results

Phosphate solubilisation from tricalcium phosphate

Numerous bacterial and fungal strains from distinctly distant genera have been reported in the literature to have phosphate solubilising capabilities [30]. In this study, all isolates tested released phosphate into solution from insoluble TCP. The phosphate concentration in the media rapidly increased over the first 48 h of incubation with a continued slow increase until the termination of the experiment at 192 h. The percentage phosphate leached from the TCP varied greatly between isolates with *Aspergillus niger*, *A. tubigenis*, and *Penicillium sp.* leaching 75, 70, and 49% of the available phosphate, respectively (Fig. 1). Notably, all three fungal species demonstrated complete clearing of the media with large pellets formed. Of the bacterial species tested, *K. pneumoniae* was the most effective (57% released), followed by *E. aerogenes* (43%), *Ps. aeruginosa* (17%), *B. megaterium* (16%), *Ps. putida* (13%), and *P. agglomerans* (10%) (Fig. 1). Unlike the fungal isolates tested, all flasks inoculated with bacteria remained turbid, indicating incomplete solubilisation of the TCP in the media. There was no significant difference in the phosphate leaching of remaining strains from the abiotic controls; these include *A. brasilense* (8%), *B. glathei* (9%), *B. subtilis* (7%), *P. polymyxa* (8%), *S. marscesnes* (9%). Abiotic

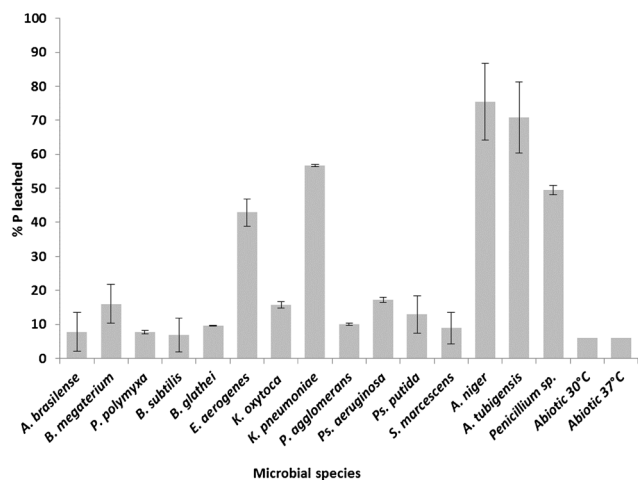


Fig. 1 Percentage of phosphate solubilised from tricalcium phosphate after 192 h incubation with microbial isolates. Data are averages ± SE of two experiments each with three biological replicates

controls at both 30 and 37 °C leached 6% of the available phosphate into solution. These isolates were excluded from further studies on monazite ores.

P solubilisation from monazite bioleaching

Upon addition of the monazite source to the modified PVK media, spontaneous release of P at time 0 ranged from 0.8 to 1.7 mg L⁻¹. After 144 h incubation with a microbial isolate, P levels dropped to an average of 0.43 mg L⁻¹ ± 0.11 and remained steady until the end of the experiment (data not shown).

Elemental composition of monazite ores

The composition of the two monazite ores is shown in Table 2. While they had similar percentages of the REEs, Ce, La, and Nd, the Cable Sands monazite contained significantly greater levels of Th and U. The MWM contained three times the levels of Fe as the Cable Sands monazite. XRD analysis of the ores determined monazite—((Ce, La, Nd, Th)PO₄) and zircon (ZrSiO₄) composition for CSM and monazite ((Ce, La, Nd, Th)PO₄) and Florencite (Ce, La, Nd)Al³⁺(PO₄)₂(OH)₆ for MWM.

REE release during bioleaching (REE solubility)

Concentrations of REE in the leachate after biotic incubation were assessed to determine release rates and solubilising potential of the microorganisms. The final concentration of REE leached from the MWM is shown in Fig. 2. *Penicillium sp* was the most efficient at leaching REEs with a total of 12.32 mg L⁻¹ REE (Ce, La, Nd, and Pr) released after incubation for 192 h. Concentrations of REE leached by the other fungal species, *A. niger* and *A. tubigenis*, were 24 times lower than *Penicillium sp*. (0.44 and 0.43 mg L⁻¹, respectively). Bacterial species, *P. agglomerans*, *E. aerogenes*, and *Ps. putida* released bound REEs into solution at similar concentrations (1.63, 1.93, and 1.45 mg L⁻¹, respectively). The remaining bacterial species released lower levels of total REEs: *K. pneumoniae* (0.56 mg L⁻¹), *K. oxytoca* (0.42 mg L⁻¹), and *B. megaterium* (0.58 mg L⁻¹). When CSM was provided as a phosphate source, all species showed preferential leaching of thorium and iron (Fig. 3) over REE, with no concentration of Ce, La, or Nd recorded above 0.02 mg L⁻¹ for any species tested. Between 4 and 30 mg of MWM ore was lost over the duration of the experiment when incubated with the bacterial isolates.

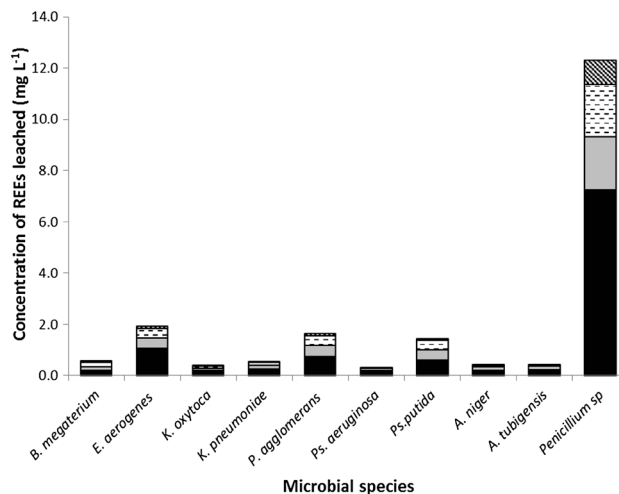


Fig. 2 Total concentration of REEs, Cerium (black coloured box), Lanthanum (gray coloured box), Neodymium (box with single line), and Praseodymium (slashed box) released into leachate after 192 h inoculation with microbial species. Data are averages of three experiments each with three biological replicates

Table 2 ICP-EOS analysis for elemental composition of Monazite ores used in this study

Sample ID	Ce	La	Nd	Th	U	Y	Pr	Fe	P	Ca	Mg	Si	Ti	Zr	
Concentration content (%)															
Mt weld monazite	12.6	10.1	6.25	0.162	<0.003	0.165	2.10	1.23	9.93	1.75	0.199	1.96	0.554	0.031	
Cable sand monazite	14.0	9.41	5.46	4.98	0.194	0.851	0.184	0.458	9.45	0.693	0.079	1.72	0.325	1.80	

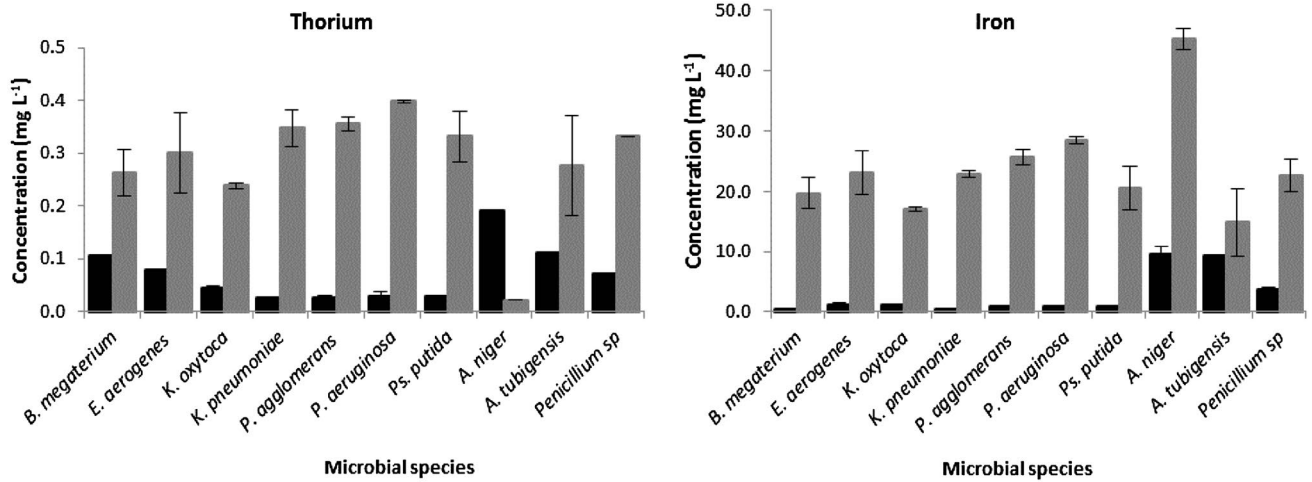


Fig. 3 Final levels of thorium and iron released into solution from Mount Weld Monazite (black coloured box) and Cable Sands Monazite (gray coloured box) after incubation with microbial species for

192 h. Data are averages \pm SE of three experiments each with three biological replicates

Fig. 4 Scanning electron micrographs of undiluted Cable Sands Monazite particles demonstrating visual structural differences amongst particles of the same sample. **a** Rare-earth element phosphate with surface iron and pitting. **b** Zircon. **c** Clay-like particle

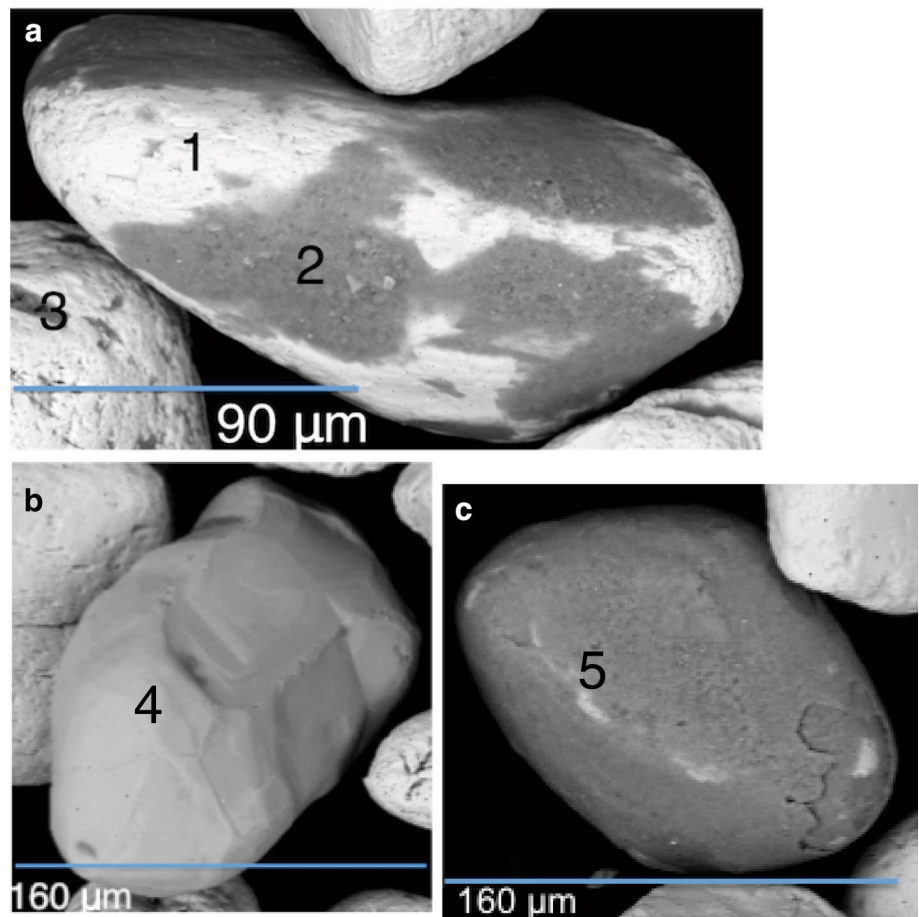
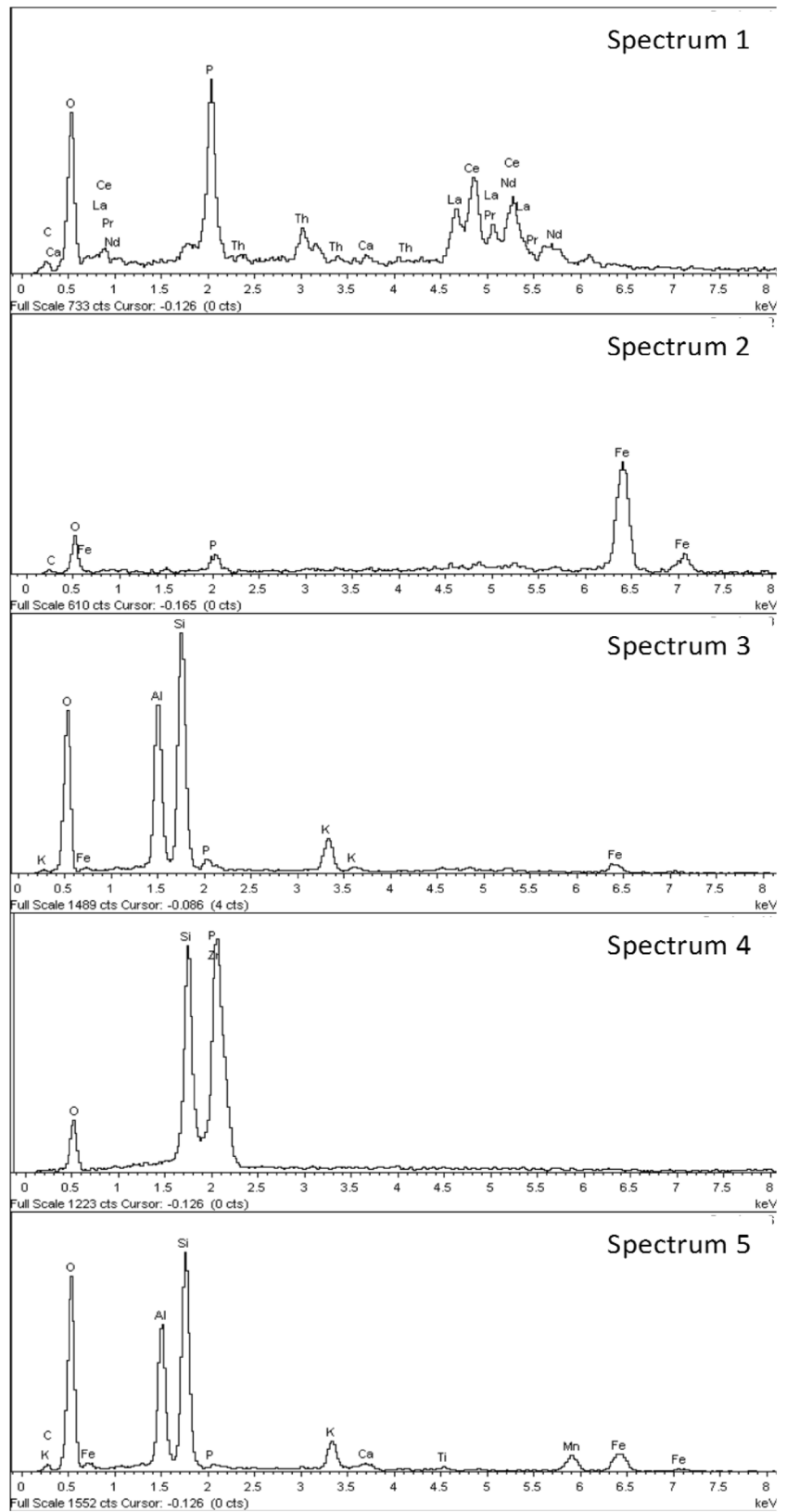


Fig. 5 Scanning electron microscopy with energy dispersive X-ray (SEM-EDX) spectral markers 1, 2, and 3 located on surface of Cable Sands monazite REE particle (Fig. 4a). SEM-EDX of Zircon particle (Fig. 4b, spectrum #4) and aluminium silicate clay mineral (Fig. 4c, spectrum #5). *Y-axis* represents number of counts, and the *X-axis* depicts the energy level (keV) of those counts



SEM examination of monazite ores

The surface compositions of the two ores were examined by SEM-EDX. The CSM is a heterogeneous ore (Fig. 4a), as along with phosphate bound REEs (Fig. 5, spectrum #1), numerous iron coverings (spectrum #2), and pits (spectrum #3) were detected on the surface of the ore particle. Additional particles containing no surface REEs were also recorded (Fig. 4b), composed of Zr and Si (spectrum #4) with other particles demonstrating clay, such as elemental compositions (Fig. 4c) with aluminium silicates trace amounts of iron, calcium, titanium, and manganese (spectrum #5). Comparatively, analysis of the MWM showed no zircon, silicates, or iron coverings (Fig. 6a). All particles examined had a homogenous surface elemental composition (Fig. 6b) of REE and P. The lack of surface iron or traces of thorium with the MWM reflects the low levels detected in the leachate after microbial incubation.

pH changes

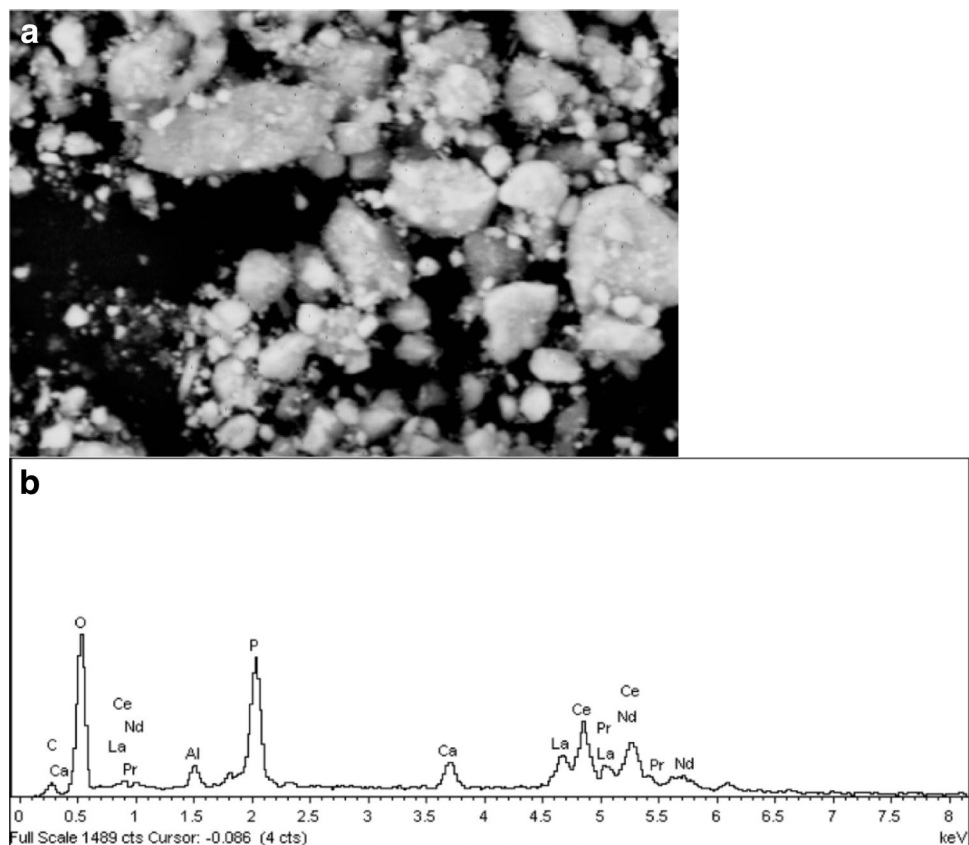
After autoclaving and addition of sterile glucose, the initial pH of all isolates on either MWM or CSM was 6.6 ± 0.1 at time 0. After 24 h with CSM, all bacterial species demonstrated a decrease in pH to below 4.6, finishing between 2.6 and 4.12 by 192 h (192 h data depicted in Fig. 7). Fungal

isolate pH remained high after 24 h (between 5 and 6), but steadily decreased reaching 1.75–3 by 192 h (Fig. 7). Bacterial isolates on MWM after 24 h had pH ranges from 3.9 to 5.7 demonstrating a greater difference in pH change than observed on CSM; however, after 192 h, pH levels were similar across the strains (3.1–3.8) (Fig. 7). Fungal isolate pH ranged from 4.6 to 5.5 after 48 h, falling to 1.8–2.17 after 192 h.

Organic acid production

All microorganisms used in this study produced gluconic acid at varying concentrations. The suite of other organic acids produced, and the concentrations they were produced at differed based on the monazite source provided. In addition to gluconic acid, the bacterial species produced acetic, formic, malic, and citric acids. When grown on MWM, the fungal species produced gluconic and acetic acids at higher concentrations than the bacterial species (Table 3). Growth on CSM resulted in production of fewer organic acids, with no formic acid detected for any microbial species. The concentration of gluconic acid produced by the bacterial species when grown on CSM was greater than when grown on MWM; however, there was a greater variation in the range of organic acids produced by the bacterial species when

Fig. 6 a, b Scanning electron micrograph of Mount Weld monazite particles (**a**) and corresponding scanning electron microscopy with energy dispersive X-ray output (**b**). *Y-axis* represents number of counts, and the *X-axis* depicts the energy level (keV) of those counts



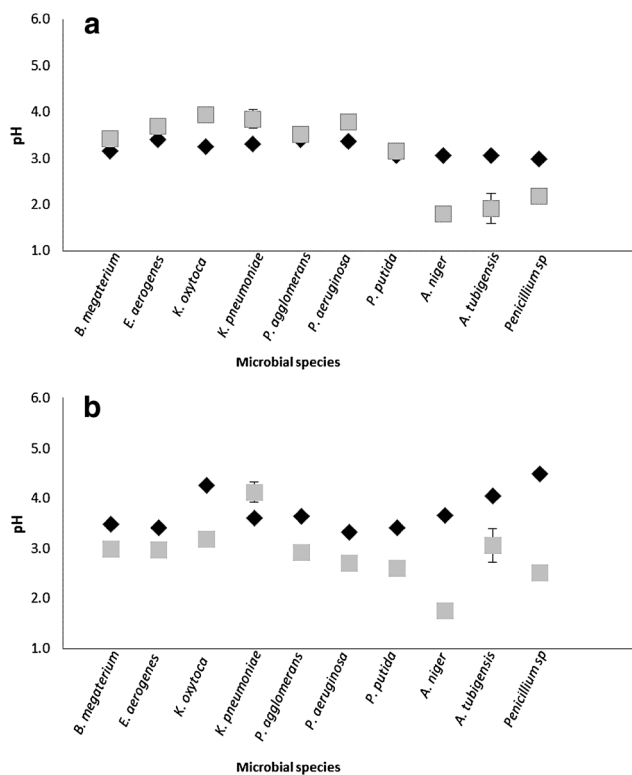


Fig. 7 Comparison of observed pH (gray coloured box) with the calculated pH (filled diamonds) based on the concentration of the organic acids detected by HPLC. Analysis of organic acids after 192 h, incubation with either Mount Weld monazite (a) or Cable Sands monazite (b). Data are averages \pm SE of three experiments (a) or two experiments (b) each with three biological replicates

grown on MWM. For the fungal species, citric acid was only detected in *Penicillium sp.* grown on MWM.

Concentrations of organic acids identified by HPLC were converted to $[H^+]$ generated and assessed against observed pH at 192 h to determine whether enough acid had been produced to reach pH levels recorded.

Calculated $[H^+]$ generated within the bacterial systems inoculated on MWM demonstrated production of high enough concentrations of acid to reach the measured pH levels. Bacterial species inoculated on CSM produced less calculated acid with the pH not reaching the observed levels at 192 h. The greatest difference between calculated pH and observed pH was demonstrated by all three fungal species, each of which had a much lower actual pH than what was calculated based on organic acid productions on both MWM and CSM (Fig. 7).

Abiotic leaching with organic acids

Abiotic leaching with the same ratios of organic acids produced by the fungal species resulted in 0.6 mg L^{-1} of REEs in total detected in the leachate, with 0.04 mg L^{-1} of thorium, and undetectable levels of iron ($<0.02 \text{ mg L}^{-1}$) (Fig. 8). Total REEs with abiotic organic acid leaching are greater than those seen with *A. niger* (0.44 mg L^{-1}) and *A. tubigensis* (0.43 mg L^{-1}), but 18 times less than what was recorded with the *Penicillium* species. The thorium levels (0.04 mg L^{-1}) mobilized from the MWM by abiotic leaching were 3.8 times lower than when incubated with *A. niger* and 2.75 times lower than *A. tubigensis*. Abiotic addition of organic acids based on those produced by bacteria resulted in lower REE leachate levels than recorded with any bacterial species tested, with the highest amount (0.208 mg L^{-1}) resulting from organic acid mix number 1. Organic acid mix #2 resulted in only 0.096 mg L^{-1} total REEs detected in the leachate. As seen with the abiotic fungal system, low Th levels were detected with organic mix #1 (0.08 mg L^{-1}) and undetectable levels with organic acid mix 2 ($<0.02 \text{ mg L}^{-1}$). Both organic acid mix #1 and #2 had undetectable levels of iron ($<0.02 \text{ mg L}^{-1}$) in the leachate.

Table 3 Maximum organic acid concentration (mM) reached after 192 h days of incubation in the presence of Mount Weld monazite (MWM) and, Cable Sands monazite (CSM). Results are the mean of three biological replicates each with three technical replicates

Microbial species	Gluconic		Acetic		Formic		Citric		Malic	
	MWM	CSM	MWM	CSM	MWM	CSM	MWM	CSM	MWM	CSM
<i>B. megaterium</i>	0.0037	0.0642	0.438	0	0.0021	0	0.0934	0	0.285	0.012
<i>E. aerogenes</i>	0.0071	0.114	0	0	0.0078	0	0.0336	0	0.1012	0.13
<i>K. oxytoca</i>	0.0017	0.0123	0	0	0.0037	0	0.0307	0	0.474	0
<i>K. pneumoniae</i>	0.005	0.0045	0	0.1082	0.0037	0	0.084	0	0.081	0
<i>P. agglomerans</i>	0.0073	0.054	0	0.069	0.0042	0	0.0336	0	0.1	0
<i>Ps. aeruginosa</i>	0.0049	0.0467	0	0.0825	0.0053	0	0.0352	0.059	0.16	0
<i>Ps. putida</i>	0.0042	0.0349	0.129	0.086	0.0042	0	0.226	0.027	0.169	0
<i>A. niger</i>	0.274	0.1955	1.4	0	0.0029	0	0	0	0.038	0
<i>A. tubigensis</i>	0.237	0.0321	2.256	0	0	0	0	0	0	0
<i>Penicillium sp.</i>	0.162	0.0122	1.47	0	0	0	0.112	0	0	0

MWM Mount Weld monazite, CSM Cable Sands monazite

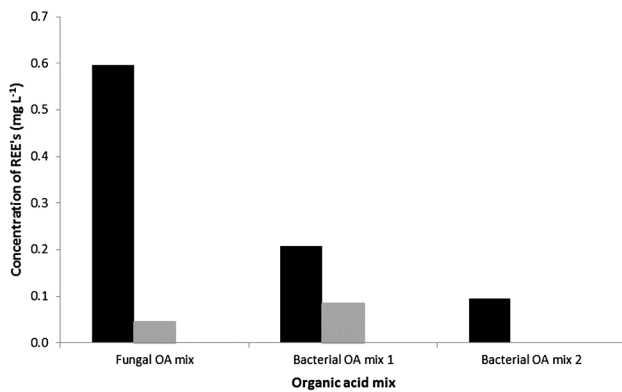


Fig. 8 Final levels of total REEs (black coloured box), thorium (gray coloured box), and iron (slashed box) released into solution from Mount Weld monazite after incubation with three abiotic organic acid mixes for 192 h. Data are averages two experiments each with three biological replicates

Discussion

Traditional REE retrieval methods enable high levels of REE recovery from various ore concentrates (>90%); however, newer and greener methods of REE extraction and recycling [31] are in high demand with a focus on bioleaching, solid-phase extraction [32], and UV light separations [33]. Examination of the use of PSM for REE recovery is a newly emerging field with a number of studies demonstrating varying degrees of success with a range of REE ores, microorganisms, temperatures, and lengths of experimental time resulting in leaching efficiencies in between 0.1–25% [19–21].

This study successfully demonstrated the release of REE from a weathered monazite source when incubated with both bacterial and fungal isolates. Cerium was leached in the highest concentration by all microbial species tested on MWM (as was expected due to its high percentage in the ore). While Lanthanum constituted 10.1% of the MWM and Nd, 6.25%, the levels of the two elements in the leachate after incubation with *E. aerogenes*, *P. agglomerans*, *Ps. putida*, and *Penicillium sp.* for 192 h were often similar, with Nd levels approximately 87–97% of those recorded for La. Incubation with *B. megaterium* and *Ps. aeruginosa* leached Nd at levels 20% greater than La, even though the total amounts of leached REEs were low. The only isolates that produced REEs leachate ratios according to their composition in the ore were the *Aspergillus* isolates, as levels of Nd were just under half those recorded with La. Similarly, a discrepancy was also recorded with the concentration of Praseodymium in the leachate for all species bar *Penicillium sp.* The isolates that leached higher levels of REEs recorded preferential leaching of Ce, La, and Nd over Pr, whereas those isolates where only low quantities of REEs

were detected in the leachate and levels of all REEs were proportional to their concentrations in the ore. As Pr is chemically similar to the other rare-earth elements, the difference in leaching ratios is interesting.

Levels of REEs (Ce, La, and Nd) leached from the CSM were never greater than 0.02 mg L⁻¹. This may be attributed to the 10:1 dilution with silica flour that was necessary to work within radiation safety levels or the larger particle size of CSM. MWM surface area was greater than the CSM due to smaller particle sizes, preferentially allowing more colonization and microbial attachment for P mobilisation and consequently greater release of REE into the leachate. Not only was the release of REE into the leachate noticeably different between the two ores, but also the release of ‘contaminants’, such as thorium and iron. As the thorium concentration in the CSM matrix is 5%, it was unsurprising to discover it at detectable levels in the leachate in comparison with the MWM where it only constituted 0.16% of the total mass. Although Ce, La, and Nd concentrations in the CSM ore were all greater than 5% (Table 2) and should have resulted in larger amounts detected in the leachate, the ore matrix has had a strong effect on elemental release during solubilisation resulting in no REE concentration above 0.02 mg L⁻¹ for any species tested. Contrastingly, Fe makes up 0.46% in the CSM and 1.23% in MWM, but much greater Fe levels were detected in the CSM leachate, up to 45 mg L⁻¹ with *A. niger* (Fig. 3). As can be seen occasionally with gold particles from pyritic sources, hydrated iron oxides form natural coatings around the particles, which are detrimental to gold recovery [34]. The presence of surface iron in the CSM (Fig. 4a) and release of bound Fe during P mobilisation or by microbial organic acid production [35] may explain why iron concentrations in the CSM leachate were so high in comparison with all other elements measured. With continued microbial acid production, these soluble Fe levels would further increase as pH decreased. As the presence of iron during REE solvent extraction is undesirable [36] with high concentrations inhibiting precipitation of REEs [37], the removal of iron via bioleaching with PSM prior to REE recovery may aid in subsequent REE precipitation. In addition, during the solvent extraction process, thorium is co-precipitated along with the REEs [38] resulting in contamination of the leach liquor. By preventing the co-precipitation of Fe with the REEs during recovery, we propose that this preferential bioleaching of thorium and iron over the REEs may provide a treatment step prior to solvent extraction, potentially decreasing environmental hazards associated with these radioactive materials and decreasing rates of iron obstruction during REE precipitation. It may also be applicable to the recovery of REE from scrap iron or for the removal of iron from neodymium–iron–boron magnets during REE recycling [39] in place of volatile, flammable solvents.

Heterotrophic metabolism by cells during growth resulted in a decreased pH of the leachate due to organic acid production or in response to respiratory acidification and NH_4^+ assimilation [40]. Species incubated with CSM produced fewer organic acids and at lower concentrations compared to those produced when incubated with MWM. As bacterial metabolism of organic compounds can be altered by P availability [41], a switch to cellular respiration over metabolism would have resulted in the observed lowering of the pH over 192 h with no corresponding organic acid production. Numerous CSM particles consisted of zircon, clay minerals, and iron, resulting in reduced access to surface P levels compared to MWM, affecting numerous biological pathways. Low-molecular weight organic acids, including acetic, formic, citric, malic, and oxalic acids, can increase P availability from inorganic sources [42] with oxalic acid used to precipitate REEs from monazite ores [43]; however, no species tested either on MWM or CSM produced detectable levels of oxalic acid. It is possible that some oxalic acid has been lost due to oxalate-REE precipitates forming; however, as industrial processes often recover surplus oxalic acid [44] after processing, trace amounts remaining in the leachate should have been detectable by HPLC. In this instance, it is unlikely that oxalate-REE complexes have formed, thereby lowering the REE content determined in the leachate. The acids detected in this study are known to form salts with lanthanum; however, lanthanum gluconate, lanthanum acetate, and lanthanum formate [45] are all water soluble, thereby not precipitating and lowering the REE leachate content. Cerium (Ce^{4+}) gluconate forms a stable water soluble complex at basic pH [46] precipitating with OH^- as the pH increases, however, remains soluble at acidic pH as was recorded in these experiments.

Numerous isolates produced formic and acetic acid after MWM incubation, but carboxyl 1 group organic acids have poor metal complexing abilities, whereas malic and citric have a high affinity for trivalent metals [47], such as the REEs. As all the bacterial isolates studied here produced malic acid, it is important to note that malic acid is used as an eluent during the ion-exchange process in rare-earth separation [48] and may have contributed to the matrix solubilisation and REE release from the MWM. Aliphatic ligands of REEs with citrate are stable [49], and as other REEs have similar valencies, it is likely that other REE salts formed from complexing with these acids are also water soluble. We are confident that the levels of REE in the MWM leachate are accurate and, therefore, have not been unduly underestimated; however, due to the heterogeneous nature of the CSM, precipitation of REEs with other minerals during solubilisation should not be excluded as a theoretical reason as to why total REE leachate concentrations were low. In addition, as REEs are strongly bound in

the monazite lattice and the available surface area variable, leaching kinetics could be extremely slow.

No citric acid was detected in leachates from the *Aspergillus* sp. As a number of low molecular weight organic acids have short half lives (2–6 h for citric) [50], degradation of citric acid generated by *Aspergillus* prior to sampling is a possible alternative to it being produced at levels below detection. By 192 h, all fungal isolates that still had non-limiting glucose concentrations (>10%) and had favourable pH levels (<pH 2) for citric acid production [51]. However, the addition of manganese sulfate (0.002 g L^{-1}) in the growth media may have been an inhibiting factor for the production of citric acid by both *Aspergillus* strains. Even though concentrations were low, pellet clumping was visible in all flasks and previous research states that even very low concentrations of manganese ($0.0004\text{--}0.002 \text{ mg L}^{-1}$) [52] can decrease production of citric acid. Unlike *Aspergillus*, some *Penicillium* species have been demonstrated to produce citric and oxalic acid while leaching manganese ores containing high levels of Mn (25.7%) [53]. In this study, it is unlikely that the levels of Mn in the growth media, or released from the MWM, had a negative impact on the production of citric acid by *Penicillium* sp. Conversely, production of citric acid by either *Aspergillus* isolates or *Penicillium* in the presence of Mn may have resulted in the formation of an insoluble manganese citrate complex that would adhere to the ore [54] reducing the overall detectable levels of citric acid in solution. The high concentration of Fe^{2+} (10 mg L^{-1}) released by *Aspergillus* from the MWM is likely to have caused the inhibition of citric acid production, as media deficient in iron is used for commercial citric acid manufacture [55]. As the *Penicillium* sp. released less iron during leaching of MWM than either *Aspergillus* isolate, it is possible that the action of aconitate hydratase [56] and conversion of isocitric acid were suppressed, resulting in a lower production of citric acid. In this case, itaconic or itartartaric acid may have been produced [57] and two standards that were not used in this study. Citric acid can release REEs at concentrations less than 1 mM [49]. Therefore, if small amounts were continuously produced by the *Aspergillus* strains, a greater impact on P release would have been seen than by other low molecular weight organic acids [58] which would have resulted in higher amounts of REEs in the leachate. Citric acid mediated dissolution of monazite would see REEs released concomitantly to P discharge [47] further accelerated by decreases in pH. As *Penicillium* sp. and all the bacteria tested produced citric acid ($0.0307\text{--}0.22 \text{ mM}$) on MWM, but REE leachate levels varied greatly and it would imply that other microbial interactions and experimental conditions are having an impact on solubility of the monazite. HPLC analysis of the leachate resulted in a number of small unidentified peaks. A limited number of standards

were used in the analysis; however, these small peaks could be accounted for by either the amino acids present in the yeast extract added to the media or other microbially produced organic acids. It is likely that unidentified acids in this study were important in solubilising the MWM resulting in the higher REE levels detected and lower pH in the leachate of *Penicillium* sp. than for other species.

Abiotic leaching of REEs from MWM was tested using organic acid combinations produced by *Penicillium* sp., *E. aerogenes* or *Ps. putida* and *P. agglomerans*. Leaching by organic acid combinations based on *Penicillium* sp. production released the greatest concentration of REEs from the MWM, in proportion to their concentrations within the ore matrix, as was seen previously with biological leaching. Neither organic acid combination based on those produced by *E. aerogenes*, or *Ps. putida* and *P. agglomerans* resulted in REEs release from the matrix at levels higher than any bacterial isolate tested. In comparison with the biologically produced acids, abiotically added acids released undetectable levels of Fe^{2+} ($<0.02 \text{ mg L}^{-1}$) after 192 h incubation and Th levels in the leachate were $<0.08 \text{ mg L}^{-1}$ for all three combinations trialed. As none of the abiotic organic acid mixes resulted in the release of similar levels of REE from the MWM matrix, it appears that additional compounds secreted by the microorganisms or possible attachment to mineral surface for phosphate utilisation are playing additional roles in solubilisation of the monazite ore.

It is apparent that testing phosphate solubilisation of PSMs on an ideal P source (TCP) did not necessarily reflect the microorganism's capabilities when provided with an REE ore. *Klebsiella pneumoniae* and *K. oxytoca* both solubilised TCP but were unable to release bound REEs in high concentrations, even though they produced similar organic acids to *Ps. putida* and *E. aerogenes*, both of which were able to release REEs into solution. These two strains of *Klebsiella* have been shown to solubilise inorganic phosphate for plant growth promotion [59, 60]; however, in this instance, both lacked the ability to obtain P from an ore source. When incubated with monazite, *Klebsiella* sp. production of malic acid, combined with the lowering of the pH, may have increased Al^{3+} solubility [47] from the MWM, thereby increasing metal toxicity. As Al^{3+} organic ligand complexes inhibit microbial metabolic activities of *Klebsiella* sp [61], inhibition of P mobilisation from the ore surface is also likely, thereby lowering the sum of REE detected in the leachate inhibiting respiration and propagation. *Pseudomonas* and *Pantoea* are known to be more tolerant of Al^{3+} [62, 63] and successfully released REEs in this study. However, as the exact concentration of Al^{3+} in the ore is currently unknown and only determined to be present by XRD, this is currently speculation. The release of bound Mn by malic and citric acid when $\text{pH} < 5$ [64], is also known to contribute to increased extracellular

Mn levels and possibly inhibited growth [65]. Both these organic acids are produced by the *Klebsiella* sp., whereas other species that produced these same acids remained unaffected by increases in soluble Mn levels.

Numerous strains of *B. megaterium* are known to solubilise phosphate [66] and are actively applied in agriculture for promoting plant growth. While in this study, *B. megaterium* was one of the less efficient microbes at leaching REEs with a total of 0.577 mg L^{-1} in the leachate, and Shin et al. [50] did not observe any leaching of REEs from a monazite ore with *B. megaterium*. However, the monazite ore in that study had significantly lower levels of REEs and a higher level of Fe than the two monazite samples tested here, possibly explaining the different result.

It was also noted that the levels of soluble P levels in the leachate were not correlated with the release of REEs into solution. During glucose fermentation, microbial incorporation of inorganic phosphorus from the surrounding medium for conversion to organic phosphoric compounds is common as it would be appropriated for immediate use in various metabolic processes. This provides an explanation as to why P levels in the leachate were very low, while some levels of REEs were high and as such why the measurement of P should not be relied upon as an indicator of ore solubilisation.

Conclusion

This study identified a number of potential PSM capable of solubilising Western Australian monazite ores with preferential leaching of REEs from the weathered MWM over iron and thorium. In contrast, leaching of CSM resulted in the release of iron and thorium rather than REEs when inoculated with a PSM. This partisan leaching of REEs from the MWM compared to the CSM reflects the differences in ligand stability constants and REE coordination within ore lattice [49] and should be considered important when choosing both a PSM and an ore source for mineral leaching. REE release appears to not only be dependent on the ore lattice but also the type and concentration of organic acid secreted during glucose fermentation, and as some acids form REE salts, precipitation of released REEs may occur lowering the overall concentration of REEs in solution. Phosphate uptake by the microbial isolates for continued metabolic cycling occurring during monazite solubilisation, and as such the impact that phosphate precipitation could have on the system is diminished, reducing the loss of REEs to secondary mineral precipitates in the leachate.

This study successfully demonstrates the concept that PSMs can be applied in bioleaching systems to release bound REEs, but to be competitive with conventional

procedures, the recovery rates via this process need to be increased without significantly increasing operational overheads. Further examination is required to identify and understand the effects of microbial produced organic acid and enzymes on different REE bearing ores and to optimize leaching rates with higher slurry concentrations.

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