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# Better together: Potential of co-culture microorganisms to enhance bioleaching of rare earth elements from monazite

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ABSTRACT

The aim of this study was to develop continuous bioleaching of monazite by combining heterotrophic and autotrophic acidophilic microorganisms. The results showed that a co-culture of autotrophic, acidophilic *Acidithiobacillus ferrooxidans* and heterotrophic *Enterobacter aerogenes* was more effective in bioleaching rare earth elements (REEs) from monazite than either species alone. This was likely due to a synergic interaction through the biogenic generation of both organic acids and sulfuric acid. In conclusion, the consortium of *E. aerogenes* and *A. ferrooxidans* solubilised REEs (Ce, La, Nd, Pr, and Y) up to a final concentration of 40 mg $L^{-1}$.

## 1. Introduction

The shift to a low carbon future is expected to accelerate the deployment of rare earth elements (REEs) in the wind and solar energy sectors. Therefore, countries rich in REEs resources (i.e., Australia) can establish long-term benefits through sustainable REE mining. Besides the primary REEs bearing minerals (i.e., monazite), large rare-earth bearing ores hosting iron-rich minerals (Fe-oxide phosphate) including goethite and hematite (Hoatson et al., 2011), may contribute to global REEs supply (Faris et al., 2017). Currently, industrial extraction of REEs from monazite involves either a basic process that uses concentrated sodium hydroxide or an acidic process that uses concentrated sulfuric acid. These generate large amounts of hazardous waste containing thorium and uranium (Abreu and Morais, 2010). Biohydrometallurgy has been studied as a more environmentally sustainable alternative to extract REEs from phosphate minerals including monazite (Keekan et al., 2017). Previously reported REEs bioleaching efficiencies from monazite with both bacteria and fungi have been very low compared to chemical leaching (Brisson et al., 2016; Shin et al., 2015). Recently, bioleaching of REEs from bastnasite-bearing rock by Actinobacteria has been investigated (Zhang et al., 2018). These authors have reported that in a nutrient-rich growth medium, the total concentration of bioleached REEs ranged from 56 to 342 μg $L^{-1}$, whereas in an oligotrophic medium, only one strain (*Streptomyces* sp.) grew in the presence of the bastnasite (0.5% w/v), and leached up to 548 μg $L^{-1}$ of total REEs (Zhang et al., 2018). Coincidentally, a combination of the low solubility of bastnasite, a lack of nutrients from the mineral, the precipitation of REEs minerals, and re-sorption of leached REEs to cell and residual mineral surfaces may have contributed to the observed low leaching efficiency (0.008–0.08%) (Zhang et al., 2018). However, compared to the conventional extraction of REEs, bioleaching can be considered as an "eco-friendly technology" which minimizes the high cost and negative environmental impact.

In phosphate-based environments "phosphate solubilising microorganisms" (PSMs) can be introduced to enhance the solubilisation of insoluble inorganic phosphate via acidification, chelation, and exchange reactions (Son et al., 2006). As a consequence, heterotrophic PSMs such as *Enterobacter aerogenes* can be used for the solubilisation of REEs from a phosphate mineral such as monazite via secretion of organic acids (Corbett et al., 2017). Earlier studies demonstrated that the recovery of REEs using heterotrophic microorganisms is possible, although, the bioleaching mechanisms are not yet clearly and explicitly understood (Brisson et al., 2016).

It has been demonstrated that optimizing microbial community

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structure in co-culture systems are an effective way of improving microbial community function (Ma et al., 2017). The continuous requirement of heterotrophic bioleaching microorganisms for a carbon and energy source to maintain microbial activity is problematic at the industrial level, however the addition of acidophilic autotrophic bioleaching microorganisms (e.g. *Acidithiobacillus ferrooxidans*) to these systems can potentially improve their performance. Autotrophic acidophiles require small amounts of inorganic nutrients, such as ferrous iron and reduced sulfur compounds for bio-oxidation (Zhuang et al., 2015). In addition, the ability of acidophilic bacteria to tolerate toxic heavy metal ions, enhances their capacity for the bioleaching of metals. *A. ferrooxidans* is the most studied obligate chemolithoautotrophic bioleaching bacterium. It gains energy from the aerobic oxidation of ferrous iron and/or reduced sulfur compounds to ferric iron and sulfuric acid, respectively (Watling, 2016). Although *A. ferrooxidans* has been used to leach phosphorous from different types of rock phosphates (Bhatti and Yawar, 2010), to the best of our knowledge, despite the commercial application of acidophilic bioleaching for a diverse range of elements from sulfide minerals, the acidophilic bioleaching of REEs-bearing minerals has not been previously studied. It has been demonstrated that microbial consortia have greater bioleaching rates than pure cultures (Johnson, 2001), we therefore propose a two-step bioleaching system where the metabolites generated by *E. aerogenes* result in pH reduction negating the need for manual pH adjustment required for *A. ferrooxidans*.

In this context, the aim of this work was to investigate the bioleaching of REEs from monazite by a co-culture of autotrophic, acidophilic *A. ferrooxidans* and heterotrophic *E. aerogenes* and compare the efficiency to those of individual pure cultures.

## 2. Material and methods

### 2.1. Phosphate and sulfide minerals

The high grade weathered monazite ore was collected from the Mount Weld Mine (Lynas Corporation), and is hereafter referred to as MWM (Mt. Weld Monazite). The ore was ground by a rod mill, pulverized in a ring mill and finally sieved to < 38 μm in particle size. The elemental composition of the MWM was analysed by inductively coupled plasma optical emission spectrometry (ICP-OES, CSIRO Minerals, Waterford, Western Australia). The ore contained (%): 10.1 La, 12.6 Ce, 2.10 Pr, 6.25 Nd, 0.165 Y, 0.162 Th, 1.23 Fe, 9.93 P, 1.75 Ca, 0.199 Mg, 1.96 Si, 0.554 Ti, 0.031 Zr, and < 0.003 U. Pyrite concentrate (p80 passing 120 μm) used as a source of Fe and S was obtained from Kalgoorlie Consolidated Gold Mines Pty Ltd. (KCGM), Australia (Bryan et al., 2015). The mineralogical composition of MWM was determined by X ray diffraction (XRD) at CSIRO Minerals, Waterford, Western Australia. The XRD analysis of MWM revealed that samples were mainly constituted by 51% monazite, 41% florencite, and 8% nontronite. Pyrite concentrate contained 60% pyrite, 12.5% quartz, 9% albite and 7.5% dolomite. Before bioleaching experiments, the mineral samples were sterilized by gamma irradiation at 50 kGy for 11 h (ChemCentre, Bentley, Western Australia).

### 2.2. Bioleaching experiment

*Entrobacter aerogenes* (ATCC 13048, obtained from ATCC) was grown to exponential phase at 30 °C in National Botanical Research Institute Phosphate (NBRIP) medium (Nautiyal, 1999), with shaking at 120 rpm, and harvested by centrifugation (3600 × *g*, 10 min). Cells were resuspended in sterile Tris-HCl buffer (100 mM, pH 7.2), centrifuged (3600 × *g*, 5 min) and washed twice more to remove any trace of phosphate. *Acidithiobacillus ferrooxidans* (ATCC 23270, obtained from DSMZ) was grown to exponential phase at 30 °C, with shaking at 120 rpm, in the basal salt media (BSM) at pH 2.0 which is described elsewhere (Zammit et al., 2011). Cells were resuspended in sterile Tris buffer (20 mM, pH 2.0), centrifuged (3600 × *g*, 5 min) and washed twice more to remove any trace of phosphate.

All bioleaching experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of the relevant media, in triplicate at 30 °C with shaking at 120 rpm in an orbital shaking incubator (RATEK, Model No: OM11) over 12 days.

The ability of *A. ferrooxidans* to bioleach MWM as a phosphate source, was evaluated in BSM (pH 2.50 ± 0.15), supplied with either $FeSO_4$ (13.9 g $L^{-1}$) and $K_2S_4O_6$ (1.51 g $L^{-1}$) (filter sterilized 0.20 μm, Satorius) or sterilized pyrite (1% pulp density), with 1% v/v bacterial inoculum (initial density $1 \times 10^6$ cells $mL^{-1}$) and 1% pulp density of sterilized monazite.

In the co-culture experiment, *E. aerogenes* was first cultivated in modified NBRIP media (3% w/v glucose and pH 7.00 ± 0.25) with 1% v/v bacterial inoculum (initial density $1 \times 10^7$ cells $mL^{-1}$) and 1% pulp density of sterilized monazite. Three days later, when the pH dropped to < 3.5, a 10 mL aliquot of *A. ferrooxidans* (initial density $1 \times 10^6$ cells $mL^{-1}$ before inoculation) in BSM was added to the leachate, and the combined culture supplied with $FeSO_4$ (13.9 g $L^{-1}$) and $K_2S_4O_6$ (1.51 g $L^{-1}$) (filter sterilized 0.20 μm, Satorius).

Cell-free abiotic controls were carried out under the same conditions. Samples were taken at 0, 2, 3, 6, 9, 12 d and pH measured using a pH meter (Ionode IJ series pH probe). Samples were then filtered with disposable syringe filters (0.20 μm, Satorius) and assayed for REEs, Y, Th, U, Fe, and P concentrations by inductively coupled plasma-mass spectrometry (ICP-MS) Agilent Technologies 7700 series, Bureau Veritas Australia Pty Ltd., Canning Vale, Western Australia) and the average values were reported.

### 2.3. Comparison of the phosphate and iron regulation of E. aerogenes and A. ferrooxidans

In order to investigate the potential metabolic pathways involved in inorganic phosphate solubilisation by both strains in the co-culture system, a genome-based comparison of phosphate pathways was carried out. The genomes of *E. aerogenes* (ATCC 13048 – KCTC 2190) and *A. ferrooxidans* (ATCC 23270) were downloaded from the NCBI ftp site (ftp://ftp.ncbi.nlm.nih.gov/). For the purpose of this comparison, the genomes were annotated using the Rapid Annotation using Subsystem Technology (RAST) server (http://rast.nmpdr.org/) using the ClassicRAST annotation scheme (Overbeek et al., 2013). Comparisons were performed using the SEED and RAST servers and Geneious v.10.2.3 bioinformatic software (Kearse et al., 2012).

### 2.4. Synchrotron analysis

Synchrotron radiation is a powerful technique that can be used to determine elemental oxidation state of REEs for a wide range of environmental samples.

#### 2.4.1. Sample preparation

Ce $L_{III}$-Edge X-ray absorption spectroscopy (XAS) data were collected on two solutions of monazite leachate from the co-culture supplied with $FeSO_4$ and $K_2S_4O_6$ at day 3 and 6 after *A. ferrooxidans* addition, as well as the MWM residue at end of bioleaching experiment. The leachates were prepared with 30% glycerol, and flash frozen with liquid nitrogen cooled iso-pentane, into 1 mm × 3 mm × 23 mm acrylic sample cuvettes. The cuvettes were covered and closed with metal free Kapton adhesive tape, which served as an X-ray transparent window. The powder sample was ground with mortar and pestle to a fine homogenous powder, and then adhered as a thin film to metal free Kapton adhesive tape.

#### 2.4.2. XAS data collection

Ce $L_{III}$-Edge XAS data were collected at beamline 7-3, at the Stanford Synchrotron Radiation Lightsource (SSRL). The beamline