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# Bioleaching of rare earth elements from monazite sand

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## ABSTRACT

Three fungal strains were found to be capable of bioleaching rare earth elements from monazite, a rare earth phosphate mineral, utilizing the monazite as a phosphate source and releasing rare earth cations into solution. These organisms include one known phosphate solubilizing fungus, *Aspergillus niger* ATCC 1015, as well as two newly isolated fungi: an *Aspergillus terreus* strain ML3-1 and a *Paecilomyces* spp. strain WE3-F. Although monazite also contains the radioactive element Thorium, bioleaching by these fungi preferentially solubilized rare earth elements over Thorium, leaving the Thorium in the solid residual. Adjustments in growth media composition improved bioleaching performance measured as rare earth release. Cell-free spent medium generated during growth of *A. terreus* strain ML3-1 and *Paecilomyces* spp. strain WE3-F in the presence of monazite leached rare earths to concentrations 1.7–3.8 times those of HCl solutions of comparable pH, indicating that compounds exogenously released by these organisms contribute substantially to leaching. Organic acids released by the organisms included acetic, citric, gluconic, itaconic, oxalic, and succinic acids. Abiotic leaching with laboratory prepared solutions of these acids was not as effective as bioleaching or leaching with cell-free spent medium at releasing rare earths from monazite, indicating that compounds other than the identified organic acids contribute to leaching performance. *Biotechnol. Bioeng.* 2016;113: 339–348. © 2015 Wiley Periodicals, Inc.

## Introduction

Rare earth elements (REEs) are in demand for a variety of technologies including efficient batteries; permanent magnets; high efficiency electric lights; and a variety of consumer electronics (Alonso et al., [2012](#); Bauer et al., [2011](#)). The REEs include the naturally occurring lanthanides (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu, but not Pm) (atomic numbers 57–60 and 62–71), as well as Sc and Y (atomic numbers 21 and 39), which have similar chemical behavior to lanthanides (Cotton, [2006](#); Gupta and Krishnamurthy, [1992](#)). The three main REE ores that are currently mined for production are bastnasite (REE-FCO<sub>3</sub>), monazite (light REE-PO<sub>4</sub>), and xenotime (heavy REE-PO<sub>4</sub>), together representing approximately 95% of

known REE minerals (Gupta and Krishnamurthy, [1992](#); Rosenblum and Fleischer, [1995](#)). In addition to REE-PO<sub>4</sub>, monazite ore usually contains Th and sometimes U, both of which are radioactive, presenting a challenge for separation and disposal when they are extracted along with REEs (Gupta and Krishnamurthy, [1992](#)). Th is usually present as cheralite (ThCa(PO<sub>4</sub>)<sub>2</sub>) or huttonite (ThSiO<sub>4</sub>) (Nitze, [1896](#); Rosenblum and Fleischer, [1995](#)). Conventional methods of REE extraction from ores involve high temperature (≥140°C) chemical leaching with either concentrated sulfuric acid or with concentrated NaOH followed by acid treatment (Gupta and Krishnamurthy, [1992](#)). Another method first heats the monazite with CaCl<sub>2</sub> and CaCO<sub>3</sub> at very high temperatures (≥975°C), prior to leaching with 3% HCl (Merritt, [1990](#); Peelman et al., [2014](#)). The use of high temperatures and harsh chemicals results in high energy usage and the production of toxic waste streams which, depending on the process, can contain Th and U from the monazite (Alonso et al., [2012](#); Gupta and Krishnamurthy, [1992](#)).

Phosphate solubilizing microorganisms (PSMs), which include both bacterial and fungal species, are capable of releasing phosphate from otherwise low solubility phosphate minerals (Rodríguez and Fraga, [1999](#)). Most research with PSMs has focused on agricultural applications with the objective of understanding microorganisms' effects on phosphate bioavailability to plants and for development of approaches to enhance the effectiveness of phosphate fertilizers (Arcand and Schneider, 2006; Asea et al., [1988](#); Braz and Nahas, [2012](#); Chai et al., [2011](#); Chuang et al., [2007](#); Gyaneshwar et al., [2002](#); Illmer and Schinner, [1992](#); Morales et al., [2007](#); Osorio and Habte, [2009](#); Rodríguez and Fraga, [1999](#); Vassilev et al., [2006](#)). In addition to agriculturally important studies, there has also been research on using PSM's to extract phosphate from apatite ores (Costa et al., [1992](#)) and to remove phosphates from iron ores to make these ores more suitable for iron production (Adeleke et al., [2010](#); Delvasto et al., [2009](#), [2008](#)). Most PSM studies have focused on calcium phosphate minerals including tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate (Altomare et al., [1999](#); Illmer and Schinner, [1992](#); Illmer and Schinner, [1995](#); Rodríguez and Fraga, [1999](#)), but a few have addressed other phosphate minerals, including AlPO<sub>4</sub>, FePO<sub>4</sub>, and turquoise (CuAl<sub>6</sub>(PO<sub>4</sub>)<sub>4</sub>(OH)<sub>8</sub>·4H<sub>2</sub>O) (Chai et al., [2011](#); Chuang et al., [2007](#); Delvasto et al., [2008](#); Illmer et al., [1995](#); Souchie et al., [2006](#)).

Several mechanisms have been proposed to explain phosphate solubilization by PSMs, but the production of organic acids is thought to be a major contributor (Gyaneshwar et al., [2002](#); Nautiyal et al., [2000](#); Rodríguez and Fraga, [1999](#); Scervino et al., [2011](#)). In addition to reducing pH, which somewhat increases the solubility of phosphate minerals, some organic acids can form complexes with the cations released from phosphate minerals and thus improving overall

solubilization (Arcand and Schneider, 2006; Bolan et al., [1994](#); Gadd, [1999](#); Gyaneshwar et al., [2002](#)). Complex forming properties of some organic acids with REEs have been analyzed. For example, the stability constants for 1:1 REE citrate complexes have been estimated around  $10^9$  (Goynes et al., [2010](#); Martell and Smith, [1974](#)), and a number of other REE citrate complexes have been proposed (Wood, [1993](#)). One study evaluated citrate, oxalate, phthalate, and salicylate complexation of REEs from monazite in the context of metal mobilization in soils (Goynes et al., [2010](#)). Beyond organic acids, other chelating molecules may also be important for solubilization of REEs. For instance, some siderophores, metal complexing molecules produced by many bacteria and fungi, have been found to chelate REEs (Christenson and Schijf, [2011](#)).

Bioleaching is potentially more environmentally friendly than conventional extraction of REEs from ores. Bioleaching of REEs has been investigated in a small number of studies, including an investigation of red mud bioleaching by *Penicillium* (Qu and Lian, [2013](#)), and two recent studies addressing bioleaching of monazite concentrate (Hassanien et al., [2013](#)) and monazite bearing ore (Shin et al., [2015](#)). Since monazite is a REE phosphate mineral, the goal of this project is to investigate factors affecting the use of using PSMs to solubilize monazite for REE extraction and to characterize the contributions of excreted organic acids to bioleaching.

## Materials and Methods

### Enrichment and Isolation of REE Solubilizing Microorganisms

REE-phosphate solubilizing enrichment cultures were established in National Botanical Research Institute phosphate growth medium (NBRIP medium), a commonly used medium for phosphate solubilization studies (Nautiyal, [1999](#)), with 10 g/L glucose (added as carbon and energy source) and insoluble  $\text{NdPO}_4$  (phosphate source) [Sigma–Aldrich, St. Louis, MO]. See Table [I](#) for medium composition and Supplementary Materials for enrichment conditions.

**Table I.** Growth media compositions

Medium Component	NBRIP medium	PVK medium <sub>a</sub>	PVK medium <sub>a</sub> without Mn and Fe	AMS medium <sub>b</sub>
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	5 g/L	–	–	–
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.25 g/L	0.1 g/L	0.1 g/L	1.0 g/L

Medium Component	NBRIP medium	PVK medium <sup>a</sup>	PVK medium <sup>a</sup> without Mn and Fe	AMS medium <sup>b</sup>
KCl	0.2 g/L	0.2 g/L	0.2 g/L	0.2 g/L
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.1 g/L	0.5 g/L	0.5 g/L	0.66 g/L
NaCl	–	0.2 g/L	0.2 g/L	–
MnSO <sub>4</sub> · H <sub>2</sub> O	–	0.002 g/L	–	–
FeSO <sub>4</sub> · 7H <sub>2</sub> O	–	0.002 g/L	–	–
Trace elements stock (1000x) <sup>c</sup>	–	–	–	1.0 mL/L
Stock A (1000x) <sup>d</sup>	–	–	–	1.0 mL/L

- <sup>a</sup> This is a modification of the original Pikovskaya medium, with yeast extract omitted.
- <sup>b</sup> This is a modification of the original ammonium salts medium, with phosphate buffer omitted.
- <sup>c</sup> Trace elements stock contained FeSO<sub>4</sub> · 7H<sub>2</sub>O (0.5 g/L), ZnSO<sub>4</sub> · 7H<sub>2</sub>O (0.4 g/L), MnSO<sub>4</sub> · H<sub>2</sub>O (0.02 g/L), H<sub>3</sub>BO<sub>3</sub> (0.015 g/L), NiCl<sub>2</sub> · 6H<sub>2</sub>O (0.01 g/L), EDTA (0.25 g/L), CoCl<sub>2</sub> · 6H<sub>2</sub>O (0.05 g/L), and CuCl<sub>2</sub> · 2H<sub>2</sub>O (0.005 g/L).
- <sup>d</sup> Stock A contained FeNaEDTA (5 g/L) and NaMoO<sub>4</sub> · 2H<sub>2</sub>O (2 g/L).

REE-phosphate solubilizing microorganisms were isolated from enrichment cultures on selective plates containing NBRIP medium solidified with 1.5% agar and containing powdered  $\text{NdPO}_4$  as the phosphate source and 10 g/L glucose as carbon and energy source. See Supplementary Materials for isolation details.

## DNA Extraction, Amplification, Sequencing, and Sequence Analysis

Biomass was collected from cultures grown on potato dextrose agar plates, frozen under liquid nitrogen, and crushed with a mortar and pestle to disrupt cell walls. DNA was extracted using the Qiagen DNeasy Plant Minikit. Fungal 18S and 5.8S genes and ITS regions were amplified by PCR using Qiagen Taq DNA polymerase with the NS1, NS3, NS4, NS8, ITS1, and ITS4 universal fungal primers (White et al., [1990](#)). PCR products were purified by polyethylene glycol precipitation followed by ethanol wash, drying, and re-suspension in sterile water (Sánchez et al., [2003](#)). Sequencing was performed at the UC Berkeley DNA sequencing facility.

## Bioleaching Growth Conditions

Bioleaching experiments were conducted in 250 mL Erlenmeyer flasks with foam stoppers. Each flask contained 0.5 g monazite sand [City Chemical LLC, West Haven, CT] ground to  $< 75 \mu\text{m}$  (200 mesh) and 50 mL growth medium. In conventional monazite processing, NaOH treatment requires grinding to  $45 \mu\text{m}$  (325 mesh), whereas sulfuric acid and  $\text{CaCl}_2/\text{CaCO}_3$  treatments do not require fine grinding (Gupta and Krishnamurthy, [1992](#); Peelman et al., [2014](#)). Growth medium composition and carbon source varied with each experiment. Four different media were used: NBRIP medium (Nautiyal, [1999](#)), modified Pikovskaya medium (PVK medium) (Nautiyal, [1999](#); Pikovskaya, [1948](#)), PVK medium without Mn and Fe, and modified ammonium salts medium (AMS medium) (Parales et al., [1994](#)). See Table I for media compositions. One of five carbon sources (glucose, fructose, sucrose, xylose, or starch) was added to the medium prior to sterilization.

Conidia (asexual spores) were collected from plates in sterile deionized water and diluted to a spore concentration to  $10^7$  CFU per mL (determined using calibration curves relating optical density at 600 nm to CFU concentration). Each flask was inoculated with 1 mL of spore suspension. Flasks were incubated at room temperature (25–28°C) and stirred at 250 rpm for the duration of the six day bioleaching experiments. All bioleaching experiments were conducted with three biological replicates for each condition unless otherwise noted.

## Abiotic Leaching Conditions

All abiotic leaching experiments (HCl, organic acid, and spent medium) were conducted in 50 mL flat bottomed polypropylene tubes. Each tube contained 0.1 g monazite sand ground to < 75  $\mu\text{m}$  (200 mesh) and 10 mL of the desired leaching solution. Tubes were incubated at room temperature (25–28°C) and stirred at 250 rpm for 48 h.

For abiotic leaching with organic acids and hydrochloric acid, acid solutions were prepared with deionized water and filter sterilized through 0.2  $\mu\text{m}$  filters prior to leaching. Acetic, citric, itaconic, oxalic, and succinic acids [Sigma–Aldrich] were tested at two concentrations each: 2 mM and 20 mM, while gluconic acid [Sigma–Aldrich] was tested at 1.8 mM and 18 mM. HCl solutions were prepared to provide a range of pH from 1.8 to 3.7. Three experimental replicates were done for each acid concentration.

For abiotic spent medium leaching, spent medium was collected after six days of bioleaching and filter sterilized through 0.2  $\mu\text{m}$  filters. To remove REEs, 15 mL of filtered spent medium was added to a 50 mL tube containing 0.5 g Amberlite IR120 resin [Sigma–Aldrich] and shaken horizontally for one hour. 10 mL of treated spent medium was then used for leaching. Six biological replicates were done for each organism.

## Biomass Measurements

Volatile solids (VS) were determined as a measure of biomass production. VS was used rather than dry weight because monazite sand becomes entrapped in the biomass during bioleaching. By measuring VS, the organic portion of the total dry weight could be measured. Samples comprising the entire contents of a bioleaching flask were filtered on glass microfiber filters, dried overnight at 105°C, cooled to room temperature, and weighed. Samples were then ashed at 550°C for four hours, cooled to room temperature, and weighed again. The difference between the dried weight and the ashed weight was determined as VS.

## Analytical Methods

Supernatant samples were collected and filtered with 0.2  $\mu\text{m}$  filters for all analyses. To quantify sugars and organic acids, 1.5 mL samples were acidified with 10  $\mu\text{L}$  6 M sulfuric acid [ACS grade, Fisher Scientific, Pittsburgh, PA] and analyzed on a Waters 2695 HPLC system using a BioRad Aminex HPX-87H carbohydrate/organic acids analysis column with 5 mM  $\text{H}_2\text{SO}_4$  as the mobile phase at a flow rate of 0.6 mL/min. Sugars were detected using a Waters 2414 refractive index detector. Organic acids were detected using a Waters 2996 UV absorption detector monitoring absorption at 210 nm. Calibration curves were prepared for concentration ranges of 0.1 to 10 g/L for sugars and 0.5 mM to 20 mM for organic acids, with the exception of acetic

acid, which could only be detected to a minimum concentration of 1 mM. Sugar standards prepared included glucose, fructose, sucrose, and xylose. Organic acid standards included acetic, citric, gluconic, itaconic, lactic, oxalic, and succinic acids.

To quantify REEs, Th and U concentrations, samples were diluted 100-fold in deionized water acidified with 1.5% nitric acid [70%, trace metals grade, Fisher Scientific, Pittsburgh, PA] and 0.5% hydrochloric acid [36%, ACS Plus grade, Fisher Scientific, Pittsburgh, PA], and were analyzed on an Agilent Technologies 7700 series ICP-MS. pH was measured using a Hanna Instruments HI1330B glass pH electrode and HI 2210 pH meter. Phosphate concentrations were determined using the BioVision Phosphate Colorometric Assay Kit.

## Statistical Analyses

Statistical analyses were performed in Python using the StatsModels module. A significance level of  $\alpha = 0.05$  was used for all analyses. When multiple comparisons were performed in a single analysis, the Šidák correction (Šidák, [1967](#)) was used to adjust *P*-values to maintain an overall significance level of  $\alpha = 0.05$ . See Supplementary Materials for details of statistical analyses and Supplementary Table SI for *P*-values from analyses. Average concentrations and amounts are reported as mean  $\pm$  standard deviation.

## Results and Discussion

### Enrichment, Isolation and Identification of Bioleaching Microorganisms

Source inoculation materials for establishing REE-phosphate solubilizing enrichment cultures were collected from two locations: tree root associated soil from the UC Berkeley campus and sand and sediment samples from Mono Lake. We hypothesized that root associated soil might yield PSMs because root associated microbial communities are known to support plant growth by improving nutrient availability (Rodríguez and Fraga, [1999](#)), and that since Mono Lake contains high concentrations of heavy metals and REEs (Johannesson and Lyons, [1994](#)), it might serve as a source of microorganisms that are tolerant of high REE concentrations.

Enrichment cultures capable of utilizing monazite as their sole phosphate source were successfully cultivated from both source materials. Initial screening of organisms isolated from these enrichments, along with known PSMs from culture collections (*Aspergillus niger* ATCC 1015, *Burkholderia ferrariae* FeG101, *Microbacterium ulmi* XIL02, *Pseudomonas rhizosphaerae*IH5, *Pseudomonas fluorescens*, *Sterptomyces youssoufiensis* X4), identified the

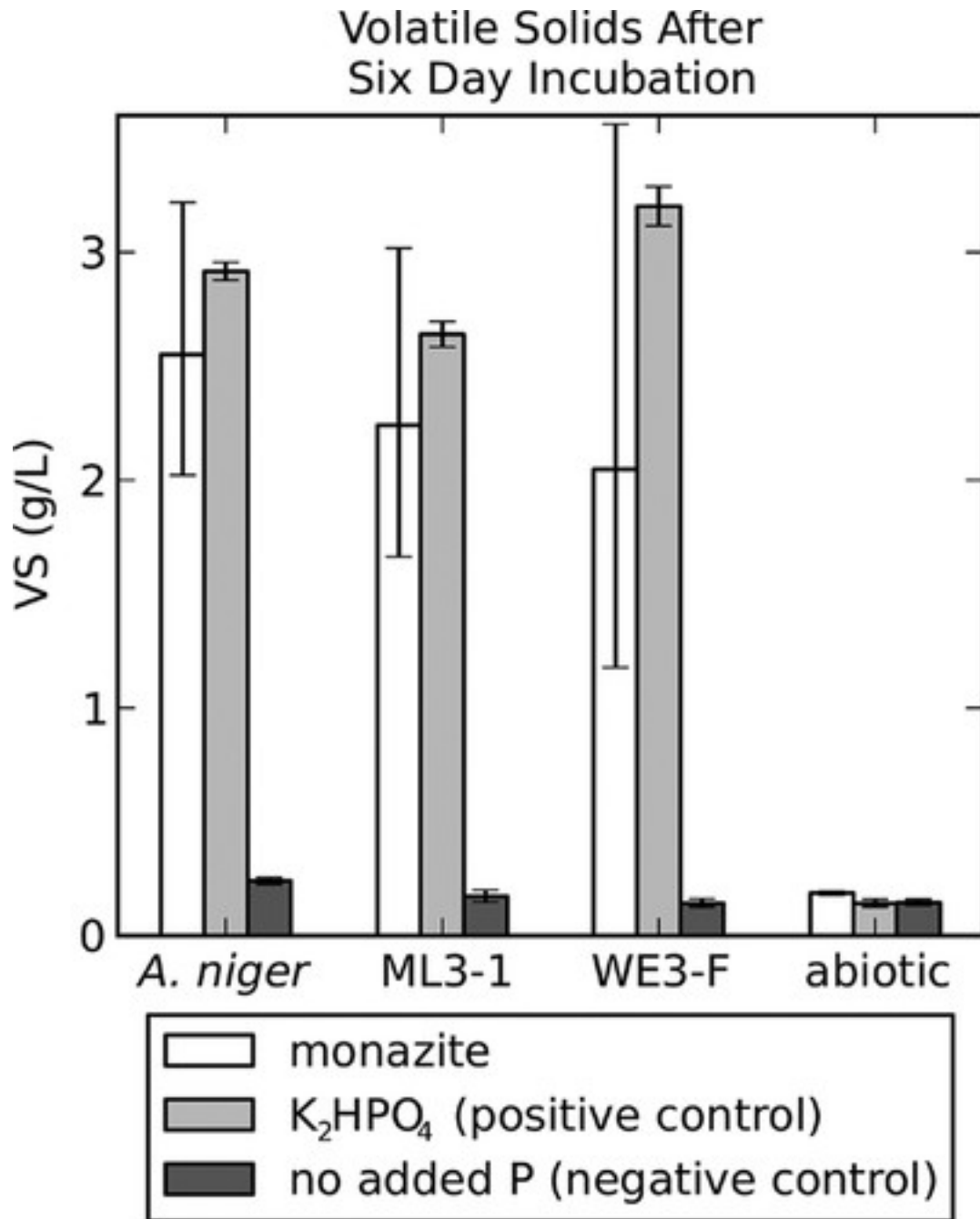


three most promising bioleaching organisms for further study, all of which were fungi: *Aspergillus niger* ATCC 1015, isolate ML3-1 from a Mono Lake enrichment culture, and isolate WE3-F from a tree root soil enrichment culture.

Sequences of 18S, 5.8S, and ITS regions of ML3-1 and WE3-F were determined and have been deposited in Genbank with accession numbers KM874778, KM874779, KM874780, and KM874781. Based on BLAST comparisons of these sequences to the NBRIP nucleotide database, ML3-1 and WE3-F showed high sequence similarity ( $\geq 99\%$ ) to *Aspergillus terreus* and *Paecilomyces* sp. respectively. Previous studies have reported phosphate solubilizing activity for some strains of both the *Aspergillus* and *Paecilomyces* genera (Ahuja et al., [2007](#); Braz and Nahas, [2012](#); Chuang et al., [2007](#); Mendes et al., [2013](#)).

## Biomass Growth During Bioleaching

Biomass production after 6 days of growth with monazite as sole phosphate source was compared to growth with 0.4 g/L  $K_2HPO_4$  as phosphate source (positive control) and to growth without added phosphate (negative control) (Fig. [1](#)). For all three organisms, growth on monazite resulted in average VS concentrations approximately tenfold greater than the negative control. Significant differences in VS production were not observed between growth on monazite and growth on  $K_2HPO_4$ , demonstrating that these organisms can utilize monazite as a phosphate source for growth.



**Figure 1**

[Open in figure viewerPowerPoint](#)

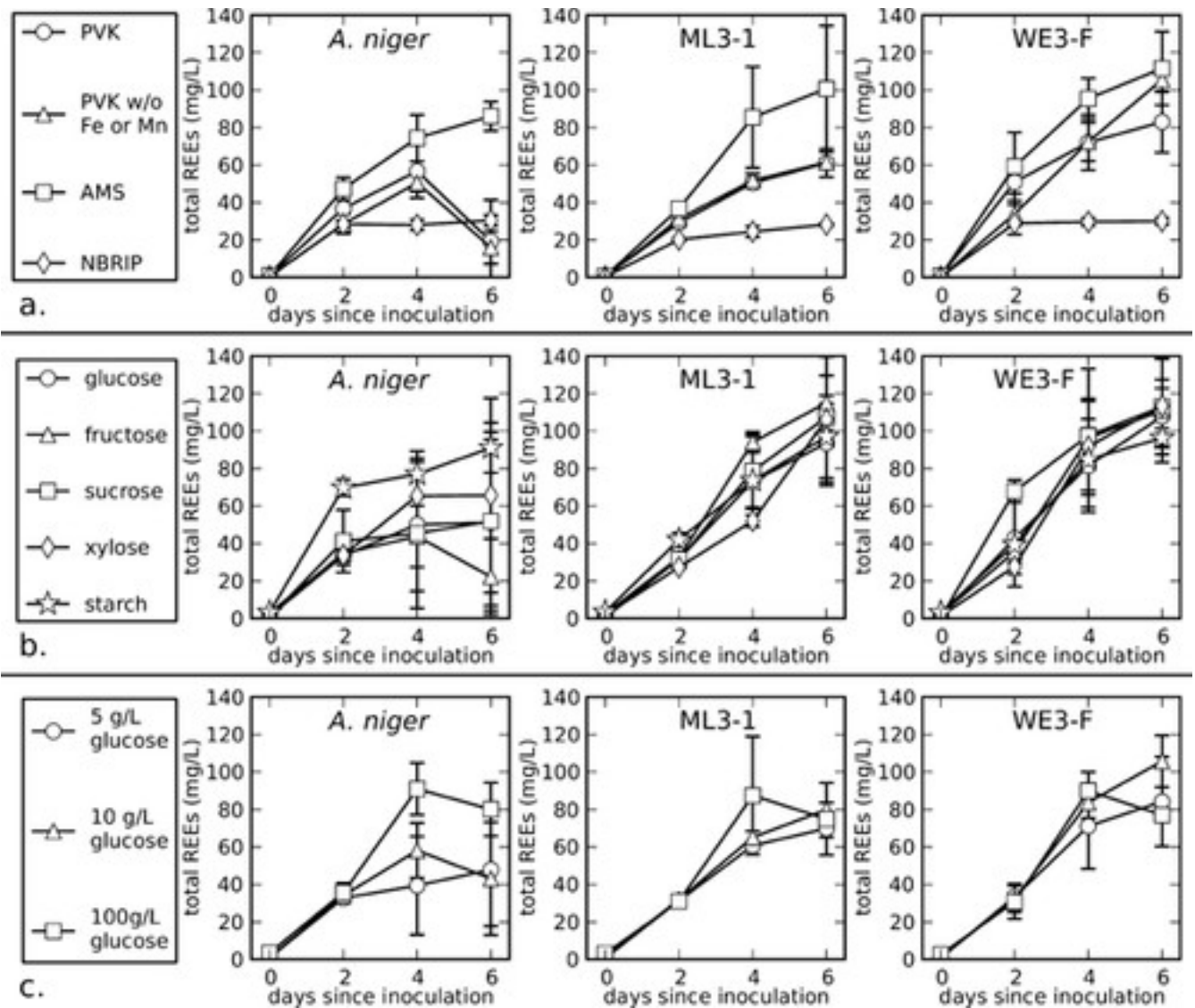
Biomass production measured as VS after six days incubation with different phosphate sources: 10 g/L monazite, 0.4 g/L K<sub>2</sub>HPO<sub>4</sub> (positive control), or no added phosphate i.e., growth on trace phosphate contamination in medium and inoculum (negative control). All incubations were in AMS medium with 10 g/L glucose. Error bars indicate 95% confidence intervals around the geometric means.

[Caption](#)

Bioleaching Performance Under Different Growth Conditions

Several previous studies have tried to optimize the solubilization of phosphate minerals by PSMs (Asea et al., 1988; Chai et al., 2011; Chuang et al., 2007; Nautiyal, 1999; Nautiyal et al., 2000). Factors addressed include carbon source, nitrogen source, and medium composition, including variations in metals concentrations. In general, carbon source and medium composition were found to have significant effects, which varied between different organisms (Chai et al., 2011; Nautiyal, 1999; Nautiyal et al., 2000). Studies addressing nitrogen source found either minimal effect or a preference for ammonium, particularly for studies involving fungal PSMs (Asea et al., 1988; Chai et al., 2011; Chuang et al., 2007; Nautiyal et al., 2000). Therefore, in this study, medium composition and carbon source were the focus of growth condition optimization while the nitrogen source was fixed as ammonium.

Varying growth media influenced REE solubilization performance (Fig. 2a), with the greatest REE solubilization for all organisms occurring with AMS medium (average final REE concentrations:  $86 \pm 6$ ,  $101 \pm 27$ , and  $112 \pm 16$  mg/L for *A. niger*, ML3-1, and WE3-F respectively). Growth on NBRIP medium consistently resulted in poor solubilization performance (average final REE concentrations:  $30 \pm 2$ ,  $28 \pm 1$ , and  $30 \pm 2$  mg/L for *A. niger*, ML3-1, and WE3-F respectively). The difference in REE solubilization between AMS medium and NBRIP medium was statistically significant for *A. niger* and WE3-F, and was marginally significant for ML3-1 ( $P = 0.065$ ), likely due to high variability and low sample size.



**Figure 2**

[Open in figure viewerPowerPoint](#)

Bioleaching of REEs from monazite under different growth conditions. Error bars indicate standard deviations around the means. **(a)** Different growth media: PVK medium, PVK medium without Fe or Mn, AMS medium, and NBRIP medium, all containing 10 g/L glucose as carbon source. **(b)** Different carbon sources: glucose, fructose, sucrose, xylose, and starch, all in AMS medium with initial carbon source concentrations of 10 g/L. **(c)** Different starting glucose concentrations: 5 g/L, 10 g/L, and 100 g/L, all in AMS medium.

[Caption](#)

Phosphate concentrations observed during bioleaching were much lower than REE concentrations. The molar ratio of REEs to phosphate in the monazite sand is expected to be  $\approx 1$ . However, the observed molar ratio of REEs to phosphate in solution at the end of bioleaching was well above one (Table II), ranging from  $5 \pm 1$  for ML3-1 grown on NBRIP medium to  $170 \pm 30$  for *A. niger* grown on AMS medium. These data indicate that much of the phosphate

associated with REEs in the monazite was either not released or was removed from solution. As indicated by the biomass measurements, some of this phosphate was used for biomass production. Estimates of phosphate incorporated into biomass (assuming 3% dry weight biomass phosphorus content (Rittmann and McCarty, 2001)) suggest that  $\sim 70$  mg/L phosphorus (i.e.,  $\sim 210$  mg/L phosphate) would need to be taken up to support  $\sim 2.2$  g/L biomass generated during bioleaching. Assuming equimolar release of REEs and phosphate from the monazite, the amount of phosphate required to support biomass growth is two to six times greater than the REE quantities released, suggesting that phosphate consumption for growth accounts for the low phosphate concentrations in solution. The apparently low REE concentration compared to the estimated phosphate in the biomass may be due to the inherent uncertainty in the estimation of biomass as VS and the assumption of 3% phosphorus concentration in cells. Since growth is occurring under low phosphate conditions, the biomass would be expected to have a lower phosphorus content. Phosphorus levels of 0.5% to 1.5% dry weight would correspond to the measured REE and biomass concentrations. This discrepancy may also be due to the removal of REEs from the system by other processes including re-precipitation (e.g., as REE-oxalates) or adhesion to microbial cells. REE loss due to re-precipitation or adhesion could not be directly confirmed or quantified in this study due to the intermingling of the fungal biomass with monazite sand particles and with any potential precipitates.

**Table II.** Molar ratio of total REEs to phosphate measured after bioleaching with different media compositions (mean  $\pm$  standard deviation)

Medium	<i>A. niger</i>	ML3-1	WE3-F
PVK	29 $\pm$ 23	11 $\pm$ 1	126 $\pm$ 8
PVK without Mn for Fe	62 $\pm$ 98	14 $\pm$ 2	68 $\pm$ 45
AMS	166 $\pm$ 25	9 $\pm$ 4	102 $\pm$ 78
NBRIP	8 $\pm$ 4	5 $\pm$ 1	5 $\pm$ 0

Previous PSM studies of  $\text{Ca}_3(\text{PO}_4)_2$  solubilization have reported phosphate concentrations in liquid cultures on the order of 250 to 520 mg/L (Chai et al., 2011; Chen et al., 2006; Chuang et al., 2007; Rodríguez and Fraga, 1999; Scervino et al., 2011). In contrast, the maximum phosphate concentration observed in this study during monazite solubilization with different media compositions was 15 mg/L (ML3-1 grown on AMS medium). Differences in solubilization between different phosphate minerals has been previously reported, even when using the same PSMs (Adeleke et al., 2010; Chuang et al., 2007; Delvasto et al., 2008; Illmer and Schinner, 1995; Rodríguez and Fraga, 1999; Souchie et al., 2006). Also, REE-phosphates are known to have particularly low solubilities in water, on the order of  $10^{-13}$  M ( $10^{-11}$  g/L) (Firsching and Brune, 1991), whereas the solubility of  $\text{Ca}_3(\text{PO}_4)_2$  is  $3.9 \times 10^{-6}$  M (0.0012 g/L) (Haynes, 2015).

With AMS medium and both versions of PVK medium, glucose was completely or almost completely consumed (final concentration  $\leq 0.6$  g/L) (Supplementary Fig. S1a). In contrast, with NBRIP medium, glucose concentrations were only reduced to  $6.3 \pm 0.1$ ,  $7.4 \pm 0.1$ , and  $7.1 \pm 0.0$  g/L for *A. niger*, ML3-1, and WE3-F respectively. Growth on NBRIP medium also resulted in smaller reductions in pH than growth on other media (Supplementary Fig. S2a).

In the study by Nautiyal introducing NBRIP medium, several versions of the medium were compared with several modifications of Pikovskaya medium, including the yeast extract free version used in this study (PVK) (Nautiyal, 1999). They showed significantly enhanced solubilization of  $\text{Ca}_3(\text{PO}_4)_2$  by a variety of bacterial strains (five *Pseudomonas* and three *Bacillus* strains) with NBRIP medium. However, the poor performance of NBRIP medium in this study with fungi indicates that despite its widespread use in phosphate solubilization studies, NBRIP medium is not well suited for some PSMs and/or solubilization of some phosphate minerals.

Among the five carbon sources tested, there was no clear over-performer (Fig. 2b). For ML3-1 and WE3-F, REE solubilization profiles were similar for all carbon sources tested. REE solubilization performance for *A. niger* was much more variable between replicates with the same carbon source. In contrast to the variability in REE solubilization, pH and carbon source consumption profiles were similar for *A. niger* for all carbon sources tested, as they also were for the other two isolates (Supplementary Fig. S1b and S2b).

For the glucose concentrations tested (5, 10, and 100 g/L), higher glucose concentrations did not correspond to improved REE solubilization for ML3-1 and WE3-F (Fig. 2c). For *A. niger*, the performance was again quite variable, and although the average REE concentration was highest

for 100 g/L glucose, this difference was not statistically significant. The pH reduction was comparable for all glucose concentrations tested (Supplementary Fig. S2c). Interestingly, for the lowest glucose concentration (5 g/L), the glucose was consumed by the fourth day (Supplementary Fig. S1), but REE concentrations continued to rise through the end of the experiment. For the highest glucose concentration (100 g/L), glucose levels remained above 10 g/L for the entire experiment. These data indicate that glucose availability was not the limiting factor for bioleaching under the conditions tested.

In a 2013 study, Qu and Lian examined bioleaching of REEs from red mud, a byproduct of bauxite ore processing for alumina production, by *Penicillium tricolor* RM-10 (Qu and Lian, [2013](#)). They reported total REE concentrations in the leachate of 20 to 60 mg/L, compared to 60 to 120 mg/L for monazite bioleaching by ML3-1 and WE3-F in this study. These concentrations corresponded to leaching efficiencies of 20%–40%, compared to 3%–5% found in this study. Note that the red mud efficiency numbers are higher even though the overall concentrations are lower due to the lower starting concentrations of REEs in the red mud. Given the differences in the ores (red mud vs. monazite) and experimental time scales (50 days vs. 6 days), it is not surprising that leaching efficiencies differ.

Two recent studies addressing monazite bioleaching reported widely ranging leaching efficiencies. Hassanien et al. reported efficiencies of up to 75% for bioleaching of monazite concentrate (Hassanien et al., [2013](#)), while Shin et al. reported leaching efficiencies of only 0.1% for bioleaching of monazite ore (Shin et al., [2015](#)). Differences in ores, bioleaching organisms, experimental conditions, and measurement methodologies may have contributed to the varying results.

Given the relatively low leaching efficiency of monazite bioleaching in this study, further optimization is necessary to achieve an economically viable process. There are several potential avenues for improving overall leaching efficiency. The results of the growth conditions comparisons suggest some important factors. In addition to lacking several trace minerals, NBRIP medium also had the lowest concentration of  $(\text{NH}_4)_2\text{SO}_4$  of all media tested (0.1 g/L), while AMS had the highest (0.66 g/L), suggesting that nitrogen concentration may be an important factor. Increasing the leaching time may also be effective. Over 6 days of bioleaching, REE concentrations did not appear to have entirely leveled off (Fig. [2](#)), and a longer leaching time may increase REE yield. Other process designs beyond leaching in a single batch should also be considered to further increase yield. The same monazite could be leached several times with fresh medium and organisms to extract more REEs, or a continuous flow process could be applied in which the monazite is retained via settling while the leachate is continuously

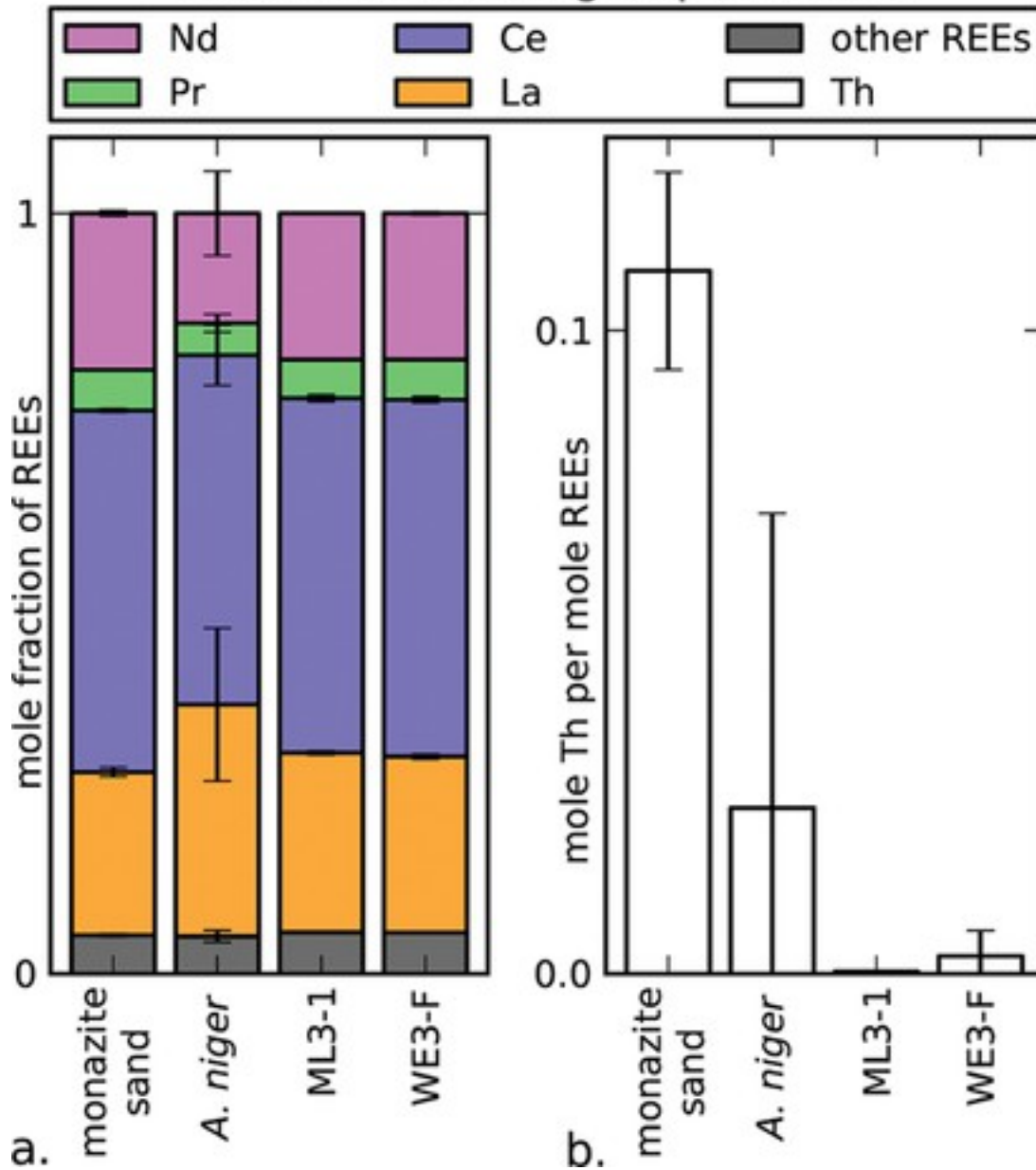
recovered. Other potentially important factors not considered here include monazite grain size, aeration, and temperature.

## Proportional Release of REEs and Thorium During Bioleaching

Proportions of REEs and Th in monazite and in bioleaching supernatant are shown in Fig. 3. The monazite sand used in this study is dominated by Ce, La, Nd, and Pr, and the bioleaching supernatant reflected this composition (Fig. 3a). Release of Th during bioleaching was low in proportion to REEs. For standard growth conditions (AMS medium, 10 g/L glucose), averages for released Th were  $0.026 \pm 0.046$ ,  $0.0003 \pm 0.0001$ , and  $0.0028 \pm 0.0039$  mole Th per mole REEs for *A. niger*, ML3-1, and WE3-F respectively (nine replicates each). In comparison, the monazite contained  $0.11 \pm 0.02$  mole Th per mole REEs (seven replicates), indicating preferential release of REEs over Th relative to the amounts present in the monazite ore.



## Proportion of REEs and Th in Monazite and in Bioleaching Supernatant



**Figure 3**

[Open in figure viewerPowerPoint](#)

Proportions of (a) REEs and (b) Th in monazite and in bioleaching supernatant after six days of bioleaching. Concentrations are normalized to total REE content of samples. Error bars indicate standard deviations around the means. Bioleaching samples are from growth on AMS medium with 10 g/L glucose.

[Caption](#)

In comparison, Th release in conventional monazite processing varies. In the commonly used NaOH treatment process, the majority of Th is leached along with REEs and must be separated

in downstream processing (Gupta and Krishnamurthy, [1992](#); Peelman et al., [2014](#)). The CaCl<sub>2</sub>/CaCO<sub>3</sub> process leaves most Th in the residual, although this process also has less favorable REE yields and requires much higher temperatures (Merritt, [1990](#); Peelman et al., [2014](#)). Th release during the sulfuric acid process depends on the specific leaching conditions (Gupta and Krishnamurthy, [1992](#); Peelman et al., [2014](#)).

## Organic Acid Production During Bioleaching

Organic acid production was observed for all organisms, with each organism producing a different set of acids. For a given organism, organic acid production was variable, and not all acids were detected in all biological replicates. Table III lists the maximum observed concentrations for identified organic acids during bioleaching experiments along with the percentage of bioleaching flasks for which each acid was detected.

**Table III.** Maximum observed concentrations of identified organic acids produced by three fungal isolates during bioleaching and percentage of bioleaching flasks for which each acid was detected

Organic Acid	<i>A. niger</i>		ML3-1		WE3-F	
	Maximum concentration	Percentage of flasks (%)	Maximum concentration	Percentage of flasks (%)	Maximum concentration	Percentage of flasks (%)
Acetic		0		0	3.8 mM	8
Citric	15.9 mM	78		0		0
Gluconic	5.3 mM	17		0	1.2 mM	67
Itaconic		0	>20 mM	97		0

Organic Acid	<i>A. niger</i>		ML3-1		WE3-F	
	Maximum concentration	Percentage of flasks (%)	Maximum concentration	Percentage of flasks (%)	Maximum concentration	Percentage of flasks (%)
Lactic		0		0		0
Oxalic	2.0 mM	17		0		0
Succinic	1.6 mM	56	4.0 mM.	28	5.4 mM	11

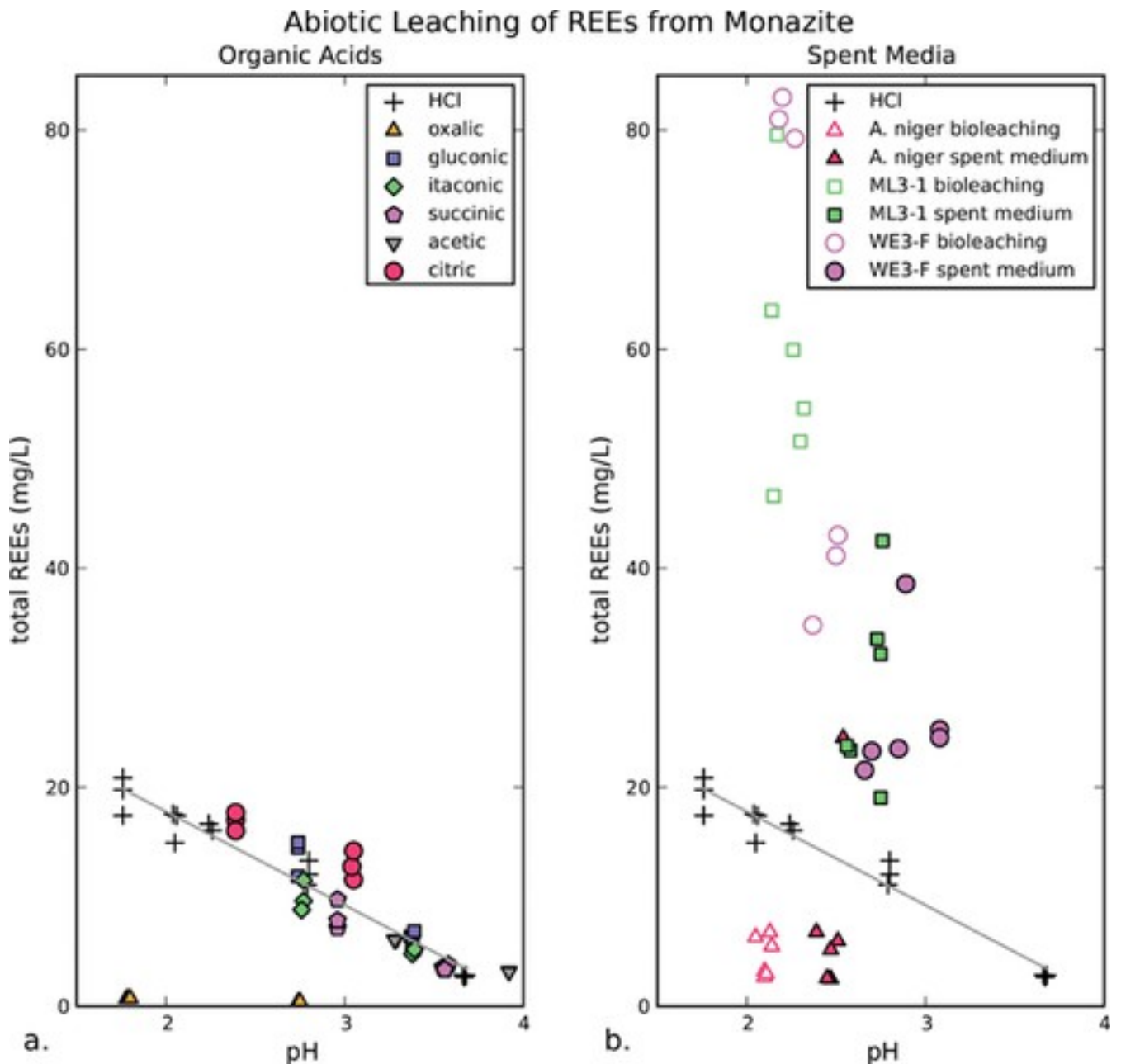
*A. niger* produced citric, gluconic, oxalic, and succinic acids. *A. niger* is known to produce these acids and is used industrially to produce citric and gluconic acids (Magnuson and Lasure, 2004; Papagianni, 2004). Optimization of *A. niger* acid production has revealed that low pH (<2) favors citric acid production while higher pH (>4) favors gluconic and oxalic acid production (Magnuson and Lasure, 2004; Ramachandran et al., 2006). In this study, the pH of the *A. niger* bioleaching cultures ranged from 2.0 to 2.8 in the later part of the bioleaching process ( $t = 4$  or 6 days), closer to the optimal conditions for production of citric acid. The production of higher concentrations of oxalic acid corresponded with lower concentrations of REEs, which is consistent with the known low solubility of REE-oxalates (Gadd, 1999).

ML3-1 produced primarily itaconic and succinic acids and WE3-F produced acetic, gluconic, and succinic acids. As noted above, ML3-1 showed high sequence similarity to *A. terreus*, some strains of which have been used industrially to produce itaconic acid (Magnuson and Lasure, 2004). *A. niger* and WE3-F also produced some compounds that generated large peaks in the HPLC UV absorbance chromatogram, but could not be identified based on the available standards. Other PSM studies have also observed additional compounds presumed to be other organic acids potentially involved in phosphate solubilizing activity (Chen et al., 2006).

## Abiotic Leaching With Hydrochloric Acid and Organic Acids

For all abiotic leaching experiments, leaching was performed for 48 h. Preliminary experiments indicated that this was sufficient time to reach equilibrium REE concentrations.

Leaching with *inorganic* HCl solutions representing a range of acidities (pH 1.8–3.7) indicated an approximately linear ( $r^2 = 0.96$ ) inverse correlation between pH (final pH after leaching) and REE solubilization within the range tested (Fig. 4a). Leaching with the most acidic solution (pH 1.8) resulted in the greatest REE solubilization, achieving a concentration of  $19 \pm 2$  mg/L. In their study examining monazite solubility in HCl acidified water, Oelkers and Poitrasson found a similar inverse relationship between pH and REE solubilization (Oelkers and Poitrasson, 2002).



## Figure 4

### [Open in figure viewer](#) PowerPoint

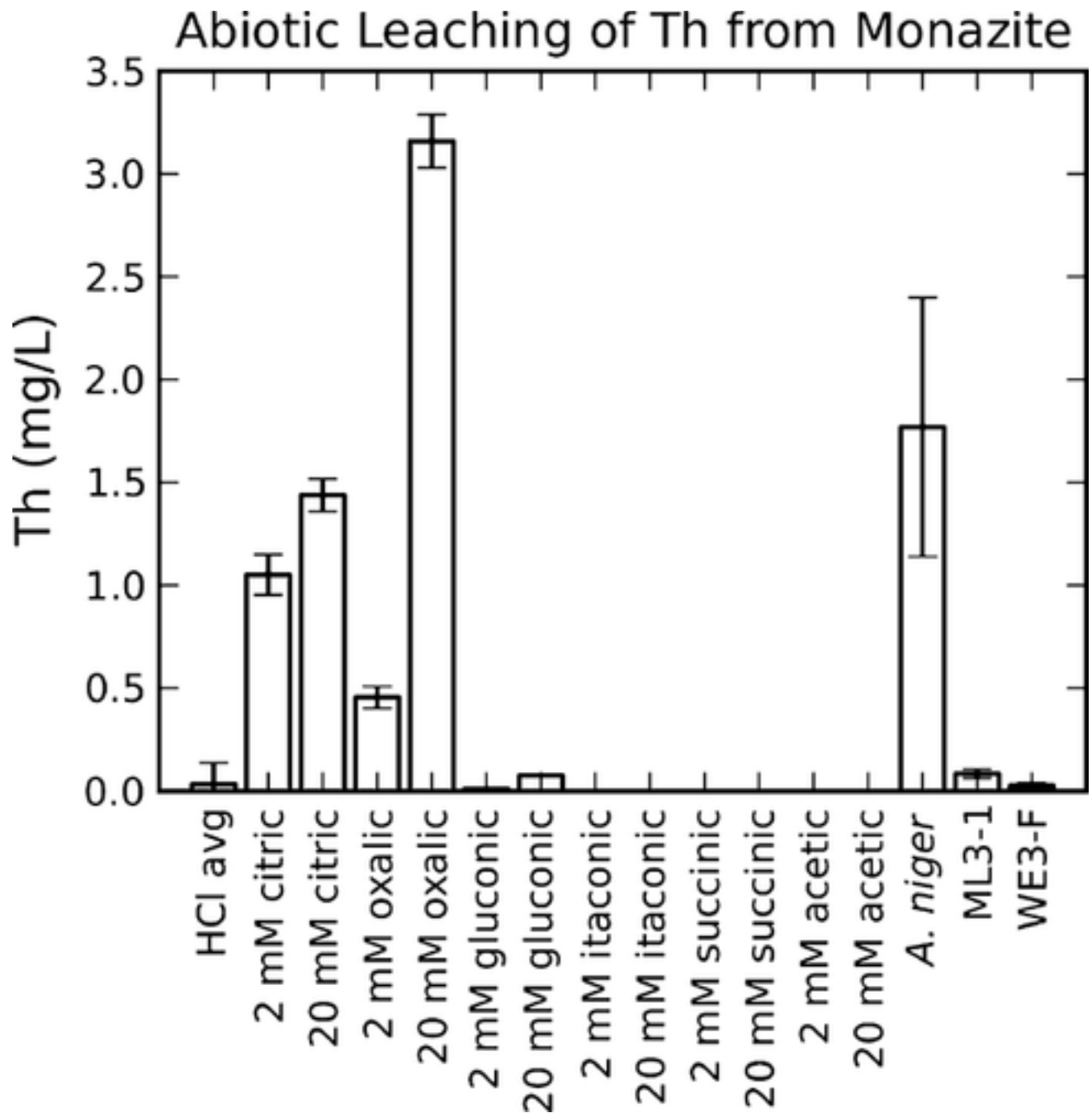
Abiotic leaching of REEs from monazite. Grey lines show the least squares fit to the HCl data ( $r^2 = 0.96$ ). The pH reported is the final pH after leaching. **(a)** Leaching with organic acids compared to HCl. **(b)** Leaching with spent medium from bioleaching compared to HCl. REE concentrations observed at the end of bioleaching are shown with unfilled markers for comparison.

### [Caption](#)

All *organic* acids tested, with the exception of oxalic acid, leached REEs from monazite to concentrations  $>1$  mg/L (Fig. 4a). The low observed REE solubilization with oxalic acid is consistent with the known insolubility of REE-oxalates. Because this behavior is known and is particular to oxalic acid, oxalic acid was excluded from the statistical analysis of abiotic leaching of REEs by other acids and spent supernatant.

For acetic, gluconic, itaconic, and succinic acids, the solubilization of REEs was not significantly different from what would be expected for the direct effect of pH (final pH after leaching). However, for citric acid, REE solubilization was slightly higher (approximately 3 mg/L, statistically significant) than would be expected based solely on final pH, suggesting that complex formation contributes to REE solubilization by citric acid. Goyne et al. found that citrate leached more REEs from monazite than salicylate, phthalate, and oxalate (Goyne et al., 2010). However, the observed REE solubilization levels for all organic acids tested ( $\leq 18$  mg/L) were substantially lower than those observed for the active cultures (averages 60–120 mg/L for ML3-1 and WE3-F depending on growth conditions).

A correlation was not detected between final pH and radioactive Th solubilization (Supplementary Fig. S3) and solubilization of Th was low overall in HCl solutions ( $\leq 0.01$  mg/L in 14 of 15 samples). Citric and oxalic acids solubilized Th significantly more than HCl solutions ( $1.0 \pm 0.1$ ,  $1.4 \pm 0.1$ ,  $0.5 \pm 0.1$ , and  $3.2 \pm 0.1$  mg/L for 2 mM citric, 20 mM citric, 2 mM oxalic, and 20 mM oxalic respectively), and had correspondingly higher ratios of Th to REEs ( $0.05 \pm 0.0004$ ,  $0.05 \pm 0.0005$ ,  $0.65 \pm 0.05$ , and  $2.6 \pm 0.07$  mole Th per mole REEs for 2 mM citric, 20 mM citric, 2 mM oxalic, and 20 mM oxalic respectively) (Fig. 5). Acetic, gluconic, itaconic, and succinic acids did not solubilize Th, resulting in Th concentrations below 0.1 mg/L and a Th to REEs ratio of  $<0.0035$  mole Th per mole REEs, comparable to bioleaching by ML3-1 or WE3-F.



**Figure 5**

[Open in figure viewer](#) [PowerPoint](#)

Abiotic leaching of Th by different organic acids and by spent supernatant from three bioleaching organisms. Error bars indicate sample standard deviations around the means. (n = 15 for HCl, n = 3 for each concentration of organic acid, n = 6 for spent supernatant for each organism).

[Caption](#)

Abiotic Leaching With Spent Medium From Bioleaching

After 6 days of growth with monazite, medium from the fungal bioleaching experiments (six biological replicates for each organism) was filtered to remove cells and treated with Amberlite IR-120 resin to remove REEs from solution, reducing total REE concentrations to <0.8 mg/L. Spent medium samples were then tested for monazite solubilization capabilities using the same two day abiotic leaching protocol used above to leach with organic acid solutions. Spent medium from ML3-1 and WE3-F solubilized REEs to levels above what would be expected based on the low pH of the spent medium (final pH after leaching) (Fig. 4b). Furthermore, citric acid was not detected in medium from bioleaching with these organisms (Table III), so no additional REE solubilization could be attributed to citric acid. Spent medium from *A. niger* was not effective at leaching REEs from monazite, likely due to the presence of oxalic acid, a known REE precipitant (Gadd, 1999). Spent medium from *A. niger* solubilized Th significantly more than the HCl solutions while spent medium from ML3-1 and WE3-F did not (Fig. 5). The corresponding Th to REE ratios were high for *A. niger* ( $0.21 \pm 0.12$  mole Th per mole REEs) and low for ML3-1 and WE3-F ( $0.0019 \pm 0.001$  and  $0.024 \pm 0.0004$  mole Th per mole REEs respectively).

The ability of spent medium to leach REEs from monazite indicates that the presence of microorganisms is not necessary for at least some portion of the observed solubilization. However, the higher REE concentrations observed for active bioleaching compared to spent medium indicate that the microorganisms' presence promote the most effective leaching. The metabolic activity of the fungi may enhance leaching effectiveness. One contributing factor may be consumption of phosphate by the microorganisms that hinders precipitation. As noted above, the high molar ratios of REEs to phosphate during bioleaching indicate that the majority of phosphate released from monazite during bioleaching is removed from solution for incorporation into biomass.

These data indicate that both ML3-1 and WE3-F release as yet unidentified compounds into solution that are more effective than the identified organic acids at solubilizing REEs from monazite. Based on these results, ML3-1 and WE3-F are more promising organisms for the development of bioleaching for processing monazite than *A. niger*.

This study provides a proof of concept for such a bioleaching process. Further study is needed to understand bioleaching mechanisms, to isolate and identify bioleaching compounds, and to optimize the process to achieve an economically viable alternative to conventional REE extraction processes. REE recovery efficiencies in conventional high-temperature chemical monazite processing range from 89% for the  $\text{CaCl}_2/\text{CaCO}_3$  process to 98% for the NaOH process (Peelman et al., 2014), as compared to 3%–5% recovery for the single-pass bioleaching in this study. Although Th is of concern in the NaOH process, the  $\text{CaCl}_2/\text{CaCO}_3$  process leaches low

levels of Th, similar to bioleaching (Merritt, [1990](#); Peelman et al., [2014](#)). Although bioleaching of REEs is promising and more environmentally friendly than the currently applied chemical processes, in order to develop a viable bioleaching process that can be competitive with conventional processing, REE recovery needs to be significantly increased.

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