1 Title: **Bioleaching of Rare Earth Elements from Waste Phosphors and Cracking Catalysts**

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Abstract

Microbial cultures were evaluated for organic acid production and their potential utility for leaching of rare earth elements (REE) from retorted phosphor powder (RPP) and spent fluid catalytic cracking (FCC) catalyst. Two bacterial and one fungal strain were isolated from environmental and industrial materials known to contain REE and compared to the industrially important bacterium *Gluconobacter oxydans*. Gluconic acid was the predominant organic acid product identified in all of the cultures. Maximum REE leaching (49% of the total REE) from the FCC material was observed using cell-free culture supernatants of *G. oxydans*, with preferential recovery of lanthanum over cerium. The phosphor powder was more difficult to leach; only about 2% of the total REE was leached with *G. oxydans*. Leaching experiments with the RPP material indicated that the extent of REE solubilization was similar whether whole cell cultures or cell-free supernatants were used. Abiotic control experiments showed that increasing gluconic acid concentrations increased leaching efficiency; for example, total REE leaching from FCC catalyst increased from 24% to 45% when gluconic acid was increased from 10 mM to 90 mM. However, *G. oxydans* cell-free culture supernatants containing 10-15 mM gluconic acid were more effective than abiotically prepared leaching solutions with higher gluconic acid concentrations, suggesting that other exudate components were important too. Our results indicate that microorganisms producing gluconic and other organic acids can induce effective leaching of REE from waste materials, and that increasing organic acid production will improve recovery.

1. Introduction

The recovery of rare earth elements (REE) from recyclable materials is an important component of the United States strategy for addressing anticipated future shortages of metals

critical for our modern technological society (DOE, 2011). The REE neodymium, europium, terbium, dysprosium, and yttrium are used in everyday products such as computers, smartphones and lighting, and in advanced clean energy technologies such as hybrid cars and wind turbines (Binnemans et al., 2013; DOE, 2011; Graedel et al., 2013). The DOE-identified “short term near-critical” elements cerium and lanthanum are key components of the fluid catalytic cracking (FCC) catalyst used in petroleum refineries to produce gasoline from higher weight crude oil (DOE, 2011). Global supply risks for REE are high because approximately 90% of all REE are currently obtained from a single nation, China (Binnemans et al., 2013).

Wastestreams that represent targets for REE recycling include permanent magnets, nickel metal-hydride batteries, and lamp phosphors (Tunsu et al., 2015). Fluorescent lamp wastes in particular present a promising opportunity because of their availability as recycling feedstocks, as they are being generated at increasing volumes throughout the world due to demand for more efficient lighting. Collection systems are already in place for the recovery of some components from fluorescent lamp wastes. Mercury is currently the main driver of the recycling effort because of environmental concerns, but other components (aluminum end caps, plastics, circuit boards, Ni-Cu wire, glass) are also routinely salvaged (DOE, 2011). The phosphor powders that line the bulbs constitute about 2-5% of the total weight of fluorescent lamp waste and are made up of pulverized apatite dust containing REE such as Y, La, Tb, Ce, and Eu (Yang et al., 2013).

To recover REE from solid materials (ores or wastes), physicochemical or biological processes may be employed. Conventional physicochemical methods for REE extraction and separation include dissolution, electrochemical technologies, fractional crystallization, ion exchange, and solvent extraction (Kronholm et al., 2013). These methods are often inefficient when concentrations of the target metals are low. Bioleaching is a hydrometallurgical process

that relies on the ability of microorganisms to solubilize and release metal elements from a solid matrix, and it has gained attention in recent years because of its potential for lowering costs and improving environmental sustainability (Brierley and Brierley, 2013). Generally, microorganisms facilitate the leaching of metals (including REE) by the production of a mineral or organic acid. The most notable mineral acid-based process is the leaching of copper from ore heaps. This process is accomplished by microorganisms that promote the conversion of insoluble metal sulfides into soluble metal sulfates. Today as much as 18-20% of global copper production uses this technique (Brierley, 2016).

Most REE-containing recycling feedstocks do not contain sulfidic material, and bioleaching by heterotrophic organisms producing organic acids presents an alternative approach. These organisms require organic carbon substrates to support growth, but these substrates could be derived from municipal or agricultural wastes (Jain and Sharma, 2004). The organic acids would dissolve the solid matrix, and in some cases, enhance metal solubility by complexation (Burgstaller and Schinner, 1993; Gräfe et al., 2011; Jain and Sharma, 2004; Schippers et al., 2014; Uroz et al., 2009). Bioleaching with heterotrophic organisms has been used to recover REE from red mud (a byproduct of aluminum production) and monazite ore. *Penicillium tricolor* RM-10 was able to leach 20-80% of the REE from red mud at a pulp density as high as 10% (Qu and Lian, 2013). Both fungi and bacteria are capable of heterotrophic leaching of monazite, but reported recovery efficiencies have varied widely. For example, Shin et al. reported 0.1% or less for total REE recovery for a number of heterotrophic bacteria (Shin et al., 2015), while Brisson et al. reported recovery values ranging from 3 to 5% for fungal isolates (Brisson et al., 2015). In contrast, Hassanien et al. reported that *Aspergillus ficuum* and

Pseudomonas aeruginosa released up to 75% and 64%, respectively, of the REE in monazite (Hassanien et al., 2014).

In this study microbial processes were evaluated for leaching REE from end-of-life waste products, specifically fluorescent lamp wastes and spent petrochemical FCC catalyst. The FCC materials, like the RPP, are considered “low-value” feedstocks, i.e., materials from which REE might not be economically harvested with conventional chemical, electrical, or mechanical methods because of low REE concentrations (Ferella et al., 2016). Microorganisms were enriched and isolated directly from the waste and REE ore materials and three isolates were selected for further study of organic acid production and compared to a known organic acid producing strain. Leaching of REE from RPP and FCC materials using the microbially produced organic acids was compared to leaching using abiotically prepared solutions of gluconic acid, the predominant acid produced by the four microorganisms tested. In addition, the effect of the presence or absence of whole cells during the leaching stage on REE solubilization was examined.

2. Materials and Methods

2.1. Substrates for enrichment and leaching

REE ore materials were obtained from the Rare Earth Resources (RER) Bull Hill Mine, located in the Bear Lodge District, north of Sundance, WY. Samples were collected from the main carbonatite dyke zone borehole 82.9 meters below land surface. Spent FCC catalyst (average particle size 80-90 μm ; 55-60% Al_2O_3 and 35-40% SiO_2) was obtained from Valero (Houston, TX) and RPP (retorted at 475°C for 8 hr under negative pressure; >90% of particles between 37 to 210 μm ; 85-95% apatite mineral and 5-15% ceramic mixture) was provided by

AERC Recycling Solutions (Allentown, PA). The REE content of the FCC catalyst and RPP is presented in Table 1; values were determined by acid digestion of the solids using 70% hydrochloric and 30% nitric acid, at 125°C for 8 h two times, followed by inductively coupled plasma mass spectrometry (ICP-MS) analysis.

Table 1. REE content in FCC catalyst and RPP.

REE	FCC catalyst ¹		RPP ²	
	Mean (µg/g)	RSD ³ (%)	Mean (µg/g)	RSD (%)
Y	14.0	5.4	50047.4	2.9
La	13617.3	2.4	8915.2	6.8
Ce	651.8	6.1	6328.0	6.1
Pr	14.7	2.9	<4.2	1.8
Nd	32.1	1.7	0.5	7.1
Sm	24.2	2.0	<0.2	1.8
Eu	0.7	6.0	3389.2	2.9
Gd	3.8	2.5	104.0	2.3
Tb	0.3	9.8	2575.8	7.9
Ho	1.4	1.8	0.2	15.0
Dy	0.3	1.4	<2.0	1.9
Er	0.7	2.0	<1.8	1.9
Tm	<0.1	3.7	0.9	3.9
Yb	0.4	4.0	0.6	5.1
Lu	0.1	6.0	31.9	6.5
Th	5.7	1.5	<2.6	1.9

¹ FCC, Valero fluid catalytic cracking catalyst (spent)

² RPP, AERC retorted phosphor powder

³ RSD, Relative standard deviation

2.2. Microorganisms and media

Gluconobacter oxydans strain NRRL B58 was obtained from the Agriculture Research Service, USDA (Peoria, IL). Isolation and organic acid analysis media: yeast-extract peptone glucose medium (YPG; 0.5 g/L yeast extract, 0.5 g/L peptone, 1 g/L glucose) and Pikovskaya phosphate medium (Pikovskaya, 1948). A modified Pikovskaya medium (Pkm) included a replacement of the calcium phosphate with CaCl₂·7H₂O (0.3 mM) and KH₂PO₄ (2.7 mM). Pkm

was modified to PkmT by including tryptone (10 g L⁻¹). Solid media was prepared with agar (15-20 g L⁻¹). Enrichment and isolation of microorganisms from the Bull Hill ore used 10% (w/v) sediment slurry in sterile phosphate buffered saline that was diluted and plated on YPG or Pikovskaya agar and incubated at 30°C for 10 days. For RPP, 0.5 g of material was suspended in 50 mL Pkm prior to incubation and plating on Pikovskaya agar for isolation. Isolates growing on Pikovskaya agar plates were evaluated for calcium phosphate clearing zones (>3-5 mm beyond the edge of the colony).

2.3. Molecular DNA techniques for phylogeny determination

Genomic DNA (gDNA) was extracted from the selected isolates using an UltraClean Microbial DNA Isolation kit (Mo Bio, Carlsbad, CA). Bacterial 16S ribosomal RNA (rRNA) genes were amplified by polymerase chain reaction (PCR) using <1 ng gDNA, TaqPlus Precision polymerase (Agilent Technologies, Santa Clara, CA), and primers 8F and 1492R; the fungal 18S rRNA gene was amplified using primer pairs F566 with F1200 and nu-SSU-0817 with nu-SSU-1536 (Borneman and Hartin, 2000; Hadziavdic et al., 2014; Reed et al., 2002). PCR products were purified with a QIAquick PCR Purification kit (QIAGEN) and sequenced (Pocatello, ID; www.mrcf.isu.edu). A consensus nucleic acid sequence was generated for each isolate and aligned pairwise, using the National Center for Biotechnology Information (NCBI) BLASTN tool to identify the closest sequence matches (Morgulis et al., 2008; Zhang et al., 2000). Gene sequences were submitted to GenBank (Accession No. KP216500, KP216504, KP216499).

2.4. Growth and organic acid production

Microbial isolates were grown overnight in Pkm medium at 30°C with shaking (180 rpm), and sub-cultured to 50 mL Pkm or PkmT medium in a 250 mL flask and incubated with

shaking at 150 rpm for up to 4 days. PkmT medium was included based on preliminary data suggesting that tryptone enhanced growth. Cultures in Pkm were incubated at 20, 30, or 40°C to test the effect of growth temperature; cultures in PkmT were grown only at 20°C. The bacterial subcultures were inoculated at 0.5×10^7 to 2×10^7 cells/mL; initial cell numbers were determined by acridine orange direct count (Ghiorse and Balkwill, 1983). For the fungal isolate A1, a 1.5 mL 2 day-old culture (6.2 ± 0.8 mg dry weight) was used as inoculum. All growth experiments were performed in triplicate. Aliquots (1-2 mL) were collected at 1, 2, and 4 days for measurement of optical density at 600 nm (for the bacteria only) and pH as well as organic acid composition.

2.5. Bioleaching studies

Most leaching studies were conducted using 4 day cultures, either with microbes present or with culture supernatants. Culture supernatants were obtained after centrifugation ($10,000 \times g$, 20 min.) and filter sterilization (0.22 μ m pore size). Cultures or sterile supernatants were added to 50 mL sterile tubes with feedstock (RPP or FCC material) at 1.5 % (w/v); the solids were prepared by autoclaving three times. The solids were leached for 24 h at 30°C with 150 rpm shaking. Treatments were in triplicate. Control treatments were the feedstocks incubated with sterile media alone or with gluconic acid added at final concentrations of 10, 30, or 90 mM.

2.6. Biosorption of soluble REE.

The possibility of REE biosorption occurring during leaching was evaluated. Solutions were prepared by chemically leaching RPP and FCC material with 35 mM gluconic acid. The solubilized REE from RPP and FCC catalyst were centrifuged and filtered (0.22 μ m pore size) to remove insoluble materials prior to addition of cells. Washed suspensions of bacterial isolates

BH1 and BH24 were added to the solutions at 5×10^7 cells/mL; for fungal isolate A1 a 1 day-old culture (2.5 ± 1.0 mg dry weight) was added. The suspensions were incubated at 30°C for 1 h at 150 rpm prior to centrifugation, filtration, and REE measurement. The extent of adsorption was determined by comparing the soluble REE concentrations before and after the incubation with cells.

2.7. Analytical methods

Organic acid analysis was performed by high-performance liquid chromatography (HPLC) with a BioRad Aminex HPX-87H column (300 mm $\times$ 7.8 mm, 5 μ m particle size) using 0.004 M sulfuric acid as the mobile phase at 0.6 mL/min and a column temperature of 35°C; detection was by photodiode array and quantitation at 210 nm. Microbial cultures were centrifuged and filtered (Millex-LH 0.22 μ m or Millex-LG 0.45 μ m PTFE) prior to HPLC analysis. Organic acid concentrations were determined from a standard curve using a BioRad organic acid analysis standard mixture (sodium salts of oxalate, citrate, malate, succinate, formate, and acetate) and separate gluconic and 2-keto gluconic acid standards.

REE concentrations were measured using ICP-MS. The instrument (iCAP Q, Thermo Scientific) was operated in accordance with **manufacturer** instructions. The filtered (0.22 μ m Millex-GP PES) samples and the commercial standard stock solutions were acidified with ultrapure concentrated nitric acid to a concentration of 1% HNO₃ (v/v).

3. Results and Discussion

3.1 Microbial isolates

As we have described elsewhere, microbial strains were isolated from the ores and other REE-containing materials for this study (Reed et al., 2016). Isolates were screened and selected for heterotrophic growth on glucose, dissolution of calcium phosphate in the Pikovskaya agar (indicator of acid production), and quantity of organic acids produced. Because they consistently produced the highest concentrations of organic acids and had the highest percent of REE leaching from the feedstocks in the initial screening tests, three isolates (BH1, BH24 and A1) were selected for further study, including phylogenetic characterization (Table 2).

Table 2. Microbial isolate identification.

Isolate	Source	Accession No.	rRNA gene sequence homology ¹	Identity (%)
BH1	Bull Hill	KP216500	<i>Acinetobacter calcoaceticus</i> GSN8	100
BH24	Bull Hill	KP216504	<i>Pseudomonas frederiksbergensis</i> 37	99.6
A1	RPP	KP216499	<i>Talaromyces purpureogenus</i>	100

¹Closest genus-species match according to NCBI BLAST

The BH1 16S rRNA gene sequence showed highest homology to the genus *Acinetobacter* which has been studied with respect to its phosphate release and uptake (Deinema et al., 1985; Kim and Pagilla, 2002; Momba and Cloete, 1996; Streichan et al., 1990) and metal uptake activities (Boswell et al., 2001). Isolate BH24 was identified as a *Pseudomonas* species. Many *Pseudomonads* are known to solubilize inorganic phosphate through the production of organic acids, including gluconic acid (Hoberg et al., 2005; Miller et al., 2010), and *Pseudomonas aeruginosa* was reported to leach REE from monazite through 2-ketogluconic acid production, an oxidized form of gluconic acid (Hassanien et al., 2014). The A1 fungal isolate was closely related to *Penicillium* and *Talaromyces*. Previously reported *Penicillium* strains have high tolerance for heavy metals such as Cu and are effective in leaching REE from red mud and carbonaceous shale (Amin et al., 2014; Qu and Lian, 2013; Romero et al., 2006). The release of

Mg and Ni from serpentine minerals by *Talaromyces* was linked to production of organic acids (Li et al., 2013). In addition to the isolates shown in Table 2, the *G. oxydans* strain B58, a member of the *Acetobacteraceae* family which includes a number of well-studied and industrially important acid producing microorganisms (Macauley et al., 2001), was included in the organic acid production and leaching studies.

3.2. Microbial growth and organic acid production

Growth was monitored for the bacteria by optical density measurements at 600 nm (OD_{600}); average values after 4 days are shown in Table 3. Fungal growth (strain A1) could not be monitored by optical density due to hyphae production. Strains BH24 and B58 grew better in Pkm at 30°C than at 20°C, while strain BH1 appeared unaffected by incubation temperature. Incubation at 40°C in Pkm was detrimental to all three bacterial strains. The presence of tryptone (PkmT) significantly increased final cell density (OD_{600}) for all of the cultures. Based on these results subsequent experiments were conducted with growth at 30°C for Pkm and 20°C for PkmT.

Table 3. Average and standard deviation of measured optical density at 600 nm at 4 days growth ($n = 3$).

Media and temperature	Average OD_{600} at 4 days		
	BH1	BH24	B58
Pkm at 20°C	0.388 ± 0.120	0.344 ± 0.021	0.555 ± 0.072
Pkm at 30°C	0.387 ± 0.058	0.442 ± 0.021	0.683 ± 0.008
Pkm at 40°C	0.096 ± 0.005	0.001 ± 0.001	0.014 ± 0.001
PkmT at 20°C	1.924 ± 0.012	2.060 ± 0.012	1.153 ± 0.012

During the four day incubation period, cultures were monitored for organic acid production. By day 4, B58 produced the highest total concentration (approximately 20 mM) of

the organic acids monitored, followed by BH1 and A1 (5 to 8 mM), and finally BH24 (<3 mM). Gluconic acid was the predominant organic acid for all of the strains. Low concentrations of 2-keto gluconic acid were also detected in the BH1 cultures (~ 1 mM) and B58 cultures (~ 3 mM); traces of acetic acid were also detected in the B58 cultures. For all of the cultures, >60% of the identified organic acids were produced in the first 24 hours. This observation was generally consistent with the measured pH values. For BH1, the pH was constant at ~3.3 during this period, for BH24 the pH dropped from 3.8 to 3.5, and for A1 the pH dropped from 2.7 to 2.4. The B58 culture was slow to start growing; by day one the OD₆₀₀ had only risen to 0.127, compared to over 0.350 for the BH1 and BH24. However, by the second day the OD₆₀₀ and pH for B58 had essentially plateaued at approximately 0.68 and 2.3 (the pH at day one was 2.9), respectively.

In PkmT, as noted above, the cultures grew to greater densities, even at a lower temperature (20°C vs 30°C), and generally the total amounts of organic acids produced were several (4 to 7) fold greater, except for B58 where the organic acid production was similar with the different media (data not shown). The relative amounts of organic acids produced when the cultures were grown in PkmT medium were similar to Pkm.

3.3. Abiotic leaching tests with RPP and FCC catalyst

Because the organic acid analyses indicated that gluconic acid was the predominant acid produced by the microorganisms, abiotic tests of REE leaching were conducted with gluconic acid at three different concentrations (10, 30 and 90 mM). Figure 1a shows the REE solubilization results for the RPP, where the effect of leaching duration was also tested. Increasing the concentration of gluconic acid improved the solubilization of total REE from the

236 RPP, from < 0.05% with 10 mM gluconic acid to approximately 12% with 90 mM gluconic acid.
 237 However, increasing the length of the contact time between the RPP and the organic acid
 238 solution beyond 1 day did not appear to impact the REE yield for the 10 and 30 mM gluconic
 239 acid treatments. The pH trends (Figure 1b) are consistent with this observation, as no pH changes
 240 were observed after the first day. For the 90 mM gluconic acid treatment, greatest REE recovery
 241 was observed after 3 days, although the pH did not change much after 1 day.

Figure 1. (a) Percent of the total REE leached from RPP with gluconic acid after 1, 3, and 9 days. (b) Change in pH over time in gluconic acid leaching of RPP. Error bars indicate standard deviations of triplicate experiments (error bars not seen fall within the symbol).

Abiotic leaching of REE from FCC catalyst was far more effective than from RPP, and as in the RPP experiments, increasing the gluconic acid concentration enhanced leaching. Total REE leaching increased from 24 to 36 to 45% for the 10 mM, 30 mM and 90 mM gluconic acid, respectively. For the FCC material, only one day leaching tests were conducted.

The results for the different feedstocks are likely related to the fact that the REE are present within very different matrices in the two materials. The limited extraction of REE from the RPP may be related to the high abundance of calcium phosphate in the RPP that would have consumed much of the acidity; the Material Safety Data Sheet for the RPP stated that it was 40-60% $\text{Ca}_3(\text{PO}_4)_2$ by mass. In contrast, cracking catalysts are composed primarily (>80%) of silica and alumina (Wu et al., 2003).

Published studies of organic acid based leaching from end-of-life materials are limited. However, Tunsu et al. (2014) reported on studies of abiotic chemical leaching of phosphor powders using acetic acid at 25% v/v, (~4.4 M); this acid concentration is more than two orders of magnitude higher than those used in our studies and the recovery was accordingly much higher: >50% of the Y and Eu were leached (Tunsu et al., 2014). High acid concentrations have also been used in mineral acid based abiotic leaching studies. De Michelis *et al.* (2011) reported >80% recoveries of Y from phosphors using 4 N HCl or H_2SO_4 at 90°C (De Michelis et al., 2011). Similarly, Rabah (2008) reported >90% REE recovery from phosphor powders using a 4 M mixture of sulfuric and nitric acid at 125°C and 5 MPa (Rabah, 2008).

3.4. Bioleaching of REE from RPP and FCC catalyst

Because the cultures were producing organic acid concentrations of 30 mM or less, and the abiotic leaching tests suggested no additional benefit from leaching longer than one day for gluconic acid concentrations at those levels, contact times of 1 day were used for the leaching tests with biologically produced organic acids. Leaching experiments were conducted using filtered supernatants from a culture grown for 4 days in Pkm or PkmT. For RPP, leaching experiments were also conducted using unfiltered 4 day cultures in order to see the effect of the presence of microbial cells during contact with the REE containing material. For the FCC material, leaching experiments were limited to the filtered supernatants of the bacterial cultures, since the results with RPP showed limited impacts from the presence of cells (see below) and previous screening studies had suggested that the fungal isolate A1 was only minimally effective in leaching REE from the spent FCC catalyst.

Figure 2 presents the total REE leached from the RPP under the different conditions. In the figure it is shown that the presence of the microbial cells did not markedly alter the solubilization of the REE from the RPP in either medium, except for BH1 and to a lesser extent BH24 in PkmT medium, where both exhibited decreased REE concentrations with microbial cells present. As expected, due to the higher organic acid concentrations seen with growth in PkmT, REE yields were generally higher in the PkmT compared to the Pkm media, except in the case of organism B58 which showed no difference between the two medium types. In keeping with the trend for the total organic acid concentrations measured in the cultures, where B58 produced the highest concentrations followed by BH1 and A1, and then BH24, average total leaching of REE from the RPP followed the same order (e.g. B58 > BH1, A1 > BH24). Still, in all cases total REE leaching was low, with a maximum of ~2% for organism B58.

Figure 2. REE leached from RPP at 24 h using microbially produced organic acids.

Microorganisms included BH1, BH24, B58 and A1 and were grown on either Pkm (a) or PkmT (b). Four-day old cultures or filtered supernatant were incubated with the RPP. A negative control of media without microorganisms was incubated in parallel, (gray bar, Neg). Error bars indicate standard deviations of triplicate experiments.

As observed in the abiotic leaching tests, REE recovery from the FCC material was far greater than from RPP. The results of the leaching tests with FCC catalyst (Figure 3) showed similar trends to the RPP with respect to the different leaching abilities of the culture supernatants, with greatest leaching (as high as 49% total REE) obtained using the B58 supernatant, followed by BH1 and finally BH24. Also as seen with the RPP, growth in PkmT did not confer an advantage in leaching to B58, although it did for the other two bacterial strains.

Figure 3. Percent REE leached from FCC catalyst after 1 day with microbially produced organic acids. Isolates BH1, BH24, and B58 grown in either Pkm or PkmT media. A negative control of media without microorganisms was incubated in parallel (gray bar, Neg). Error bars are standard deviations of triplicate experiments.

Relative yields of the individual REE in the leachates were not necessarily consistent with the measured compositions of the RPP. Figure 4 shows the relative REE composition of the RPP, compared to the relative proportions of the individual REE in the leachates. Y accounts for approximately 70% of the REE in the RPP, but for more than 80% of the REE in all of the

leachates. In addition, the RPP contains about twice as much Ce or La as Eu, but significantly more Eu than Ce or La was solubilized by the microbial supernatants. Tb was hardly released at all. Similar leaching trends were seen in the abiotic leaching experiments (data not shown). These observations are likely attributable to the presence of Y and Eu as oxides in phosphors, while La, Ce and Tb are primarily present as phosphates which are more difficult to dissolve by acid (Peelman et al., 2014; Yang et al., 2013).

Figure 4. Relative distributions of REE in the untreated original RPP (first column) and in the REE leached by isolates (BH1, BH24, B58, and A1) after 1 day. To effectively graph the REE distributions it was necessary to present each REE as a fraction of the total. Yttrium was 60% of the REE content in all conditions.

3.5. Biosorption of REE

Because we noted a slight difference between PkmT supernatants and cultures for BH1 and BH24 with respect to RPP leaching efficiency, and because many researchers have reported

on the sorption of rare earths to microbial cells (Andrès et al., 2003; Ozaki et al., 2006; Takahashi et al., 2005; Takahashi et al., 2010; Yoshida et al., 2004), and sorption of leached REE could lead to underestimation of leaching efficiency, we evaluated whether biosorption might be occurring in our experiments. Fresh cultures of microbes were contacted with filtered solutions containing REE leached abiotically with gluconic acid from the RPP and FCC materials. Incubation with aqueous REE at room temperature for 10 min was reported by Takahashi *et al.*, to be sufficient to reach equilibrium with respect to REE sorption by *Bacillus subtilis* and *Escherichia coli* (Takahashi et al., 2005). In our study, after 1 h incubation with microbial cells no significant sorption of the REE leached from RPP was detected, as measured by removal of REE from the aqueous media. However, it is still possible that slow biosorption led to the observed differences in leaching efficiency for the BH1 and BH24 supernatants and cultures.

3.6. Role of gluconic acid

Gluconic acid was the predominant acid produced by all four of the microbial cultures included in our study, and the abiotic experiments demonstrated that this organic acid was effective for releasing REE from the RPP and FCC materials. Microbial production of gluconic acid has been acknowledged in the literature by others as a likely contributor to biologically mediated phosphate mineral dissolution (Delvasto et al., 2008; Miller et al., 2010; Pérez et al., 2007). *Klebsiella* species that produced gluconic acid along with oxalic and citric acid were reported to dissolve Senegal rock phosphate (Rajput et al., 2013); the latter is a siliceous phosphate ore (Zhang et al., 2006). Gluconic acid based bioleaching of metals from a tungsten-rich hydrocracking catalyst has also been reported for a *Penicillium* strain; the fungus was able to

extract 10-70% of the heavy metals in the catalyst when grown for 30 days with 1-5% of the catalyst (Amiri et al., 2011).

It is important to note however that our data indicate that the gluconic acid concentration is not necessarily the prime determinant of leaching efficacy. Figure 5 shows that the *Gluconobacter* B58 supernatants that were determined to contain an average concentration of 12.5 mM gluconic acid were actually more effective at solubilizing REE from FCC material than abiotically prepared solutions containing 30 and 90 mM gluconic acid. Limited data for the non-REE metals Al, V, Fe, Ni, Cu and Sb (not shown here) indicated that the B58 supernatants were also more or at least as effective as the abiotic gluconic acid solutions at leaching these metals from the spent FCC. A better predictor of REE leaching efficiency is pH; prior to contact with the solids, the B58 supernatant with 12.5 mM gluconic acid had an average pH of 2.3, while the 10, 30 and 90 mM gluconic acid solutions had pH values of 3.2, 2.9 and 2.5, respectively. These data suggest that other components of the microbial exudates contribute to acidity; in the case of *Gluconobacter*, these likely include the 2,5-diketo gluconic acid along with the 2-keto gluconic acid (Krajewski et al., 2010; Macauley et al., 2001). Unfortunately a 2,5-diketo gluconic acid standard was not commercially available and therefore we were not able to verify the occurrence of this acid. Miller *et al.* reported that the phosphate-solubilizing *Pseudomonas* species they studied also produced 2-keto gluconic acid. In addition to simple dissolution of the solids by acidity, chelation by biologically produced ligands may also be occurring. Researchers working with the fungus *Aspergillus niger* found that both bioleaching (organisms present) and spent medium (supernatant) leaching of Al, V, Sb, Fe and Ni from spent FCC catalyst were more effective than abiotic leaching using mixtures of citric, gluconic and oxalic acids, and they suspected that other metabolites were likely contributing to the leaching performance (Aung and

Ting, 2005). Improvement in REE solubilization under biological leaching conditions compared to chemical leaching has also been reported for monazite and red mud (Hassanien et al., 2014; Qu and Lian, 2013), and it is likely that in those studies unknown components contributed to leaching as well.

Figure 5. Percent REE leached from FCC catalyst after 1 day using a 4 day B58 culture supernatant (12.5 mM gluconic acid produced) and abiotic solutions of 10, 30 and 90 mM gluconic acid in sterile Pkm. Error bars are standard deviations of triplicate experiments.

4. Conclusions

This research shows that utilizing microorganisms that produce gluconic and other organic acids can result in effective leaching of REE from end-of-life waste materials. Such organisms can be readily isolated from REE-containing materials themselves, indicating that the

microbes are tolerant of metals (REE and heavy metals) in the samples. In this study however we found a previously described strain of *G. oxydans* (B58) to be more effective at leaching REE than the isolates. Further evaluation of *G. oxydans* for REE recovery applications is warranted; in addition to the robust leaching performance observed in this study, *G. oxydans* is also an attractive candidate for metabolic modeling and genetic engineering due to the availability of an annotated genome sequence (Prust et al., 2005) and developed genetic systems (Herrmann et al., 2004; Merfort et al., 2006; Prust et al., 2005; Schleyer et al., 2008; Zhang et al., 2010), as well as a recent developed metabolic network model (Wu et al., 2014). Genetic modifications and or manipulation of cultivation conditions to increase acid production and achieve even higher REE recovery will be considered. Follow up studies by our group will also evaluate biological heap leaching of REE containing waste materials, similar to what is already done industrially for biological leaching of copper and gold ores. The objective is to improve our overall understanding of which factors are most important for controlling REE recovery from wastes by bioleaching, to enable scale-up of the approach and assessment of its economic viability.

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410 **References**

- 411 Amin, M.M. et al., 2014. Fungal leaching of rare earth elements from lower carboniferous carbonaceous
412 shales, southwestern Sinai, Egypt. *Romanian Journal of Biophysics*, 24(1): 25-41.
- 413 Amiri, F., Yaghmaei, S. and Mousavi, S.M., 2011. Bioleaching of tungsten-rich spent hydrocracking
414 catalyst using *Penicillium simplicissimum*. *Bioresource Technology*, 102(2): 1567-1573.
- 415 Andrès, Y., Texier, A.C. and Le Cloirec, P., 2003. Rare earth elements removal by microbial biosorption:
416 A review. *Environmental Technology*, 24(11): 1367-1375.
- 417 Aung, K.M.M. and Ting, Y.-P., 2005. Bioleaching of spent fluid catalytic cracking catalyst using
418 *Aspergillus niger*. *Journal of biotechnology*, 116(2): 159-170.
- 419 Binnemans, K. et al., 2013. Recycling of rare earths: a critical review. *J Clean Prod*, 51: 1-22.
- 420 Borneman, J. and Hartin, R.J., 2000. PCR primers that amplify fungal rRNA genes from environmental
421 samples. *Appl. Environ. Microbiol.*, 66(10): 4356-4360.
- 422 Boswell, C.D., Dick, R.E., Eccles, H. and Macaskie, L.E., 2001. Phosphate uptake and release by
423 *Acinetobacter johnsonii* in continuous culture and coupling of phosphate release to heavy metal
424 accumulation. *J. Ind. Microbiol. Biotechnol.*, 26(6): 333-340.
- 425 Brierley, C.L., 2016. *Biological Processing: Biological Processing of Sulfidic Ores and Concentrates—*
426 *Integrating Innovations, Innovative Process Development in Metallurgical Industry*. Springer, pp.
427 109-135.
- 428 Brierley, C.L. and Brierley, J.A., 2013. Progress in bioleaching: part B: applications of microbial
429 processes by the minerals industries. *Applied microbiology and biotechnology*, 97(17): 7543-
430 7552.
- 431 Brisson, V.L., Zhuang, W.Q. and Alvarez-Cohen, L., 2015. Bioleaching of rare earth elements from
432 monazite sand. *Biotechnol. Bioeng.*
- 433 Burgstaller, W. and Schinner, F., 1993. Leaching of metals with fungi. *Journal of Biotechnology*, 27(2):
434 91-116.
- 435 De Michelis, I., Ferella, F., Varelli, E.F. and Vegliò, F., 2011. Treatment of exhaust fluorescent lamps to
436 recover yttrium: Experimental and process analyses. *Waste Manage.*, 31(12): 2559-2568.
- 437 Deinema, M.H., van Loosdrecht, M. and Scholten, A., 1985. Some physiological characteristics of
438 *Acinetobacter* spp. accumulating large amounts of phosphate. *Water Sci. Technol*, 17(12): 119-
439 125.
- 440 Delvasto, P. et al., 2008. Diversity and activity of phosphate bioleaching bacteria from a high-phosphorus
441 iron ore. *Hydrometallurgy*, 92(3-4): 124-129.
- 442 DOE, 2011. *Critical Materials Strategy*, U.S. Department of Energy.
- 443 Ferella, F., Innocenzi, V. and Maggiore, F., 2016. Oil refining spent catalysts: A review of possible
444 recycling technologies. *Resources, Conservation and Recycling*, 108: 10-20.
- 445 Ghiorse, W.C. and Balkwill, D.L., 1983. Enumeration and morphological characterization of bacteria
446 indigenous to subsurface Sediments. *Developments in Industrial Microbiology*, 24: 213-224.
- 447 Graedel, T.E., Harper, E.M., Nassar, N.T. and Reck, B.K., 2013. On the materials basis of modern
448 society. *Proceedings of the National Academy of Sciences*.
- 449 Gräfe, M., Power, G. and Klauber, C., 2011. Bauxite residue issues: III. Alkalinity and associated
450 chemistry. *Hydrometallurgy*, 108(1-2): 60-79.
- 451 Hadziavdic, K. et al., 2014. Characterization of the 18S rRNA gene for designing universal eukaryote
452 specific primers. *PLoS One*, 9(2): e87624.
- 453 Hassanien, W.A., Desouky, O.A. and Hussien, S.S., 2014. Bioleaching of some rare earth elements from
454 Egyptian monazite using *Aspergillus ficuum* and *Pseudomonas aeruginosa*. *Walailak Journal of*
455 *Science and Technology (WJST)*, 11(6).
- 456 Herrmann, U., Merfort, M., Jeude, M., Bringer-Meyer, S. and Sahm, H., 2004. Biotransformation of
457 glucose to 5-keto-D-gluconic acid by recombinant *Gluconobacter oxydans* DSM 2343. *Applied*
458 *microbiology and biotechnology*, 64(1): 86-90.

- Hoberg, E., Marschner, P. and Lieberei, R., 2005. Organic acid exudation and pH changes by *Gordonia* sp. and *Pseudomonas fluorescens* grown with P adsorbed to goethite. *Microbiological Research*, 160(2): 177-187.
- Jain, N. and Sharma, D.K., 2004. Biohydrometallurgy for nonsulfidic minerals—a review. *Geomicrobiol. J.*, 21(3): 135-144.
- Kim, H.J. and Pagilla, K.R., 2002. Verification of enhanced phosphate removal capability in pure cultures of *Acinetobacter calcoaceticus* under anaerobic/aerobic conditions in an SBR. *Biotechnology and Bioprocess Engineering*, 7(6): 335-339.
- Krajewski, V. et al., 2010. Metabolic Engineering of *Gluconobacter oxydans* for Improved Growth Rate and Growth Yield on Glucose by Elimination of Gluconate Formation. *Appl. Environ. Microbiol.*, 76(13): 4369-4376.
- Kronholm, B., Anderson, C.G. and Taylor, P.R., 2013. A Primer on Hydrometallurgical Rare Earth Separations. *Jom-U.S.*, 65(10): 1321-1326.
- Li, Z. et al., 2013. Bioleaching of Lizardite by Magnesium- and Nickel-Resistant Fungal Isolate from Serpentine Soils—Implication for Carbon Capture and Storage. *Geomicrobiol. J.*, 32(2): 181-192.
- Macauley, S., McNeil, B. and Harvey, L.M., 2001. The genus *Gluconobacter* and its applications in biotechnology. *Critical reviews in biotechnology*, 21(1): 1-25.
- Merfort, M. et al., 2006. Modification of the membrane-bound glucose oxidation system in *Gluconobacter oxydans* significantly increases gluconate and 5-keto-D-gluconic acid accumulation. *Biotechnology Journal*, 1(5): 556-563.
- Miller, S.H. et al., 2010. Biochemical and genomic comparison of inorganic phosphate solubilization in *Pseudomonas* species. *Environ. Microbiol. Rep.*, 2(3): 403-411.
- Momba, M. and Cloete, T., 1996. Biomass relationship to growth and phosphate uptake of *Pseudomonas fluorescens*, *Escherichia coli* and *Acinetobacter radioresistens* in mixed liquor medium. *J. Indust. Microbiol.*, 16(6): 364-369.
- Morgulis, A. et al., 2008. Database indexing for production MegaBLAST searches. *Bioinformatics*, 24(16): 1757-1764.
- Ozaki, T. et al., 2006. Interactions of rare earth elements with bacteria and organic ligands. *Journal of Alloys and Compounds*, 408: 1334-1338.
- Peelman, S., Zhi, H., Sietsma, J. and Yang, Y., 2014. Leaching of rare earth elements: past and present. *ERES2014: 1st European Rare Earth Resources Conference*.
- Pérez, E., Sulbarán, M., Ball, M.M. and Yarzabal, L.A., 2007. Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. *Soil Biol. Biochem.*, 39(11): 2905-2914.
- Pikovskaya, R., 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologiya*, 17: 362-370.
- Prust, C. et al., 2005. Complete genome sequence of the acetic acid bacterium *Gluconobacter oxydans*. *Nat Biotech*, 23(2): 195-200.
- Qu, Y. and Lian, B., 2013. Bioleaching of rare earth and radioactive elements from red mud using *Penicillium tricolor* RM-10. *Bioresource Technology*, 136(0): 16-23.
- Rabah, M.A., 2008. Recyclables recovery of europium and yttrium metals and some salts from spent fluorescent lamps. *Waste Manage.*, 28(2): 318-325.
- Rajput, M.S., Kumar, G.N. and Rajkumar, S., 2013. Repression of oxalic acid-mediated mineral phosphate solubilization in rhizospheric isolates of *Klebsiella pneumoniae* by succinate. *Arch. Microbiol.*, 195(2): 81-88.
- Reed, D.W. et al., 2016. Microbially mediated leaching of rare earth elements from recyclable materials, *International Mineral Processing Congress*, Quebec City, Quebec, Canada.
- Reed, D.W. et al., 2002. Microbial communities from methane hydrate-bearing deep marine sediments in a forearc basin. *Appl. Environ. Microbiol.*, 68(8): 3759-3770.

- Romero, M.C., Reinoso, E.H., Urrutia, M.I. and Moreno Kiernan, A., 2006. Biosorption of heavy metals by *Talaromyces helicus*: a trained fungus for copper and biphenyl detoxification. *Electronic Journal of Biotechnology*, 9(3): 0-0.
- Schippers, A. et al., 2014. Biomining: metal recovery from ores with microorganisms, *Geobiotechnology* I. Springer, pp. 1-47.
- Schleyer, U., Bringer-Meyer, S. and Sahm, H., 2008. An easy cloning and expression vector system for *Gluconobacter oxydans*. *International Journal of Food Microbiology*, 125(1): 91-95.
- Shin, D., Kim, J., Kim, B.-s., Jeong, J. and Lee, J.-c., 2015. Use of Phosphate Solubilizing Bacteria to Leach Rare Earth Elements from Monazite-Bearing Ore. *Minerals*, 5(2): 189-202.
- Streichan, M., Golecki, J.R. and Schön, G., 1990. Polyphosphate-accumulating bacteria from sewage plants with different processes for biological phosphorus removal. *FEMS Microbiol. Ecol.*, 73(2): 113-124.
- Takahashi, Y., Chatellier, X., Hattori, K.H., Kato, K. and Fortin, D., 2005. Adsorption of rare earth elements onto bacterial cell walls and its implication for REE sorption onto natural microbial mats. *Chem. Geol.*, 219(1-4): 53-67.
- Takahashi, Y., Yamamoto, M., Yamamoto, Y. and Tanaka, K., 2010. EXAFS study on the cause of enrichment of heavy REEs on bacterial cell surfaces. *Geochim. Cosmochim. Acta*, 74(19): 5443-5462.
- Tunsu, C., Ekberg, C. and Retegan, T., 2014. Characterization and leaching of real fluorescent lamp waste for the recovery of rare earth metals and mercury. *Hydrometallurgy*, 144-145(0): 91-98.
- Tunsu, C., Petranikova, M., Gergorić, M., Ekberg, C. and Retegan, T., 2015. Reclaiming rare earth elements from end-of-life products: A review of the perspectives for urban mining using hydrometallurgical unit operations. *Hydrometallurgy*, 156: 239-258.
- Uroz, S., Calvaruso, C., Turpault, M.-P. and Frey-Klett, P., 2009. Mineral weathering by bacteria: ecology, actors and mechanisms. *Trends in Microbiology*, 17(8): 378-387.
- Wu, J.-H., Wu, W.-L. and Hsu, K.-C., 2003. The effect of waste oil-cracking catalyst on the compressive strength of cement pastes and mortars. *Cement and Concrete Research*, 33(2): 245-253.
- Wu, X., Wang, X. and Lu, W., 2014. Genome-scale reconstruction of a metabolic network for *Gluconobacter oxydans* 621H. *Biosystems*, 117: 10-14.
- Yang, F., Kubota, F., Baba, Y., Kamiya, N. and Goto, M., 2013. Selective extraction and recovery of rare earth metals from phosphor powders in waste fluorescent lamps using an ionic liquid system. *J. Hazard. Mater.*, 254-255(0): 79-88.
- Yoshida, T., Ozaki, T., Ohnuki, T. and Francis, A.J., 2004. Adsorption of rare earth elements by gamma-Al₂O₃ and *Pseudomonas fluorescens* cells in the presence of desferrioxamine B: implication of siderophores for the Ce anomaly. *Chem. Geol.*, 212(3-4): 239-246.
- Zhang, L., Lin, J., Ma, Y., Wei, D. and Sun, M., 2010. Construction of a novel shuttle vector for use in *Gluconobacter oxydans*. *Molecular biotechnology*, 46(3): 227-233.
- Zhang, P., Weigel, R. and El-Shall, H., 2006. Phosphate rock. *Industrial minerals and rocks: commodities, markets, and uses* 7th edn. Society for Mining, Metallurgy, and Exploration Inc., Littleton: 703-722.
- Zhang, Z., Schwartz, S., Wagner, L. and Miller, W., 2000. A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology*, 7(1-2): 203-214.