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# MICROBIALLY MEDIATED LEACHING OF RARE EARTH ELEMENTS FROM RECYCLABLE MATERIALS

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## ABSTRACT

Bioleaching offers a potential approach for recovery of rare earth elements (REE) from recyclable materials, such as fluorescent lamp phosphors or degraded industrial catalysts. Microorganisms were enriched from REE-containing ores and recyclable materials with the goal of identifying strains capable of extracting REE from solid materials. Over 100 heterotrophic microorganisms were isolated and screened for their ability to produce organic acids capable of leaching REE. The ten most promising isolates were most closely related to *Pseudomonas*, *Acinetobacter* and *Talaromyces*. Of the acids produced, gluconic acid appeared to be the most effective at leaching REE (yttrium, lanthanum, cerium, europium, and terbium) from retorted phosphor powders (RPP), fluidized cracking catalyst (FCC), and europium-doped yttrium oxide (YOEu). The *Acinetobacter* isolate, BH1, was the most capable strain and able to leach 33% of the total REE content from the FCC material. These results support the continuing evaluation of gluconic acid-producing microbes for large-scale REE recovery from recyclable materials.

## KEYWORDS

Bioleaching, Recyclable materials, Gluconic acid, Rare earth elements

## INTRODUCTION

An enhanced recovery, substitute, reuse or recycle paradigm is important for balancing the supply and demand of critical materials (DOE, 2011). The recovery of rare earth elements (REE) from recyclable materials is an important component in this model. REE are used in a variety of modern products. For example, neodymium, europium, terbium, dysprosium, and yttrium are important for applications such as permanent magnets, lamp phosphors, rechargeable batteries, etc. (Binnemans, Jones, Blanpain, Van Gerven, Yang, Walton, & Buchert, 2013; DOE, 2011; Graedel, Harper, Nassar, & Reck, 2013). Cerium and lanthanum are used in glass polishers and additives in fluidized cracking catalysts (FCC).

The economic practicality of recovering REE from end-of-life solid materials depends on the value of the recovered metals relative to the cost of recovery. Bioleaching relies on the ability of microorganisms to solubilize and release elements from a solid material, and compared to conventional hydrometallurgical approaches it can have lower capital and operating costs while providing greater environmental sustainability (Brierley & Brierley, 2013). Organic acid based bioleaching of metals utilizes heterotrophic microorganisms, which require organic carbon substrates to support growth (Jain & Sharma, 2004). The metabolites produced by these microorganisms can dissolve the solid matrix through acidity and in some cases also enhance metal solubility by complexation (Burgstaller & Schinner, 1993; Gräfe, Power, & Klauber, 2011; Jain & Sharma, 2004; Schippers, Hedrich, Vasters, Drobe, Sand, & Willscher, 2014; Uroz, Calvaruso, Turpault, & Frey-Klett, 2009).

For this study we evaluated microbial processes for leaching REE from recyclable materials, namely retorted phosphor powders (RPP) derived from fluorescent lamp wastes and spent FCC. Harvesting REE from these types of materials with conventional chemical, electrical, or mechanical methods has generally not been considered economically viable because of their low REE content. Although bioleaching has not been reported previously for phosphor powders, other researchers have shown that the powders can be treated with acidic solutions or ionic liquids to recover the REE (Rabah, 2008; Tunsu, Ekberg, & Retegan, 2014; Yang, Chen, Li, Chen, Chen, & Liu, 2014). Bioleaching of heavy metals from spent hydrocracking catalysts has been discussed in the literature, but those studies did not target REE (Amiri, Yaghmaei, & Mousavi, 2011; Brandl, Bosshard, & Wegmann, 2001; Ilyas & Lee, 2013; Santhiya & Ting, 2005; Yang et al., 2014; Zhu, Xiang, Zhang, Wu, Dang, Li, & Wu, 2011).

We enriched and isolated microorganisms directly from the waste materials, and we also obtained microbial isolates from ore samples collected from a prospective REE mine and an operating phosphate mine. We characterized the microorganisms phylogenetically and assessed their ability to produce organic acids and leach REE from the recycling feedstocks.

## METHODS

### Microbiological Source, Growth Media, Enrichment, and Isolation

We obtained REE ore materials from the Rare Earth Resources (RER) Bull Hill Mine, located in the Bear Lodge District, north of Sundance, Wyoming, USA. Samples were collected from the Bull Hill main dyke zone at three depths of a single borehole on October 21, 2013, identified by the feet below land surface (fbls): 43.6 mbls, 60.7 mbls, and 82.9 mbls. They were shipped overnight to the Idaho National Laboratory (INL; Idaho Falls, Idaho, USA). Phosphate ore was obtained from the Monsanto Blackfoot Bridge Mine located 16 kilometers northeast of Soda Springs, Idaho on November 13, 2013. The mine is in the Permian Phosphoria Formation, known to contain REE (Emsbo, McLaughlin, Breit, du Bray, & Koenig, 2015). Ore samples were collected from the top and bottom seams and gangue materials. FCC was obtained from Valero (Houston, Texas, USA) and RPP was obtained from AERC Recycling Solutions (Allentown, Pennsylvania, USA). Research grade purity (99%) europium-doped yttrium oxide red phosphor (YOEu; $Y_{1.92}Eu_{0.08}O_3$; CAS No. 68585-82-0) was purchased from Sigma Chemicals and used as a control substrate for comparison to the RPP.

Media used for enrichment and selection of organic acid producing microorganisms included Horikoshi ATCC 2162 (Qu & Lian, 2013), Czapek ATCC 312 (Deng, Chai, Yang, Tang, Wang, & Shi, 2013), Bacillus ATCC 573 (without pH adjustment), Luria-Bertani Miller's medium with 5 g/L enzyme hydrolyzed casein (LB-C), yeast-extract peptone glucose medium (0.5 g/L yeast extract, 0.5 g/L peptone, 1 g/L glucose) and Pikovskaya's phosphate medium (Pk) (Pikovskaya, 1948). A modified Pikovskaya medium (Pkm) included a replacement of the calcium phosphate with $CaCl_2 \cdot 7H_2O$ (0.07 g/L) and $KH_2PO_4$ (0.37 g/L). Pkm was modified to Pk2 by including tryptone (2 g/L) along with more yeast extract (2 g/L rather than 0.5 g/L) and less glucose (6 g/L rather than 10 g/L). Solid media was prepared with agar (15-20 g/L). In some Pikovskaya agar media, phosphor powder was included in the agar at 0.5% (w/v) to examine if it was conducive or inhibitory to microbial growth. Glucose-based general aerobic heterotrophic bacterial medium with phenol red (GHB) was purchased in individual 10 mL vials from Biotechnology Solutions (Houston, Texas).

To enrich microorganisms from each ore sample we used 10% (w/v) sediment slurry prepared in sterile phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$, 1.8 mM $KH_2PO_4$, pH 7.4) that was serially diluted and plated on the various agar as described above; one mL of slurry was added to liquid GHB medium. All of the enrichment cultures were incubated at 30°C for 10 days. For each GHB culture that showed acid production (red to yellow color change), we plated 100 µL of sample on Pk agar for microbial isolation. For microbial enrichment and isolation from the RPP and FCC materials, 0.5 g of material was suspended in 50 mL Pkm, Pk2, or LB-C medium and incubated at 30°C with shaking (100 rpm) for 5 days prior to plating (100 µL) on Pk agar for isolation. All isolates were transferred to Pk agar and evaluated for clearing zones beyond the edge of the colony, indicating calcium phosphate dissolution. We used a dissecting microscope (Bausch & Lomb) to evaluate colony morphology and visualized individual cells with an Olympus BX51 light microscope. We named isolates according to their origin (BH, Bull Hill; BB, Blackfoot Bridge; A, AERC) and numbered them sequentially.

**Microbial Organic Acid Production and Feedstock Leaching**

The RPP, FCC, and YOEu were used as feedstocks for leaching studies, after tyndallization or autoclaving three times in succession. Microbial isolates were grown aerobically overnight in LB-C or Pk2 medium at 30°C with shaking (180 rpm). For each experiment, we subcultured the selected culture to 50 mL Pkm (pH 5.7) or Pk2 (pH 6.4) medium in a 250 mL flask containing the prepared feedstock (1.5 +/- 0.04 % (w/v)) and incubated them at 30°C with shaking at 150 rpm from 1 to 14 days. Experiment volume was maintained by measuring mass and adding sterile nanopure water. The initial concentration for each bacterial culture ranged from $0.5 \times 10^7$ to $2 \times 10^7$ cells/mL; cell numbers were determined using the acridine orange direct count method (Ghiorse & Balkwill, 1983). For the fungal isolate, cell numbers were not determined; a 3 mL 2 day-old culture was used as inoculum for each treatment. We prepared treatments of Pkm and Pk2 media without added microbial cultures as abiotic controls; to a subset of the Pkm controls we added gluconic acid (35 mM) to examine the effect of the acid in the absence of the microorganisms.

We collected one mL samples from each culture, and filtered (0.22 µm pore size) and stored them at -20°C until analysis by high-performance liquid chromatography using a BioRad Aminex HPX-87H column (300 mm × 7.8 mm, 5 µm particle size) with 4 mM sulfuric acid as the mobile phase, a flow rate of 0.6 mL/min and column temperature of 35°C. Organic acids were quantified using a photodiode array detector at 210 nm with BioRad organic acid analysis standards (sodium salts of oxalate, citrate, malate, succinate, formate, and acetate) and solutions of gluconic acid at known concentrations.

REE concentrations were measured using inductively coupled plasma mass spectrometry (ICP-MS). The instrument (iCAP Q, Thermo Scientific) was operated in accordance with the manufacturer's instructions. Filtered (0.22 µm pore size) samples and commercial standard stock solutions were acidified with ultrapure concentrated nitric acid to a concentration of 1% $HNO_3$ (v/v). The REE content of the FCC and RPP was determined after acid digestion (70% hydrochloric and 30% nitric acid, two rounds of heating at 125°C for 8 h). Indium (mass 115) at 10 ppb was used as the internal standard and introduced into the

samples and standards by online injection. The $R^2$ value of the calibration curve was >0.999. Percent recovery was determined for the leaching experiments based on the sum of the predominant REE present in the original solid (i.e., Y, La, Ce, Eu, and Tb for the RPP; Y and Eu for YOEu; La and Ce for the FCC).

**Phylogenetic Determination**

We extracted genomic DNA from the selected isolate cultures using an UltraClean Microbial DNA Isolation kit (Mo Bio, Carlsbad, CA). Bacterial 16S ribosomal RNA genes (rRNA) were amplified from the template (<1 ng DNA) by polymerase chain reaction (PCR) using primers 8F and 1492R as described previously (Reed, Fujita, Delwiche, Blackwelder, Sheridan, Uchida, & Colwell, 2002). For fungal strains, 18S rRNA was amplified using primer pairs F566 with F1200 and nu-SSU-0817 with nu-SSU-1536 (Borneman & Hartin, 2000; Hadziavdic, Lekang, Lanzen, Jonassen, Thompson, & Troedsson, 2014). PCR products were purified with a QIAquick PCR Purification kit (QIAGEN) and sequenced at Idaho State University's Molecular Research Core Facility (Pocatello, ID; www.mrcf.isu.edu) using the primers mentioned above as well as 519R and 907R (Reysenbach, Longnecker, & Kirshtein, 2000) and 704F (GTAGCGGTGAAATGCGTAGA). Consensus nucleic acid sequences were determined as described previously (Reed et al., 2002).

We identified the closest sequence matches for the nucleic acid sequences by a pairwise alignment using the National Center for Biotechnology Information (NCBI) BLASTN tool (Morgulis, Coulouris, Raytselis, Madden, Agarwala, & Schäffer, 2008; Zhang, Schwartz, Wagner, & Miller, 2000). Gene sequences were submitted to GenBank with accession numbers KP216499 to KP216509. Approximately 1345 to 1360 base pairs (bp) were used for bacterial alignment and 850 bp for the fungus.

## RESULTS

**Microbial Enrichment, Isolation, and Characterization**

To identify microorganisms effective at leaching REE, we targeted recyclable materials, REE ores, and phosphate ores as enrichment sources. The goal was to isolate organisms capable of tolerating REE and extracting them from the source materials by production and exudation of organic acids. From the different source materials and enrichment media, we isolated and discriminated (by colony morphology and cell morphology) >100 microbial strains.

We selected isolates for further evaluation based on heterotrophic growth on glucose, dissolution of calcium phosphate in the Pk agar (presumably by acid production), and the measured production of organic acids. Fifteen (14 bacterial, 1 fungal) of the selected isolates produced large zones of clearing (e.g., >3 mm beyond the edge of the colonies) in the Pk agar plates. Of the microorganisms that originated from the phosphor powders and cracking catalyst only one, A1, was able to induce clearing in the Pk agar. We monitored the liquid cultures for pH and the production of organic acids during incubation in Pkm and Pk2 media. Those that lowered the pH to below 5 and/or produced total organic acid concentrations greater than 20 mM were chosen for further evaluation.

For phylogenetic characterization of the most promising organic acid producing organisms, we obtained sequences for the DNA coding for the 16S ribosomal RNA gene (rRNA) for the bacteria or the 18S rRNA gene for the fungus and compared them to gene sequences available in the NCBI databases (Table 1).

Table 1 - Results of 16S and 18S rRNA gene sequencing for selected microbial isolates

| Isolate | Accession No. | rRNA gene sequence NCBI BLAST homology match | Identity (%) |
|---|---|---|---|
| BH1 | KP216500 | *Acinetobacter calcoaceticus* str GSN8 | 100 |
| BH4 | KP216501 | *Pseudomonas* sp. AgResearch_C9 | 99.9 |
| BH21 | KP216503 | *Pseudomonas* sp. WR4-40 | 100 |
| BH24 | KP216504 | *Pseudomonas frederiksbergensis* str 37 | 99.6 |
| BH41 | KP216506 | *Pseudomonas fluorescens* str CPO 4.0100 | 100 |
| BH46 | KP216507 | *Yersinia enterocolitica* str FE81536 | 99.8 |
| BB46 | KP216509 | *Pseudomonas fluorescens* str M-T-TSA_84 | 100 |
| A1 | KP216499 | *Talaromyces purpureogenus* | 100 |

**Bioleaching of REE from Recyclable Materials**

We incubated the microbial isolates for two weeks in the presence of the REE-containing materials and evaluated organic acid production and the amount of REE solubilized. The REE contents of the FCC and RPP are shown in Table 2. Several of the microorganisms isolated in this study were shown to leach REE from the feedstocks. Gluconic and citric acid were the primary organic acids produced by the bacterial isolates able to solubilize >1% REE. In samples with active leaching, the media pH decreased from 5.7 (Pkm) or 6.4 (Pk2) to between 2 and 5 for the FCC and RPP and to between 5 and 6 for YOEu.

Table 2 - REE content in recyclable materials

| REE | FCC Mean[1] (µg/g) | FCC RSD[2] (%) | RPP Mean (µg/g) | RPP RSD (%) |
|---|---|---|---|---|
| Y | 14.0 | 5.4 | 50047 | 2.9 |
| La | 13617 | 2.4 | 8915 | 6.8 |
| Ce | 652 | 6.1 | 6328 | 6.1 |
| Eu | 0.7 | 6.0 | 3389 | 2.9 |
| Tb | 0.3 | 9.8 | 2576 | 7.9 |

[1]Mean, Average of three replicates
[2] RSD, Relative standard deviation

We observed a positive correlation between the amount of REE solubilized and the total amount of citric and gluconic acid produced by the various isolates (Figure 1). In general, REE were most effectively solubilized from the FCC, followed by YOEu and RPP. The same trend was observed with the abiotic gluconic acid (35 mM) control (data not shown). The only exception to this trend was observed with the fungal isolate A1, which proved to be more effective at solubilizing REE from YOEu than from FCC. In the abiotic controls to which no organic acid was added, REE leaching was <0.03% in all cases except for the FCC in Pk2 media where total REE leaching was 1.2%.

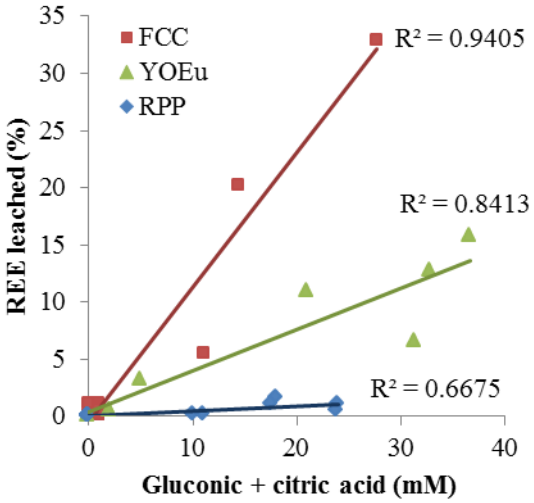


Figure 1 - Bioleaching of REE from feedstocks after two weeks as a function of total gluconic and citric acid produced; data points represent different organisms and treatments. Lines are linear regressions

The percent of REE leached also correlated well with gluconic acid concentration alone. Gluconic acid was the major organic acid present in the biotic experiments where greater than ~3% of the total REE was leached from the feedstocks. In particular, microbial strains BH1 and A1 produced predominantly gluconic acid and A1 showed a strong correlation between gluconic acid production and REE dissolution (Figure 2).

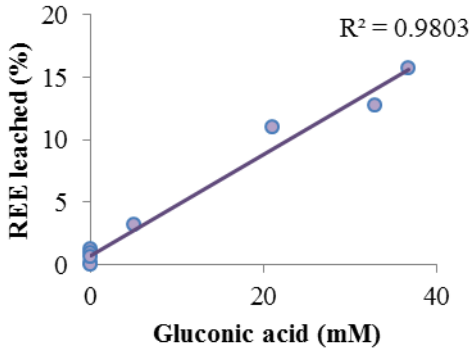


Figure 2 - Bioleaching of REE from feedstocks by the *Talaromyces* fungal isolate, A1, as a function of total gluconic acid produced. Linear regression was performed

Relative solubilization of the individual REE was not necessarily consistent with the measured elemental compositions of the feedstocks. This was most evident for RPP where twice as much Ce or La compared to Eu was present in the feedstock, yet more Eu was solubilized by the isolates; the relative REE contents of the three RPP leachates containing the most total REE are shown in Figure 3.

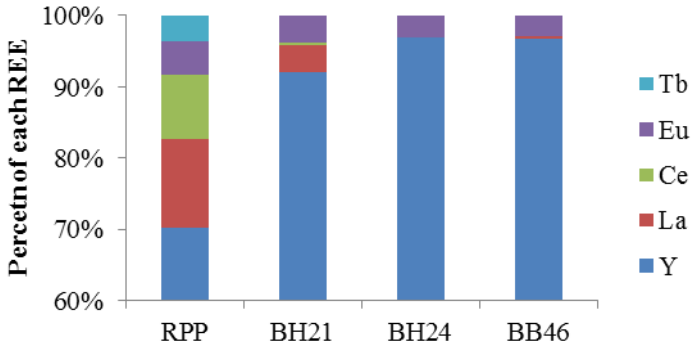


Figure 3 - Cumulative relative distributions of REE in original RPP (first column) and in leachates produced by three isolates (last three columns) after 2 weeks of bioleaching. Vertical axis scale configured to facilitate visualization of REE distribution; first 60% of REE content in all cases consisted of yttrium

## DISCUSSION

This work was conducted to support the development of an economical and environmentally friendly process for recovering REE from end-of-life products, an important component in balancing the supply and demand of critical materials. Organic acid producing microorganisms that could effectively leach REE from waste materials were the targets for this study. Most of the isolates identified here were closely related to bacterial species belonging to the γ-*Proteobacteria* phylum; several were related to *Pseudomonads* previously described as able to solubilize inorganic phosphate through the production of organic acids, including gluconic acid (Hoberg, Marschner, & Lieberei, 2005; Miller, Browne, Prigent-Combaret, Combes-Meynet, Morrissey, & O'Gara, 2010). *Pseudomonas* strains have also been reported to leach REE from monazite by producing 2-ketogluconic acid, an oxidized form of gluconic acid (Hassanien, Desouky, & Hussien, 2014). One of the most prolific producers of gluconic acid isolated here, BH1, was able to leach ~33% of the total REE content from the FCC material. This isolate was identified as an *Acinetobacter* strain. *Acinetobacter* are present in contaminated soils and are reportedly tolerant of heavy metals and REE (Boswell, Dick, Eccles, & Macaskie, 2001; Stoner & Tien, 1995). The A1 isolate was more effective at leaching REE from YOEu than from the other feedstocks and the amount leached was greater than the yield from the abiotic control and from the isolate BH1 with similar amounts of gluconic acid. The 18S rRNA gene sequence for A1 was homologous to rRNA genes from the soil fungi *Penicillium* and *Talaromyces* (recently reclassified from *Penicillium*). 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, El-Aassy, El-Feky, Sallam, El-Sayed, Nada, & Harpy, 2014; Qu & Lian, 2013; Romero, Reinoso, Urrutia, & Moreno Kiernan, 2006).

This research shows that utilizing microorganisms that produce organic acids, particularly gluconic acid, can result in effective leaching of REE from waste materials. The relationships between gluconic and citric acid and total REE leached from the different feedstocks (Figure 1) suggested that RPP was the most recalcitrant to bioleaching. We observed much less leaching than the >50% Y and Eu solubilization reported by Tunsu et al. (2014) for abiotic leaching of phosphor powders with acetic acid. The primary reason for the discrepancy is likely related to acid quantities; Tunsu et al. (2014) used acetic acid at a concentration of 25% (v/v), or ~4.4 M, while in our experiments the organic acid concentrations were two orders of magnitude lower. Most of the acidity was probably consumed or neutralized by reaction with the calcium phosphate comprising 40-60% of the RPP (estimate from the material safety data sheet received from the manufacturer). Recovery of REE by bioleaching was easier from the YOEu, and even more so from the FCC, with the lone exception of leaching by the fungus A1.

The REE were not released equally into solution from the feedstocks under the mild acidic conditions. Y and Eu were released most readily from the RPP; this is likely related to the presence of Y

and Eu as oxides, while La, Ce and Tb are present as phosphates (Peelman, Zhi, Sietsma, & Yang, 2014; Yang, Kubota, Baba, Kamiya, & Goto, 2013). A recent study showed that REE solubilization from monazite and apatite increased generally with increased organic acid concentration, but not all REE were recovered equally (Goyne, Brantley, & Chorover, 2010). Goyne et al. (2010) postulated that the particular minerals hosting the REE may be more important in determining dissolution rates than the identity of the organic acid, although they observed that citric acid was especially effective at leaching REE from phosphate minerals. More effort is required to elucidate the functional roles of the various organic acids and of the microbes themselves in heterotrophic bioleaching of REE-containing materials.

## CONCLUSIONS

We readily isolated organic acid producing organisms from REE-containing ores as well as REE wastes, suggesting that "exotic" or difficult to maintain species are not required for effective leaching properties. This result bodes well for the robustness of REE recycling technologies built on bioleaching. Among the more than 100 strains isolated in this study, the *Acinetobacter* sp., BH1, and the fungus *Talaromyces* sp., A1, appear to be particularly promising candidates for the development of biologically-based industrial approaches for REE recovery from recyclable materials.

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