Contract No:

This document was prepared in conjunction with work accomplished under Contract No. 89303321CEM000080 with the U.S. Department of Energy (DOE) Office of Environmental Management (EM).

LDRD-2022-00108: Biomining Rare Earth Elements PI: Robin L. Brigmon
LDRD External Report Summary

Summary Statement

Rare earth elements (REEs) are crucial to many high-tech devices and maintaining a competitive modern economy, thus they are of critical and strategic importance. Currently, mining approaches include rock grinding, strong acid or base extraction, separation, and concentration. The objective of this project was to develop cost-effective, environmentally friendly technologies for bioextracting REEs from mined materials.

Introduction

Problem: The United Stated government has identified that the REEs are critical elements, used extensively in consumer electronics, and military and national security hardware, which are in short supply. **Relevancy:** In response to increasing geopolitical tensions with key critical element producers, several agencies are issuing funding opportunity announcements related to improved recovery of these elements. **Objective.** Here we tested biomining, the process of microbiologically extracting REEs from water, rock ores, and mine waste to fill this need. Currently, these elements are mined by mineral grinding, followed by solvent extraction, separation, and then reconcentration. This work aimed to develop technologies for bioextracting these REEs from mined ores. Bioextraction technology focused on 1) utilizing natural microbes that generate exudates that enhance the extraction of elements, 2) evaluating microbes to dissolve the materials, and 3) and biosurfactant producing microbes. **Results.** Electrochemical impedance spectroscopy and voltammetric techniques were combined with molecular analysis to determine optimum microbe-mineral interactions for critical element biorecovery. Select microorganisms were tested and proved useful for REE uptake. REEs were successfully extracted at the part per million level from select mined materials. A partnership has been developed with Mosaic, a mining company with REE rich mine tailings tested by SRNL and they have requested a proposal for future work based on our results. The study culminated in a proof-of-concept demonstration, manuscripts, partnership with a major mining company to move forward, and the data needed to develop proposals for scaled-up biomining of critical elements. UGA Placeholder

Approach REEs are an essential part of many high-tech devices, thus of critical and strategic importance. Currently, the major mining approach of REEs is and creates large waste streams. The objective of this project is to develop cost-effective, environmentally friendly technologies for bioextracting and bioaccumulating REEs from mined bastnaesite (Ce, La)CO_3F), rhyolite (silica rich felsic volcanic rock), and apatite ($Ca_5(PO_4)_3(OH,F,Cl)$) and phosphogypsum using microorganisms. Microorganisms tested for bioextraction include *Bacillus cereus* (ATCC 14579), *Cupriavidus basiliensis* SRS, a bacterium isolated from the Savannah River Site, and BioTiger®, a microbial consortium patented by SRNL. *B. cereus* and BioTiger® exude biosurfactants (e.g., rhamnolipids) that enhance the extraction of multivalent cations. With the bastnaesite *C. basiliensis* SRS extracted REEs cerium (Ce), lanthanum (Ln), neodymium (Nd), significantly higher (>20ppb) than the other cultures compared to uninoculated samples using biostimulation. Bioextracting worked better using the natural mined material rather that autoclaved mining material, suggesting a potential role for the native microbiota of the material in biomining success. Next-generation sequencing of 16S rRNA genes was used to monitor microbial population changes associated with biomining. When added in combination with nutrient broth to natural (unautoclaved) mining material, the isolate *Cupriavidus* SRS was highly competitive, dominating 99% of the microbial population in the extracted material. In biosorption studies, *C. basiliensis* SRS sorbed >90% Ce and Ln from solution over controls. Using bioaugmentation along with heat treatment, it was demonstrated that *Cupriavidus* SRS can bioextract select REEs from mined materials at ppm concentrations from two different materials.

Accomplishments

- Non-Disclosure Agreement (NDA-2021-00032) and a Mutual Confidentiality Agreement were made with the Mosaic Company to work on their mining materials and exchange information.
- Screening of microbes with biomining potential demonstrated that *Cupriavidus basilensis SRS*, a novel microbe isolated from SRS, was the strongest candidate.
- Of the minerals/mining materials tested, Bastnaesite Ore from MP Materials, Mountain Pass CA, and the Apatite & Phosphogypsum from Mosaic Company, Plant City, FL, showed the greatest potential for bioextraction of REEs up 200ppm. (Figures 1, 2, and 3). This was a significant improvement over Year 1 where we only got to the ppb level of REE bioextraction efficacy.
- Heating the material before bioextracting REEs gave the most productive results.
- Characterization of *Cupriavidus basilensis SRS* demonstrated its novelty (Table 1 & Figure 4).
- *C. basilensis* SRS was shown to produce siderophores that can facilitate biomining REEs (Figure 5). Siderophores are low-molecular-weight molecules that chelate metals with a very high and specific affinity.
- When added to mine materials in REE extraction experiments, *C. basiliensis* dominated showing a competitive edge over indigenous microorganisms using molecular techniques (Figures 6 & 7).
- We developed techniques to monitor bacterial biomining activity in real time using the cost-effective technique that links electronic signals to physiological status of cells (Figure 8).
- Mosaic Company has expressed interest in future work with SRNL

Peer-reviewed Publications

Fu, H., A. Kugler, P. Huyck, R. L. Brigmon, E. Ottesen. 2022. Draft Genome Sequence of *Cupriavidus basilensis SRS*, a Bacterium Isolated from Stream Sediments. *Microbiology Resource Announcements*. In Press. https://journals.asm.org/doi/epub/10.1128/mra.00691-22

Kugler, A., F. M. Coutelot, A. Friedman, S. W. Polson, . C. Seaman, W. Simpson, and R. L. Brigmon. 2022. Bioremediation of Metals in Constructed Wetland Sediments with *Cupriavidus SRS*. *Nature Scientific Reports*. In Press.

Teel, H.R., K. Likit-anurak, S. Shimpalee, C. E. Turick. 2022. Imaginary admittance and charge transfer resistance correlate to the physiological status of *Shewanella oneidensis* cultures in real time. *Bioelectrochemistry*. 147, 108210

Electrochemical Impedance Spectroscopy Correlates Microbial Growth Status to Mineral Reduction. 2022. Teel, H.R., S. Shimpalee, A. J. Kugler, C. E. Burckhalter, R. L. Brigmon, and C. E. Turick. *Journal Industrial Microbiology & Biotechnology*. In prep.

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Teel, H.R., S. Shimpalee, A. J. Kugler, C. E. Burckhalter, R. L, Brigmon, and C. E. Turick. 2022. Electrochemical Impedance Spectroscopy Correlates Microbial Growth Status to Mineral Reduction. In prep.

Kugler, P., Fu, H., A. Kugler, D. Kaplan, R. L. Brigmon, A.N. Depoy, E. Ottesen. 2022. Solubilization of Critical Elements from Unprocessed Ores by Bacterial Processes. *Frontiers in Microbiology: Microbiological Chemistry and Geomicrobiology.* In prep.

A.N. Depoy, Fu, H., A. Kugler, P. Huyck, D. Kaplan, R. L. Brigmon, E. Ottesen. 2022. Regulation od Siderophores Bioprocessing Rare Earth Elements. Applied Environmental Microbiology. In prep.

Presentations

Teel, H.R., S. Shimpalee, A. J. Kugler, C.E. Turick, and C. E. Burckhalter. 2021. Use of Dielectric Spectroscopy to Correlate Admittance and Charge Resistance to Microbial Growth Status. Poster. Geophysical Union Annual Meeting. New Orleans, LA

Brigmon, R.L., A. Kugler, D. Kaplan, J. Seaman, H. Fu, E. Ottesen. 2022. Bioextraction of Rare Earth Elements with Novel Bacteria. American Society for Microbiology Meeting. Washington, DC.

Manager presentation: *Biomining – Critical Minerals and Materials Recovery Using Microorganisms* – SRNL L3000 Managers Meeting Presentation, 16Dec2021 (Talk)

Intellectual Property

No invention disclosures, copyright disclosures, patent applications, and patents granted.

Total Number of Post-Doctoral Researchers
Two post-doctoral researchers supported.

Alex Kugler. SRNL. On site
He Fu, University of Georgia, Off-site

Total Number of Student Researchers
Five students supported by the project.

Jackson DeVault, Undergraduate from University of Toledo. On site LDRD research.

Yasmi Chibber Undergraduate from University of Maryland. Funded by DOE Science Undergraduate Internship (SULI) Program. On site LDRD research.

Amber Depoy, Undergraduate from University of Georgia. Off-site LDRD research.

Patrick Huyck, Graduate student from University of Georgia. Off-site LDRD research.

Harris Teel, Undergraduate from University of South Carolina. Off-site LDRD research.

Figures & Table

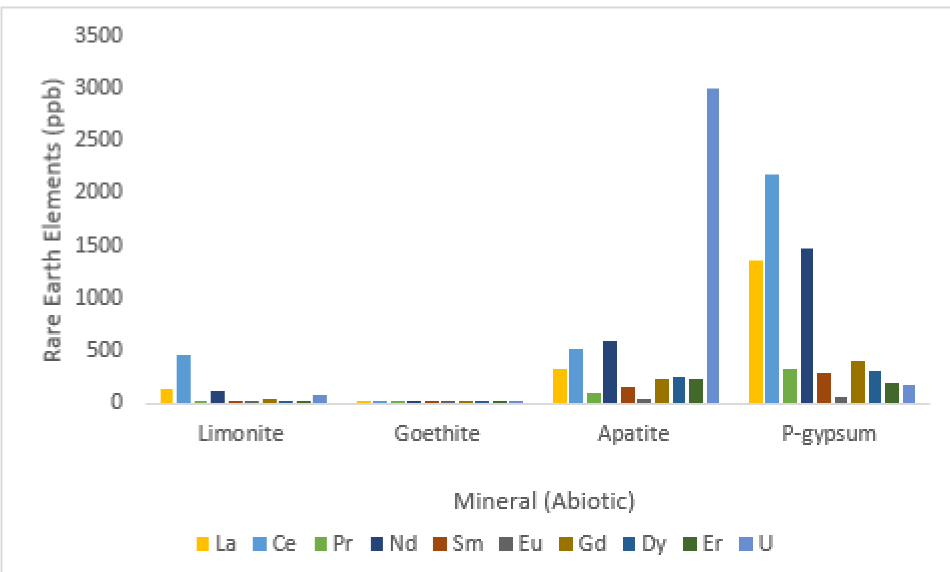

Figure 1: Extraction of REES with heating alone.

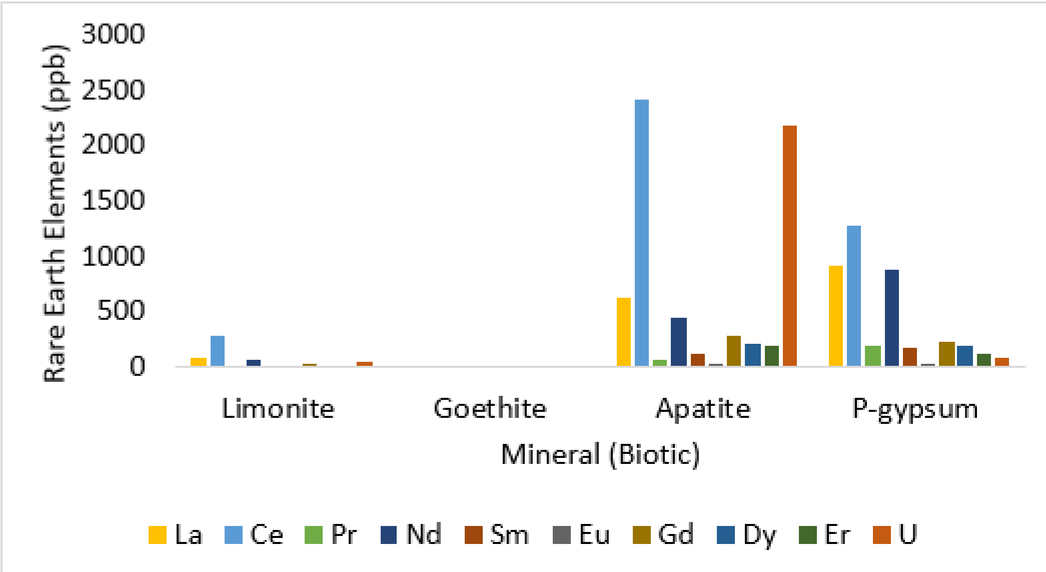

Figure 2: Bioextraction of REEs with combined heating and *Cupriavidus basilensis SRS*.

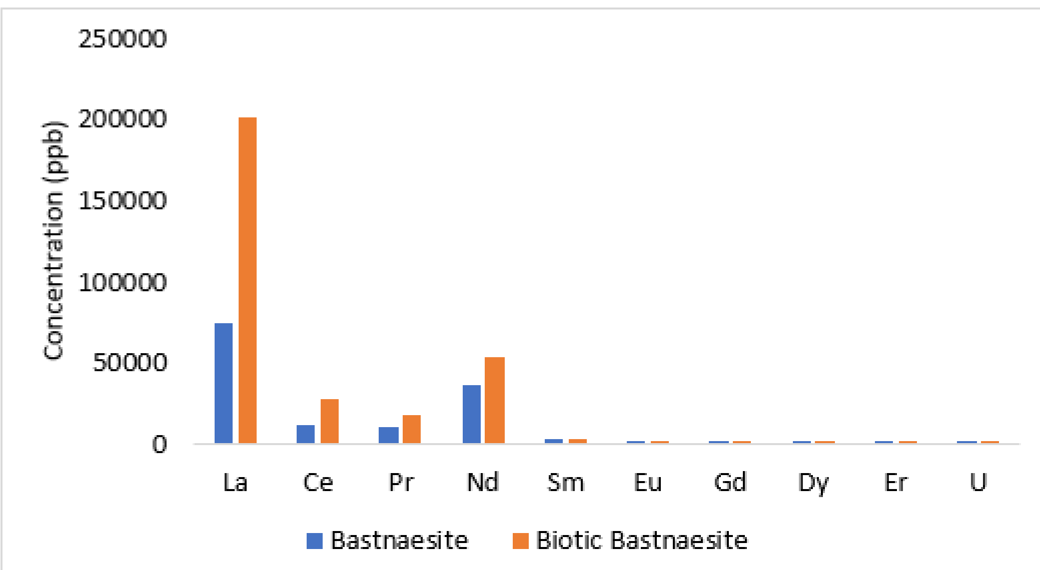

Figure 3: Bioextraction of REES from Bastnaesite with heating and *Cupriavidus basilensis SRS*.

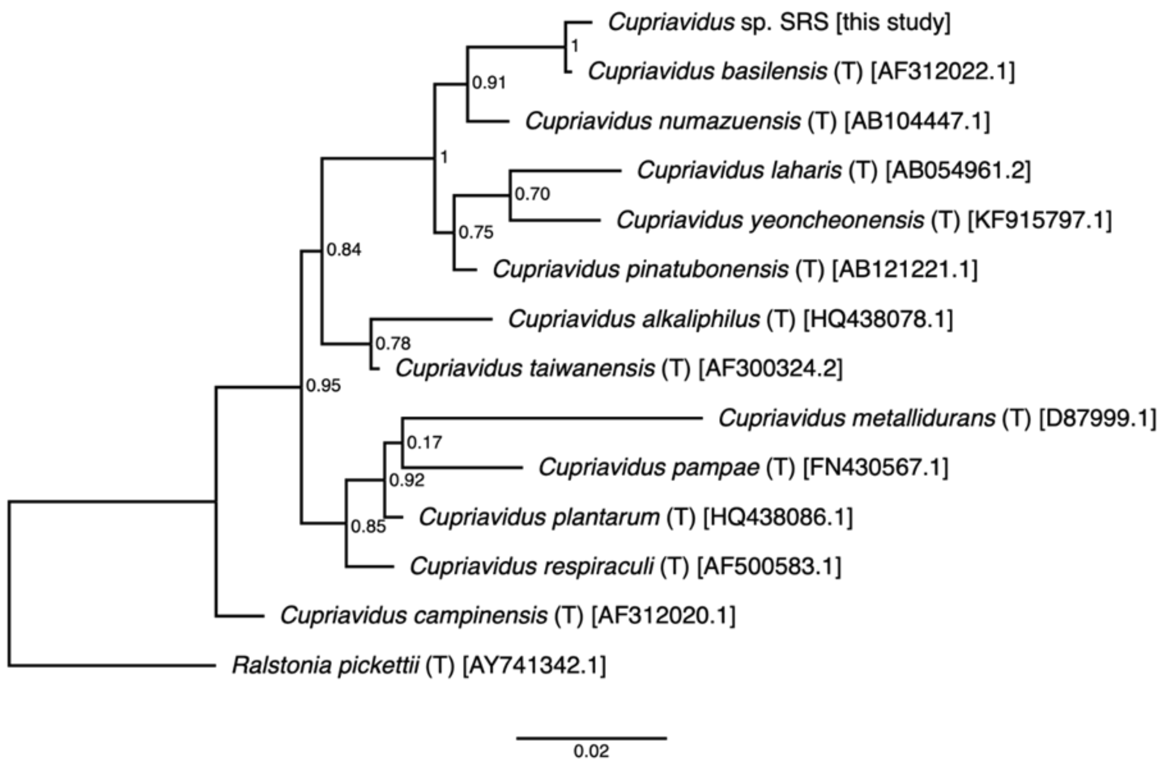

Figure 4. Phylogenetic tree of 16S rRNA relating *Cupriavidus basiliensis SRS* to other closely aligned Cupriavidus spp, resulting from a near-full length nt sequence indicating a near 100% confidence that the *Cupriavidus basiliensis SRS* belongs to the *Cupriavidus* genus.

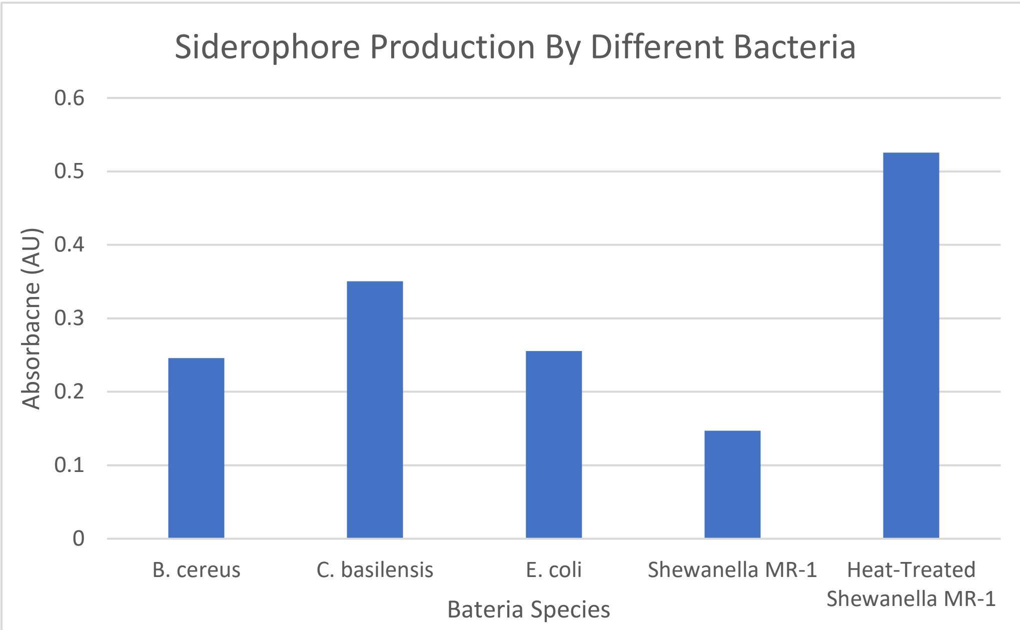

Figure 5: Siderophore presence in different bacteria as measured by absorbance

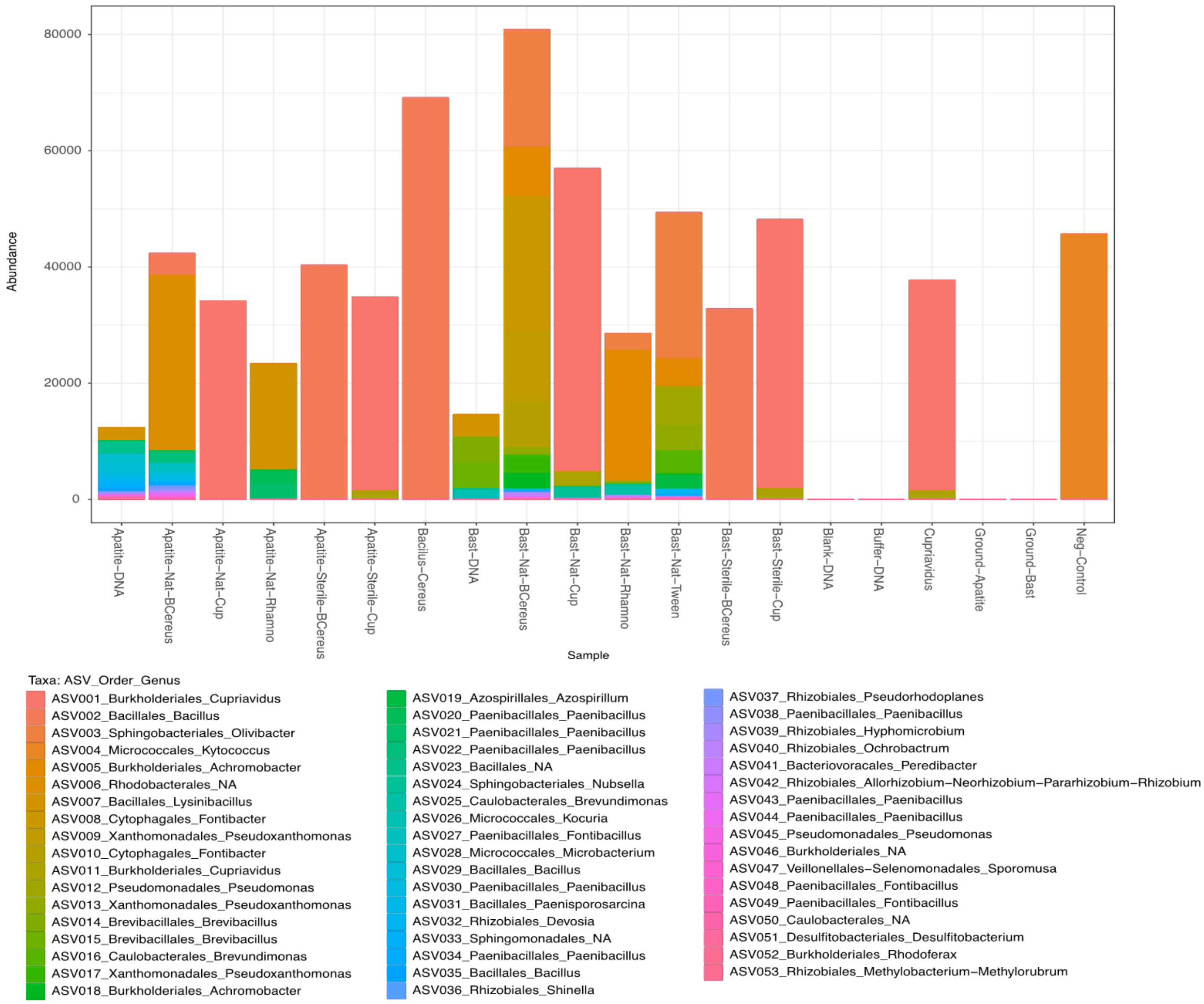

Figure 6. Molecular composition of mine treatments after 2-week incubation. Bact=Bastnaesite, Sterile indicates substrate autoclaved pre incubation.

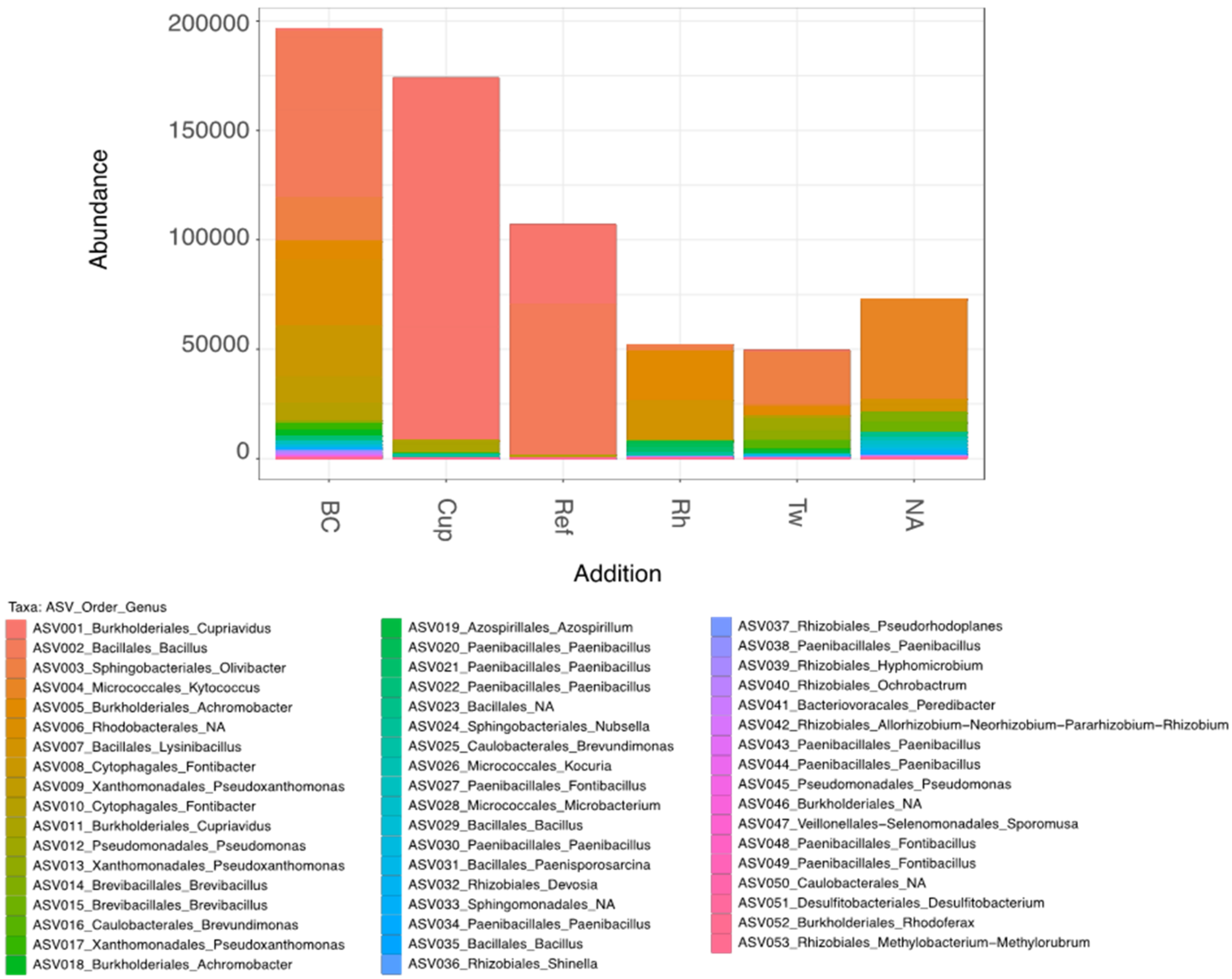

Figure 7. Shifts of bacterial profile based on experimental surfactant treatment. Bc=Bacillus cereus, Cup=Cupriavidus, Rh=rhamnolipid, Tw=Tween 20, Rf=Reference culture of Bacillus cereus and Cupriavidus, NA=non added.

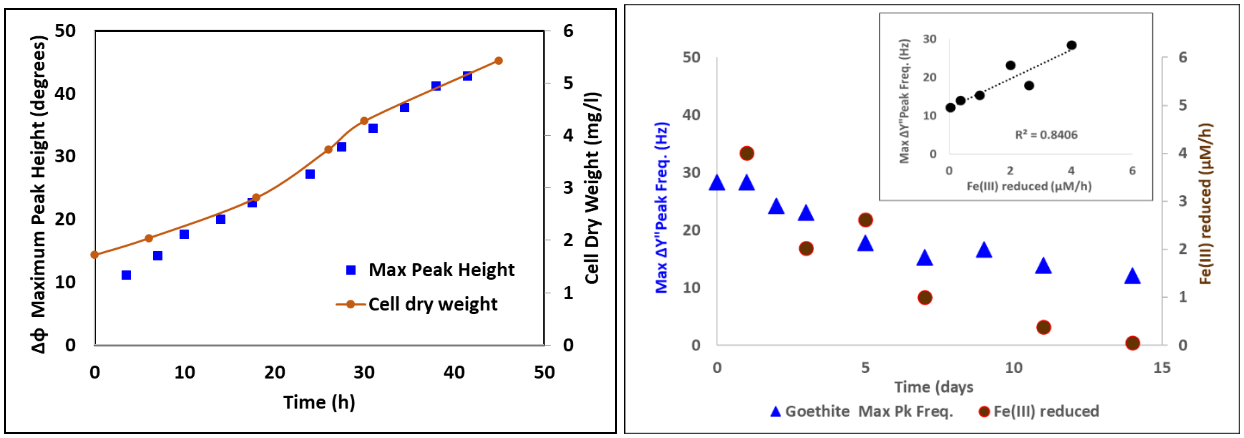

Figure 8. Remote, in-situ detection of bacterial growth (left) and Fe(III) oxide reduction (right). Bacterial cells are surrounded by lipid membranes that have a relatively high electrical resistance. The change in the phase angle (Δϕ) reflects increased resistance (impedance in an AC signal) and the increase in Δϕ correlates well to increased cell density during growth. The bacteria we used send electrons extracellularly to Fe(III) minerals in order to grow and is reflected in the rates of Fe(III) mineral reduction.

Table 1. General features of the draft genome of *Cupriavidus basilensis* SRS

Size	G+C content (%)	No. of Contigs	N50 (bp)	Coverage (x)	CheckM Completeness (%)	CheckM Contamination (%)
8.92 Mb	65.2	176	123,203	411	99.94	2.68

SRNL-STI-2022-00570

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REVIEWS AND APPROVALS

1. Principal Investigator:

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Name and Signature   Date

2. Technical Review:

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Name and Signature   Date

3. PI's Manager Signature:

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Name and Signature   Date

4. PI's Division Director Signature:

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Name and Signature   Date

5. Intellectual Property Review:

This report has been reviewed by SRNL Legal Counsel for intellectual property considerations and is approved to be publicly published in its current form.

SRNL Legal Signature

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Name and Signature   Date