Contract No:

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LDRD-2021-00108
LDRD External Report Summary

Title of Project
Biomining Critical Elements and Metals

Project Start and End Dates
Project Start Date: 10/1/20
Project End Date: 9/30/22

Project Highlight
Critical elements play a vital role in consumer electronics including computers, cell phones, medical equipment, as well as military hardware that are critical to the country's economic and national security. Strategies are needed to reduce our dependency on critical element imports. This project is developing approaches for biomining critical elements from geological materials.

Project Team
Robin L. Brigmon (PI)
Charles Turick (retired 9/30/21), Daniel I. Kaplan, Anna Knox (co-PIs)
Alex Kugler, a SRNL Post-doc starting his second year on the project.
Jason Westrick and Andrew Fu (Post-doc working at UGA on the project)
Elizabeth Ottesen (University of Georgia)
Sirivatch Shimpalee (University of South Carolina)
John Seaman (Savannah River Ecology Laboratory)
Jackson Devault (Undergraduate Student, University of Toledo)
Kris Likit-Anurak, (Graduate), and Hunter Teel, (Undergraduate), Students, University of South Carolina

Abstract
The US government has identified 17 critical elements, including most rare earth elements (REEs), and metals, which are used extensively in consumer electronics as well as military and national security hardware. We are developing techniques for biomining these elements, the process of using microorganisms to extract critical elements from water, ores, and mine waste to fill these needs. Bioextraction is focusing on: 1) building on our first-year successes of identifying microbes that generate exudates that enhance the extraction of multivalent cations, 2) evaluating available phosphate solubilizing microbes to dissolve apatite and monazite, and 3) and biosurfactant producing microbes. Bioaccumulation efforts involves testing microbes that: 1) naturally release complex exudates that contain organic chelating agents, and 2) naturally accumulate or hyperaccumulate (wt-% levels) metals. Results from year one includes proof of concept that biosurfactant producing microorganisms can release REEs from select ores and that release of REEs is increased by the addition of glucose. Molecular results for microorganism profiling proved a population shift after addition of microorganisms. The study will culminate in a proof-of-concept demonstration, manuscripts, and the data needed for scaled-up biomining of REEs.

Objectives
- Develop a technology for bioextracting of REEs from minerals known to be high in REEs.
- Develop a technology for the bioaccumulation of REEs.
- Demonstrate electrochemical monitoring of microbial activity could be applied for biogeochemical interactions.
- Conduct a proof-of-concept demonstration of the most effective biomining processes

- Create an industrial partnership

Introduction

While rare earth elements (REE) are a critical US resource, there is currently no domestic infrastructure to process mine REEs into individual elements. REEs are widely dispersed throughout the environment making extraction and isolation a costly endeavor. For example, Cerium (Ce) is the 26th most abundant element in the upper crust making it more abundant than various commonly mined metals such as lead, cobalt, molybdenum, uranium, and all the precious metals. There are abundant domestic REE resources available, however due to the wide dispersion and complex industrial mining processes, new methods must be developed to make these options viable. In order to develop biomining methods for enhanced extraction and reconcentration, we are applying physical, chemical, and microbiological methods. First, appropriate mining materials were selected for testing as biomining candidates. Second, culture conditions were identified using known biosurfactant producing microorganisms and/or bioprecipitating organisms. Third, we conducted initial screening tests, to quickly identify the most effective microbes and then do more involved testing to define the limits of the optimal biological, chemical, and mineral conditions for bioextraction and bioaccumulation. These latter, more in-depth, studies included microbiological laboratory techniques, microscopy, spectroscopy, genomic, and electrochemical techniques to optimize process efficiency and yields using the most promising organism/organisms. Figure 1 shows biosurfactant production being screened for select cultures to be applied in biomining efforts.

While it has been observed that microbes can interact with metals including REEs, the mechanisms with respect to extraction are not well understood. As part of our efforts to develop and optimize biomining, we applied molecular techniques to: 1) improve our understanding of bioaugmentation mechanisms, 2) identify shifts in microbiological populations as a function of biomining efforts, and 3) optimize the process to achieve an economically viable alternative to conventional metal and REE extraction processes. Experiments are being conducted with raw materials acquired from the one of the largest REE mines in the country, Mountain Pass Mine (CA), and apatite from the Florida phosphate industry. These materials are being ground to ~1 mm to simulate the feed used in present mining operations for inorganic leaching (e.g., sulfuric acid). Figure 2 demonstrates molecular level changes observed in biomining extractions. This information can be used to optimize the process in future extractions.

Methods are currently being developed to recover critical elements from key environments, bioremediation efforts, and additional biomining processes. Biosurfactant, surfactants of microbial origin, producing microbial consortia were found to release REEs from selected mine ores (Figure 3). Current efforts include modifying the media to increase biosurfactant production and correlate with REE extraction. In year 2, we plan to apply organic acid producing microorganisms to further increase REE release.

Approach

The resulting information from this work identified common genetic characteristics of microbes that are correlated to bioprecipitation and/or bioaccumulation, examination of the composition of microbial communities in REE enriched environments, as well as of consortia showing promise, and key microbes correlated with improved biomining performance. This approach includes not only observing and tracking changes in which microbes interacted with REEs, but also using community transcriptomics to observe changes in microbial gene expression over time in our lab microcosms. By tracking microbial behavior both *in situ* and *ex situ* with REE rich materials we can develop successful biomining protocols. Through a current transcriptional analysis of active cultures, we are identifying mechanisms of action and potential inhibitors.

Our biomining approach for release of critical element is illustrated in Figure 4. We are leveraging key microbial products including enzymes, organic acids, ligands, reductases, and polysaccharides that can facilitate release of critical elements *ex situ.* Thus, biomining can be accomplished in a controlled manner by regulating culture conditions including speciation, nutrients, temperature, time, and respiration rate. Electrochemical methods were being tested to monitor microorganism in controlled biogeochemical interactions (Figure 5).

Accomplishments

- Proof of principle was achieved demonstrating that REEs could be bioextracted with biosurfactant producing microorganisms.
- Screening with varying mine ores found bastnasite and apatite from Florida to have critical element potential.
- Molecular analysis showed that the added microorganisms for bioextraction were resilient in the mining environment.
- Screening with varying mine ores found bastnasite and apatite from Florida to have critical element potential.
- Discovered that biosurfactant release of REEs could be enhanced by the additional glucose as measured experimentally.
- A Non-disclosure Agreement (NDA) has been established with the mining company Mosaic, Inc (https://www.mosaicco.com), for technical data and mining-sample exchanges (Figure 6).
- Electrochemically monitoring microbial activity & biogeochemical interactions to provide an indirect assessment of biomining.

Future Directions

- In year 2, we will test redox factors and organic acid producing species for REEs bioextraction in combination with biosurfactant producing microorganisms.
- Will work with the new industrial partner Mosaic to evaluate materials from their phosphate mining facility in Florida.
- Based on testing results, a CRADA may be pursued with Mosaic.
- In 2022, we will test additional materials including Monazite for REE extraction.
- Develop regenerable bio-based extraction modules for specific removal of REEs from mixtures. These modules, e.g., continuous culture reactors, would allow biomining cultures to be maintained cost effectively.
- Integrate REE biomining pilot plants with existing geo- and civil engineering infrastructure with an industrial partner (e.g., Mosaic).
- Most proficient microbes at bioaccumulation, REEs amenable to biomining, and bioprocesses, (e.g., bioextraction configurations) will be sequenced and optimized for future work.
- We plan on at least four additional peer-reviewed publications this next FY based on the results.

- The manuscripts and the data will be used to develop external proposals for biomining of REEs with D0D and DOE.

FY 2021 Peer-reviewed/Non-peer reviewed Publications

- Alex Kugler, Robin L. Brigmon, Fanny M. Coutelot, Abby Friedman, Shawn W. Polson, John C. Seaman, and Waltena Simpson. 2021. "Bioremediation of Cu and Zn in Constructed Wetland Sediments using the novel bacteria Cupriavidus SRS" is in review at the journal *Biodegradation.*
- Two additional manuscripts are in preparation for submission to journals including "Electrochemical Techniques for Real Time, *In situ* Detection of Physiological Status of Shewanella oneidensis Throughout Growth" for *Bioelectrochemistry* and "Bioextraction of Critical Elements from Mine Waste with Biosurfactants" for *Applied Microbiology and Biotechnology*.
- SRNL personnel are primary authors on all submitted and in process manuscripts listed.
- Hunter R. Teel, Sirivatch Shimpalee, Alex J. Kugler, Courtney E. Burckhalter, and Charles E. Turick. 2021. "Use of Dielectric Spectroscopy to Correlate Admittance and Charge Transfer Resistance to Microbial Growth Status", Abstract accepted by the American Geophysical Union Fall Meeting in New Orleans, LA.

Intellectual Property

N/A

Post-Doctoral Researchers (3)

Dr. Alex Kugler is a Post-Doctoral Researcher working on this project at SRNL. He is starting his second year at SRNL.

Drs. Jason Westrick and Andrew Fu are Postdoctoral Fellows who worked on the project at UGA as part of their subcontract.

Student Researchers (2)

Mr. Jackson Devault is an undergraduate researcher working on the project at SRNL. Mr. Devault is a Bioengineering Major at the University of Toledo.

Mr. Hunter Teel is an undergraduate Mechanical Engineering Major at USC who worked on the project there.

Figures

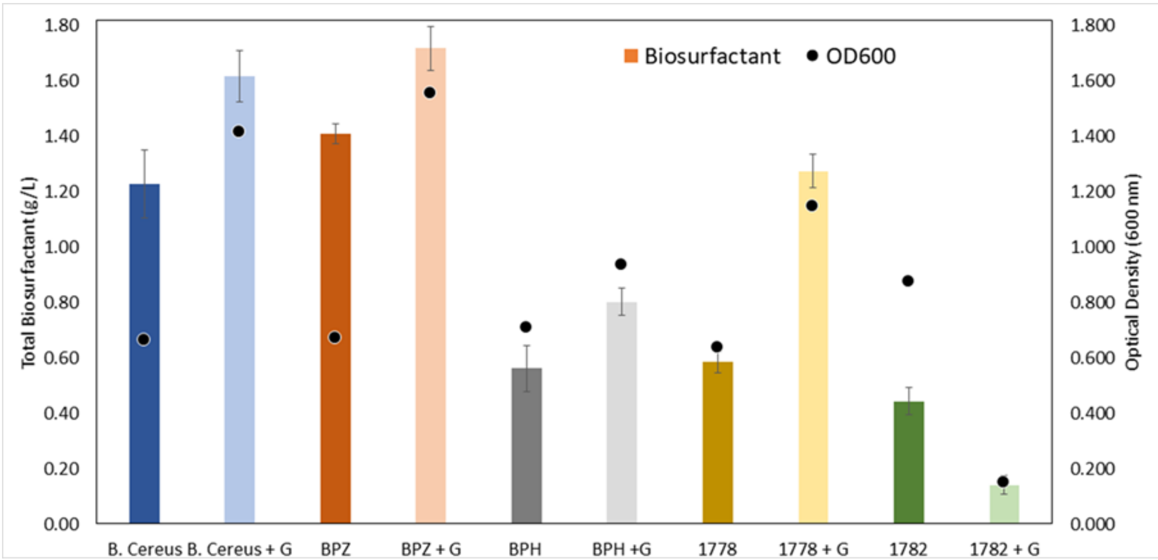

Figure 1: Environmentally resilient microorganisms being screened for biosurfactant production for use in biomining applications. *Bacillus cereus* has been tested previously but BPZ (*Alcaligenes piechaudii*) and BPH (*Sphingomonas SP. S37)* are part of the SRNL BioTiger consortia and proven to grow in extreme environments. Both 1778 and 1782 are mutants of BPH).

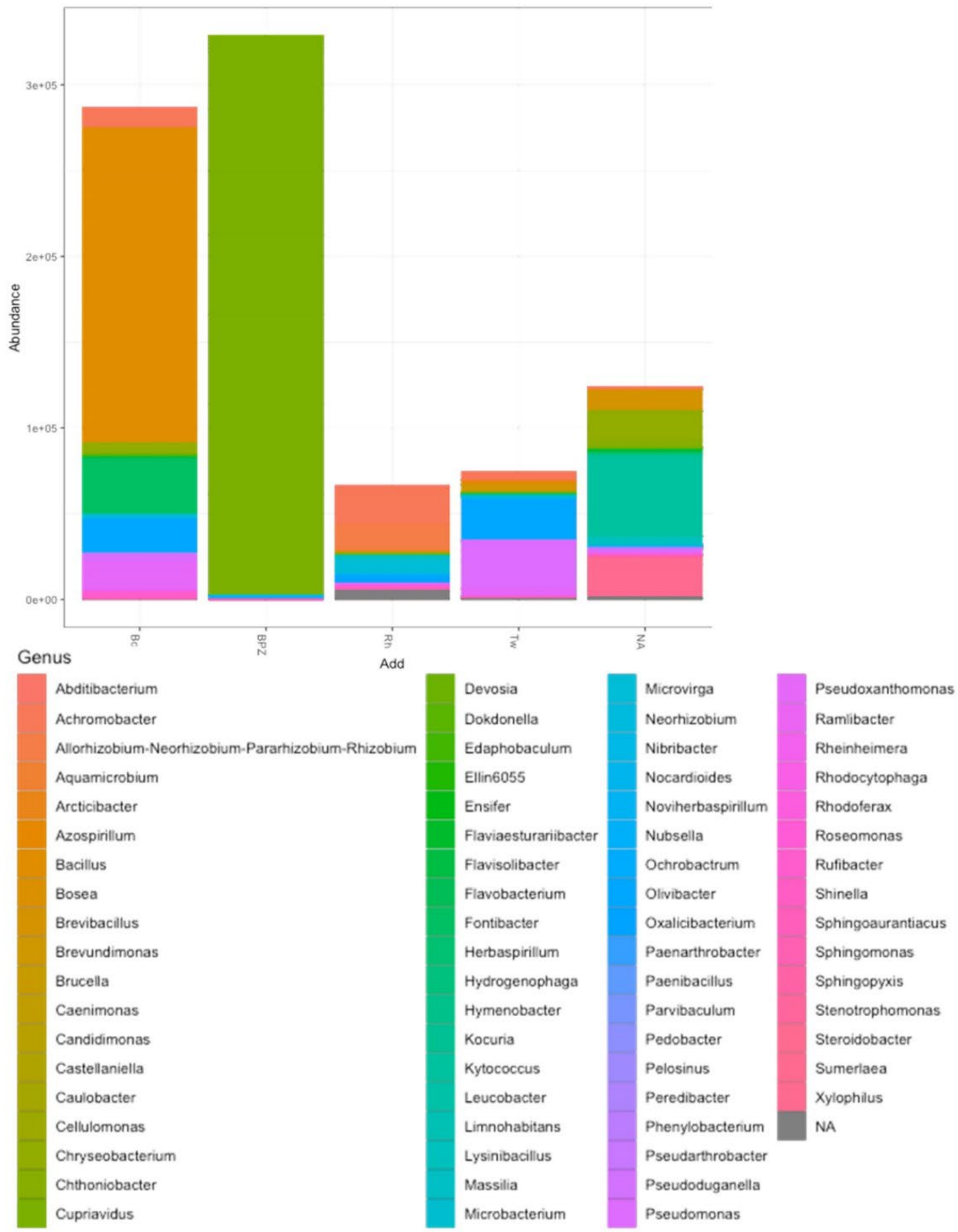

Figure 2. Changes in the bacterial profile based on experimental surfactant treatment show domination of several genus's, while rare genus appear such as *Abditibacterium*, an extremophile known for living in nutrient poor envrionments. These molecular techniques can be used to help demonstrate the stability and metabolic capacity of the biomining technology.

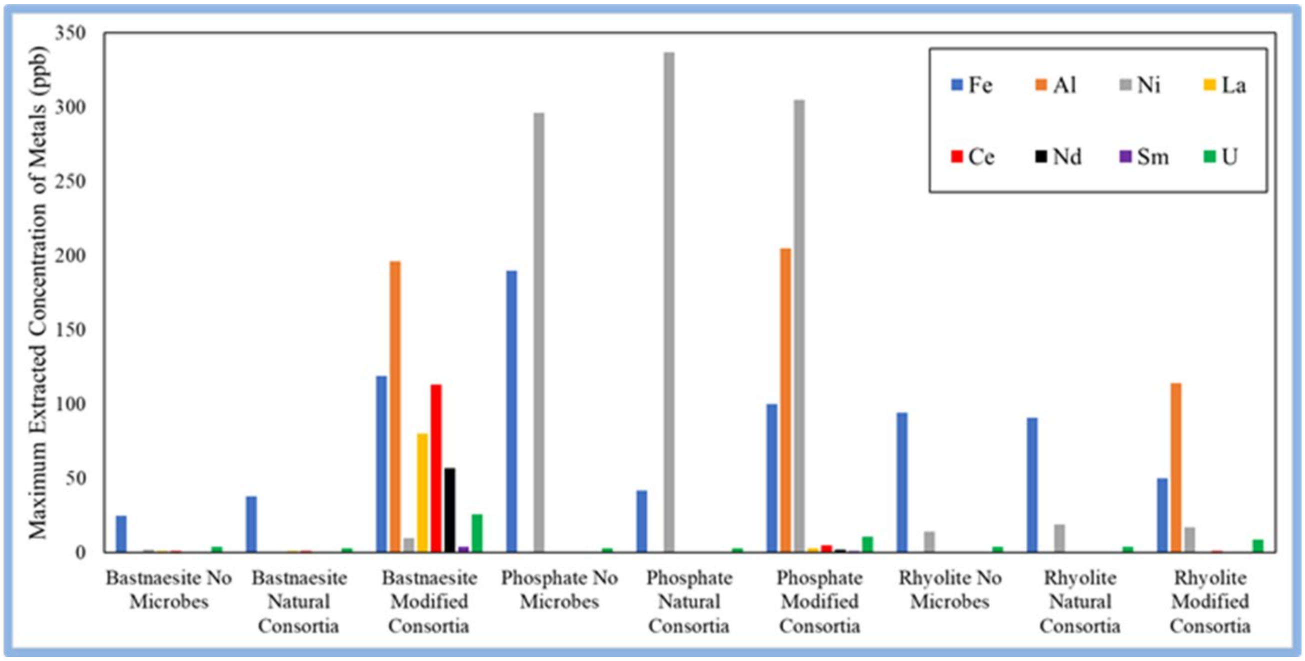

Figure 3. Maximum concentration of bioextracted metal ions (ppb) including REEs from mine ores. Modified microbial consortia were added to make biosurfactants enabling REE release of interest including Cerium (Ce), Lanthanum (La) Neodymium (Nd), and Samarium (Sm) as shown.

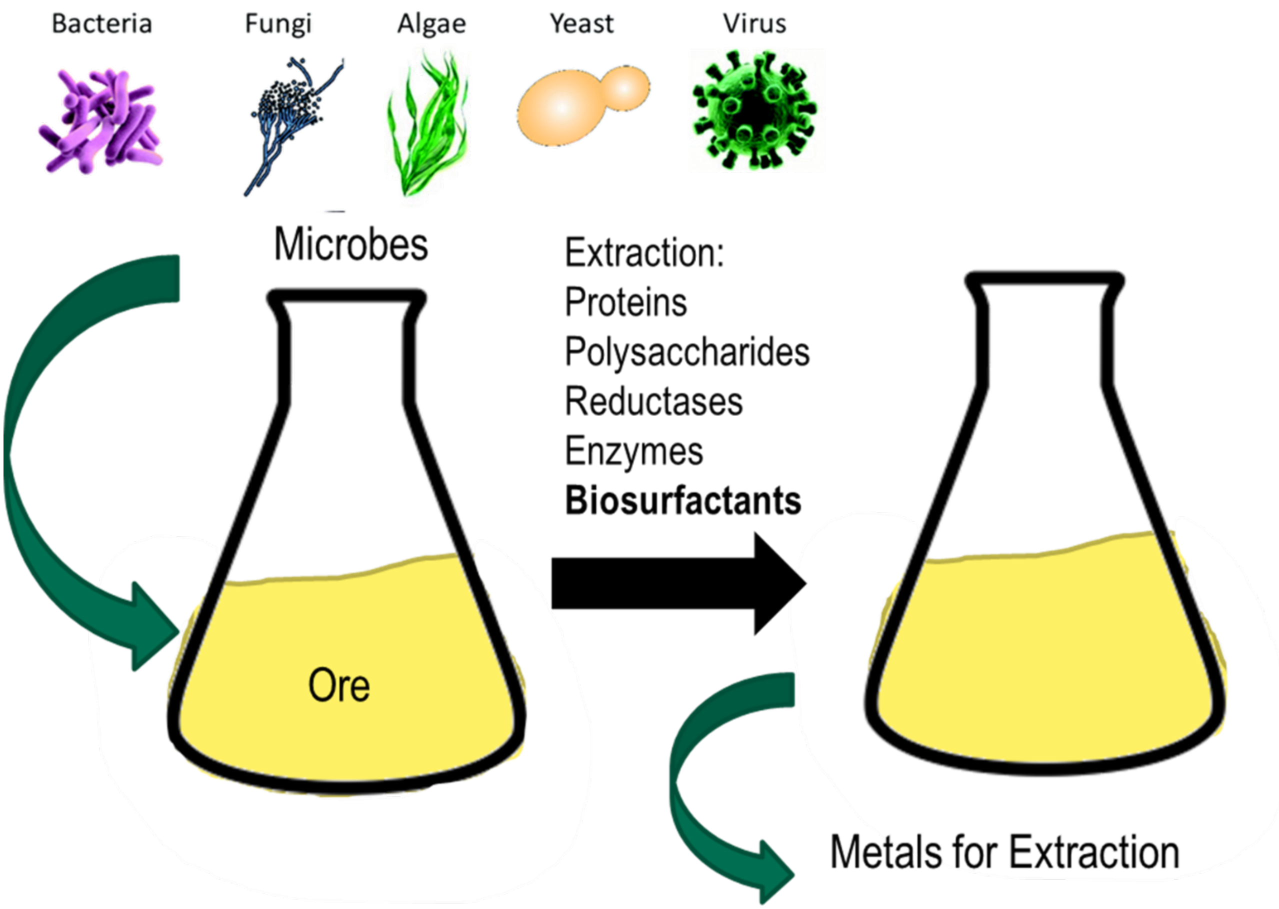

Figure 4. Approach using natural microorganisms and byproducts for critical element release. This method allows screening of varying cultures, environmental conditions, and media to optimize biomining.

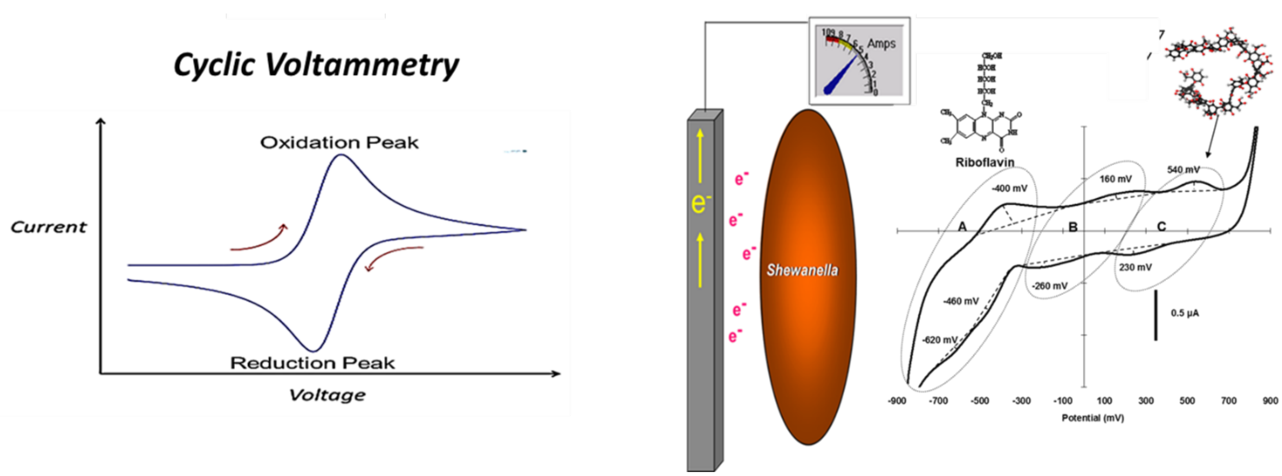

Figure 5. Electrochemical methods were proven to be a means of studying and monitoring microorganisms in metal shuttling by cofactors including riboflavin. These techniques allow real-time testing of microbial activities.

Figure 6. A Non-Disclosure Agreement has been established with The Mosaic Company (Tampa, FL) to provide an exchange of information and mining samples for further testing in FY22 (Image: *Jim Stem /* Bloomberg *via Getty Images*).