UNLOCKING MICROBIAL POTENTIAL TO DEVELOP INNOVATIVE AND ENVIRONMENTALLY RESPONSIBLE TECHNOLOGIES IN SUSTAINABLE MINING

Dan Stigers, Karrie Radloff, Michaeline Albright, Bianca Cruz, Tom Lankiewicz,
Elizabeth Deyett, Dayal Saran, Kent Sorenson
Allonnia, Boston, MA

Abstract

Biology opens a new frontier for the mining industry. With rising environmental concerns, the need to couple conventional chemical and physical technologies with sustainable approaches is pressing. At Allonnia, we harness microbial processes to create innovative and environmentally responsible technologies with broad applications. The native microbial community (microbiome) remains largely unexplored at most mining sites, unlike the geochemical environment. The exploration of complex biological reactions that could transform mining technology is just starting and holds vast possibilities, spanning bio-cementation, carbon sequestration, ore beneficiation, water mitigation, bioleaching, metals recovery, and more. Allonnia has collected samples from mine sites in Australia, Africa, and North America to characterize the microbiome and uncover the genetic potential present in mineral-rich environments. We are evaluating microbial metabolites that selectively solubilize gangue from various ores. In addition, biological reactions hold promise as tailings stabilizers. Our early efforts illustrate exciting prospects for harnessing biology's potential in mining.

Introduction

Our mission is to utilize known and novel biological processes to tackle some of the world's most pressing environmental challenges. We identified several processes essential to the mining industry that are naturally occurring at the microscale in microbial communities across the globe and have used this as both an inspiration and a starting point for transforming how these processes can be done at scale – to unlock untapped potential and to bring about more environmentally responsible technologies. To do this, we maintain and continue to build an industry-leading database of genetically sequenced and functionally characterized microbes and enzymes for biotransformation or biogeochemical sequestration of target compounds in various environments and media. We have identified two areas within the mining industry where known microbial processes play a key role: the solubilization of target elements and the controlled agglomeration of particles through the precipitation of carbonates.

The natural weathering of silicate and other materials by microbes present in the environment provides the starting point for the development of unique bio-solvents comprised of bacterial and/or fungal metabolites (Castro et al. 2000; Jain and Sharma, 2004; Torres et al., 2019, Li et al. 2019; Lamerand et al., 2020). The bio-solvents have several distinct advantages over conventional mineral acids such as sulfuric or hydrochloric acid (Dong et al. 2022). First, in addition to lowering the pH and promoting acidolysis, the bio-solvents provide ligands and chelators that form strong complexes and can potentially improve solubilization rate and extent by thermodynamically favoring the reaction towards increased solubility. Second, the bio-solvent can be produced via fermentation and potentially recovered through less environmentally costly methods than conventional mineral acids. We are developing a range of bio-solvents that can be tuned based on material (e.g., ores, concentrates, tailings) and selective removal needs (e.g., gangue or penalty elements) to act as targeted extractants. We are focusing on tuning bio-solvents that can effectively remove silica, aluminum, magnesium, and calcium present in silicates, such as kaolinite and other secondary alumino-silicates. Gangue removal has the potential to improve metal recovery (e.g., through improved separation via flotation), upgrade ores and concentrates, and remove components that negatively impact downstream processing, such as smelting.

The natural generation of calcite minerals is a ubiquitous process found in a wide range of environments (Naveed et al. 2020). One such process is known as microbially-induced carbonate precipitation (MICP). Bio-cementation is rapidly expanding in the field of environmental engineering and relevant applications of this technology include soil stabilization, erosion control, dust suppression, groundwater remediation, construction material fabrication and restoration, toxic metal immobilization, and CO_2 sequestration (Mujah et al. 2017, Proudfoot et al. 2022, Song et al. 2022). We are developing both enzymatic and microbial approaches for agglomerating and controlling the strength and geotechnical stability of a range of materials, including ore stockpiles and tailings.

Experimental

Microbiome Database/Biobank

While the geochemical profile at mining sites is usually extensively characterized, the microbiome is largely unexplored even from a basic diversity standpoint, let alone in terms of understanding the genetic functional potential of microbes. Allonnia has obtained samples from mine sites in Australia, Africa, and North America to characterize the microbiome in these mineral-rich environments. We extracted DNA for amplicon sequencing and taxonomic analysis not only for bacteria and archaea but also for fungi. In addition, we have isolated hundreds of organisms on various media types to facilitate whole genome sequencing and build Allonnia's bio-bank. Living in extreme environments, microbes evolve incredible genetic mechanisms to utilize and/or cope with high levels of elements such as copper, cobalt, iron, aluminum, etc. that would be toxic to most forms of life. Cataloging this microbial diversity, deciphering the genetic potential, and building a biobank of isolated organisms gives us a starting point for harnessing these microbes and furthering their capabilities to transform and sequester target compounds or perform targeted processes across a range of applications.

Bio-solubilization

Allonnia is evaluating a range of bio-solvents for their ability to selectively solubilize gangue minerals, and remove aluminum (Al), silica (SiO_2), calcium (Ca), and magnesium (Mg) from the solids. The bio-solvents are composed of microbial metabolites present within individual bacteria, fungi, and/or microbial consortia that have shown potential for selective solubilization in natural and laboratory conditions.

We have extended solubilization experiments across a range of ore types, tailings, and individual minerals to understand baseline bio-solvent performance and key operational parameters. The results presented in this paper include an ore material that has been pulverized to a P95 of 106 microns and silica-rich tailings with a similar size distribution. Operational parameter evaluations have included solid loading (5 to 30% solids by weight) and a range of temperatures (30 to 80°C) under well-stirred conditions.

The data presented in this paper were analyzed by a commercial analytical laboratory using lithium borate fusion disks measured on a wavelength dispersive X-ray fluorescence spectrometer (WD-XRF) and semi-quantitative estimates of mineralogy were determined by X-ray diffraction (XRD) using the Rietveld refinement method. Both methods included industry-standard QA/QC reporting requirements.

Bio-cementation

Allonnia is evaluating promising microbes from our bio-bank for MICP ability with a goal of also limiting urea and Ca additions to create more economic, sustainable, and environmentally friendly bio-cement formulations. In addition, we are exploring applications for enzyme-induced calcite precipitation (EICP) and the production of microbial enzymes at scale for mining applications. As part of our experimental work, we have been testing baseline reaction conditions and developing cementation formulas and deployment strategies. We are exploring key factors such as curing time, moisture content, enzyme activity, temperature, and pressure on unconfined compressive strength achieved through the bio-cementation of mining materials. Further, we are testing the ability of the ore and tailings bio-cement to withstand variable environmental conditions (e.g., heat and moisture) and exploring how different materials may inhibit these carbonation reactions. These factors will determine the efficacy of the calcite precipitation process for various mining applications including stockpile protection from the elements, dust control, and liquefaction control for tailings and cargo stabilization. Lastly, we are testing additional biological additives to reach target values for parameters such as strength and moisture content.

Results and Discussion

Microbiome Database/Biobank

Allonnia's work on the isolation and sequencing of microbes from mine tailings ponds has demonstrated the wide genomic diversity present in "inhospitable" mineral-rich locations. To date, we isolated over 350 fungal and 450 bacterial species (Figure 1). We identified bacteria using 16S amplicon sequencing and fungi using ITS sequencing, in addition to whole genome sequencing on select isolates. In samples from several tailings sites, we identified over 25 unique bacterial phyla (the grouping below kingdom in taxonomic classification) and 11 fungal phyla. Remarkably, for the fungi over 65% of the isolated species have not been described in published taxonomy databases, including some of the most abundant (Figure 2).

Figure 1. Bacteria and Fungi Isolated from Mining Sites around the World.

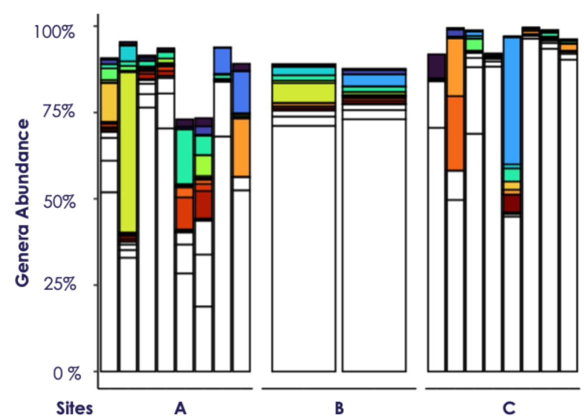

Figure 2. Abundance of Specific Fungal Genera at Several Tailings Sites. Colored bar segments show known genera. Uncharacterized genera are shown with white bars.

Bio-solubilization

Allonnia's bio-solvents can effectively solubilize silicate materials resulting in the removal of gangue components such as Al, Ca, Mg, and SiO_2, while minimizing the loss of the mineralogical phases with value metals such as lithium (Li), copper (Cu), nickel (Ni), and iron (Fe). We report a subset of our results with an ore material and with silicate-rich tailings here.

Ore Material

The target elements for solubilization from this material were alumina (Al_2O_3) and SiO_2, which were present in multiple phases including kaolinite and quartz, as determined by XRD. Testing was conducted on four samples with moderately different grades and compositions from similar ore bodies. One strong performing bio-solvent (Bio-solvent 1A) was able to achieve the removal of 19 to 31% of total $Al_2O_3$and 22 to 40% of total SiO_2 (as measured by XRF) after two hours of treatment of the pulverized ore under semi-optimized conditions. This was a single contact test and additional solubilization is likely with additional contacts. Further, upon the removal of the alumina and silica, the target metal increased by 2% to 5% over the same time (Figure 3).

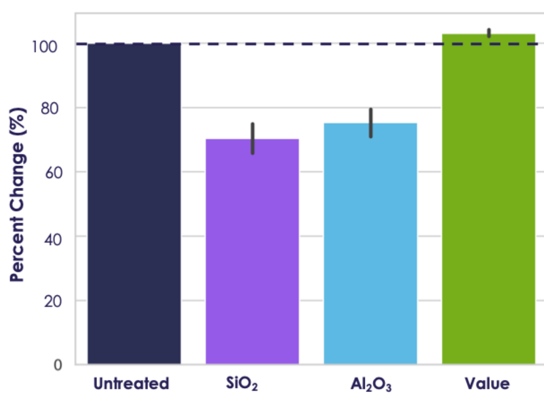

Figure 3. Gangue Removal from Ore using Bio-solvent 1A. Beneficiation of 4 ore materials showed significant removal of SiO_2 and Al_2O_3 and an increase in the value metal after just 2 hours of treatment and with only a single contact. Error bars based on measurement of 12 samples (3 of each ore type).

Based on preliminary data, the relationship between percent gangue removal and ore grade suggests that removal is increased with lower ore grades at a solid loading of almost 40% by weight, similar to typical processing steps (Figure 4).

In addition, we can refine the bio-solvent composition and the operational parameters to fine-tune the solubilization of the gangue while retaining the value metal. One of the clearest examples of the "tunability" of the bio-solvents is the ability to adjust the ratio of metabolites to target a penalty element. Figure 5 demonstrates how a subtle change in the composition of a single bio-solvent resulted in similar performance for alumina and silica removal and upgraded the value metal, whereas one version (Bio-solvent 1A) resulted in significantly improved removal of a penalty element. Similar optimization has also been observed with temperature controls and with the amount of solid loading to the bio-solvents. This work is on-going.

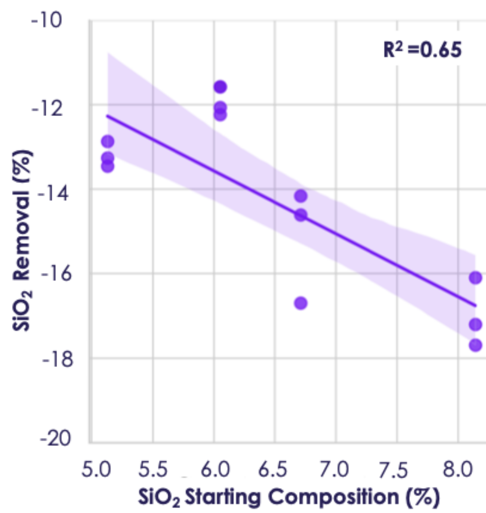

Figure 4. Increase in Gangue Removal with Decreasing Ore Grade. Beneficiation was greater for ore materials with a lower starting grade (higher gangue content). Grade differences are between 4 ore materials, with 3 samples each.

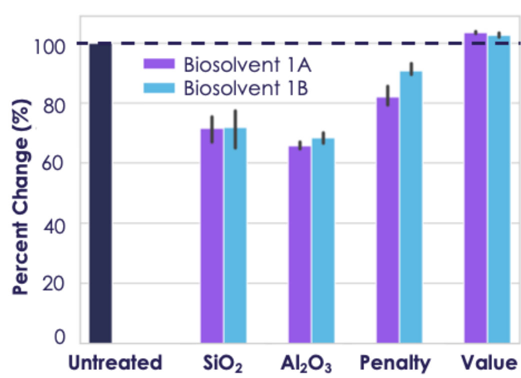

Figure 5. Improved Penalty Element Removal with Minor Adjustment of the

Bio-solvent. A small change in the ratio of bio-solvent components resulted in improved penalty element removal for one ore type, while other positive impacts remain unchanged (removal of gangue and upgrading of value metal). Error bars based on measurement of 3 samples.

Tailings

The target elements for solubilization of the tailings materials were Mg and Ca, which were present in a range of silicates including pyroxenes, chlorite, and feldspar, as determined by XRD. Numerous studies promote the carbon capture potential present within mining tailings (such as Bullock et al., 2021), and the search for viable methods for economically processing tailings at the scale required for carbon capture is ongoing. Testing was conducted on tailings from three different mine sites, but generally, the same type of ore was extracted and processed in the same manner, and very small amounts of valuable metals like Cu or Ni remained in the tailings. Several bio-solvents showed a high affinity for Ca and Mg removal. The decrease in the percent of CaO and MgO was similar to the reduction achieved by sulfuric acid after four days of treatment (Figure 6 a,b).

Similar results were also observed for alumina and silica (Figure 6 c,d). Importantly, the best-performing bio-solvents did not impact the low concentrations of Cu and Ni present in the tailings (reduced by less than 10%) showing the capability for the bio-solvent to selectively dissolve the Mg and Ca species over the value metal (Figure 6 e,f). This selective solubilization is unlike the solubilization using sulfuric acid which reduced the concentrations of Cu and Ni by nearly 60% and 25%, respectively (Figure 6 e-f).

The bio-solvents were as effective as mineral acids, such as sulfuric acid, at cation (Mg and Ca) removal, and also present a significant environmental advantage. Unlike strong acids, the Mg and Ca are still labile in solution and can be readily precipitated (Figure 7). The photograph shows the loaded bio-solvent solution after mixing with the tailings on the left and the precipitates formed after base adjustment on the right. The bio-solvents are not degraded during the solubilization. We are currently refining our methods for bio-solvent recovery.

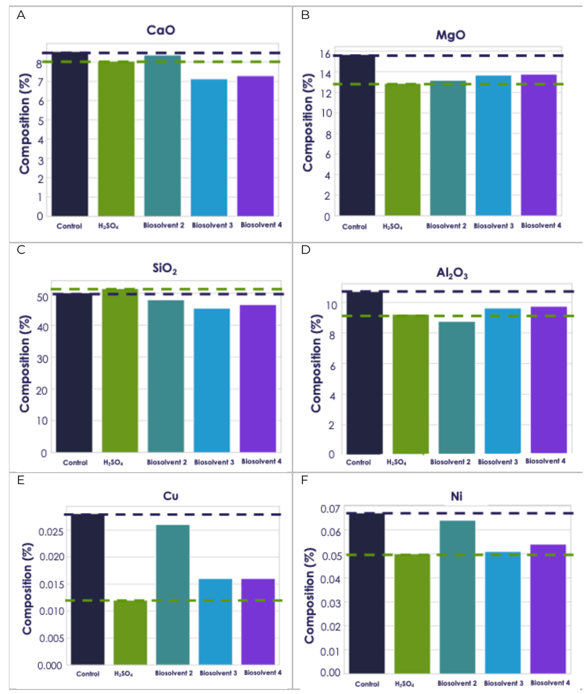

Figure 6. Three Bio-solvents Demonstrated Similar Silicate Solubilization of Tailings as Sulfuric Acid. Solubilization of three bio-solvents is shown compared to a water control and sulfuric acid after 4 days of treatment. The bio-solvents were selected to target CaO and MgO removal (A, B). SiO_2 and Al_2O_3 (C,D) removal was similar. The bio-solvents demonstrated better retention of value metals, Cu and Ni (E, F).

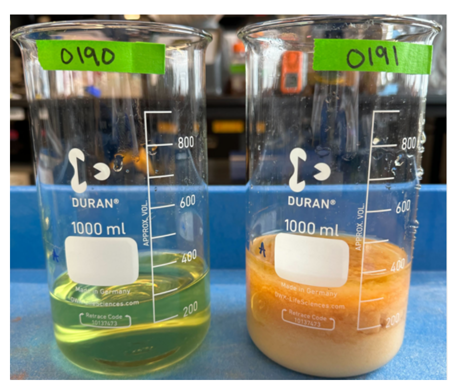

Figure 7. Cations are Readily Precipitated out of the Bio-solvents with Base Adjustment. While

this process is unoptimized, it demonstrates that the cations are viable for other applications.

Bio-cementation

With our bio-informatics database, we identified several isolates with the genetic potential to perform MICP pathways and have been testing the ability of these organisms in our lab (Figure 8).

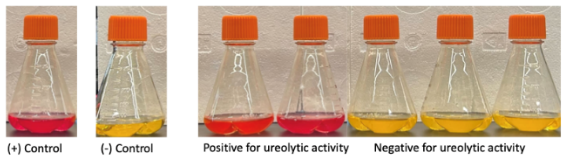

Figure 8. Enzymatic activity observed in select microbes within Allonnia's biobank. Utilization of urea results in a yellow to red color change.

Using the same enzymatic formulation and by altering treatment conditions, we achieved differing amounts of unconfined compressive strength for the same material, as compared to a water control (Figure 9). The conditions tested in Trial 2 resulted in a strength that was over 5-fold higher than Trial 1 and was achieved through altering a few key conditions. This suggests that the strength of the agglomerated material can be readily tuned based on the application requirements. In addition to testing several different ore and tailings materials, we are currently working on evaluating other factors such as the resistance of our bio-cemented material to variable temperature and moisture conditions.

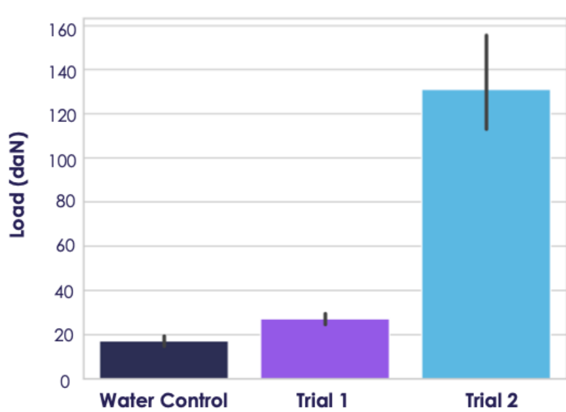

Figure 9. Tunability of the Unconfined Compressive Strength of the Mining Material. By varying the treatment conditions but using the same enzymatic formulation, the unconfined compressive strength was altered to fit different application requirements. Data are averages of 7 or 8 samples for each treatment.

Conclusion

Allonnia's ever-growing microbial and enzyme database and bio-bank include the genetic sequences for and the isolates/enrichments of many individual and consortia of bacteria and fungi from unique environments across the world. We believe that characterizing the microbiome present at mining sites will be key to uncovering the genetic potential present in mineral-rich environments and unlocking novel approaches for solving mining's greatest challenges. We are currently utilizing this resource to inform our work in the selective solubilization of gangue minerals using bio-solvents and in developing microbial approaches for agglomerating and controlling the strength and geotechnical stability for a range of mining-associated materials.

Our current work on bio-solvents has identified multiple formulations that were able to produce the targeted removal of silicate minerals in both ore materials and tailings. For a range of ore materials, approximately 30% of the gangue alumino-silicates were removed after just 2 hours of treatment with one bio-solvent. Over the same time, the target metal composition increased by approximately 3%. Three bio-solvents solubilized tailings material to a similar extent as sulfuric acid. Subtle differences were present between the bio-solvents, with one only minimally solubilizing the low level of Ni and Cu present in these tailings relative to sulfuric acid. These preliminary results suggest that we are able to fine-tune the bio-solvents to target the removal of specific minerals and prevent others from dissolving from both the ore and tailings tested. We are continuing to evaluate an expanded range of materials and bio-solvents.

Our work in material agglomeration also utilizes the microbial and enzyme database and bio-bank to harness and improve the ability of microbes to enable carbonate precipitation. In parallel, our laboratory-scale application experiments have demonstrated the ability to achieve significant increases in material strength for a range of mining-associated materials, including ores and tailings. We are also beginning to successfully tailor our microbial products and processes to meet chemical and physical specifications relevant to targeted mining applications including stockpile protection from the elements, dust control, and tailings stabilization. For example, we are developing bio-cementation formulations that work well in low moisture systems as would be required for iron and nickel cargo liquefaction control.

Allonnia's exploration of complex biological reactions that could transform mining technology is just starting and holds vast possibilities. Our early efforts, shared here, illustrate exciting prospects for harnessing biology's potential in mining for selective solubilization which has direct applications within ore beneficiation via gangue removal and the bio-cementation of tailings and stockpiles.

References

1. Bullock, L.A., et al. (2021) "Global carbon dioxide removal potential of waste materials from metal and diamond mining." *Frontiers in Climate* 3: 694175.
2. Castro, I., et al. (2000) "Bioleaching of zinc and nickel from silicates using Aspergillus niger cultures." Hydrometallurgy 57.1: 39-49.
3. Dong, et al. (2022) "A critical review of mineral–microbe interaction and co-evolution: mechanisms and applications." *National science review* 9.10: nwac128.
4. Jain, N., and D. K. Sharma. (2004) "Biohydrometallurgy for nonsulfidic minerals—a review." Geomicrobiology Journal 21.3: 135-144.
5. Lamérand, C, et al. (2020) "Olivine dissolution and hydrous Mg carbonate and silicate precipitation in the presence of microbial consortium of photo-autotrophic and heterotrophic bacteria." Geochimica et Cosmochimica Acta 268: 123-141.
6. Li, Z-b., et al. (2019). "Specificity of low molecular weight organic acids on the release of elements from lizardite during fungal weathering." Geochimica et Cosmochimica Acta.
7. Mujah, D. et al. (2017) "State-of-the-art review of biocementation by microbially induced calcite precipitation (MICP) for soil stabilization." Geomicrobiology Journal 34.6: 524-537.
8. Naveed, M. et al. (2020) "Application of microbially induced calcium carbonate precipitation with urea hydrolysis to improve the mechanical properties of soil." Ecological Engineering, 153, 105885.
9. Proudfoot, D. et al. (2022) "Investigating the potential for microbially induced carbonate precipitation to treat mine waste." Journal of Hazardous Materials, 424, 127490.
10. Song, M. et al. (2022) "A review on the applications of microbially induced calcium carbonate precipitation in solid waste treatment and soil remediation." Chemosphere, 290, 133229.
11. Torres, M. A., et al. (2019). "The kinetics of siderophore-mediated olivine dissolution." *Geobiology* 17.4