Bioprocess ASABE start-up Competition
Clemson University, Biosystems Engineering
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Problem identified

Arsenic is a metalloid found in nearly every environment; however, anthropogenic inputs of arsenic have led to increasingly higher concentrations in soil and groundwater worldwide in recent years. As a result, in 1993, the World Health Organization (WHO) provisional guideline for arsenic in water was reduced from 50 μ/L to 10 μ/L. The Environmental Protection Agency (EPA) federal guidelines for standards of concern start at 390 parts per billion (ppb) in soils. Of particular interest to this design is the agricultural arsenic contamination due to its impact on food safety. Currently, rice grown in the United States contains 1.4 to 5 times more arsenic than rice from Europe, India, and Bangladesh. (Peplow, 2005)

This higher average of arsenic in rice could be because rice is a crop that can be rotated with or replaced with cotton. Rice production can then be adversely impacted by the previous arsenical herbicide and pesticide use in the area due to arsenic remaining in the soil or contaminated water sources. The most significant anthropogenic input of arsenic to agricultural soils is arsenic-based pesticides and herbicides during most of the 20th century. (Punshon et al., 2017) Inorganic arsenic compounds were the principal compounds used in agriculture and included calcium arsenate and lead arsenate. (Bencko et al., 2017) Calcium arsenate and lead arsenate were used extensively until the 1950s, primarily as a pesticide. (Punshon et al., 2017) One of the primary regions of inorganic and organoarsenic herbicide use in the United States includes the cotton-belt states of Alabama, Arkansas, Louisiana, Mississippi, **Texas**, and South Carolina. (Bednar et al., 2002)

These increased arsenic levels are adverse to rice production in America's fields. Currently, American rice production is almost entirely produced in four regions of the country. These regions include the Arkansas Grand Prairie, the Mississippi Delta (parts of Arkansas, Mississippi, Missouri, and Louisiana), the **Gulf Coast** (Texas and Southwest Louisiana), and California's Sacramento Valley. (USDA 2021) Barring the Sacramento Valley of California, these regions previously saw cotton production before the decline of American cotton. Rice is often rotated with or replaced cotton, so any legacy effects of arsenic herbicide/pesticide use would impact the rice fields. American rice is produced in irrigated areas, where producers often seed aerially in the flooded fields. (USDA 2021)

Bioleaching as a solution

Bioleaching is a method of remediation that solubilizes arsenic in the soil allowing for capture and removal. The traditional process of bioleaching metals from ore often uses the bacteria *T. ferroooxidans,* and the bacteria has been shown to bioleach heavy metals from the soil. Fungi can also remove metalloids from the soil; *Penicillium chrysogenum* has been shown to extract metals such as Cu, Pb, Zn, and Cd from soils. (Deng et al., 2012) Another fungus, *A. niger*, has been effective at bioleaching waste printed circuit boards and heavy metals from soil. (Faraji et al., 2018 and Ren et al. 2008)

In our research at Clemson University, we have done lab-scale, batch reactor bioleaching experiments to test the efficacy of local soils both with and without inoculation of *A. niger*. Glucose was added as a carbon source in the experiments and was required to see significant bioleaching. In these batch experiments, between 10-20% of arsenic was leached from the solids to the soil solution. This percent removal was reached regardless of inoculation, suggesting that local soils can solubilize arsenic based on their local microbial biome if the microbes' growth is encouraged by adding a carbon source such as glucose.

In-situ/Ex-situ remediation

Ex-situ

For off-site remediation of arsenic-contaminated soils, CSTRs, PFRs, or batch reactors can be designed and sized to move insoluble arsenic to the soluble phase. The liquid effluent can then be collected, and the arsenic precipitated out. The most common methods of removing arsenic from aqueous process streams are precipitation as arsenic(III) sulfide, calcium arsenate, or ferric arsenate (Robins et al.).

In situ

It is believed that on-site treatment can be achieved by adding a carbon source and collecting the soil solution via underground drainage. Like ex-situ treatment, the effluent can be collected, and the arsenic precipitated out. The system's design would depend on the soil characteristics and the microbial biome present. *A. niger* is a ubiquitous fungus and is capable of solubilizing arsenic. Its presence will then be a key indicator of the local biomes' ability to bioleach arsenic without inoculation of another species.

Predicted outcomes

The target market for our company would be the many farmers currently crowing rice in Texas. Currently, Texas ranks fifth in rice production, with over 150,000 acres of rice planted on 272 farms in 2019 (usarice). The characteristics we would look for in clients would be land with high arsenic concentrations. Based on historical data collected and the large concentration of arsenic in American rice, a large customer base is predicted. Other customer bases are also predicted because arsenic contamination isn't solely unique to farmlands.

The outcomes predicted by our design would positively impact Texas by providing better, healthier growing conditions for the state's crops. This outcome has a two-fold positive outcome.

Not only will the healthier soil provide increased production in remediated fields, but chronic exposure to arsenic will be lessened or eliminated in the local human population.

Environmental and Economic impacts

Bioleaching is an environmentally friendly method of remediation. The principal mechanism behind the concept is acidolysis, resulting in solubilizing the metal of interest. This does leave the field at low pHs but can easily be remedied with pH-increasing compounds such as calcium carbonate. The remediation cost can also quickly be recouped by the increased production seen in remediated fields and the decreased cost of medical care for chronic arsenic exposure. This situation sees both the client and the state benefit from our process design.

Ethical and professional responsibilities

This technology is currently in its infancy, so our ethical and professional responsibilities entail rigorous and robust experimentation and modeling to confirm that the impact of our remediation is, in fact, low impact. We have identified issues with pH after remediation. While extensive experimentation has been done thus far, due to the complexity and variability of soil matrices, further testing is needed to confirm that other physical and chemical alterations that follow from the remediation do not potentially negatively impact the soil in the future.

Future Competitors and our competitive advantage

Our future competitors can be anyone from a similar educational background. In fact, due to how academia works, any student we work with could become a future competitor. Our competitive advantage currently lies in the fact that we believe we are delving into solutions that others aren't looking at as much. Consequently, we are steps ahead of any group that would like to compete. If we enter the industrial scene before any other company, we believe we can grab a dominant share of the market while other competitors are still weeks or months behind.

Members

Zachary Gilstrap (he/him)-Graduate student at Clemson University is currently researching bioleaching arsenic-contaminated soils via the growth of *A. niger*. I spent the last two years researching bioleaching using lab-scale batch reactors. Results from experimentation will greatly behoove the company.

Frank Jeffries (he/him)-Undergraduate student at Clemson University in Biosystems engineering. His focus area is bioprocess engineering, and she participated in Zachary Gilstrap's research through Creative Inquiry (CI) at Clemson University. Frank is incredibly hard-working and keen on bioleaching in general. He very often independently researched solutions to problems that arose in Creative Inquiry. His initiative, drive, and current knowledge would greatly help our company.

Natalie Whitaker (she/her)-Undergraduate student at Clemson University in Biosystems Engineering. Her Focus area is bioprocess engineering, and she participated in Zachary Gilstrap's research through Creative Inquiry (CI) at Clemson University. Natalie is charismatic and works well in multidisciplinary environments. Her contribution to previous bioleaching research. Her work ethic, personality, and continuing education will only help the company.

Start-up and funding

Because starting up a company is an iterative process, we still need to perform intense and robust experimentation. It might even be best to add a non-engineer to the team for marketing objectives. Because experimentation still needs to be done, it might be more realistic for us to approach a company whose objective lines up with ours to fund our research and design. In this case, our company model is to function more like consultants to those who know they need arsenic remediation. The start-up money will be used on lab-scale experiments needed to model solutions, which can then be applied on-site. So the go to market plan is to seek investors.

Diversity/Inclusion

We have no plans of not being inclusive, but we want to foster inclusivity when possible. Depending on its economics, the company will have a diversity and inclusivity office similar to the many we see on our local University Campuses. When the start-up is successful, extending internship opportunities to marginalized students has been discussed and has the entire team behind it.

Advancement in the field of Agricultural and Biological Engineering

The advancement in Agricultural and Biological engineering is creating and implementing a method of environmentally friendly arsenic remediation. The outcomes have been discussed before, but this would increase the health of the soil and, consequently, productivity. The process itself is wholly biological, so the advancement in Biological engineering is self-evident.

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