

LAB REPORT

Programmable microbial biocrusts for desert soil stabilization via carbonic anhydrase-mediated calcium carbonate precipitation

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In drylands, wind-driven soil erosion is a major driver of desertification. Desertification is characterized by reduced soil stability and accelerated land degradation, both of which damage local infrastructure and ecosystems. Conventional soil stabilization methods, such as those that rely on cement, polymers, or mechanical vibration, are often energy-intensive and environmentally unfriendly. Microbially induced calcium carbonate precipitation (MICP) offers a bio-mediated alternative by binding soil particles with calcite. Unfortunately, urease-mediated MICP generates ammonia as a byproduct, conferring phytotoxic risks that limit its large-scale application. Thus, this project proposes a low-cost engineered biocrust based on carbonic anhydrase-mediated MICP that mitigates ammonia release. Together, the co-culture is intended to be deployed in dryland regions and to independently form a biocrust in designated areas. However, our design has limitations. Although the carbonic anhydrase in our system is highly efficient, it can only conduct MICP under basic, calcium-rich conditions. By inducing carbonic anhydrase expression in wild soil bacteria, this system achieves regulated, enhanced calcite precipitation, thereby forming a thin, erosion-resistant surface crust. Further development of this project may yield a scalable, programmable, carbon-sequestering method to sustainably mitigate desertification.

Keywords: Desertification, bioconversion, soil-stabilization, MICP, synthetic biocrust



The authors were mentored by David Doyle, Concordia International School Shanghai, Shanghai, China. Please direct correspondence to: David Doyle, david.doyle@concordiashanghai.org. This is an Open Access article, which was copyrighted by the authors and published by BioTreks in 2026. It is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

DRYLANDS make up a vast portion of the Earth's terrestrial surface: roughly 52 million square kilometers, making up almost half of all non-ice-covered land. As shown in *Figure 1*, soil degradation is a widespread global phenomenon that affects extensive regions across Africa, Asia, Europe, the Americas, and Australia, with many areas experiencing moderate to severe damage (Middleton et al., 1997). According to the United Nations Convention to Combat Desertification (UNCCD), about 36 million km² of land is already affected by desertification, and 250 million people are directly impacted. If current trends persist, as many as 135 million people could be displaced by 2045 due to land degradation (United Nations, 2019).

Unfortunately, in many parts of the world, soil with poor engineering and technological development is unsuitable for large-scale construction, primarily due to its unstable structure and low mechanical strength. Moreover, specific environmental factors, such as ex-

treme temperatures, freeze-thaw cycles, and localized seismic activity, will increase soil susceptibility to desertification and hinder industrial efforts to combat it (Patil et al., 2021).

This global crisis significantly affects local agricultural processes, limiting many national economies. In China, for example, the Loess Plateau, covering an area of about 640,000 square kilometers, is important to the ecological, economic, and social development of China. However, due to its unique geological and topographic characteristics, it faces serious soil erosion. According to a survey by researcher XuanShuo Zhang in 2024, numerous reservoirs of various sizes were constructed in the loess hilly region for the purpose of water storage and soil conservation, which utilize local soil as a base material to improve the ecological environment. However, heavy rains and floods have resulted in numerous landslides due to destabilized soil, posing serious threats not only to the safety of residents in the vicinity,

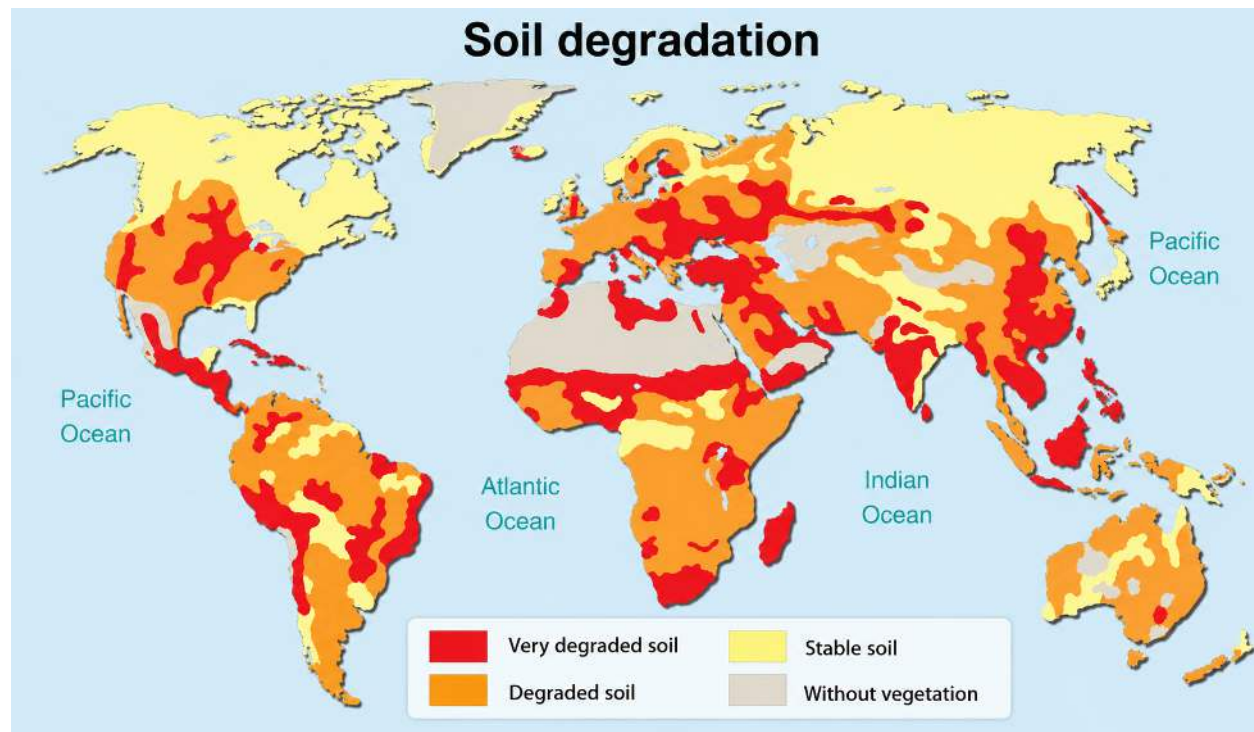


Figure 1. Global soil degradation map (Source: UNEP, International Soil Reference and Information Centre, World Atlas of Desertification, 1997)

but also exerting a severe impact on the local economy, infrastructure, and ecological environment. Thus, improving engineering technology to stabilize soil structure and prevent erosion through human intervention and reinforcement technology is meaningful and necessary (Zhang et al., 2024).

Microbial induced carbonate precipitation (MICP) & efficiency optimization

Various soil improvement techniques have been commercially developed to enhance soil mechanical strength. Techniques such as preload, synthetic polymers, and mechanical vibration have been widely used. However, the widespread use of material pathways requires significant energy and artificial materials, resulting in high engineering costs and various post-effects. Meanwhile, engineering polymer materials through chemical pathways will also lead to long-term soil and groundwater contamination, adversely affecting the project's purpose. Nowadays, injecting fine cement slurry into the soil's empty space to form cemented soil is the most common method (Dubey et al., 2023).

Most notably, mixing and "one-phase" injection procedures delay nucleation, spreading reagents more

evenly. According to the results, this procedure added approximately 3% polycarboxylic acid, postponed the onset of CaCO_3 by more than 2 hours, and achieved complete Ca^{2+} conversion within 24 h, yielding about 2.76 MPa after five treatment cycles (Dubey et al., 2023).

Meanwhile, active nanomaterials have also been applied to improve the efficiency of many engineering processes due to their high specific surface area and well-known pozzolanic activity. Nano-MgO has been proven effective in improving soil strength, cohesion, and durability, thereby resisting soil erosion. Unfortunately, this method confers alarming toxic risks to human health and chronic health problems from long-term exposure, so it cannot be applied to most agricultural sites and communities. Thus, there is an obvious and urgent need to investigate an eco-friendly, applicable, and efficient soil structure stabilization method. (Chen et al., 2022)

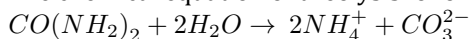
Among the methods of preventing desertification, the formation of a biocrust through calcium carbonate precipitation is one of the most promising, effective, and sustainable solutions. Calcium carbonate precipitation can be achieved through a biological process called Microbial Induced Carbonate Precipitation (MICP). This technique is essential for sand fixation by stabilizing the loose and silty sand in the surface layer.

The end product of MICP, calcite crystals, can bridge loose sand in the crustal sand layer, forming a tough crust through cohesion and improving its mechanical strength to prevent soil erosion. (Cao et al., 2024)

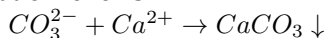
Two pathways of MICP have been explored: the decomposition of urea by urease and the conversion of carbon dioxide and water into calcium carbonate by carbonic anhydrase. *Figure 2* accurately portrays the pathways of the two conversions. In urease-mediated MICP, urea hydrolysis generates both carbonate ions and ammonium as byproducts, increasing local pH. In contrast, carbonic anhydrase accelerates the hydration of free carbon dioxide into bicarbonate and carbonate ions, increasing mineral precipitation while limiting environmental impact (Castro-Alonso et al., 2019). Both methods offer unique benefits and tradeoffs.

Most research papers present designs advancing urease engineering technology. This process utilizes urease-producing bacteria, such as *Bacillus* and *Sporosarcina pasteurii*, to hydrolyze urea into ammonium (NH_4^+) and carbonate ions (CO_3^{2-}). Then, the generated carbonate ions from ureolysis react with free calcium ions (Ca^{2+}) to form calcium carbonate. Occurring at an optimal pH of 7-9, this overall process depends on bacterial mineralization capacity and the geochemical and environmental conditions of a specific site.

The chemical equation of ureolysis follows:



The chemical equation of calcium carbonate precipitation follows:



(Source: Liu et al., 2025)

The CaCO_3 crystals nucleate heterogeneously on microbial cell walls or soil sediment particles, progressively cementing soil grains, filling pore spaces, and strengthening soil particles overall (Wu et al., 2022). Meanwhile, compared to the spontaneous reaction occurring in nature, enzyme-facilitated hydrolysis of urea is approximately 1014 times faster, increasing the shear strength and conversion efficiency of treated soil (Jabri et al., 1995). After the urea hydrolysis pathway of MICP, wind erosion was reduced by 63.9–97.7% in the wind tunnel experiment (Han et al., 2020). Therefore, this lab outcome serves as crucial evidence of MICP's anti-erodibility in aeolian soils and its leading-edge technology role in combating desertification.

A recent paper shows ureolysis MICP transitioning from lab scenario to field application with progress in uniformity, strength, and erosion control. Field validations in Ahoskie (USA) and Sanya (China) demonstrated the efficacy of MICP in coastal erosion control through tailored delivery systems and environmental adaptations. Sanya's studies highlighted seawater-

compatible MICP solutions, achieving a maximum 1743 kPa penetration resistance in the atmospheric zone and layered "M-shaped" CaCO_3 precipitation in tidal regions. (Liu et al., 2025)

Figure 3 presents the mechanism of conventional urease-driven MICP. Urea hydrolysis by urease-producing bacteria first generates carbonate ions and ammonium, then leads to calcium carbonate nucleation and CaCO_3 crystal growth on the bacterial cell surface. However, high NH_4^+ concentrations often have negative effects on ecosystems. In particular, the accumulating toxicity can result in leaf chlorosis and suppressed root growth despite it being an inorganic N source for plant species. Furthermore, ammonium triggers deeper physiological and morphological responses, including changes in cytosolic pH, gene expression, protein modification, ion transport, redox and phytohormone status, as well as root system architecture, which may ultimately have the opposite effect of the goal of MICP. (Yang et al., 2023)

Especially at the expense of higher cost and difficulty in urea availability, this introduces the research to a more natural, sustainable, and efficient pathway: microbial-induced calcite precipitation facilitated by carbonic anhydrases. Carbonic anhydrases achieve MICP by hydrating carbon dioxide, forming carbonic acid, losing two protons to become carbonate ions, and binding to calcium ions to form calcium carbonate.

Recent laboratory studies have shown a significant increase in the reaction rate of carbon dioxide hydration (108 times faster), which facilitates a rapid hydration to form a substantial amount of calcium carbonate compared to urease-facilitated MICP (Liu et al., 2024).

Other benefits of carbonic anhydrase on MICP's performance on sandy soil were also systematically examined through a series of macroscopic and micro-fabrication tests. The results mainly show that: 1) During microbial consolidation of sandy soils, carbonic anhydrase (CA) significantly increases the production of cement by 105.3% compared with conventional MICP, with an optimum dose of carbonic anhydrase-producing bacteria reaching approximately 4%. 2) By incorporating CA, the compressive strength and resistance of the cured soil showed obvious improvements. Based on experiments, the unconfined compressive strength of solidified soil increases with the increase in CA bacteria content, reaching 1.915 MPa at a content of 4%. (8.54 times the strength of the cured sample via conventional MICP). 3) The grain size of the calcite is larger after changing the enzyme to CA, the shape of the hexahedron is more uniform, and the overall mechanical properties are improved. 4) When both urease and CA are present to co-precipitate calcium carbonate to cure sandy soil, CA enormously accelerates

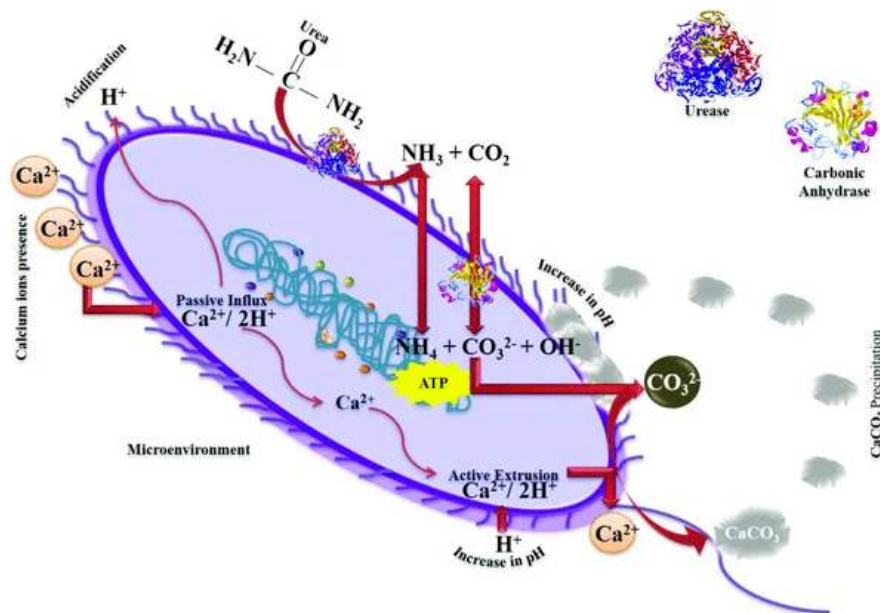


Figure 2. Schematic diagram of MICP formation mediated by urease and carbonic anhydrase (Source: Castro-Alonso et al., 2019, used under the Creative Commons CC BY license)

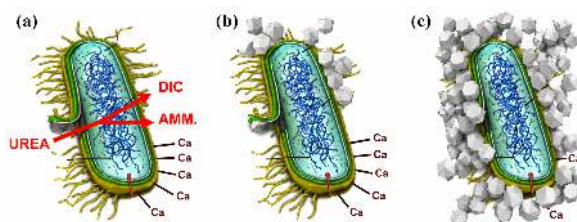


Figure 3. Diagram of calcium carbonate precipitation induced by urease-producing bacteria (Source: Tang et al., 2020, used under the Creative Commons CC BY license)

the rate of urea-generated carbon dioxide hydrate formation to produce the end product. This eventually provides a more favorable condition for mineralization (Liu et al., 2024).

MICP & engineered biocrust restrictions & advancement

The wide application of MICP to restore degraded dryland is still not fully developed. The knowledge gaps have hindered advances in soil solidification technology and possibilities in MICP, with different factors being manipulated and maximized.

Research has shown that bacteria, soil conditions (soil content, temperature, salinity, pH, etc.), and cementation material (type and concentration) all di-

rectly affect microbial and enzyme activity, thereby influencing the efficiency of MICP. (Song et al., 2022) In addition to engineered bacteria, soil bacteria that are sites for nucleation during calcium carbonate precipitation will also impact crystal growth, affecting its size, durability, and effectiveness. (Gao et al., 2014) Furthermore, soil conditions directly determine cementation sites and reaction space. Research shows that soil particles with more open spaces for free movement of bacteria achieve optimal crust hardness, stiffness, compressibility, and permeability. (Liang et al., 2022) Among all, the presence of salt helps provide more nucleation sites for MICP and weave calcium carbonate networks through bacteria. (Dhami et al., 2016)

Although MICP through CA doesn't produce high concentrations of ammonium, nor does it require increased cost due to the addition of a calcium source (calcium chloride), the process still reveals many limitations. Large-scale application of calcium chloride may contribute to soil salinization and calcium chloride runoff, potentially affecting vegetation, microbial communities, and groundwater quality (Szklairek et al., 2022). Meanwhile, CA is highly dependent on the availability and optimization of inorganic carbon, Ca^{2+} , pH, and the nucleation environment, which can be inconsistent without specifically controlled delivery substrates and local pH. Its activities and kinetics vary greatly across ecosystems and communities, and it often becomes immobilized due to harsh environmental

conditions. Without optimization, carbonic anhydrase is not flexible enough to be widely applied. Ultimately, it is clear that CA's role in MICP has been less studied than urease, leaving room for deeper investigation and the possibility to maximize its capability (Tang et al., 2020).

A successful MICP biocrust will save O&M costs and water, recover lost energy, and offer a simple recipe for land surface management on-site for renewables. The same technique can stabilize road shoulders, construction sites, and other drylands where dust is a health and climate problem.

The 2025 iGEM team meduCA presented a similar project, inducing bio-cementation with carbonic anhydrase, which includes the process of MICP. meduCA conducted a phylogenetic analysis, characterizing many types of carbonic anhydrases (CAs). One of the categories describes Periplasmic alpha CAs, which catalyze rapid hydration of carbon dioxide for extracellular pH regulation and carbonate equilibrium (Supuran & Capasso, 2017). Periplasmic alpha CAs match the desired function of our group, so a specific coding sequence (CDS) for a Periplasmic alpha CA is selected.

Materials and methods

Design

We designed an expression device that encodes *Sulfolobus solfataricus* (Saz) (Aguir et al., 2004) α -carbonic anhydrase (Saz α CA) tagged with a signal peptide. TorA encodes a signal peptide that transports Saz α CA to the periplasmic space. TorA is linked to Saz α CA via a flexible peptide linker, BBa_K416001. Upstream of TorA are a medium-strength ribosome binding site (RBS), RBS B0032, and inducible promoter araC. Downstream of Saz α CA is terminator B0015. 6 bp spacers were added between the promoter and RBS, RBS and TorA, and Saz α CA and the terminator. Two BsaI recognition sites flank the entire device and will create sticky ends during Golden Gate Assembly (GGA). This device was ordered online and synthesized by Genscript (Figure 4).

Our device will be inserted into the plasmid backbone pJUMP23-1A(sfGFP) (Valenzuela-Ortega & French, 2021) using Golden Gate Assembly with the BsaI restriction enzyme (Figure 5). The plasmid is available from Addgene at <http://n2t.net/addgene:126970>.

Initially, we selected the gram-negative, non-pathogenic, and resilient soil bacterium *Pseudomonas putida* KT2440 to be the chassis for our design (Martin-Pascual et al., 2021; Yunus et al., 2024). However, we do not have access to *P. putida* KT2440 in our lab. *P. putida* KT2440 is typically transformed

via electroporation and does not respond well to heat shock, which is the preferred transformation method in our lab. Because of these accessibility and technical challenges, we chose to work with *Escherichia coli* TOP10 and *E. coli* DH5 α as proof-of-concept chassis. *E. coli* TOP10 was used to propagate and amplify pJUMP23-1A(sfGFP) because of its high plasmid yield. *E. coli* DH5 α was selected to host the recombinant plasmid because of its high transformation efficiency and genetic stability.

Amplifying plasmid vector pJUMP23-1A(sfGFP)

Transform *E. coli* TOP10 cells with heat shock

Start by resuspending pJUMP23-1A(sfGFP) DNA powder included in the 2025 iGEM distribution kit (iGEM) in nuclease-free water. Then, thaw a tube of competent *E. coli* TOP10 cells on ice. Set up an experimental group by adding both DNA and cells to a tube, and set up a negative control by only adding cells. After keeping both tubes on ice for 30 min, transfer them to a dry bath set to 42°C for 30 seconds, then return them to ice. After 5 min, add LB broth and incubate in a shaker for an hour.

Selection using the selection marker kanamycin

Obtain two agar plates and two 50 μ g/mL kanamycin agar plates. Label and plate a "+" and "-" on each type of plate. Incubate overnight and observe for fluorescent colonies in the +DNA kanamycin plate. Collect a colony with a loop and inoculate a fresh tube of kanamycin LB broth. Incubate the tube in the shaker.

Extract vector with miniprep Using DiaSpin Plasmid Mini-Preps Kit (Sangon Biotech), precipitate cell debris, bacterial proteins, and bacterial genomic DNA, and elute the amplified plasmid.

Run a gel and NanoDrop UV spectrophotometer to confirm DNA length and concentration Validate the mini-prep products on a NanoDrop. Record key values such as DNA concentration, A260/A280, and A260/A230 readings. Next, confirm the size of the DNA in the miniprep products with gel electrophoresis.

Assembling the plasmid via GGA

Using Golden Gate Assembly, insert Saz α CA expression device into pJUMP23-1A(sfGFP) Add Saz α CA expression device, mini-prep product, type II endonuclease BsaI, T4 DNA ligase, T4 DNA ligase buffer, BSA, and nuclease-free water into a PCR tube on ice according to Table 1. After adding the last component,

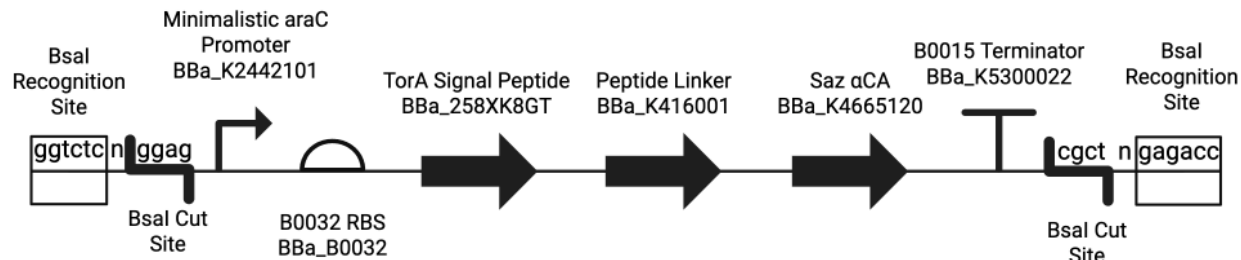


Figure 4. Diagram of custom Saz α CA expression device synthesized by Genescript (BioRender)

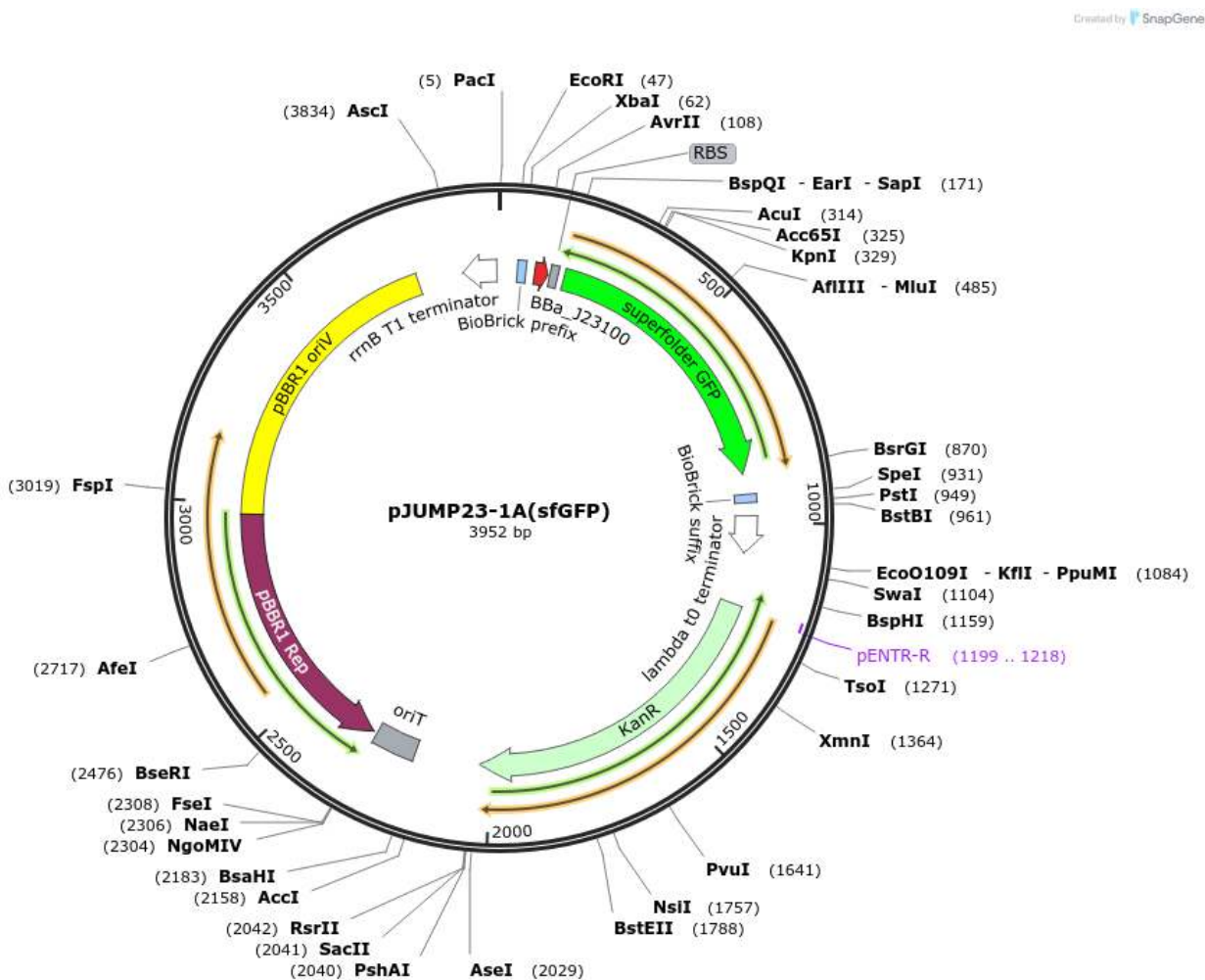


Figure 5. Overview diagram of pJUMP23-1A(sfGFP) (Addgene). pJUMP23-1A(sfGFP) was a gift from Chris French (Addgene plasmid # 126970; RRID:Addgene_126970)

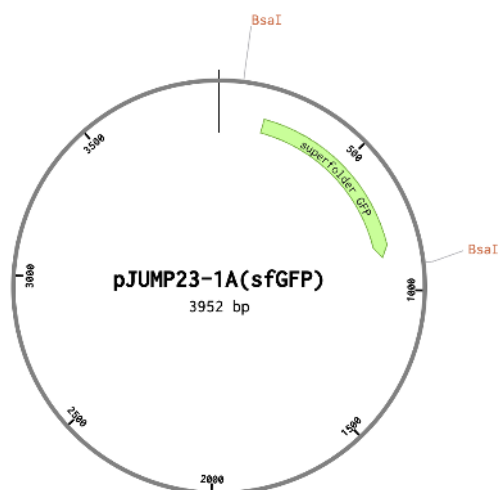


Figure 6. Diagram of BsaI cut sites on pJUMP23-1A(sfGFP) (Benchling).

mix the reaction mixture by pipetting or by flicking and centrifuging the tubes to collect the liquid at the bottom. Place it in the thermocycler and run the program shown in Table 2.

Immediately after thermocycling, transform *E. coli* DH5 α with the recombinant plasmid and verify success by plating. Follow the same procedures as transforming *E. coli* TOP10 with the vector. This time, colonies that appear non-fluorescent on the kanamycin + plate have been transformed with the recombinant plasmid. Collect one with a loop and grow in kanamycin LB broth.

Quantifying the size and function of Saz α CA

Grow and induce expression Grow the recombinant cells to mid-log phase, and then induce Saz α CA expression with isopropyl β -D-1-thiogalactopyranoside (IPTG). Add increasing amounts of IPTG to separate samples for SDS-PAGE.

Run transformed cells through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) Lyse cells with lysis buffer and perform a freeze-thaw cycle. Separate soluble and insoluble substances, and finally prepare the soluble mixture into a loading sample and run an SDS-PAGE gel to confirm the size of Saz α CA.

Be aware that the band corresponding to Saz α CA may be faint. In our design, Saz α CA is an efficient enzyme, so strong expression is unnecessary and may

TABLE 1
GGA REACTION MIXTURE

Component	Volume	Notes
BsaI endonuclease	0.2 μ L	1U for each DNA part. Dilute if necessary to reach desired volume
T4 DNA Ligase	0.2 μ L	10 CEU per DNA part. Higher concentrations of ligase are directly related to misligation.
10x T4 Ligase Buffer	10% of total reaction volume = 1 μ L	
BSA, 1mg/mL concentration	10% of total reaction volume = 1 μ L	
Coding sequence	30 fmol in 0.6 μ L	
Plasmid backbone	15 fmol in 0.75 μ L	Vector quantity (not concentration) should be half the DNA part(s)' quantity. Ideal concentration is 50 nM.
Deionized water	6.25 μ L	
Total reaction volume	10 μ L	

Note.—Enzymes must be added after buffer and water are mixed: combine and mix water, buffer, and enhancers before adding endonuclease and ligase.

TABLE 2
THERMOCYCLER PROGRAM FOR BASIC GGA (SUITABLE FOR 2-3 PARTS)

Lid Temperature: 75 $^{\circ}$ C	Temp. ($^{\circ}$ C)	Time (min)
	37	20
Repeat 10x	37	1.5
	16	3
	50	5
	80	5

Note.—The Repeat 10x cycle applies only to the three steps immediately below it, not to the initial or the final step.

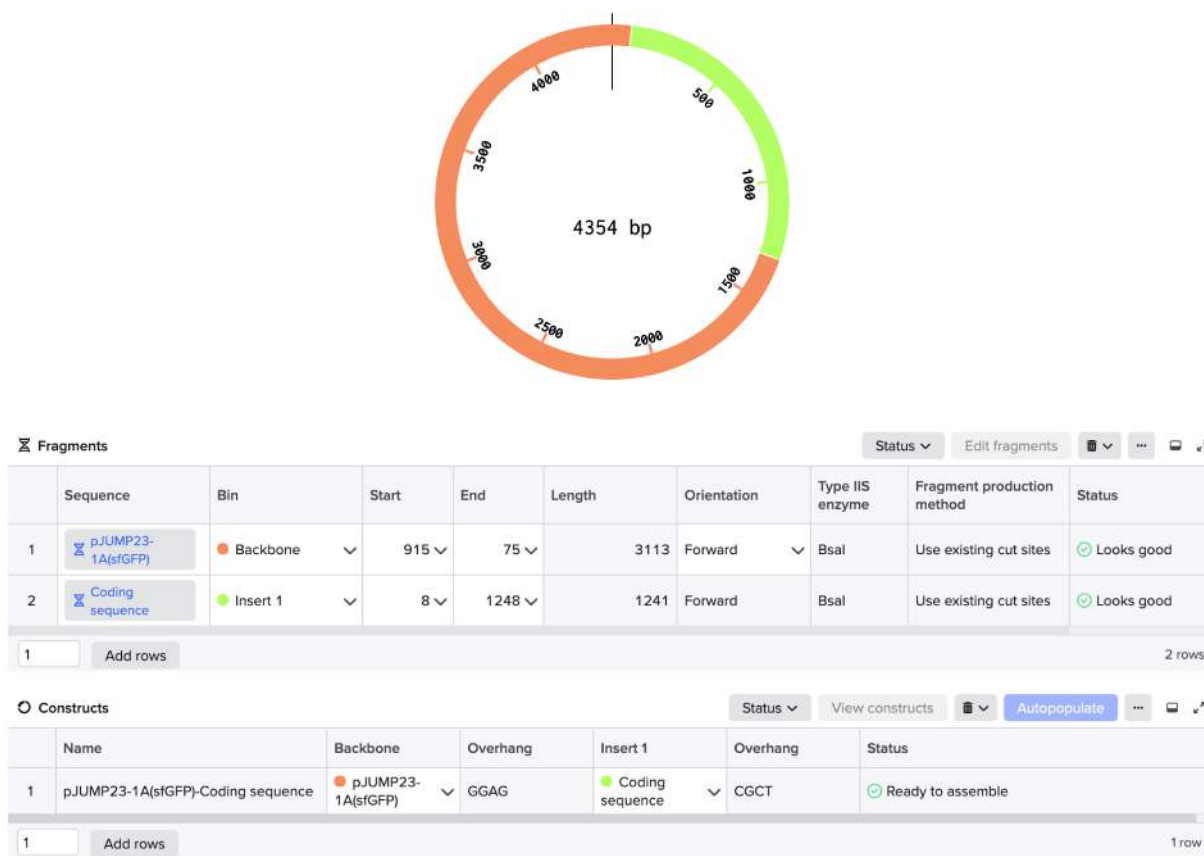


Figure 7. Benchling GGA simulation (Benchling)

cause excessive burden on the chassis. Instead, a medium-strength promoter and RBS were chosen.

Wilbur-Anderson Assay Confirm enzyme functionality by conducting the Wilbur-Anderson assay. Prepare recombinant cells and add them to Tris buffer with bromothymol blue indicator. Set up another negative control with no cells added. Rapidly add CO₂-saturated water to the sample, record the time, and observe for the color change from blue to yellow. Repeat for the negative control and determine if Saz αCA is functional.

pNPA Assay In principle, the pNPA works similarly to the Wilbur-Anderson Assay. However, pNPA is now the substrate for Saz αCA, and absorbance changes from pNPA are more accurate and responsive than those of Bromothymol blue. This assay can be conducted in cuvettes, and absorbance can be recorded with a spectrophotometer, yielding more accurate data to quantify the efficiency of Saz αCA.

Safety

All bacterial strains used in these experiments are non-pathogenic. All the above experiments have been conducted in a Biosafety Level 1 (BSL-1) lab. All members wore personal protective equipment during labs.

Both *E. coli* DH5α and TOP10 are laboratory-domesticated strains that are widely used in molecular biology and synthetic biology research. These strains are classified as BSL-1 organisms and are considered non-pathogenic to healthy humans. They have been extensively engineered for laboratory use and lack the virulence factors associated with pathogenic *E. coli* strains.

All culture media were sterilized prior to use to prevent microbial contamination. LB broth and other growth media were autoclaved at 121°C before inoculation and incubation of bacterial cultures. Following experimentation, all liquid cultures, agar plates, disposable consumables, and other materials that came into contact with genetically modified bacteria were sterilized by autoclaving at 121°C for a minimum of 30 minutes prior to disposal. No genetically modified or-

ganisms were intentionally released into natural environments, and all experimental work was performed under supervised laboratory conditions.

As an additional biosafety measure, we designed, but did not implement or experimentally test, a genetic kill switch intended to limit the survival of our system outside designated areas, shown in Figure 8. The proposed design is a double-repressor cascade kill switch repressed by cumate, an inexpensive and biodegradable chemical inducer. Under this design, the engineered bacteria would only survive and produce biocrusts in environments containing cumate. In the absence of cumate, the kill switch would express MazF and inhibit bacterial survival, thereby reducing the risk of unintended environmental consequences.

The design was inspired by the containment strategy developed by the 2024 iGEM team CAAPI, which engineered a cumate-dependent survival system for an environmental strain of *Bacillus subtilis* intended for agricultural applications (CAAPI, 2024). However, CAAPI were able to engineer the bacterial chromosome so that cumate induced an essential gene, without which the cell would die. Since we do not possess the techniques to do so, we instead opted for the lack of cumate inducing a toxin.

While this approach is theoretically capable of restricting bacterial survival to designated treatment zones, it was not constructed or tested during the course of this project. As we will discuss in the Next steps section, future work on this project will involve experimentally validating this kill switch.

Results

E. coli TOP10 transformation is a verified success

The first step in our methods was to transform our selected plasmid backbone into *E. coli* TOP10 strain for replication. As described in the Methods section, the backbone was extracted from the iGEM distribution kit and transformed into *E. coli* TOP10 cells by heat shock. 4 total plates were set up:

Two control groups were present in this experiment to ensure the validity of the results from the experimental groups. The LB (-) plate, containing only LB nutrient broth, serves as a positive control group, or the baseline level of bacterial growth without interference from the plasmid backbone or kanamycin. This can then be compared to our experimental groups to better understand the change from a baseline. The LB/kanamycin (-) serves as a positive control group, providing a baseline of bacterial growth in the absence of genetic modifications when exposed to kanamycin. This would better highlight whether the plasmid backbone affects bacterial growth by comparing bacterial

growth in the LB/kanamycin (+) and LB/kanamycin (-) groups.

As seen in Figures 9C and 9D, there is lawn growth on both plates without kanamycin, regardless of whether transformed or untransformed bacteria were used. In the plate with kanamycin, plated with untransformed bacteria, there is evidently no growth. Lastly, on the plate with kanamycin, there are two surviving bacterial colonies.

With the two control groups verifying the effectiveness of kanamycin in killing non-transformed bacteria and the normal growth of *E. coli* TOP10 without kanamycin, it can safely be concluded that the transformation was successful.

Mini-prep yields contaminated results

Plasmid extraction was performed on the transformed *E. coli* TOP10 cells. We used a spectrophotometer to measure optical density at 600 nm (OD600) and confirm that our cells were in mid-log phase. The extracted plasmid product was verified with a NanoDrop.

We struggled with low plasmid concentration and high contamination levels, so we had to repeat mini-prep multiple times. In our initial attempts, DNA concentrations were around 60 ng/uL, and A260/A230 values were around 0.5. The extraction kit procedures state that A260/A230 should be greater than 2 for sequencing or Golden Gate Assembly. Residual guanidine salts, phenol, EDTA, and ethanol can contribute to a low A260/A230.

In our final attempt, we reduced contamination by washing three times instead of two, centrifuging faster in the final wash step, and leaving the tubes open for 5 min after the final wash step. These changes eliminated more contaminants and gave ethanol time to evaporate out of the column. Our last attempt had a plasmid concentration of 48.8 ng/uL, an A260/A280 value of 2.08, and an A260/A230 value of 1.15. Despite A260/A230 still being below 2, we decided to proceed to GGA due to time constraints.

Golden Gate Assembly failed to yield recombinant cells

Following the procedures described above, we had enough reagent to prepare two tubes of reaction mixture. While preparing the reaction mixture, we found it difficult to pipette the small volumes accurately according to the procedure. After finishing GGA and transformation, we plated the cultures and observed for growth. Unfortunately, the results do not support that GGA was successful, and we were not able to obtain the recombinant cell for further testing (Figure

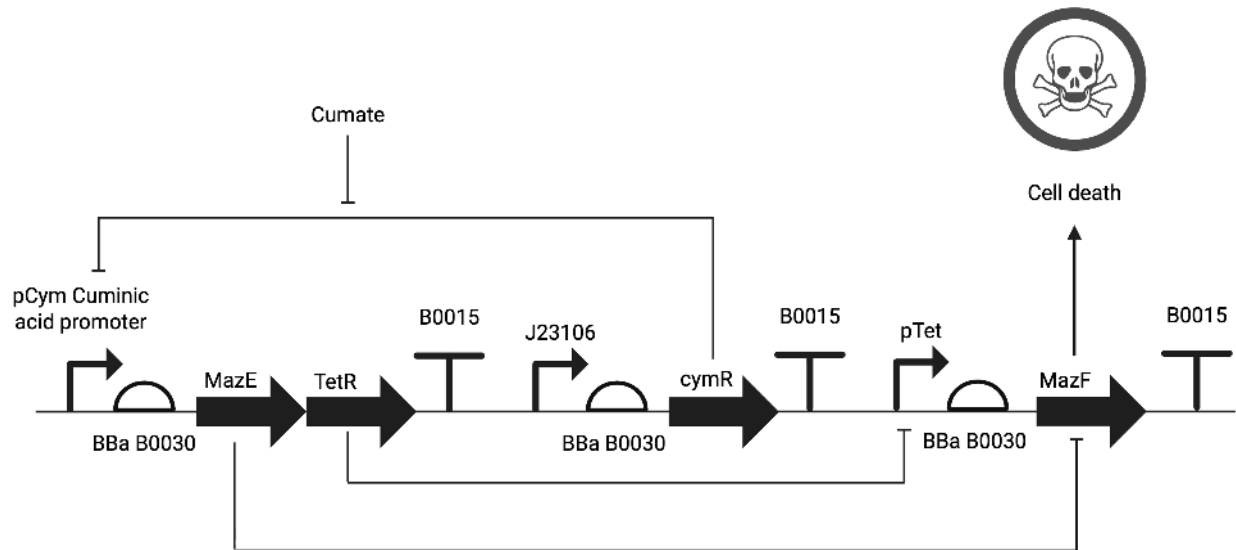


Figure 8. Diagram of kill switch repressible by the presence of cumate (BioRender)

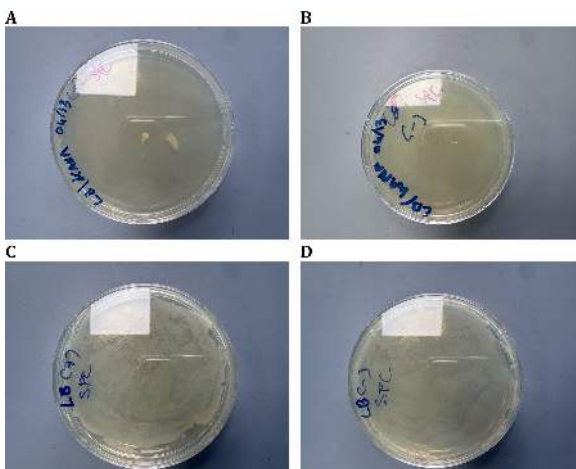


Figure 9. Qualitative data of transformation. (A) and (B): Kanamycin was added to both plates. (A) is labelled with (+), indicating bacteria with the transformed plasmid, and (B) is labelled with (-), indicating bacteria without the transformed plasmid. (C) and (D): Kanamycin was not added to either plate. (C) is labelled with (+), indicating bacteria with the transformed plasmid, and (D) is labelled with (-), indicating bacteria without the transformed plasmid.

10).

The observed growth on the LB plates validate that the *E. coli* DH5 α cells used in this transformation were competent. However, there was no growth on any of the three kanamycin plates, suggesting that no cells were transformed with either pJUMP23-1A(sfGFP) or the recombinant plasmid.

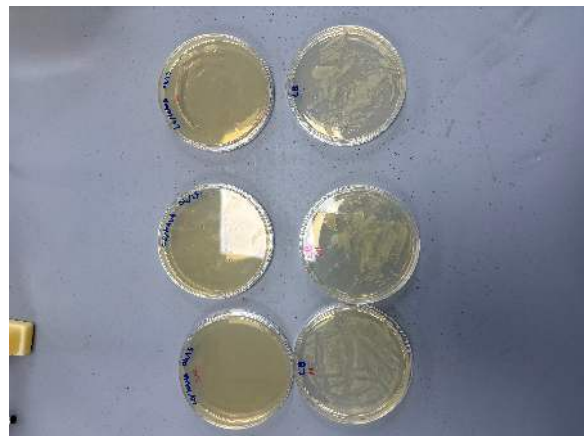


Figure 10. Golden Gate Assembly transformation plates

Due to limited lab resources and time, we were not able to conduct the remaining experiments detailed previously. We have discussed in the Next steps section about doing them in the future.

Discussions

In our final mini-prep attempt, the A260/A230 value of 1.15 suggests high levels of contamination. According to the mini-prep instructions manual, some contaminants, such as ethanol, measured by A260/A230, are known to inhibit enzymatic activity and interfere with downstream experiments. Therefore, contamination from the miniprep could be responsible for why Golden Gate Assembly did not yield recombinant cells.

Another explanation for the lack of recombinant

cells is that bacterial transformation with *E. coli* DH5 α may have failed. *E. coli* DH5 α has a similar heat shock transformation efficiency to *E. coli* TOP10 (Liu et al., 2018). Since the initial transformation of *E. coli* TOP10 with pJUMP23-1A(sfGFP) only yielded two colonies, it is possible that transformation with the recombinant plasmid failed because of random chance.

This microbial-induced MICP system is a promising step towards addressing desertification. By using synthetic biology, we replicated a natural phenomenon observed in biocrusts in a controlled, malleable *E. coli* system, enabling more cost-effective and sustainable solutions than current physical ones, and allowing others to modify and replicate our system.

The additional features in our plasmid, such as the TorA secretion protein, arabinose-induced promoter, and cumate-induced kill switch, all work to complement the core Saz α CA enzyme which makes the system more controllable, safe, and cost-effective to maintain.

Aside from desertified soil, our system may also prove to be effective in several other applications. Wastewater, for example, also provides a suitable system for MICP, forming carbonate that can precipitate with calcium or magnesium ions. However, this system cannot be used in acidic environments, as calcium carbonate dissolves when reacting with acids.

Our system could also be applied in space biology and extraterrestrial habitat development. Habitats on other planets or moons are also unstable, and frequent storms stir up large amounts of dust that can interfere with equipment, infrastructure, and human health. An MICP biocrust can contribute to stabilizing the soil, reducing dust, and supporting the development of livable habitats on other planets.

However, despite the biosafety measures incorporated into this project, there are still potential environmental and safety risks with this system. For instance, horizontal gene transfer remains a potential concern. Although the engineered strain was designed for laboratory use, plasmid-borne genetic material may be transferred to naturally occurring microorganisms through processes such as transformation or conjugation. Although the genome of our selected chassis and our coding sequence will not confer pathogenic traits, the unintended spread of our genetic constructs still could have unintended consequences.

The introduction of our engineered bacteria into natural environments may alter existing microbial communities. Our bacteria could potentially compete with native microorganisms for nutrients and space and disrupt their ecosystem.

Several iterations have been planned to improve the system. For instance, the aforementioned kill switch

can be added to our system to provide safety measures and limit unintended harm. One of the weaknesses in our system, which is the need for a calcium source and a carbon dioxide source to perform MICP, can be addressed by adding parts that allow our chassis to intake soil calcium and atmospheric CO₂ at a faster rate. Another consideration is the stability of our chassis when exposed to desert temperatures and low water levels for extended periods of time.

Next steps

To advance this study, the MICP performance will be quantified under controlled laboratory conditions by measuring carbonate precipitation, calcite formation, and resulting improvements in soil mechanical strength. In addition to repeating the unsuccessful experiments and conducting the ones we didn't have time for (such as SDS-PAGE and the Wilbur Anderson assay), the following experiment is also planned.

To test the efficiency of this biocrust, a controlled laboratory experiment is designed using treated sandy soil samples subjected to real-life simulated environmental conditions. Thirty 30 cm $\times$ 30 cm $\times$ 5 cm trays will be prepared using sandy soil and treated by spraying bacterial culture and cementation solution over the surface through three cycles over five to seven days. Three control experiments will be conducted in untreated sand, abiotic cementation solution, and a killed-cell control.

After forming biocrusts, trays will go through accelerated weathering stages. Drying will be accomplished by alternating periods of 8 to 12 hours at 40 to 50°C with 12-hour intervals at room temperature, 5 to 10 times. Wet-dry cycles will involve water spraying followed by drying and measurement of weight loss during this process. If equipment allows for it, photodegradation will be estimated by UV-C exposure. For wind erosion testing, the tray samples will be tested with a bench-top fan or homemade PVC wind tunnel at speeds of low ($\sim$ 3 m/s), medium ($\sim$ 6 m/s), and high ($\sim$ 10 m/s); the trays will be weighed before and after ten minutes, along with a downwind collecting pan to capture the eroded sediments to measure the wind erosion rate in grams per meter squared per minute. The soil strength, measured in kPa, is going to be measured from several different angles and locations to show the reduction in soil strength due to the various stresses. Since the only metric tested here is whether the system actually functions, in case standard atmospheric CO₂ concentrations are insufficient or if natural calcium concentrations of the soil samples are found to be too low, gas pumps and supplemental calcium could be added to magnify the strength and for-

mation speed of the biocrust.

The results that are to be obtained are the percentage of soil mass lost as a function of wind speed and treatment, penetration resistance after each cycle, crust integrity (intact, cracked, fragmented, and destroyed), as well as the amount of CaCO_3 in the crust layer compared to the sub-crust soil.

The effectiveness of the theoretical cumate-induced kill switch should also be assessed. This can be achieved by assembling the kill switch into a vector and transforming *E. coli* with it. Next, grow recombinant cells in a cumate + and cumate - culture tube. We can conclude that the kill switch is effective if there is only growth in the cumate + tube.

Author contributions

AM and ED drafted the initial idea of this research proposal. SJ worked on the background section. EW and AM worked on conducting experiments, collecting data, and analyzing results. EW worked on the discussion and results section. AM worked on the materials and methods, as well as graphing and designing scientific diagrams.

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