Biocementation for All, Anywhere:
A New Experiment for Introductory Soil Mechanics Courses

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ABSTRACT

This paper presents a novel laboratory experiment that can be incorporated into introductory soil mechanics courses to introduce students to the field of biogeotechnical engineering and the use of biostimulated microbially induced calcite precipitation (MICP). Applying MICP to granular soils results in an increase in peak strength and shear stiffness of the soil as a result of the precipitation of calcium carbonate on soil particle surfaces and at soil particle contacts. The authors developed protocols to treat small volumes of soil and to test the effectiveness of the treatment using a simple strength test based on ASTM D3967-16. In fall 2020, the experiment was piloted as a four-week, course-based research experience that can be conducted by students remotely or in a traditional laboratory environment. This paper provides an introduction to MICP and describes the protocols for conducting the experiment. The paper also suggests approaches for how the experiment can be incorporated into a traditional introductory soil mechanics course.

INTRODUCTION

This paper presents a novel lab experiment that can be incorporated into introductory soil mechanics courses to introduce students to the field of biogeotechnical engineering and the use of biostimulated microbially induced calcite precipitation (MICP) to cement granular soils. Cementation is the binding of soil particles as a result of the precipitation of mineral matter in the pore spaces of the soil matrix and can involve many different types of minerals (e.g., silica, calcite, iron oxides, and sodium chloride among others). MICP is a biomediated cementation process that precipitates calcium carbonate (calcite) on soil particle surfaces and at soil particle contacts. The process results in an increase in peak strength and shear stiffness of the soil. While MICP most commonly involves adding bacteria to the soil, a process known as bioaugmentation, biostimulated MICP is a process that relies on the bacterial populations that occur naturally in soil. Biostimulated MICP has been demonstrated to successfully cement soil particles together without the specialized equipment and biological processes required to grow bacteria associated with bioaugmentation.

The authors have developed protocols for an efficient and economical process to treat small volumes of soil using biostimulated MICP and to evaluate the effectiveness of the treatment using a simple strength test based on ASTM D3967-16 "Splitting Tensile Strength of Intact Rock Core Specimens." The authors piloted the protocols as a four-week, course-based experiment in an introductory soil mechanics course. While the experiment can be conducted during traditional laboratory sessions and in a traditional laboratory space, students participating in the fall 2020 pilot completed the work remotely in their residences and dorm rooms at a cost of approximately $15 per student.

Because the biostimulated MICP process relies on the community of microbes that naturally exist in soil and because microbial communities are different in different geographical locations, the authors are developing the process to facilitate a potential larger research project involving multiple institutions to determine the characteristics of soils and pore water that are suitable for biostimulated MICP. As part of the envisioned project, data produced by students from different institutions in geographically different locations would be used to initially populate a publicly available database of results. The authors hope that the project will continue to scale and be used in introductory soil mechanics courses across the country (and beyond!) and that students involved in the project will submit their results to the growing database. The database of results would be available to the larger research community to support greater understanding of the applicability of biostimulated MICP in geographically diverse locations. This vision requires that simple and reliable experimental protocols be developed for students to follow and report results in a consistent format.

This paper provides background information on the motivation for the project, an introduction to MICP, and a description of the protocols that the authors developed for conducting course-based experiments of biostimulated MICP treatment and testing of granular soils. The paper also suggests approaches for how the project can be incorporated into a traditional introductory soil mechanics course.

BACKGROUND

Motivation/Rationale for Initiative. The field of biogeotechnics involves the use of biomaterials and bioprocesses (as well as materials/processes inspired by biological organisms or processes) to address traditional problems in geotechnical and geoenvironmental engineering (Sekharan et al., 2019). This interdisciplinary field has gained momentum in the past decade and has resulted in numerous advances related to reducing damage caused by earthquakes (Kavazanjian et al., 2015) and slope stabilization (Gowthaman et al., 2019), among other engineering issues. The Center for Bio-mediated & Bio-inspired Geotechnics (CBBG) was created in 2015 as an NSF-funded engineering research center and is a nexus for biogeotechnics work.

A developing area of research within biogeotechnics is MICP. MICP processes in granular soils result in the formation of calcite on the surfaces of soil particles and in the spaces between soil particles. The calcite attaches to and connects the soil particles. Multiple researchers have shown that MICP is effective in increasing initial shear resistance, peak shear strength, and liquefaction resistance (e.g., DeJong et al., 2006; Gomez et al., 2014; Montoya et al., 2013; Montoya & DeJong, 2015; Whiffin et al., 2007). A primary application of MICP is to strengthen soils below structures that are at risk of failure during earthquakes due to the presence of saturated, loose sands—a failure of soil known as liquefaction. Additional applications of the process to increase strength include, but are not limited to, improving soil bearing capacity prior to constructing new foundations and increasing soil strength to reduce the lateral loads applied to a retaining structure. Current technologies available to accomplish similar outcomes use Portland cement and sodium silicate-based methods. These existing methods are often expensive and are associated with environmental and sustainability concerns (Gomez et al., 2017).

In addition to applications involving the increase of soil strength, MICP has also been used to reduce seepage rates into soils by creating a low-permeability crust at the soil surface (Gao et al., 2019), to decrease desiccation cracking in clayey soils (Liu et al., 2020), to improve the internal erosion resistance of gravel-sand mixtures (Jiang & Soga, 2019), and to create sustainable building materials (Bernardi et al., 2014; Cheng et al., 2020).

The majority of MICP research has involved adding bacteria to the soil, an approach known as bio-augmentation (DeJong et al., 2006). However, using a bio-augmentation approach is typically economically prohibitive for applications involving large volumes of soil and the approach can also raise environmental concerns associated with introducing bacteria into the soil (Gomez et al., 2017). In addition, some researchers have found that native bacterial populations out-compete augmented, non-native, bacterial populations after being introduced (Gomez et al., 2018).

Recent research has shown that MICP can occur in soils using bacteria that are naturally present, an approach called bio-stimulation (Gomez et al., 2017). The bio-stimulated approach poses less of a risk to the environment and, because bacteria do not have to be grown to use in the process, has the potential to be economically advantageous. However, for bio-stimulated MICP to become a viable and widespread method for soil improvement, the process needs to be shown to be effective in a broad range of soils and the engineering community needs to be educated about the processes to identify suitable soils and apply the treatment.

The authors want to create a course-based research experience (CRE) tool to educate and engage students and instructors about the new field of biogeotechnics and to support research on and increase knowledge of the effectiveness of bio-stimulated MICP in soils from diverse locations. CREs offer students experience in doing original research as well as the opportunity to contribute to the knowledge base in their discipline. CREs are becoming more common in the natural sciences, including biology; however, CREs that provide students the opportunity to contribute to the knowledge base in geotechnical engineering are rare.

In January 2021, an informal survey was sent out to all 350+ members of the United States Universities Council on Geotechnical Education and Research (USUCGER) asking for information on the use of CREs in required undergraduate courses in soil mechanics or geotechnical engineering. Two responses were received describing hands-on research projects that had been incorporated into required undergraduate geotechnical engineering courses and one response was received describing the incorporation of a lab-based research project in an elective course. However, while the described experiences creatively engaged students in conducting experiments, none of the research projects were interdisciplinary and none engaged students in research that contributed to the knowledge base of the discipline. A well-developed, easily-implemented CRE in the area of biogeotechnics has the potential to become broadly implemented in civil engineering curricula and to bring the benefits of CREs to thousands of undergraduate students each year.

Biostimulated Microbial Induced Calcite Precipitation. Microbially induced calcite precipitation (MICP) is a bio-mediated cementation process that precipitates calcium carbonate (calcite) on soil particle surfaces and at soil particle contacts increasing peak strength and shear stiffness of the soil (DeJong et al., 2006; Martinez & DeJong, 2012; Montoya et al., 2013; Whiffin et al., 2007). In the presence of urea, microorganisms containing active urease enzymes catalyze a hydrolysis reaction creating ammonia and carbonic acid (Mobley et al., 1995). In a non-acidic solution, the ammonia will react with water to create ammonium and hydroxide ions and the hydroxide "promotes the deprotonation of carbonic acid to form increased concentrations of carbonate ions, which, in the presence of sufficient soluble calcium, may supersaturate aqueous solutions with respect to calcite and initiate calcite precipitation" (Gomez et al., 2018).

Burbank et al. (2011) demonstrated that bio-stimulated MICP can be accomplished both in the laboratory and in the field and Burbank et al. (2013) demonstrated that bio-stimulated MICP created sufficient calcite precipitation to increase soil strength as measured by both cone penetration and cyclic triaxial shear tests. Gomez et al. (2017) directly compared bio-stimulated and bio-augmented processes in large-scale tank experiments (1.7 meter diameter tanks) and found similar improvements in the engineering properties resulting from the two treatments. More recently, Gomez et al. (2018) concluded that native microorganisms may be used to induce calcite precipitation at depths up to 12 meters.

However, bio-stimulated MICP in native soils has only been demonstrated in a limited number of natural soils (Burbank et al., 2011; Gomez et al., 2014). Bio-stimulated MICP depends on the presence of native bacteria that contain active urease enzyme and, if they are present, those bacteria must be successfully cultivated so that they are present in sufficient numbers to result in improvements to the engineering properties of that soil. If bio-stimulated MICP is to become a useful tool to improve soil, more information is needed on the effectiveness of the process in soils from diverse locations and with different composition, pore water chemistry, and microbiota. A study by Walters and Martiny (2020) analyzed over 27,000 samples of soil, water (saline and fresh), aerosols, and plant surfaces (among other microbial communities) from a range of habitats and geographic regions by the Earth Microbiome Project (Thompson et al., 2017) and found that the samples from soil contained the highest variety of microbes. This diversity in the microbial community will likely impact whether engineers will be able to get the desired improvements in engineering properties at these locations using bio-stimulated MICP. A CRE that allows students to collect data on the effectiveness of biostimulated MICP in soil from around the globe will provide useful and needed information on the broad applicability of this treatment method.

Course-based Research Experiences. Course-based research experiences (CREs) provide opportunities for all students to have research experiences as called for by multiple national reports (AAAS, 2011; Olson et al., 2012). A substantial amount of work has been done on the effectiveness of course-based undergraduate research experiences: CREs increase the diversity of students engaged in scientific research (Bangera & Brownell, 2014); improve retention of students in scientific research (Eagan et al., 2013); increase student interest in research (Committee for Convocation on Integrating Discovery-Based Research into the Undergraduate Curriculum et al., 2015; Davidson, 2018); increase student's interest in pursuing a Ph.D. (Russell et al., 2007); and seem to be particularly beneficial for students from historically underrepresented backgrounds (Hernandez et al., 2013). Many CREs have been developed for life science curricula – possibly the best known is the SEA-PHAGES (Science Education Alliance – Phage Hunters Advancing Genomic and Evolutionary Sciences) program jointly administered by Graham Hatfull's group at the University of Pittsburgh and Howard Hughes Medical Institute's Science Education Division and run at colleges across the country and internationally (https://seaphages.org/). In the PHAGES CRE, students collect a soil sample, isolate a bacteriophage (phage), and use multiple methods to characterize that phage.

The project described in this paper meets the expectations for a CRE since 1) students will be making discoveries that are of interest to stakeholders outside the classroom and 2) students' research results will be collected in a publicly available database (https://www.designsafe-ci.org/).

OVERVIEW OF PROTOCOL FOR MICP TREATMENT AND TESTING

The basic test set up for MICP treatment is shown in Figure 1 and uses pegboard, wood, 60 ml syringes, aquarium tubing, rubber stoppers, twist ties, and kitchen scouring pads. The cost for a set up for treating two samples as shown in Figure 1 is less than $10.

Figure 1. MICP test set up.

The treatment process includes a stimulation phase and a cementation phase. The solutions used for each phase are based on Gomez et al. (2018) with modifications based on personal communications with Dr. Jason DeJong (University of California, Davis) and Dr. Michael Gomez (University of Washington). the solutions are prepared using distilled water and the ingredients listed in Table 1.

Stimulation solution:
0.1 gm/L yeast extract
100 mM ammonium chloride
42.5 mM sodium acetate
350 mM urea (agricultural grade)
(pH 8.5)

Cementation solution:
0.1 gm/L yeast extract
100 mM ammonium chloride
42.5 mM sodium acetate
350 mM urea (agricultural grade)
250 mM calcium chloride

For both the stimulation and cementation solutions, it is possible to mix the dry ingredients ahead of time. The ingredients can then be shipped to remote students, if needed. Prior to beginning either the stimulation or cementation treatments, the appropriate volume of distilled water is added to create a concentrated solution that is kept under refrigeration (approximately 4° C). For each daily treatment, an appropriate volume of the concentrated solution is mixed with additional distilled water for a total volume equal to at least 1.5 pore volumes of the soil sample. The total cost for the stimulation and cementation treatments for two soil samples is less than $5 (assuming each soil sample is approximately one inch in diameter and two inches high).

The soil is treated with the stimulation solution daily for seven days and the pH of the effluent is measured each day to monitor for the presence and growth of ureolytic bacteria. The pH of the effluent should be at 9.0 or higher for the last two to three days of the stimulation treatment. Students involved in the CRE should be told that, while the pH will change as a result of the growth of ureolytic bacteria, pH changes only indicate biological and/or chemical activity in the soil and the observed change in pH may result from other activities occurring in the soil. After the stimulation treatments, the soil is then treated with the cementation solution daily for an additional seven days. After the stimulation and cementation treatments are completed, distilled water is gravity flushed through the sample and the sample—still in the syringe—is drained and allowed to dry.

Once the sample is dried, the sample is removed from the syringe for strength testing. (Removal of the sample from the syringe can be accomplished using the syringe plunger.) Strength testing of the cemented sample is done using a simplified version of ASTM D3967-16, “Splitting

Tensile Strength of Intact Rock Core Specimens." A loading device can be easily constructed using wood 2x4's that are connected by a door hinge such that the two arms of the device are parallel when a MICP cemented sample is placed between the arms. The simple device is shown in Figure 2 and for a CRE taught in a face-to-face classroom or laboratory, a single device can be shared by all students in the course.

Figure 2. Images of simple splitting tensile strength testing device.

By placing the device on a low friction surface and applying a load in a horizontal direction using a luggage scale or similar device (attaching the device to the cord), the applied load to the sample can be calculated:

$$P = F\left[\frac{L_{load}}{L_{sample}}\right]$$

where:

P = applied load to sample
F = force applied at the cord
L_{load} = distance between force applied at the cord and the hinge
L_{sample} = distance between the center of the sample and the hinge

The splitting tensile strength of the specimen can then be calculated (ASTM D3967-16):

$$\sigma_t = 2P/\pi t D$$

where:

s_t = splitting tensile strength
P = maximum applied load to sample
t = height of the cylindrical specimen
D = diameter of the specimen, i.e. the inner diameter of the syringe

INCORPORATING MICP INTO AN INTRODUCTORY SOIL MECHANICS LAB COURSE

Swenty and Swenty (2018) reported that approximately 90 percent of civil engineering programs require an introductory course in soil mechanics or geotechnical engineering and that the course had an average credit hour load of 3.7 implying that the majority of these courses included a laboratory component. Their findings were similar to Welker (2012) who reported that 93 percent of accredited engineering programs required an introductory course in soil mechanics or geotechnical engineering and 83% included a laboratory component as part of the course.

There is no standardized content for an introductory course. However, Wirth et al. (2017), proposed that the introductory course meet for at least 40 hours and include a 2 to 3 hour laboratory session each week. Based on their review of the curriculum and formats for introductory geotechnical engineering courses in US and European institutions, they propose that the following course content be included in a required introductory to geotechnical engineering course to prepare students for the 21st century:

- *Soil classification
- Fluid flow through soils
- *Mechanical behavior (including oedometer and triaxial tests)
- *Geostructures (including retaining walls, embankments, dams, shallow foundations)
- Hydromechanical coupling (basic introduction to consolidation)
- Compaction
- *Shallow foundation design
- Introduction to in situ testing

The above topics complement topics listed in traditional soil mechanics laboratory text books such as that written by Das (2015):

- *Determination of water content
- Specific gravity
- *Sieve analysis
- *Hydrometer analysis
- *Liquid limit test
- *Plastic limit test
- Shrinkage limit test
- *Engineering classification of soils
- Constant head permeability test in sand
- Falling head permeability test in sand
- Standard proctor compaction test
- Modified proctor compaction test
- Determination of field unit weight of compaction by sand cone method
- Direct shear test on sand
- Unconfined compression test
- Consolidation test
- Triaxial tests in clay

If local soils are used for the project and sufficient volumes of soil are gathered to support the laboratory tests to be conducted, the topics noted with an asterisk (*) in the above lists can be aligned with in-class lectures and laboratory tests associated with an MICP class project as noted in Table 1.

Table 1. Alignment of lecture and laboratory components of introductory soil mechanics or geotechnical engineering course with MICP project.

Topic		**Alignment with MICP Project**
Lecture	Soil classification	Soil classifications that are appropriate for MICP treatments
	Mechanical behavior	Strength of soils and the benefits of small amounts of cementation within soils
	Geostructures	Relationship between soil strength (including cemented soils) and the design of geostructures (including retaining walls, embankments, dams, shallow foundations)
	Shallow foundation design	Increase in shallow foundation capacity in cemented soils
Laboratory	Determination of water content	These tests can be conducted on native soils samples to be used in MICP project
	Sieve analysis	
	Hydrometer analysis	
	Engineering classification of soils	

The amount of integration of the MICP project in the course is at the discretion of the instructor. If the MICP project is to be conducted as part of the laboratory portion of the course, a tentative schedule is shown in Table 2. (The developed protocols assume that laboratory sessions occur weekly.) With the exception of the first session, the activities associated with the MICP project typically require an hour or less and can be combined with other laboratory activities at the discretion of the instructor.

RESULTS OF PILOT CRE

The authors piloted the preliminary protocols for conducting MICP as a CRE during fall 2020. The CRE was implemented in two courses at Lafayette College: an introduction to geotechnical engineering course and a microbiology course. These courses were taught remotely and students conducted the CRE wherever they were living.

Because the pilot project was conducted remotely by the students, each student was sent the materials necessary to set up and treat two soil samples. Each student was sent a sample of Ottawa 50/70 sand to act as a control for their experiment and each student obtained a sample of granular soil from a location of their choice close to wherever they were living. Students documented the locations where they obtained their samples (Figure 3) and provided a simple visual description of the soil. Each student then set up the testing apparatus and treated both the control and the local soil sample using the protocols that had been established.

Table 2. Tentative laboratory scheduled for MICP project.

Day (Laboratory Session)	Activity	Approximate Time Required
Day 0 (First laboratory session)	Obtain soil samples; conduct visual manual classification; set up and saturate samples	2 to 3 hours
Day 7 (Second laboratory session)	First stimulation treatment	30 minutes (assuming concentrated stimulation solution has been made prior to the laboratory session).
Day 8 through 13	Daily stimulation treatment	10 minutes (can be conducted by a teaching assistant)
Day 14 (Third laboratory session)	First cementation treatment	1 hour (assuming concentrated cementation solution has been made prior to the laboratory session)
Day 15 through 20	Daily cementation treatment	10 minutes (can be conducted by a teaching assistant)
Day 21 (Fourth laboratory session)	Drain and begin drying samples; clean up testing apparatus	1 hour
Day 28 (Fifth laboratory session)	Removal of samples from syringes; strength testing	Approximately 3 minutes per sample.

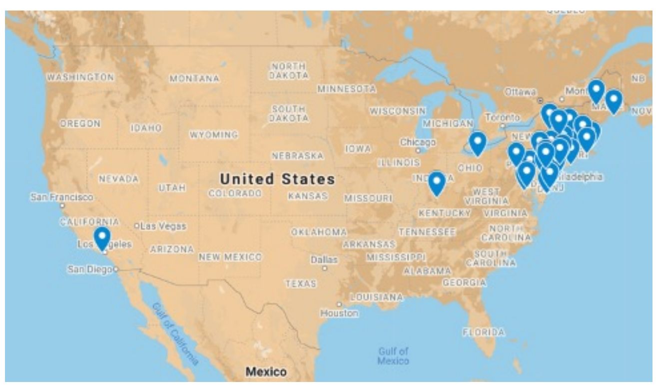

Figure 3. Locations of samples obtained by students during fall 2020 Lafayette pilot (source: Google Maps).

There was insufficient time for each student to be provided with the simple testing device. Students involved in the CRE during this preliminary pilot returned their treated and drained samples to their course instructors for strength testing. (The samples were returned in their syringes and wrapped in the supplied bubble wrap.) The authors of this paper then removed the samples from the syringes and strength-tested the cemented samples. The results of the strength tests are shown in Figure 4.

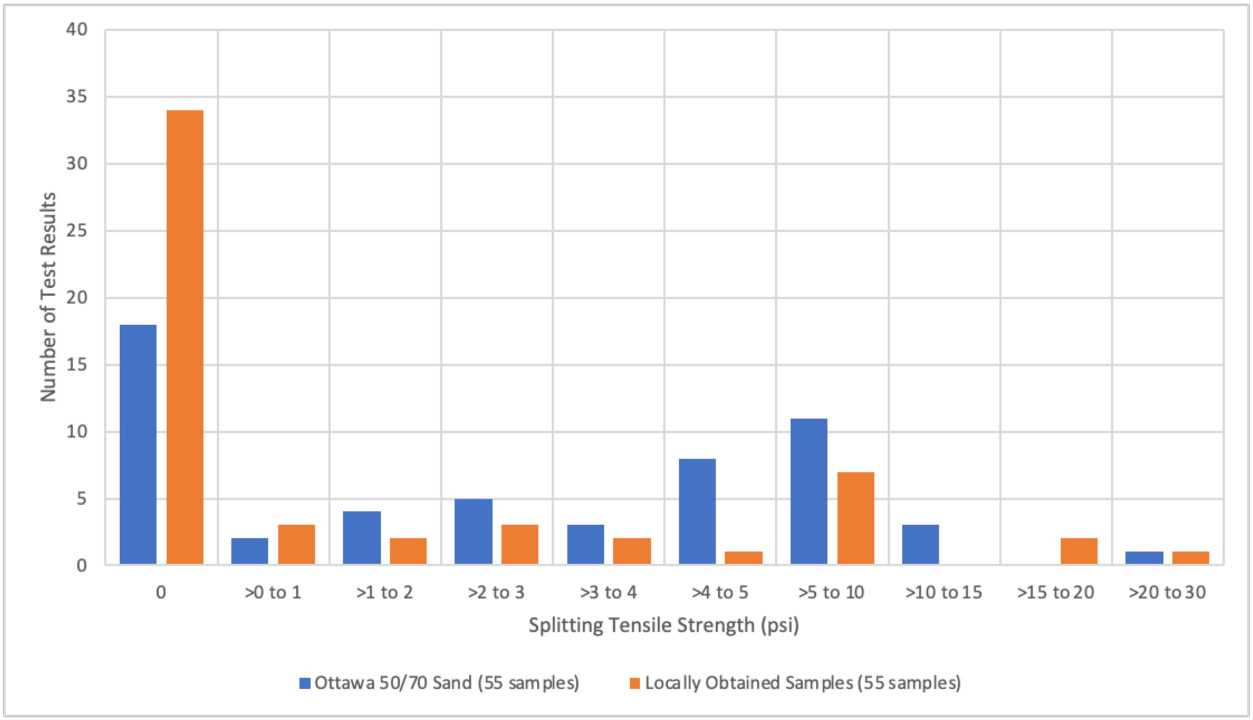

Figure 4. Splitting tensile strength results from fall 2020 Lafayette pilot.

The results from the preliminary pilot provide evidence that students can conduct the CRE in a range of non-traditional laboratory environments. However, the variability in the test results obtained from the control samples indicate that the protocols need further refinement for the data to be useful to the larger geotechnical community.

To improve our understanding of the expected variability in test results, during spring 2021 the authors conducted replication testing using the MICP protocols developed on samples of two different manufactured batches of 50/70 Ottawa sand. Ten samples from each batch of the Ottawa sand were treated in a controlled laboratory environment. The stimulation and treatment solutions used were identical for each sample and the cemented samples were not transported prior to strength testing. The samples were also rinsed with distilled water prior to draining to remove soluble salts and bio-polymers and the samples were dried for at least 48 hours in an oven set at approximately 65 to 75 degrees Celsius. While the authors obtained more consistent results from these tests, additional work is being conducted to further decrease the variability in the results. This additional work includes modifying the protocols to include multiple soakings of the samples with distilled water and testing the cemented samples with hydrochloric acid to confirm the presence of calcite in the cemented samples.

CONCLUSION

CREs have significant benefits associated with student learning, student engagement, and student persistence in STEM majors. At present no CRE is available for students in civil engineering programs in which students gather data and contribute their results to a database that is of interest to a large group of stakeholders. The CRE presented here can fill that gap and provide an opportunity for instructors teaching soil mechanics and/or geotechnical engineering to introduce the field of biogeotechnics to their students and to engage students in research as part of their introductory courses. Written materials for instructors and student to support offering the MICP CRE are available on request (send requests to rothm@lafayette.edu).

Once the testing protocols are finalized, future work is planned to create a database to gather the results of student testing and to make those results available to the public. Information on how to contribute to that database will be made available to any interested instructor.

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