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NCHRP IDEA Program

Application of Microbial Facilitated Stabilization for Sustainable Improvement of Expansive Pavement Subgrades

Final Report for
NCHRP IDEA Project 192

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EXECUTIVE SUMMARY

Expansive soils cause millions of dollars of damage each year to transportation infrastructure across the country. The main goal of this project was to evaluate the application of environment-friendly biological processes to stabilize expansive subgrades. Current stabilization options have been inadequate especially to pavement infrastructure and result in expensive rehabilitation activities. ***Concept and Innovation***: Microbial-induced calcite precipitation (MICP) is an innovative approach that uses soil bacteria to precipitate calcite and alter the behavior of the soil. Experience with MICP implementation has been primarily using bioaugmentation (introducing microorganisms into the soil), especially in case of sands where microbial populations are minimal. This method has several issues including the survivability of the bacteria in the new environment which results in uncertain treatment performance. However, in the case of clays, there is no need to introduce exogenous bacteria as clays are natural incubators for microorganisms. Hence, this research study used bio-stimulation to encourage indigenous bacteria to precipitate calcite and alter soil behavior.

The study tasks were accomplished in two stages; stage 1 involved laboratory work while stage 2 focused on preliminary fieldwork. The objectives of the laboratory work were to study the role of soil type, clay content and bacterial populations on treatment effectiveness and develop a protocol for field implementation. The field work was intended to explore the protocol's effectiveness in the field and help develop a protocol for future field applications.

The laboratory work consisted of four major tasks; a) Develop Treatment Solution Delivery System (TSDS) to construct a "mini soil microcosm" and deliver required treatment solutions to all pores in the microcosm b) Establish baseline data for both natural soils and artificial mixes, c) Culture bacteria to be added to artificial mixes d) Subject artificial and natural soils to MICP treatments and conduct macro and micro scale studies. It should be noted here that although bio-stimulation (use of indigenous bacteria) was the ultimate goal, the research team used exogenous bacteria in case of artificial mixes to control for initial bacterial populations.

Artificial Mixes and Natural Soils: Four artificial soil mixes and four natural soils with varying clay contents and plasticity characteristics were tested in this research. The artificial mixes were prepared using medium-to-coarse sand (obtained from a local quarry) and bentonite mineral powder (obtained from a commercial source). The sand/bentonite ratio was 95/5, 90/10, 85/15, and 80/20 for AM-1, AM-2, AM-3, and AM-4 mixes respectively. The four natural soils were collected from different locations in Idaho and Montana that had different plasticity characteristics. The natural soils are denoted as MS (Marsing, ID), GF (Great Falls, MT), DC (Dry Creek, MT) and BR (Bad Route, MT) indicating the location from which they were obtained. Both groups were subjected to a variety of geotechnical testing to establish the baseline data.

MICP Treatments: The treatment procedures varied between natural soils and artificial mixes as bacteria were added to artificial mixes, while indigenous bacteria were stimulated in natural soils. In artificial mixes, each soil specimen was compacted at optimum moisture content and maximum dry unit weight was injected with one treatment solution consisting of 250 mM of $CaCl_2$ and 333 mM of urea to achieve calcite precipitation. The treatment durations ranged between 6 to 8 hours for the four soils depending on the permeability of the soil. In the case of natural soils, two types of treatment solutions were used, enrichment solution containing sodium acetate (100 mM), urea (333 mM) and corn steep liquor (0.5 g/L) was used to stimulate indigenous bacteria and grow their numbers and followed up with cementation solutions containing sodium acetate (100 mM), urea (333 mM), corn steep liquor (0.5 g/L) and calcium chloride (250 mM) to achieve calcite precipitation. Treated samples were subjected to the same battery of tests the control soil samples were subjected.

Major Findings: Calcite precipitation was observed in both natural soils and artificial mixes after MICP treatments. The net calcite content ranged from 0.29 to 0.68% for **artificial soils** mixed with 10^6 cells/gm of bacteria after one treatment cycle. **Higher calcite content** was observed for soils with **lower clay contents**. In case of artificial soils mixed with 10^8 bacterial cells per gram of soil, the calcite content ranged from 0.78 to 0.99%. The net calcite precipitation in case of natural soils ranged from 0.39 to 1.56%; however, in case of **natural soils** the **calcite concentration increased** with **increase in clay content** for the four soils tested in this research. It is clear that there is contrasting behavior between natural and artificial soils, the presence of higher clay contents seems to be helping natural soils precipitate higher calcite while the same in case of artificial soils is resulting in lower calcite precipitation. This could be due to natural soils having higher bacterial concentrations in soils with higher clay contents. In artificial mixes, since the bacterial concentration remains constant across all samples the availability of pore space and access to treatment solutions is controlling calcite precipitation. The **impact of calcite precipitation** on **strength improvement** and **swell reduction** was significant. The

1-D swell strains which ranged from 0.31 to 8.84% before treatments were reduced to 0.06 to 0.47% for the artificial mixes. In the case of natural soils, the untreated swell strains were 1.15 to 17.9% and the treated swell strains ranged from 0.5 to 13.13%. The percentage improvement in UCS values after treatment ranged from 2 to 46% in case of artificial mixes while the same for natural soil ranged from 22 to 342%. It is clear that the treatment response is not consistent between natural soils and artificial mixes, these differences are attributed to the nature of bacteria present in the untreated soils, and this hypothesis was not tested in this research. However, it is evident from this research that it is possible to achieve calcite precipitation in natural soils using native soil bacteria and alter the behavior of the soils. There was considerable improvement in UCS and reduction in 1-D swell strain due to this bacterial activity.
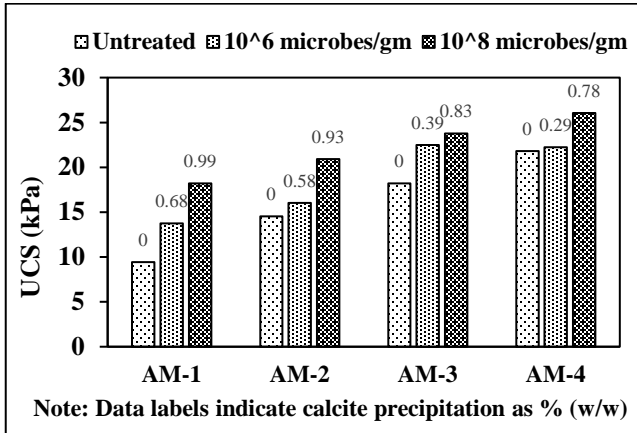
Figure 1 UCS values of artificial mixes before and after treatments
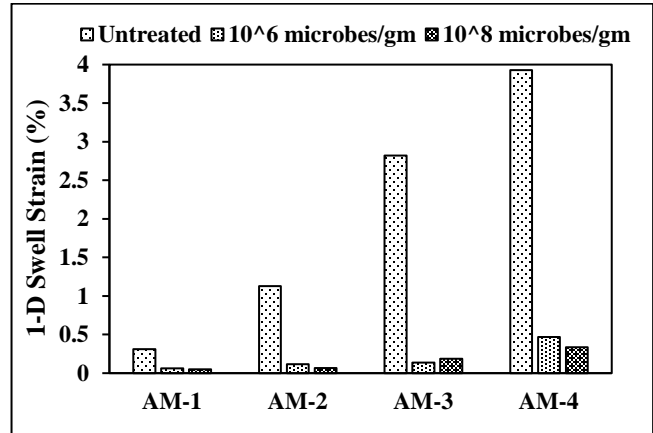
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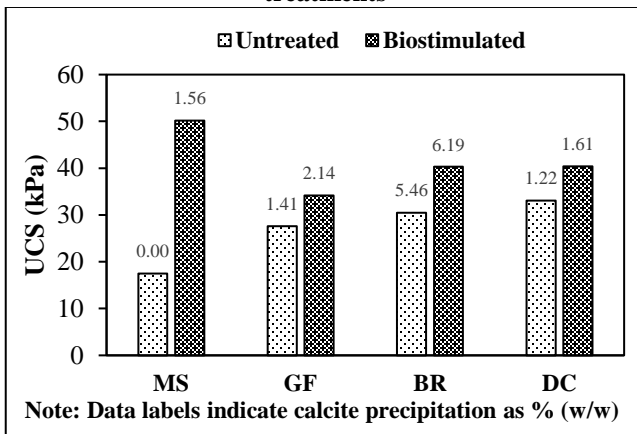
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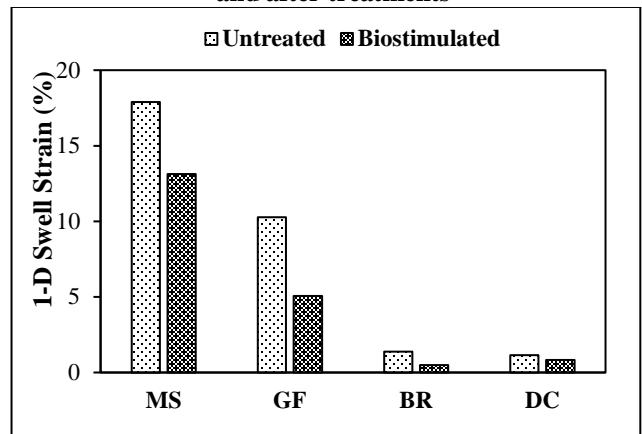
Figure 4 1-D swell strain values of natural soils before and after treatments

Preliminary field work was performed with an intent to evaluate the laboratory protocols in the field. For this purpose, a test site was selected with help from Idaho Transportation Department close to the location from which MS soil was obtained. Two different configurations were tested, one with a center-to-center distance of 16 in. (40.64 cm) between the injection points and the second with a center-to-center distance of 30 in (76.2 cm). The injections were performed using a packer system to ensure the borehole was sealed during injection and the treatment solutions do not escape from the annular space between the injection pipe and the borehole. Five rounds of treatments were performed at one-week intervals starting with one round of enrichment solutions followed by four rounds of cementation solutions. Each treatment consisted of pumping approximately 25 gallons (94.6 liters) of the treatment solution. The composition of treatment solutions was identical to that of laboratory experiments. The targeted depth of treatment was between 2 to 3 feet (0.6 to 0.9 m). Samples were collected from the bottom of borehole after each treatment to be tested for calcite content and free swell index. The results showed that calcite precipitation increased with treatments (up to 8% total) and the free swell index dropped from 114% to 29%. Based on these preliminary tests a field protocol was developed to be implemented in the field.

INTRODUCTION AND PROBLEM STATEMENT

Expansive soils, also known as swell-shrink soils, have been a problem for civil infrastructure such as roads and foundations since ancient times [1]. These soils swell and shrink with changes in moisture content, causing buildings and pavements to crack. The reason for this behavior is the presence of the heaving/swelling mineral known as Montmorillonite, which has an expanding lattice. This clay mineral expands when exposed to water. Some examples of expansive clays include high-plasticity index (high-PI) clays, over consolidated clays rich with Montmorillonite minerals, and shale. There are soils rich with these minerals in many places all over the world, especially in arid and semi-arid regions [2]. These soils are present in the majority of the states in the United States and cover about one-fifth of the land area of the country [3]. Estimated annual costs related to high plastic expansive soil damage have increased from $2.2 billion in 1973 to $15 billion in 2012 across the United States [4, 5]. Swell pressures contribute to heave movement and structural lifting in lateral and vertical directions, inducing at least some damage to structures for a majority of expansive soil sites. Problems associated with differential movements from swell are common for lightly loaded structures, including residential buildings and pavements constructed on expansive soils.

CURRENT STABILIZATION METHODS

The prevalence and annual damages caused by these soils have influenced researchers and practitioners to develop different stabilization measures to mitigate this issue. Use of chemical additives such as cement and lime to stabilize expansive soils has increased over the last few decades. The most widely used chemical additives are lime, Portland cement and fly ash [6]. Little and Nair [7] present a detailed discussion of different types of stabilizers, and their reaction mechanics during stabilization. For traditional and some nontraditional stabilizers like cement kiln dust and lime kiln dust, the stabilizers react with the soil in two steps, namely, cation exchange and pozzolanic reactions. During cation exchange, exchangeable cations present in the soil are replaced with calcium ions (Ca^{2+}) present in the stabilizer. Flocculation and agglomeration result, causing soil plasticity changes. Pozzolanic reactions occur at a slow rate due to increased soil pH and the release of reactive alumina and silica from the soil. These react with the calcium ions and form pozzolanic compounds such as calcium aluminate silicate hydrate, calcium aluminate hydrate, and calcium silicate hydrate [7]. Pozzolanic reactions are responsible for soil strength improvements, while the cation exchange reactions reduce soil volume change. Other chemical reactions such as carbonation (CaO reacts with atmospheric CO_2 and forms in $CaCO_3$) also occur at a very slow rate and further contribute to stabilized soil strength.

There are several methods of application to chemically stabilize expansive soils. These can be broadly classified as: (1) shallow stabilization, (2) deep soil mixing, and (3) injection. Engineers use shallow stabilization for roadway applications, excavating up to 1.5 m of soil and mixing it with chemicals like lime, cement, or fly ash [8]. They excavate expansive soil to the required depth, mix with the chemical or additive in wet or dry form, and re-compact the soil to suit specified density and moisture conditions. Deep soil mixing is another method where engineers mix soil in-situ with lime or cement to greater depths. This method was originally developed in Scandinavian countries to stabilize soft clays, but it has also been applied to expansive soils. Deep mixing is an important ground improvement technique for stabilizing soft and problematic soils, including expansive and stiff clays [8]. Another in-situ method that does not require excavation is the injection method [9] where engineers inject chemicals like lime slurry or potassium chloride into the soil.

Problems and Alternatives

Unfortunately, engineers have observed subgrade failure even after lime and cement stabilization, attributed to: (a) stabilizer loss over time, or (b) certain physicochemical soil properties that render the stabilizer ineffective (other soils with similar index properties may respond well to the same stabilizer). Current design guidelines often use PI values to determine stabilizer type and amount needed to enhance native soil performance. However, PI values can be misleading because two soils with different Atterberg limits can yield the same PI value [10–12]. Guidelines direct engineers to treat both soils with the same type and amount of stabilizer. However, this often leads to poor performance of one of the subgrades [13], as soils with similar PI values can have different mineralogy. Accordingly, the same stabilizer cannot effectively treat both soil types. Further, these chemical stabilizers have an adverse effect on the environment and economy. The production of cement and lime is a prime source of greenhouse gases [14]. UNEP [14] mentioned that

one ton of cement and lime production could release 1 and 1.2 ton of CO_2 into the environment, respectively. That report also concluded that annually, around 7-8% of overall CO_2 emissions result from cement production alone. It is evident that there is a distinct need to develop sustainable and eco-friendly solutions to mitigate the problems with high plastic clayey soils.

Researchers have investigated innovative alternative foundation techniques such as drilled and belled piers, granular pile-anchors [15, 16], and sand cushion technique for counteracting expansive soil problems. However, these methods can be very expensive - especially for constructing lightly loaded structures like pavements. Hence, it is important to identify both environmentally friendly and cost-effective methods, especially for lightly loaded structures. Using indigenous bacteria to stabilize expansive soils falls into this category. Bacteria are a dominant soil inhabitant with $\sim 10^6$-10^{12} bacterial cells per gram of soil and containing as many as 10^4 different genotypes [17]. Microbial metabolic activities often contribute to selective cementation by producing relatively insoluble organic and inorganic compounds both within and outside the cellular structure [18]. Microbial Induced Calcite Precipitation (MICP) is one such technique where the metabolic activity of certain types of bacteria present in the soil (e.g. *Sporosarcina Pasteurii*) results in the formation of inorganic compounds (such as $CaCO_3$) outside the cellular structure; these compounds can bind soil particles together. In MICP, one mole of urea, $(NH_2)_2CO$, is hydrolyzed into two moles of NH_4^+ and one mole of CO_3^{2-} by the microbial enzyme urease: $CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-}$. In the presence of calcium ions, CO_3^{2-} spontaneously precipitates as calcium carbonate: $Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3$. NH_4^+ generation increases local pH (~ 9.5), and importantly further increases the rate of calcium carbonate precipitation. Researchers have demonstrated the MICP method by combining the ureolytic bacterium, *Sporosarcina Pasteurii* (bio-augmentation), urea, and a source of calcium ions in laboratory and in the field [19–22]. Burbank et al. [23]demonstrated that biomineralized soils showed properties indicating that calcite precipitation increased soil resistance to seismic-induced liquefaction. In the study, researchers performed cyclic triaxial tests on the soil before and after treatments to determine the *cyclic stress ratio (CSR)* (defined as cyclic shear stress divided by initial vertical effective stress). Researchers compared results against cement-treated soil performance results from Clough et al. [24]. When Calcite precipitation ranged from 2.2–2.6%, the CSR increase to induce liquefaction was similar to results reported by Clough et al. [24] with 2% Portland cement.

CONCEPT AND INNOVATION

Researchers have shown that MICP was able to mitigate seismic-induced liquefaction, reduce permeability and compressibility, and increase shear strength [19, 25–29]. In this research, an attempt was made to expand the applicability of this technique to treat clays with varying plasticity and improve their engineering behavior. There are two application strategies for this technology; *bioaugmentation* and *biostimulation*. *Bioaugmentation* is a process where urease-producing exogenous bacteria are added to the soil, whereas *biostimulation* uses indigenous bacteria already present in the soil to precipitate calcite.

Given their electronegative nature to hold onto water, clays have a natural tendency to incubate microorganisms [30]. Clayey soils also contain larger populations of indigenous bacteria as compared to non-clayey soils, which researchers can stimulate to precipitate calcite. Hence, it would be advantageous to use these naturally occurring microorganisms (*Sporosarcina Pasteurii*) in the soil to stabilize expansive clays. In addition, Burbank et al. [23] demonstrated that it was possible to stimulate indigenous microorganisms (bio-stimulation) to precipitate calcite. Doing so might overcome challenges that develop when augmenting soil with exogenous bacteria such as bacteria clogging near the injection inlet leading to uneven calcite precipitation [27]. Further, other researchers have reported that non-indigenous microorganisms introduced into natural soils rapidly decline in numbers and rarely reproduce [31]. Therefore, bio-stimulation of indigenous bacteria generally is more practical and cost-effective as compared to bio-augmentation as there is no need to cultivate quantities of fresh bacterial culture on site. Past studies [27, 32–34] showed that biostimulation is a superior alternative as the bacteria are already accustomed to the soil environment compared to augmented bacteria. Hence, this research investigates the applicability of biostimulation to clayey soils in minimizing their swelling potential and improving the strength.

In addition, this method is capable of treating expansive soils in situ without the need for reconstruction which involve excavation and mixing as required in case of most chemical stabilization procedures. This method would offer significantly superior environmental performance. This solution meets or lowers the costs of expansive soil stabilization and can be easily applied to soil with existing construction equipment used for treating expansive soils. By simply injecting the treatment solutions to the required depth we will avoid costly reconstruction using chemically stabilized subgrades or other design alternatives used to stabilize expansive soils.

IDEA PRODUCT

This project aims at developing a new stabilization alternative for mitigating pavement distresses due to underlying expansive clays. The payoff of this research project is considerable, as federal and state governments can realize significant cost savings and positive environmental impacts in both new pavement construction and repairs of distressed pavements in regions with expansive soils. The proposed treatment methodology will provide a sustainable alternative for expansive soil treatments. Tangible *benefits* include:

- Extended Life and Reduced Cost—Fewer incidences of cracking would result in lower infrastructure maintenance costs. In addition, you could apply the method without major reconstruction to existing pavements showing subgrade heaving distress.

- Improved Health and Environment—Federal and state agencies would gain a sustainable treatment alternative for expansive soil problems beneath transportation infrastructure.

- Wide Use—Outcomes may recommend the treatment method for other problem soils like soft clays and collapsible soils, thus avoiding sinkhole damage if detected early.

Potential users of this developed treatment method include state DOTs, federal agencies, and construction industry personnel constructing pavement in areas with abundant expansive soils.

INVESTIGATIVE APPROACH

The primary goal of this project is to apply MICP technique to mitigate swelling in expansive subgrade. To achieve this goal, research team answered the following research questions:

1) Can calcite precipitation occur using indigenous bacteria from different sources?
2) What is the role of clay content on MICP effectiveness?
3) Do bacterial populations at the start of the treatment have an impact on the treatment?
4) What is the impact of plasticity characteristics on MICP effectiveness?
5) Can the laboratory performance be repeated in the field?

The study tasks were accomplished in two stages; stage 1 involved laboratory work while stage 2 focused on preliminary fieldwork. The objectives of the laboratory work were to study the role of soil type, clay content and bacterial populations on treatment effectiveness and develop a protocol for field implementation. The field work was intended to explore the protocol's effectiveness in the field and help develop a protocol for future field applications. The laboratory work consisted of four major tasks:

a) Develop Treatment Solution Delivery System (TSDS)
b) Establish baseline data for both natural soils and artificial mixes,
c) Culture bacteria to be added to artificial mixes
d) Treat soils and asses performance

DEVELOPMENT OF TSDS

TSDS was designed and developed to construct a "mini soil microcosm" and deliver required treatment solutions to all pores in the microcosm. This device consists of a chamber made from schedule 80 clear PVC tube that houses soil samples that are 2.8 in. (71 mm) diameter and 5.6 in. (142 mm) height. This device is capable of delivering treatment solutions at injection pressures as high as 20 psi (137 kPa). This chamber is sandwiched between two 5 cm thick PVC plates that are held together using threaded rods and screw caps (see Figure 1). Inside the PVC chamber, the soil sample rests on a bottom pedestal and is covered using a top cap. Latex membrane was used to wrap around the soil sample as well as the pedestal and top cap to protect it from unwanted surface erosion. Both top cap and bottom pedestal have grooves to accommodate O-rings which ensure the latex membrane is tightly in place and also restrict water from percolating from the sides. The top cap and the bottom pedestal contain tiny holes to allow the flow of treatment solutions through them into and out of the soil sample. The bottom pedestal was glued to the base plate and included holes with a puddle arrangement to collect effluent from the samples. The top and bottom PVC plates are also arranged with pressure valves

to control the flow of treatment solutions into and out of the PVC chamber. The bottom valve is connected using PVC tubing to a pressure regulated reservoir hosting the treatment solutions. The top valve is used to release any excess pressure inside the chamber.

After the chamber is pressurized, the treatment solution flows through the soil sample as that is the only path of least resistance for the fluid to escape. The escaped treatment solution after traveling through the soil sample is collected in an effluent collector. This device is capable of driving treatment solutions through the soil sample at pressures ranging from 2 psi to 20 psi (14 kPa to 137 kPa). Four separate chambers were prepared for this research at Boise State's engineering workshop. All the chambers were thoroughly checked for leaks and safety tested at a pressure of 30 psi (206 kPa).
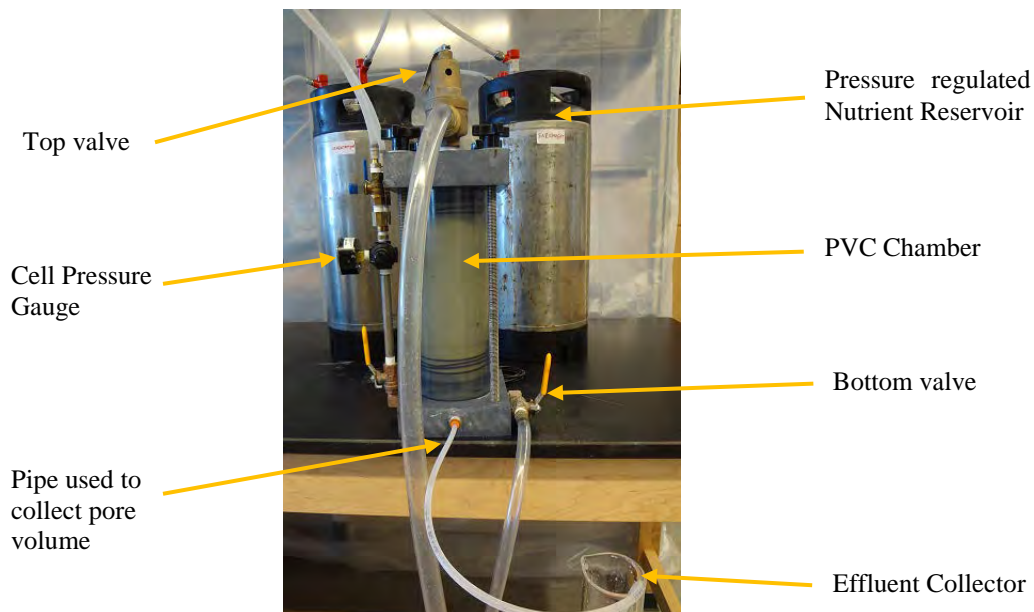
Figure 5: Photograph showing the various components of TSDS

ESTABLISH BASELINE DATA

Four artificial soil mixes and four natural soils with varying clay contents and plasticity characteristics were tested in this research. The following tests were performed on all soil samples to establish the baseline data. The American Society for Testing and Materials (ASTM) test protocol corresponding to the test is listed along with its American Association of State Highway and Transportation Officials (AASHTO) counterpart.

1. Specific Gravity Test- ASTM D854 and AASHTO T100
2. Sieve analysis and hydrometer Test-ASTM D422 and AASHTO T88
3. Atterberg Limit Test- ASTM D4318 and AASHTO T89 & T90
4. Standard Proctor Compaction Test- ASTM D698 and AASHTO T99
5. Unconfined Compression Strength Test- ASTM D2166 and AASHTO T208
6. 1-D swell test: ASTM D4546 (Method A) and AASHTO T216
7. Permeability- ASTM D2434 and AASHTO T 215
8. Calcium Carbonate Test-ASTM D4373

Artificial Mixes

Since one of the objectives of this project was to study the role of clay content, four artificial soil mixes were prepared with varying clay content. These mixes were prepared using medium-to-coarse sand (obtained from a local quarry) and bentonite mineral powder (obtained from a commercial source). Another objective of this study was to study how the initial population of bacteria impacts the treatment. To maintain control on the initial concentration of the bacteria the

research team chose to prepare artificial mixes and add predetermined amounts of bacteria to the mixes. The percentage ratios of sand and bentonite for each of the soil mixes along with soil notation are presented in Table 1.

Table 1 Composition of artificial soil mixes

Soil Notation	% Sand	% Clay (Bentonite)
AM-1	95	5
AM-2	90	10
AM-3	85	15
AM-4	80	20
AM-5	50	50

Table 2 presents the data obtained from the tests performed on the four artificial mixes. Test results indicate that specific gravity decreases with increase in clay content due to the lower unit weight of bentonite. Standard proctor tests yielded maximum dry density values around 16.6 kN/m^3 for AM-1 thru AM-4 soils. The values of optimum moisture content ranged from 15.4 to 20.0%, increasing with the clay (bentonite) content. The unconfined compressive strength (UCS) ranged from 62.0 to 178.5 kPa and it increases with the increase in clay content. This is typical for clayey soils as the presence of clayey particles improves the cohesion between particles and results in higher unconfined strength. It should be noted here that, due to confinement, high sand content would result in higher shear strength.

1-D swell test data showed that the swell strains ranged from 0.3 to 26.3% while the swell pressures ranged from 6 to 155 kPa for the four artificial mixes. The swell pressures and the swell strains increased with increase in bentonite content. It should be noted here that the time taken for 1-D swell tests ranged from two weeks to four weeks as the clay content increased. This could be attributed to the decrease in pore space as the clay content increased, and hence it took longer for water to percolate through the available pore space. The permeability tests performed on these soils corroborated this further as the permeability data ranged from 1.95 x 10^{-4} to 9.84 x 10^{-6} cm/s from AM-1 through AM-4 soil samples.

Table 2 Summary of the baseline data collected for the four soil mixes

Soil Notation	Specific Gravity	Liquid Limit	Plasticity Index	MDUW (kN/m^3)	OMC (%)	UCS (kPa)	1-D Swell strain (%)	Swell Pressure (kPa)	Permeability (cm/s)
AM-1	2.63	NP	NP	16.6	15.4	62.0	0.31	6	1.95E-04
AM-2	2.65	41	NP	16.6	17.5	139.5	1.04	27	9.85E-05
AM-3	2.59	53	NP	16.8	18.1	178.5	2.82	48	1.86E-05
AM-4	2.61	64	NP	16.6	17.2	137.2	8.84	155	9.84E-06

Natural soils

One of the main objectives of this study was to evaluate if calcite precipitation would occur using indigenous bacteria from different sources, natural soil samples from different sites in Idaho and Montana that were causing swelling related pavement distresses were collected. The natural soils were also studied to understand the effect of varying plasticity characteristics and microbial communities on MICP effectiveness. The natural soils are denoted as MS (Marsing, ID), GF (Great Falls, MT), DC (Dry Creek, MT) and BR (Bad Route, MT) indicating the location from which they were obtained. These soils were subjected to all the tests artificial mixes were subjected and the results are presented in TABLE 3. The MS and GF soils were classified as high plastic soils (CH), and DC and BR soils were classified as low plastic soils (CL) according to USCS. As per the AASHTO classification system, MS and GF are classified as A-7, while DC and BR are classified as A-6 and A-7-6 respectively. Test results show that specific gravity values ranged from 2.6 to 2.9 for the four natural soils tested in this research. Standard proctor tests yielded maximum dry unit weight (MDUW) values between 11.0 and 17.2 kN/m^3, while the optimum moisture contents ranged from 16.9 to 36.7%. The unconfined compressive strength (UCS) ranged from 156.4 to 370.2 kPa. The highest UCS was recorded for BR soil while the lowest was observed for MS soil. It should be noted here that the research team did not conduct any tests to evaluate the bacterial species in the soils, as the goal here was to establish if calcite precipitation was possible in any natural clayey soil.

Table 3 Summary of the baseline data collected for the five soil mixes

Soil Notation	Specific Gravity	Liquid Limit	Plasticity Index	MDUW (kN/m^3)	OMC (%)	UCS (kPa)	1-D Swell strain (%)	Swell Pressure (kPa)	Permeability (cm/s)
MS	2.67	111	71	11.0	32.6	156.4	17.9	287	1.15E-07
GF	2.60	103	62	12.8	36.7	159.3	10.3	210	1.38E-07
DC	2.75	37	17	17.2	16.9	166.4	1.2	40	1.13E-04
BR	2.9	42	16	16.6	19.8	370.2	1.4	50	9.13E-06

CULTURE BACTERIA

As explained in the Artificial Mixes section of this report, the initial bacterial populations were controlled by augmenting the artificial mixes with known amounts of bacteria. For this purpose, model bacterium *Sporosarcina Pasteurii* (ATCC 11859) was chosen due to its ability to hydrolyze urea. It is a soil-borne microbe which is classified as non-toxigenic and non-pathogenic. This bacterium was obtained from a commercial source and a growth curve was established to determine the growth rate and setup a protocol to grow the bacteria to a required population level. The growth media required to grow the bacteria contained Lysogeny Broth (LB), 40% urea along with tryptone, yeast extract and NaCl. LB is a rich medium to grow an immense number while the urea acts as the nitrogen source. FIGURE 6 presents a sample of LB agar plate with growth media with and without bacterial growth.
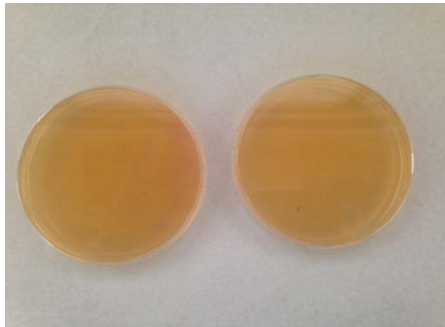
(a) Petri dishes containing LB agar without bacteria   **(b) Petri dishes containing LB agar with bacteria**

Figure 6: Photographs depicting the growth of bacteria on agar medium

The increase in cell size and cell mass during the development of microorganisms is identified in a growth curve. The growth of these organisms depends on physical and nutritional factors. The physical factors are pH, temperature, osmotic pressure, hydrostatic pressure, and moisture content of the media while the nutritional factors include, the amount of carbon, nitrogen, sulphur, phosphorus and other trace elements available in the media. To study the *S. Pasteurii* growth population, the cells of the bacterium were inoculated into the sterilized broth (LB broth and 40% urea solution in a ratio of 19:1) and incubated in an incubating shaker. The bacterial growth was studied by plotting the cell growth (absorbance) versus the incubation time or log of cell number versus time. The curve thus obtained was known as a standard growth curve. The increase in the cell mass of the organism or cell growth was determined by using a spectrophotometer. This device was used to determine the turbidity or optical density which is the measurement of the amount of light absorbed by a bacterial suspension. The degree of turbidity in the broth culture is directly co-related to the microbe population present. In this research study, the growth curve was established to determine the cell number of *S. Pasteurii* at a definite optical density.

TREAT SOILS AND ASSESS MICP PERFORMANCE

The treatment procedures varied between natural and artificial soil samples as bacteria were added to artificial soils while indigenous bacteria were stimulated in natural soils. As a result, the treatment solutions, protocols, and durations varied between the two groups of soils. This section details treatment procedures for the two groups of soils in this research.

Treatment process for artificial soils

For artificial mixes, the treatment process started with the addition of known amounts of bacterial populations (10^6 and 10^8 microbes/gm) followed by sample compaction at MDUW and OMC. It should be noted here that the soil samples were autoclaved before augmenting with bacteria to ensure that there is no contamination of bacterial species. The compacted samples were then placed in the TSDS and the chambers were filled with a treatment solution consisting of 250 mM of $CaCl_2$ and 333 mM of urea. The samples were then injected with one pore volume of the treatment solution. Each treatment lasted between 6 to 8 hours depending on the permeability of the soil sample. Figure 7 presents a pictorial representation of the treatment process for artificial soils.

1. Taking appropriate amount of solution having LB broth and Urea

2. By using inoculating loop, one colony of bacteria was collected and put in the flask

3. Then, the flasks were kept inside the shaker at 37°C and 200 rpm

6. Optical Density of solution was also determined for all serial dilution

5. Serial dilution work is shown in this figure

4. Serial dilution was performed after keeping the flasks overnight inside the shaker

7. Platings were used to count the bacterial population (CFU/mL)

8. Desired amount of concentration (106 microbes/gm) was taken to mix with the soil for treatment purpose

9. Desired amount of concentration (10^6 microbes/gm) was mixing with the soil

12. Soil sample was injected using treatment solution

11. Prepared sample was placed inside the TSDS

10. Soil with bacteria was put inside a tube and sample was prepared using static compactor

Figure 7: Pictorial representation of the treatment process for artificial soils

Treatment process for natural soils

In case of natural soils, soil samples obtained from the field were compacted to OMC and MDUW without the addition of bacteria as those samples contained indigenous bacteria. These bacteria were stimulated to assist with calcification process using treatment solutions (TS-N1 and TS-N2). The enrichment solution (TS-N1) contained sodium acetate (100

mM), urea (333 mM) and corn steep liquor (0.5 g/L). The purpose of using enrichment solution was to stimulate the growth of bacteria where acetate acted as a carbon source and urea as a nitrogen source. The second solution, cementation solution (TS-N2), consisted of sodium acetate (100 mM), urea (333 mM), corn steep liquor (0.5 g/L) and calcium chloride (250 mM). The calcium present in the cementation solution precipitates calcium carbonate in the soil pores after reaction with carbonates produced by bacterial activity.

The treatment of natural soils started by placing compacted natural soil samples in the TSDS device. After placing the soil sample in the TSDS, the PVC chamber were first filled with TS-N1, and the samples were injected with one pore volume, and the effluent was monitored for pH changes. When the pH raised above 8 and it achieved the desired pore volume, the chamber was emptied and refilled with TS-N2. After the addition of TS-N2 the calcite precipitation begins which reduces the pH. This treatment cycle was continued until one pore volume of the solution is collected as effluent and also when the pH has risen beyond 8 indicating that the sample is ready for the second round of treatment. In this stage of testing only one round of treatments was carried out to compare with artificial soils and also due to the duration of each treatment cycle. Each treatment duration ranged between 6-10 weeks depending on the type of natural soil and its permeability. Figure 8 presents a pictorial representation of the treatment process for natural soils.

1. Preparation of natural soil samples at MDUW & OMC

2. Preparing 3''x 6'' soil samples using Static Compactor

3. Extracted soil samples from Static Compactor

6. Representation of natural soil samples after treatment cycles

5. Enrichment and Cementation solutions were injected through natural soil samples

4. Prepared natural soil sample was placed inside the TSDS
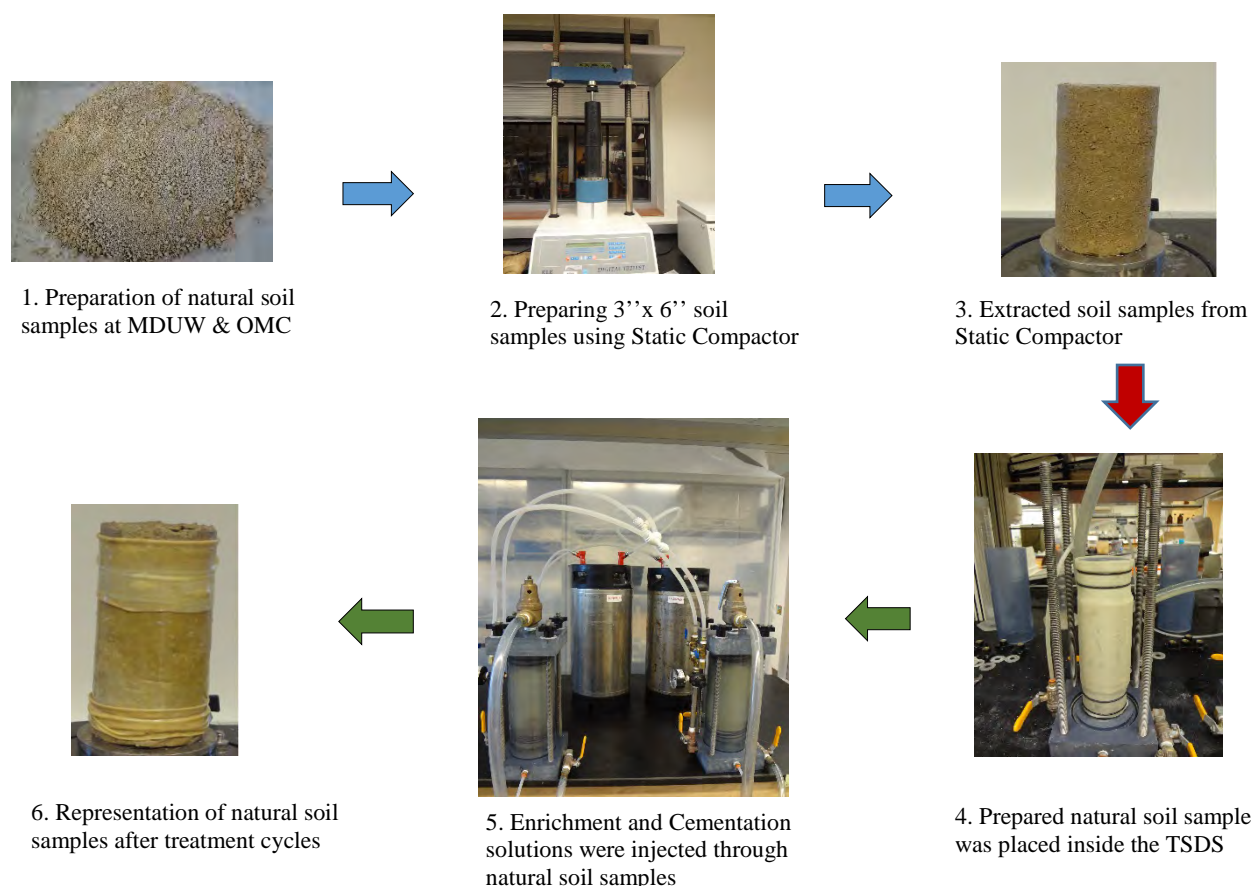
Figure 8: Pictorial representation of the treatment process for natural soil

ASSESSMENT OF MICP PERFORMANCE

As a measure of MICP treatment performance two types of studies were executed; (a) microscale studies and (b) macroscale studies. Under microscale studies, the soil samples were tested for calcite precipitation. The quantitative measure of calcite was performed using Rapid Carbonate Analyzer as per ASTM D4373. After the completion of the treatment phase, the biostimulated soil samples were oven dried and crushed into smaller particles passing #40 sieve to ensure that HCl passed into the inner structure of the soil sample. The precipitated carbonate in this soil sample was

quantified using a small portable device known as Rapid Carbonate Analyzer. This device is a rapid measurement of carbonate present in a soil specimen. This device consisted of a reaction cylinder, a cup filled with hydrochloric acid (HCl) and a pressure gauge. The reaction cylinder was closed tightly, and the small cup was tilted to create reaction between the HCl and soil samples. As a result, carbon di-oxide was released, and it was recorded using a pressure gauge. The collected pressure readings were then inserted into a calibration curve to obtain the amount of calcium carbonate. This calibration curve was prepared by using different amounts of predetermined reagent grade calcium carbonate. The amount of calcium carbonate was determined as a percentage of the dry weight of soil.

Under macroscale studies, the impact of calcite precipitation on engineering performance of the soil sample was tested using UCS (AASHTO T208) and 1-D swell tests (AASHTO T 216). In addition to these tests both Atterberg limits (AASHTO T89 & T90) and standard Proctor compaction tests (AASHTO T99) were performed to study the effect of treatment on both artificial mixes and natural soils. The following sections present the results obtained from each of these studies for artificial mixes and natural soils tested in this research. It should be noted here that all data points presented here are an average of three tests on identical soil samples in identical experimental conditions.

Microscale studies on artificial soils

There are four factors which influence the precipitation of calcite: (1) the calcium concentration, (2) the concentration of dissolved inorganic carbon (DIC), (3) the pH, (4) the availability of nucleation sites [35]. An alkaline environment is formed during the calcite production of urease producing bacteria [36]. Stocks-Fisher et al. [37] showed that pH increased from 6.0 to 10.0 in the active period of calcite precipitation but the enzyme activity was optimal at a pH around 8.0. So, in this research, a pH of 8.0 or above was targeted as an indicator of calcite precipitation.

The $CaCO_3$ quantification tests conducted on all treated and untreated soils are presented in Table 4 and Figure 9. It can be observed from this table that the %$CaCO_3$ precipitated after treatment reduced with the clay content present. This could be due to the reduction in the pore space available for the calcite precipitation. It can also be observed from Figure 9 that $CaCO_3$ precipitation increased with increase in bacterial content. The availability of larger bacterial populations is facilitating larger amounts of calcite to precipitate as higher amounts of urea is being hydrolyzed, resulting in larger amounts of carbonate presence in the soil matrix.

Table 4: Calcite content recorded before and after treatments in Artificial Soil Mixes

Soil Notation	Liquid Limit (%)	$CaCO_3$ (w/w)		
		Before Treatment	After Treatment (10^6 cells/gm)	After Treatment (10^8 cells/gm)
AM-1	NP	0	0.682	0.99
AM-2	41	0	0.584	0.93
AM-3	53	0	0.389	0.83
AM-4	64	0	0.292	0.78
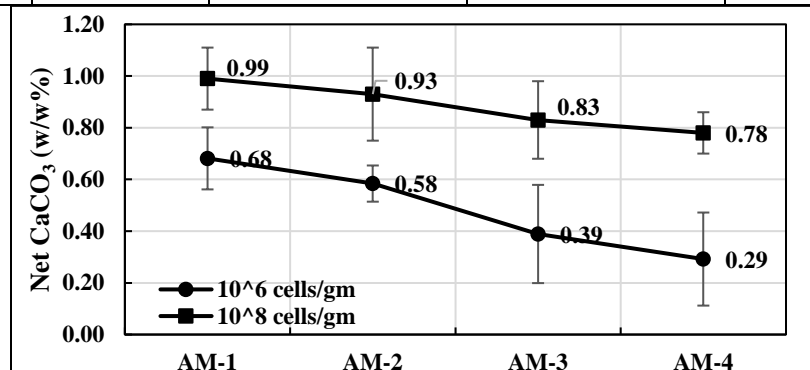
Figure 9 Calcite precipitation data for different artificial mixes after treatments

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Microscale studies on natural soils

In case of natural soils pH data was monitored during both enrichment and cementation phases of the treatment and it was noted that the pH varied from 8.0 to 9.2 over both phases. As in case of artificial soils the presence of calcite after treatments was quantified using the calcium carbonate test. This data is presented in Table 5. In case of natural soils, the untreated soils contained calcium carbonate. The amount of calcium carbonate for untreated MS soil was nearly zero, but the same for GF, BR and DC soils was considerable (See Table 5). The untreated GF soils had 1.41% (w/w) of calcium carbonate, but the biostimulated GF had 2.14% (w/w) of calcium carbonate (Table 5). The percentage increase in the amount of calcium carbonate after treatment was 52%, 13% and 32% for GF, BR and DC soils, respectively. It can be observed from Table 5 that MS soil had highest calcite precipitation (1.56%) among all the natural soils and the artificial mixes. Despite the fact that the natural soils had higher liquid limits and clay contents these soils precipitated larger quantities of calcite. This behavior is attributed to the indigenous nature of the bacteria in natural soils, where the bacteria are already accustomed to soil environment and were able to precipitate larger amounts of calcite, while in case of artificial soils the augmented bacteria need to first accustom to the home environment and hence were not able to perform on par with the natural soil bacteria.

Figure 10 presents the variation of calcite precipitation with liquid limit of the soil. It can be observed from the plot that the amount of calcite precipitated after treatment increased with increase in liquid limit. GF soil which had a liquid limit of 111, precipitated 1.56% of calcite after treatment. This indicates that the soils with higher capacity to hold water (high swelling capacity) can precipitate higher amounts of calcite. Higher liquid limits generally indicate higher swell potential and larger clay sized particles. Based on this observation it can be said that MICP is favored in soils with higher swelling potentials which are usually considered very problematic.

Table 5: Calcite content recorded before and after treatments in Natural Soils

Soil Notation	Liquid Limit (%)	CaCO$_3$ (w/w)		
		Before Treatment	After Treatment	Difference
MS	111	0.002	1.56	1.56
GF	103	1.413	2.144	0.73
DC	37	1.218	1.608	0.39
BR	42	5.459	6.19	0.73
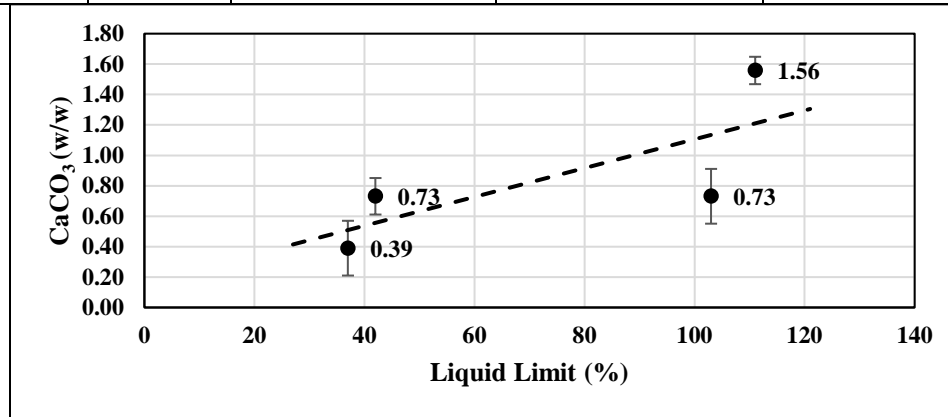
Figure 10 Calcite precipitation data for different natural soils after treatments

Macroscale studies on artificial soils

Compaction characteristics using standard effort were determined before and after treatment for all four artificial soil. The results from treated artificial soils indicated that the MDUW ranged between 17.8 to 18.5 kN/m^3 while the OMC varied from 11.3 to 13.9%. Figure 11 compares these values before and after treatments. It can be observed from these figures that all four soils showed increase in MDUW and decrease in OMC after MICP treatments. These changes are

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attributed to calcite precipitation, as calcite has higher specific gravity which increases overall unit weight of the soil. The decrease in OMC shows that the precipitation of calcite has changed the abundance of charged particles in the soil body which results in less OMC than the untreated soils (Figure 11-b).

(a) (b)
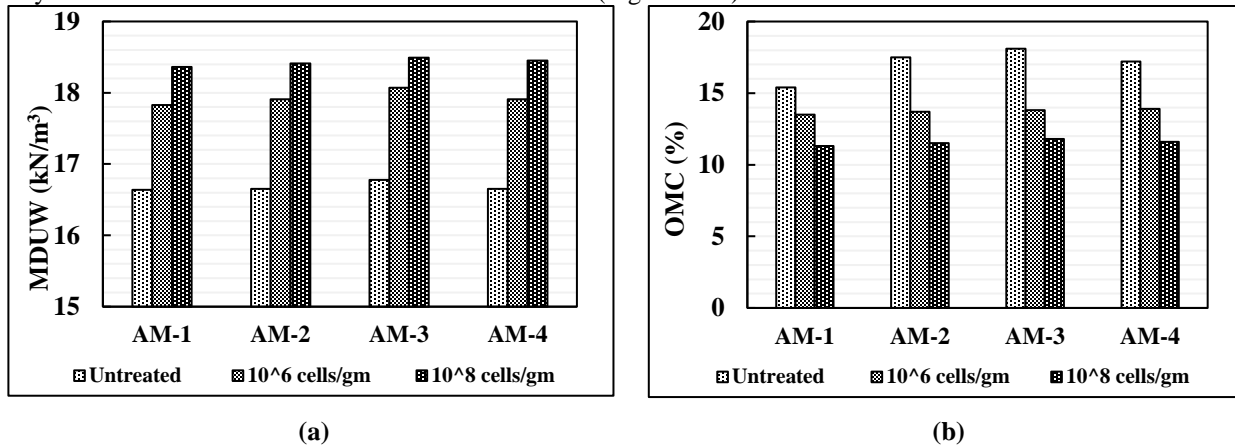
Figure 11: Variation of (a) Maximum Dry Unit Weight (MDUW) and (b) Optimum Moisture Content of treated and untreated Artificial Mixes

Unconfined Compression test

Figure 12presents a comparison of UCS data before and after treatments for artificial soils. It should be noted here that since the samples after treatments are at (or close to) 100% saturation, control soil samples were also tested at the same moisture content to be able to compare the results at the similar moisture contents. It could be observed from the Figure 12 that there is improvement due to treatments. The percentage change in UCS values after treating with 10^6 cells/gm bacteria populations ranged from 2 to 46% for the four artificial mixes. The highest improvement was observed for soil with lowest clay content (AM-1) while the lowest was observed for that with highest clay content (AM-2). A similar observation was made for treatments involving 10^8 cells/gm concentration where the percentage change in UCS ranged between 19 and 93%. The improvements corroborate with the calcite precipitation amounts noted in the earlier section. As expected, higher calcite precipitation resulted in higher UCS values.
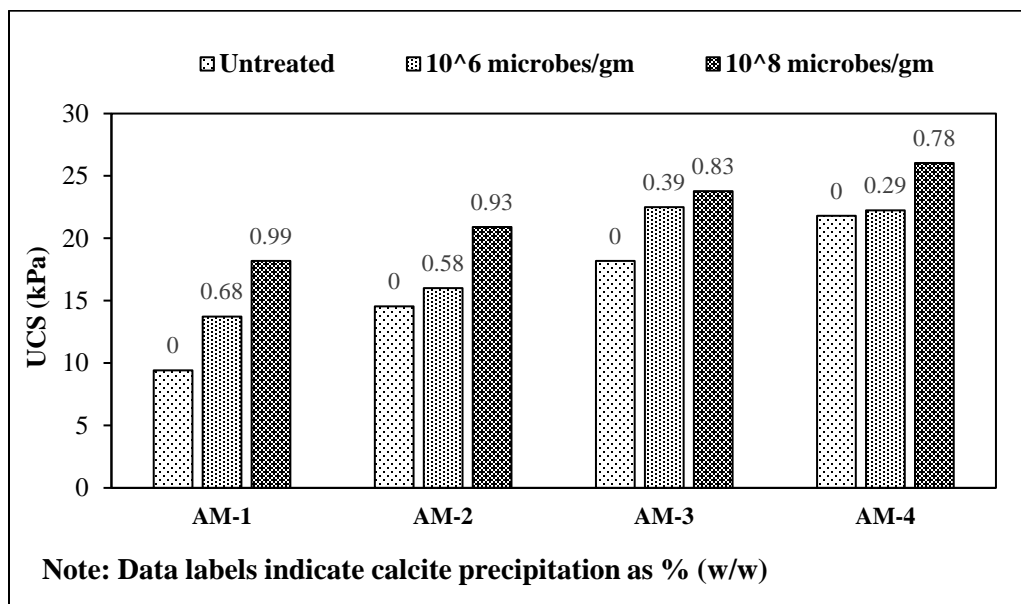
Figure 12: Variation of Unconfined Compression Strength of treated and untreated Artificial Soils Mix

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1-D Swell test

Data obtained from 1-D swell tests before and after treatments for the artificial soil mixes is presented in Figure 13. Similar to the UCS test data, swell data showed great promise for MICP treatment applications in expansive soils. It can be observed from Figure 13 that the 1-D swell strains and the swell pressures were considerably reduced after MICP treatments. This reduction in swell strain can be attributed to the precipitation of calcite which is not only changing the composition of the soil but also binding particles together thereby minimizing the swelling in the soil.

Figure 13: Variation of (a) 1-D Swell Strain and (b) Swell Pressure of untreated and treated Artificial Mixes

Macroscale studies on natural soils

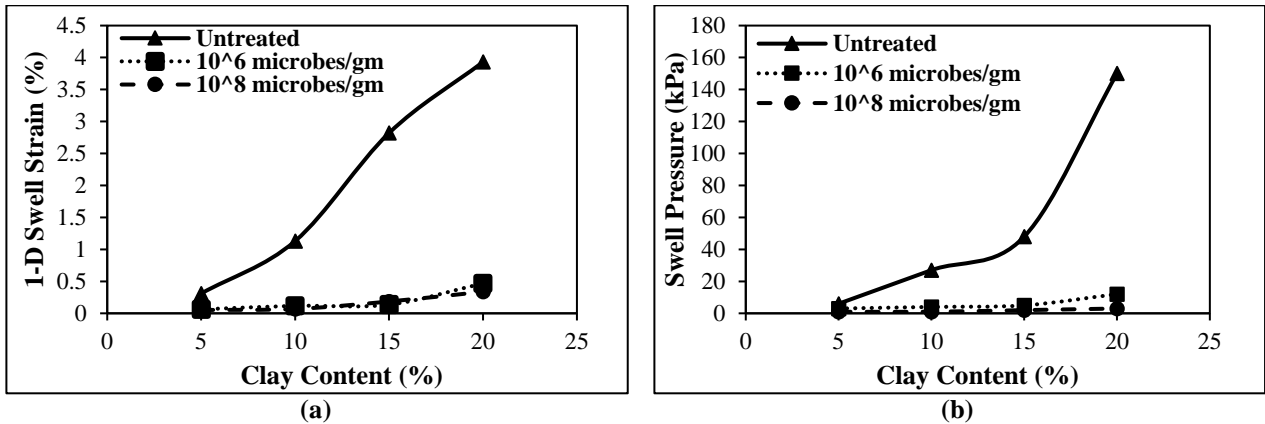This section presents the test results from the evaluation studies performed on natural soils along with a discussion on results explaining the reasons behind the changes due to treatments.

Unconfined compression strength

UCS tests were used to study the variations in undrained shear strength characteristics of treated and untreated soils. Two types of UCS tests were conducted, UCS- α and UCS- β. UCS-α was determined on samples compacted at Optimum Moisture Content (OMC) and Maximum Dry Unit Weight (MDUW) while the UCS-β was determined on samples that are close to saturation immediately after the treatments. The moisture content used for testing UCS-β of biostimulated soils were used to determine the UCS-β of untreated samples to compare the UCS values at similar conditions. In the case of UCS-α samples, the treated samples were oven dried and re-compacted at OMC and MDUW to compare with untreated samples at similar conditions. The results of UCS-α and UCS-β for natural soils are presented in Figure 14. In case of natural soils, the UCS-α increased by 66%, 10% and 51% (Figure 14a) and the UCS-β increased by 24%, 32% and 22% for GF, BR, and DC soils, respectively (Figure 14b). The reason for the appreciable increase in strength of is due to the precipitation of calcium carbonate (calcite) that binds the soil particles together and improves their strength. In the case of MS soil, the UCS-α value reduced by 6% while the UCS-β increased by over 300%. While the reduction in UCS-α is well within the coefficient of variation of this test (~20%) and hence can be considered as no change in UCS value, the increase of 300% in UCS-β is noteworthy. One of the reasons for this distinct behavior compared to the rest of the soils is the presence of high amounts of clay fraction in these soils (70%) which could have resulted in high bacterial populations which yielded larger calcite precipitation which increased the UCS-β considerably. When this treated soil was recompacted, the bonds may have been broken and resulted in a loss of that strength, and the UCS-α value returned to untreated levels. One important observation here is that all soils regardless of their bacterial origins and plasticity characteristics, showed improvement after treatments although no clear trends were observed with respect to plasticity or clay contents.
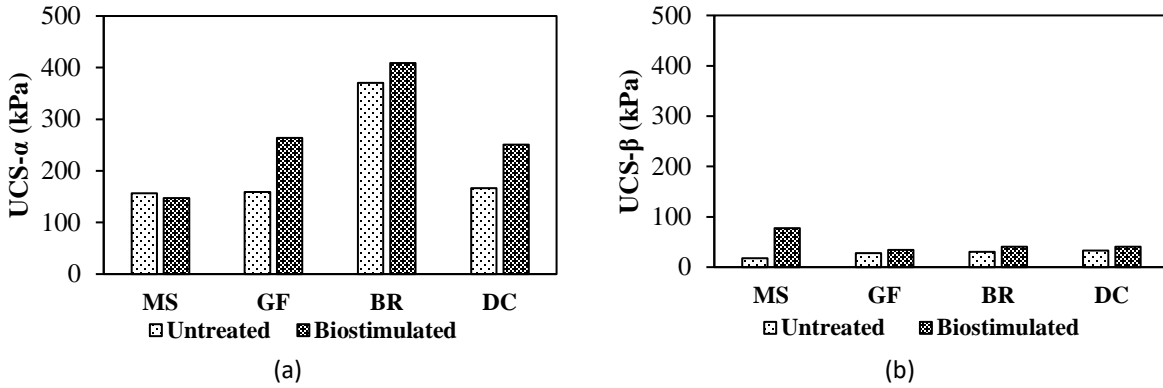
Figure 14 Variation of UCS characteristics of treated and biostimulated natural soils
(a) UCS-α data, (b) UCS-β data

1-D Swell test

The 1-D swell tests were performed on untreated and biostimulated soils to study the effect of MICP treatments on 1-D swell strain and swell pressure of these soils. All untreated and biostimulated samples were dried and remolded at MDUW and OMC and placed inside the consolidometer to determine the 1-D swell strain and swell pressures. The results of 1-D swell strain and swell pressure for MS, GF, BR, and DC soils are presented in Figure 15. The untreated MS soil showed high swell strain and swell pressure than the other natural soils. Having a high plasticity index and the presence of swelling mineral, e.g., montmorillonite could be the reason for this high swelling. The 1-D swell strain and swell pressure decreased for biostimulated natural soils. For MS, GF, BR and DC soils, the 1-D Swell strain decreased by 27%, 51%, 28% and 64% respectively (Figure 15a), while the swell pressure decreased by 38%, 36%, 18% and 70% respectively (Figure 15b). The highest reduction was observed for BR soil.

The formation of calcium carbonate might have bonded the particles, and the biofilm may have created a barrier between the charged clay particles and water molecules leading to soils with less swelling potential. While the percentage decrease compared to untreated soils was appreciable, the actual decrease in swelling strain was not satisfactory for some of the soils. The 1-D swell strain of MS soils decreased from 17.9 % to 13.13 %, similarly for GF soils, it decreased from 10.27 to 5.06 %. In the case of DC soil, it decreased from 1.15 to 0.83 % for DC and from 1.38 to 0.5 % for BR soils. Similar observations were made in case of artificial soils. The 1-D swell strains after treatment were acceptable for DC and BR soils as their values were below 1% to 2% but that for MS and GF soils was not acceptable. This does not mean that MICP will not work for these soils, but rather, these soils may need additional treatment cycles to bring the swelling strain below threshold levels.
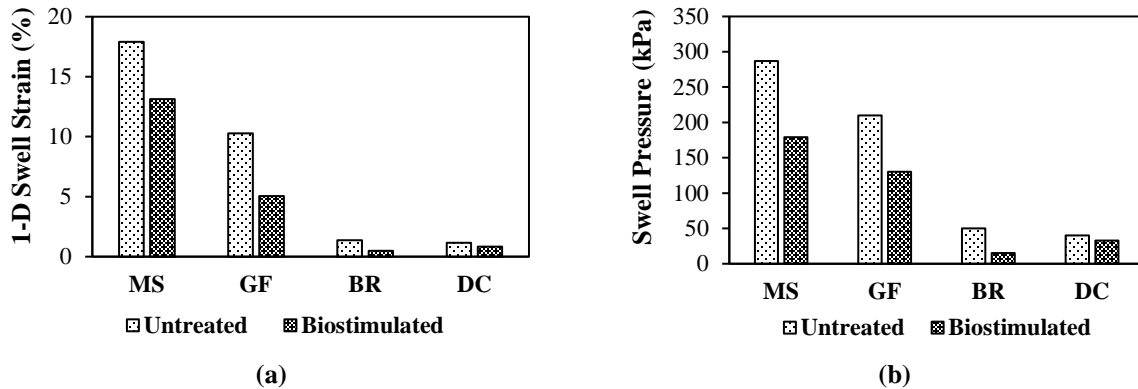
Figure 15 Variation of swelling characteristics of untreated and biostimulated natural soils
(a) 1-D Swell strain, (b) Swell Pressure

FILED TRIALS AND RESULTS

The main objective of field tests was to understand the feasibility of achieving calcite precipitation in a natural environment, which can be subject to highly variable temperature and moisture conditions. The field work was carried out to study the suitability of MICP in stabilizing expansive soils at a certain depth below the ground surface with an intent of treating existing pavement structures that are underlain by expansive soils. This was studied by injecting enrichment and cementation solutions into the soil and monitoring the soil pH and calcite concentration in the area of treatment. The swelling potential of the treated soil (from the different depths) was also measured to understand the effect of field treatments. The following sections detail the procedures followed along with the results and discussion.

Site Location

The project location for field trials of MICP was provided by the Idaho Department of Transportation's District 3. The field test site is in Marsing, Idaho along the US 95 highway. The soil from this location was tested during the laboratory phase of this study and was observed to have the highest amount of calcite precipitation. The coordinates of the location are 43°27'15.4"N and 116°51'39.1"W. Figure 16shows the location of project site. This site was about 50 miles (80.5 km) from the Boise State campus. As noted in "Natural soils" section of this report, the MS soil has very high swell potential. In the past few decades, ITD has experienced several issues regarding pavement distress on US 95 at this location.
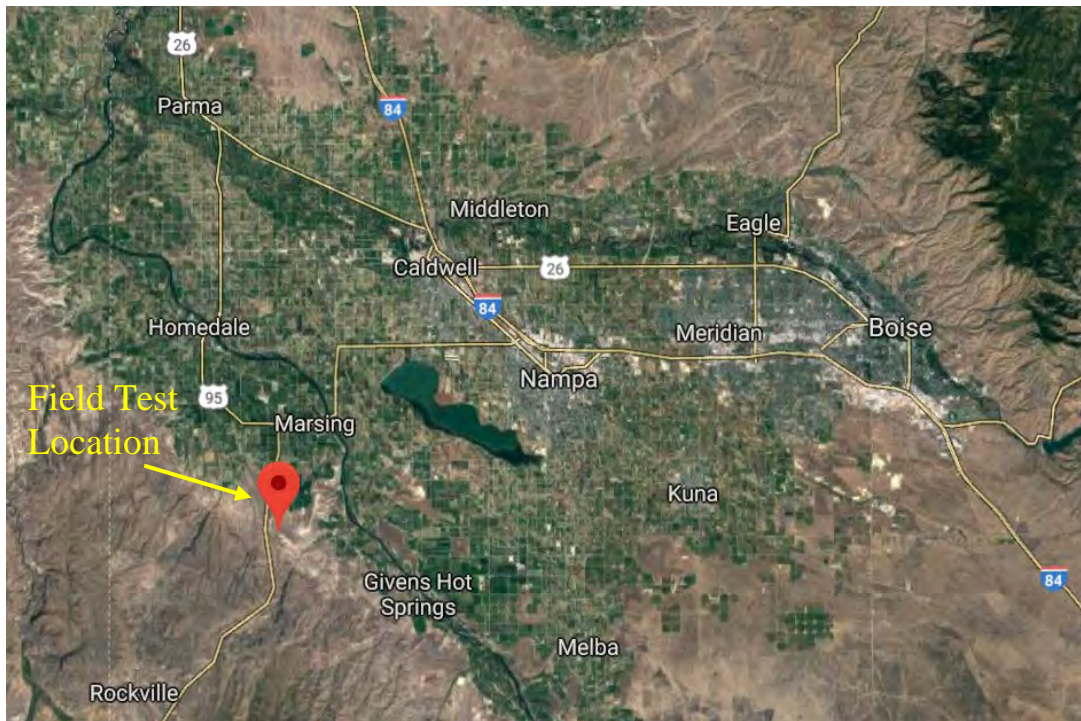
Figure 16 Map showing the locatio of the field testing site (obtained from Google Maps)

Field Test Setup

In order to inject the treatment solutions to the required depth, the research team contemplated several approaches. One of the approaches was to have an open ditch and gravity feed the treatment solutions until they reached a targeted depth. This approach was quickly rejected as this would have taken longtime and using such method on an active highway would disrupt the traffic for extended periods of time. The second alternative was to drive perforated steel tubes to the required depths and send treatment solutions using gravity feeding. While this approach still had the similar time limitations due to gravity feeding, the perforated steel tubes would give direct access to treatment solutions and avoid surface stagnation. This method was also rejected as leaving treatment solutions reservoirs on site for extended periods of time is not practical

due to concerns of theft and vandalism. The third approach was to inject the treatment solutions at select locations, to targeted depths under pressure. This approach would save time and complete the injection operation in much shorter amount of time. However, this approach had the disadvantage of the treatment solutions percolating from the sides of the steel tubes as the permeability in soil is much slower and the solutions are being pushed at high pressures. To avoid this issue, the research team finally chose the option of drilling to targeted depth and using a pneumatic packer system to seal the borehole and then inject the treatment solutions under pressure. A rubber lining present in the pneumatic packer would seal the gap between the steel pipe and the borehole and prevent solutions from percolating back to the surface. A list of the equipment used for the field trials is as follows:

1. Handheld power auger – To drill borehole to required depth
2. Pneumatic packer system – To seal the borehole
3. Water tank or reservoir – To hold treatment solutions
4. Hydraulic pump – To inject treatment solutions under pressure
5. Soil Core – To collect samples from different depths

A portable gas-powered handheld auger was used to drill injection points in the field site. A spiral auger head 2.5" (6.35 cm) in diameter was used to drill holes up to a depth of 30" (76 cm) into the ground. A photograph of the auger used in this research is presented in Figure 17a. A 25-gallon (94.6 liters) tote tank was connected to a portable water pump to inject the treatment solutions (Figure 17b). The water pump could be operated in the field by connecting it to a 12V car battery. The water pump had a rated capacity to pump 5.5 gallons per minute (20 liters per minute) at a pressure of 60 psi (4.2 bar).

(a)   (b)   (c)

(d)   (e)

Figure 17 Photographs of the field setup used in this research (a) Handheld power auger (b) 25-gallon Tote tank (c) Pneumatic packer (d) Water pump (e) Paddle mixer

The outlet from the portable water pump was connected to a pneumatic packer that injected the solution into the ground. A single point pneumatic packer was used for this project. The packer can be inflated with air through a 1/8" (3.2 mm)

outer diameter tubing that extends from the packer to the ground surface. A manual hand pump with a gauge can be used to inflate the packer. The outer diameter of the packer used for this project was 1.8" (46 mm) when uninflated.

On the surface, the inlet of the packer is connected to the outlet from the water pump. At the outlet of the packer, a PVC Tee connection was attached such that the solution would be pushed out laterally from the tube. A pressure gauge at the inlet of the packer was used to read the pressure within the injection tube.

Injection Method

The chemicals were injected into the ground through several injection points that were spread apart at fixed distances form two interlocking grids. The first grid consisted of four holes spaced at a distance of 16" (40.64 cm) on center to form a square. The second grid consisted of four holes spaced at a distance of 30" x 20" (76.2 cm x 50.8 cm) on center to form a rectangle. Figure 18 presents a photograph showing the layout of the injection locations. The handheld auger was used to drill injection holes to a depth of 36" (91.4 cm) into the ground surface. The diameter of the holes was maintained at 2.5" (6.35 cm) so that the injection system could be easily inserted into the holes. Achieving a uniform distribution of injection points was hindered by the presence of hard rock pieces in the soil that clogged the auger head. In which case the holes were moved to penetrate the full target depth.
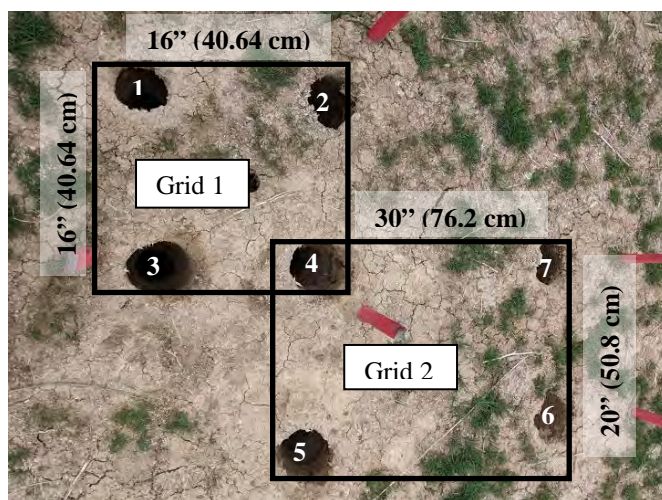
Figure 18 Layout of Injection Points

After the boreholes were drilled to the target depth, treatment solutions were injected into the holes using the packer assembly shown in Figure 17c. This is a pneumatic packer where the rubber tubing around the inlet pipe can be inflated using a manual pump. The inflation pressure rating for this rubber tubing was between 140 to 205 kPa. The packer is suitable for borehole sizes of 48 to 74 mm. The packer is retrofitted with a pressure gauge which measures the rate of inlet pressures. The inflation pressure was maintained at 10 to 15 kPa higher than the injection pressure to ensure a tight seal in the borehole. The pneumatic packer was inserted into the injection points to inject solutions into the ground. A hand pump was used to inflate the rubber lining on the packer tube and seal the holes. This prevented the solutions from easily raising back to the surface.

Table 6 Concentration of Chemicals Used in Enrichment and Cementation Solutions

Chemical	Concentration (g/l)	
	Enrichment Solution	Cementation Solution
Urea	20	20
Sodium Acetate Anhydrous	8.2	4.1
Solulys	0.5	0.5
Calcium Chloride Anhydrous	-	27.74

The treatment solutions consisted of two separate mixes for enrichment and cementation - similar to the laboratory test in case of natural soils. The concentration of various chemicals in the enrichment and cementation solution are given in Table 6. The chemicals were purchased in powder form and mixed on site in the tote tank (reservoir) using a paddle mixer. Figure 17 shows the reservoir and the paddle mixer. The tank was thoroughly cleaned between injections to ensure no calcite buildup inside the tank.

12 V Car Battery Powers the Water Pump

Solution Tank and Attached Water Pump Feed the Packer

Handpump to pressurize the rubber membrane of the pneumatic packer

Pressure Gauge attached to the packer reads injection pressure

Solution is injected below the surface through the packer tube
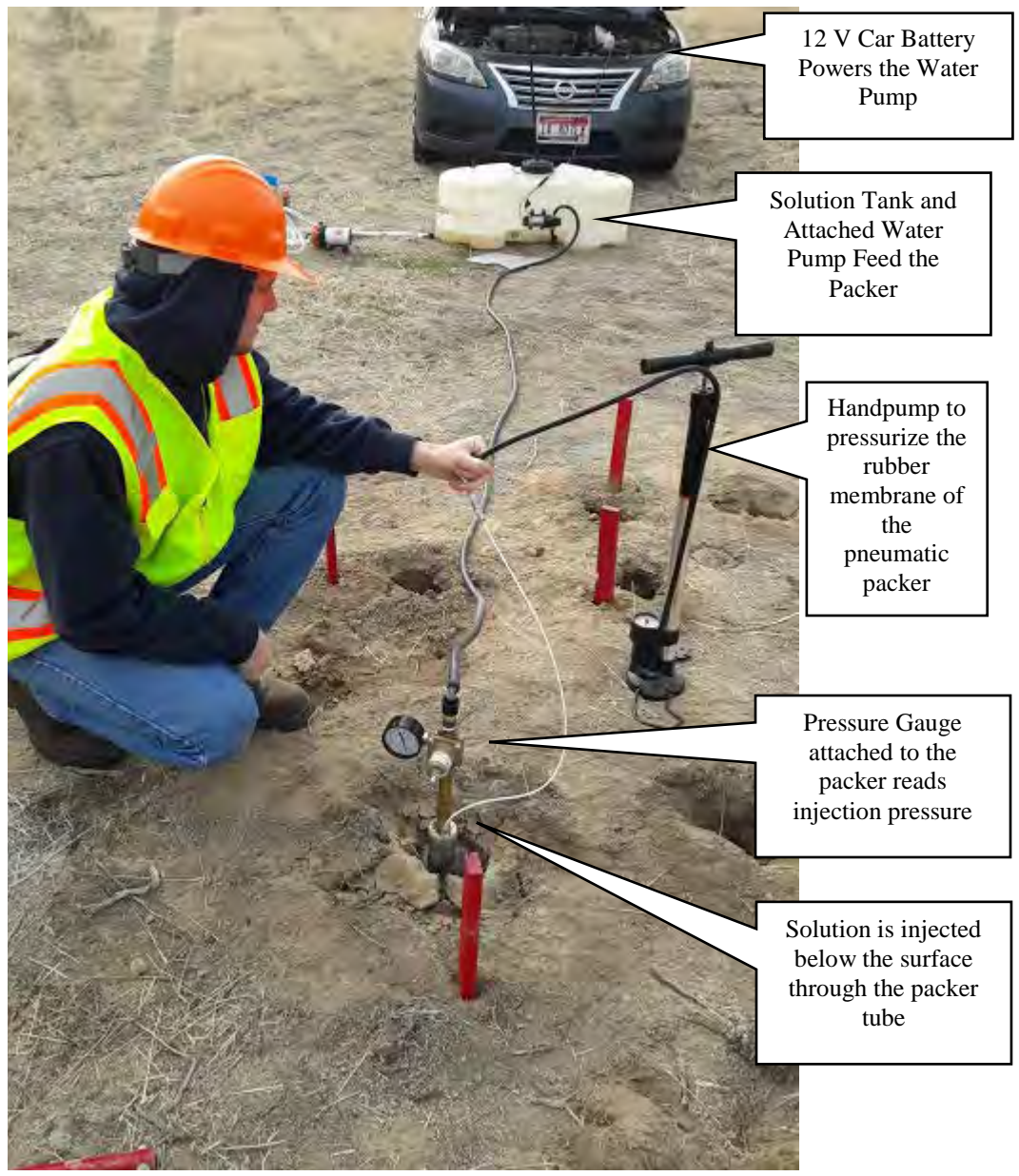
Figure 19 Equipment Setup for Injection of Solution in Field

The injections were done at a pressure of 20 psi (137 kPa) similar to the laboratory infiltration pressures. Each injection point was injected with 3 - 6 gallons (11.35 - 22.7 liters) of solution. Injection operation was stopped when the treatment solutions started rising from the sides of the packer lining on to the surface. As the injection process continued, soil surrounding the packer deformed, leaving a gap between the packer lining and the soil through which treatment solutions were escaping. The research team was able to inject a minimum of 4 gallons during each injection operation. The amount of treatment solutions required for each cycle was determined based on the pore volume of the targeted treatment section. The research team targeted to treat approximately 2 ft (60.96 cm) of soil across each grid. The approximate pore volume of the target area (16" X 16" X 24" or 40.64 cm x 40.64 cm x 60.96 cm) was determined to be 19 gallons (71.92 liters).

To ensure that all pores had access to the treatment solutions 24 gallons (90.84 liters) (6 gallons (22.7 liters) per injection point) of the treatment solutions were injected for each round treatment.

The treatment started with the injection of enrichment solutions. The enrichment solutions allow for the growth of urease producing bacteria over time. An enrichment period of 7-days was allowed before the injection of cementation solutions as it was observed that the pH was above 8.0 after this time. The consecutive injection of cementation solution was performed at 7-day intervals when pH of the soil was observed to be between 8.3 to 9.7. Three consecutive injections of cementation solutions were made at an interval of 7 days. A fourth injection of cementation solution was done 14 days after the third cementation due to weather related delay.

Observation and Results

Soil samples were collected from the bottom of the borehole before every injection operation. A manual soil core with a diameter of 2" (5 cm) was used to collect the samples (see Figure 20). This sample was taken to the laboratory to determine the calcite precipitation and swell potential. This section presents the results from these tests along with few observations made during the field-testing phase.

Figure 20 Manual soil core used to collect soil samples from the bottom of the boreholes

Lateral Influence of Injection

During the process of injection, the solution was seen to be flowing into a neighboring injection point through the soil. This observation was made in injection points within a distance of 16" (40.64 cm). This suggests that the solutions could flow laterally up to at least the distance the 16" when injected at a pressure of 20 psi (137 kPa). The following picture shows that the adjacent hole is being filled while the treatment solutions are being from a nearby borehole. It should be noted here that no lateral flow was observed in case of the injection points spread in a rectangular grid pattern (30" x 20" or 76.2 cm x 50.8 cm). This could mean that at 20 psi (137 kPa) injection pressure the lateral influence is less than 20" (50.8 cm).
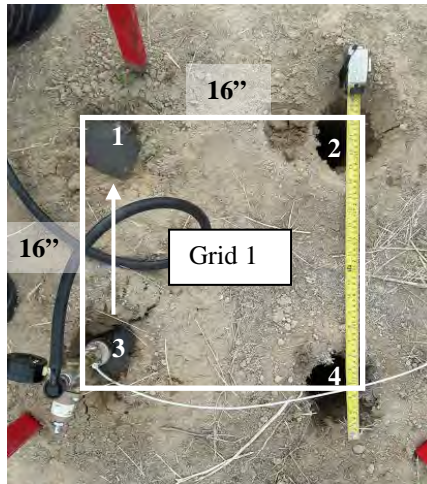
Figure 21 Flow of solution between injection points

The flow of solution into neighboring injection point was seen between point 3 and point 4 during the injection of enrichment solution and first round of cementation solution. However, during the second round of cementation solution injections, it was observed that the flow was between point 1 and point 3. The change in flow path could be due to blockage of flow lines with gradual precipitation of calcite in the soil. As calcite precipitation occurs and particles are bonded together, the initial flow path that was formed during the flow of solution from point 3 to point 4 may have been be blocked. So, a consecutive injection in the same point would result in the solution taking alternate pathways.

Calcite Concentration

The calcium carbonate content in the soil was detected by using the same procedure used to determine laboratory samples. This procedure involved mixing air-dried soil with 1N HCL in an air tight container and measuring the pressure of carbon dioxide gas produced. A calibration chart prepared from pressure readings with known amounts of calcium carbonate was used to calculate the calcium carbonate in each soil sample. Soil samples from field showed that there was a consistent rise in concentration of calcite at the injection points with every round of cementation injection as seen in Figure 22. This data clearly shows that calcite precipitation is possible in plastic soils using MICP via biostimulation.
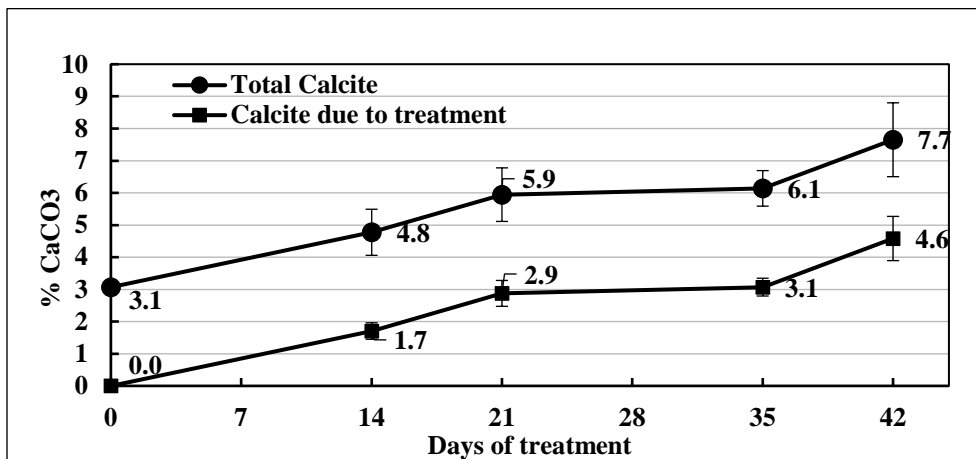
Figure 22 Change in calcite content with treatment cycles

Swelling Potential

The swelling potential of the soil was compared using a free swell index test with kerosene. The free swell index test is an experimental procedure performed to estimate the expansion potential of a given soil [*38*]. It is defined as the ratio between the difference in volumes of a soil submerged in distilled water (polar fluid) and kerosene (nonpolar fluid) without any external constraints for 24 hours to the volume of the soil submerged in kerosene after 24 hours. In this test, two representative oven-dried soil samples (passing # 40 sieve) weighing 10 grams each were poured into two graduated cylinders of 100 ml capacity. One cylinder was filled with distilled water while the other was filled with kerosene up to the 100 ml mark. Entrapped air was removed by minor shaking and stirring with a glass rod. Soil samples are allowed to attain equilibrium state (without any further change in the volume) for a duration of 24 hours [*39*]. The final volume of soil samples in both cylinders are recorded after 24 hours, and the FSI is measured using the following equation:

$$Free\ Swell\ Index\ (FSI)\ (\%) = \frac{(V_d - V_k)}{V_k} \times 100 \qquad (1)$$

Where,

V_d = Volume of the soil sample from the graduated cylinder containing distilled water.

V_k = Volume of the soil sample from the graduated cylinder containing Kerosene.

Tests with samples collected at injection points showed that the free swell index decreased significantly with each treatment at the injection points (Figure 23). This data confirms that the precipitated calcite is altering the behavior of the soil and making it less expansive.
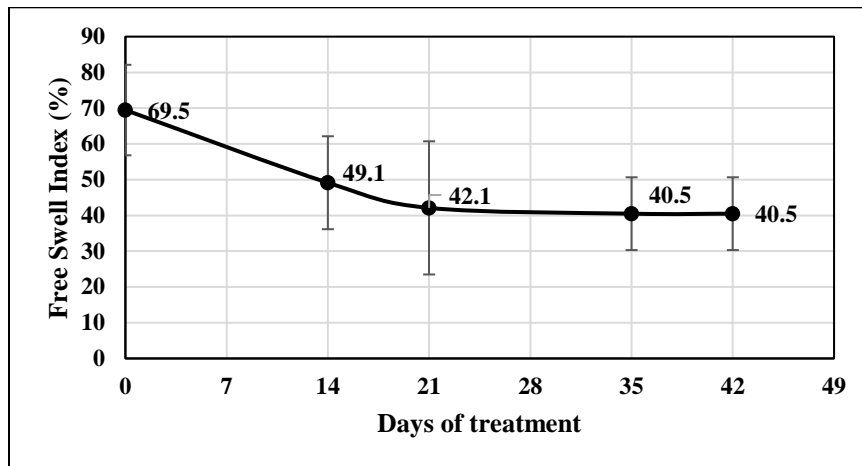
Figure 23 Change in free swell index with treatment injections

Findings

The results of the field test showed that the calcite content increases significantly with each successive injection of cementation solution and reduces swelling potential of the soil. This shows that microbial induced calcite precipitation can be successfully replicated in the field through successive injections of enrichment and cementation solutions into the soil. However, the application method needs further refinement and perfection through further research in the field on live highways to optimize the process as a regular field application.

The homogeneity of the calcite precipitation and its effects on the volumetric behavior as well as strength of expansive soils need to be studied in a larger scale to understand the full benefits of MICP. Instrumentation and on-site measuring devices will be necessary to measure and monitor the physical and chemical changes in the soil. Further research along this path could lead to a revolutionary method for stabilizing expansive soils.

PROPOSED FIELD PROTOCOL

Based on the knowledge gained through this research the following protocol is proposed for further research in field applications.

Initial Preparation

- Drill 2" (5 cm) to 2.5" (6.35 cm) diameter holes from the surface of asphalt to the expansive subgrade soil
- The holes can be spaced at 20" (50.8cm) to 30" (76.2 cm) center-to-center for injection pressures of 20 psi (137 kPa) (as per observations from the field testing in this research)
    - Actual spacing of the drilled holes will be determined by the pressure of injection. A high-pressure injection may require sparsely spread holes, but the subgrade soil may fracture under that pressure. A study of injection pressure and influence distance would help determine the best spacing for a given location.

Bio-Stimulation

- Determine the amount of treatment solutions needed based on the porosity of the soil and the targeted amount of calcification
- Prepare enrichment solution consisting of
    - Urea - 20 gm/l
    - Sodium Acetate Anhydrous - 8.2 gm/l
    - Solulys - 0.5 gm/l
- Determine the pH of the soil before injection of treatment solutions.
- Connect the solution tank to the pneumatic packer through a pump and insert the packer into the drilled hole.
- Inflate the pneumatic packer within the limits of the rated capacity of the packer lining to seal the injection point.
- Inject the solution into the ground through the packer until the volume of injection reaches the expected pore volume in the influence zone of the injection point or until the solution starts raising to the surface.
- Monitor the pH of the soil in the injection location. After seven days of the injection of enrichment solution the pH level should rise significantly.
- Cementation solution should be injected after the pH in the soil rises to around 9.

Bio-Cementation

- Prepare cementation solution consisting of
    - Urea - 20 gm/l
    - Sodium Acetate Anhydrous - 8.2 gm/l
    - Solulys - 0.5 gm/l
    - Calcium Chloride – 27.74 gm/l
- Inject the cementation solution into the soil every 7 days until the desired level of calcification is achieved.
- Monitor soil samples from the injection location to determine the amount of calcite precipitate that has occurred over time.

Instrumentation

- It is recommended that the site be instrumented with moisture probes to detect the extent of treatment solutions
- It is recommended that the site be instrumented to monitor vertical movements at different depths within the treatment zone.
- It is recommended that non-destructive strength/stiffness testing be performed at regular intervals to detect changes in strength/stiffness in situ.

IMPLEMENTATION PLANS

The project team recognizes that the implementation potential is high for this technique, and the target applications are not only in high plastic soils but any weak subgrade. The presence of bacteria is inevitable in any subgrade and as a result application of MICP is feasible in all subgrades. The implementation of such technique will require buy-in from the

agency as well as contractors. The project team recognizes the challenge to overcome institutional barriers and gain acceptance by the industry. The validation and endorsement from agencies will be essential for this acceptance and hence the project team will seek early adopters in the industry. The project team is working with ITD and Montana Department of Transportation to find possible test sites to apply this technique on a live highway. At this time no such sites are under construction, however the research team will continue to pursue other transportation agencies that experience problematic subgrades. A trial application of the technique on an existing highway section with appropriate instrumentation and field monitoring equipment is the next step needed to move this technology forward.

SUMMARY AND CONCLUSIONS

Stabilization of expansive soils through calcite precipitation using the indigenous bacteria is an innovative and promising idea that could replace traditional practice in favor of cost and sustainability. The study of MICP were done in two stages in this project: stage 1 involved of laboratory work while stage 2 focused on preliminary fieldwork. The treatment process generally involved introduction of a bio-enrichment solution to stimulate bacteria in soil and subsequent periodic injections of bio-cementation solution to help precipitate calcite. Results obtained from both laboratory as well as field tests have shown that MICP can be a very effective method in stabilizing expansive soils. In the laboratory tests, calcite precipitation was observed in both natural soils and artificial mixes after MICP treatments. Preliminary field work was performed to evaluate the laboratory protocols in the field. The following are major findings from this research:

- The net calcite content ranged from 0.29 to 0.68% for **artificial soils** mixed with 10^6 cells/gm of bacteria after one treatment cycle. **Higher calcite content** was observed for soils with **lower clay contents**. In case of artificial soils mixed with 10^8 cells/gm of bacteria the calcite content ranged from 0.78 to 0.99%.
- The net calcite precipitation in case of natural soils ranged from 0.39 to 1.56%; however, in case of **natural soils** the **calcite concentration increased** with **increase in clay content** for the four soils tested in this research.
- Increase in calcite precipitation with increasing clay content in natural soils could be due to higher bacterial concentrations in these soils. In artificial mixes, since the bacterial concentration remains constant across all samples the availability of pore space and access to treatment solutions is controlling calcite precipitation.
- The **impact of calcite precipitation** on **strength improvement** and **swell reduction** was significant.
- The 1-D swell strains which ranged from 0.31 to 8.84% before treatments were reduced to 0.06 to 0.47% for the artificial mixes.
- In the case of natural soils, the untreated swell strains were 1.15 to 17.9% and the treated swell strains ranged from 0.5 to 13.13%.
- The percentage improvement in UCS values after treatment ranged from 2 to 46% in case of artificial mixes while the same for natural soil ranged from 22 to 342%.
- There was considerable improvement in UCS and reduction in 1-D swell strain due to this bacterial activity.
- It is evident from the results that it is possible to achieve calcite precipitation in natural soils using native soil bacteria and alter the behavior of the soils.
- Field test results showed that calcite precipitation increased with treatments (up to 8% total) and the free swell index dropped from 114% to 29%.

Results show that microbial induced calcite precipitation can be successfully replicated in the field through successive injections of enrichment and cementation solutions into the soil. Based on the preliminary field tests, a field protocol has been developed for future implementations in the field. Additional field tests are necessary for improving the system and realizing the full benefits of MICP.

GLOSSARY

Atterberg Limit: Atterberg limits are a basic measure of the critical water contents of a fine-grained soil.

Autoclave: An equipment for sterilization that uses high-pressure steam.

Bentonite: a common mineral in clay that has expansive properties

Bioaugmentation: a process where urease-producing exogenous bacteria are added to the soil to precipitate calcite

Biostimulation: a process using indigenous bacteria present in the soil to precipitate calcite

Calcite: calcium carbonate

Cation exchange: an exchange of positively charged molecules or ions surrounding the soil particles

Exogenous bacteria: bacteria that is introduced to the system from outside

Expansive soils: Soils that undergo high change in volume when subject to change in moisture content

Free swell index: When the volume of the soil increases without any application of external forces or water pressure, it is called as free swell. The index will measure the increase in volume with respect to the original volume.

Genotypes: the genetic makeup of a cell or organism

Hydrometer Test: A laboratory test method used to determine the particle size distribution of fine-grained soils.

Microbial-induced calcite precipitation (MICP): A process in which urease producing bacteria precipitate calcium carbonate in soil

Microcosm: simplified ecosystems that are used to simulate and predict the behavior of natural ecosystems under controlled conditions

Permeability: A measure of the ability of a material to transmit fluids.

Plasticity index (PI): The size of the range of water contents where the soil exhibits plastic properties. The PI is the difference between the liquid limit and the plastic limit.

Plasticity: The property of a material to undergo non-reversible changes in shape or volume in response to applied forces.

Pneumatic packer: A tube lined with inflatable rubber to seal boreholes and inject pressurized fluid through the tube.

Rapid Carbonate Analyzer: A device used for measuring soil carbonate content.

Sieve analysis: A procedure used to assess the particle size distribution of a granular material by allowing the material to pass through a series of sieves.

Specific Gravity: specific gravity of an object is the ratio between the density of an object to the density of water at 20 degree Celsius

Sporosarcina Pasteurii: a bacterium with the ability to precipitate calcite and solidify soil given a calcium source and urea, through the process of microbiologically induced calcite precipitation

Standard Proctor Compaction Test: a laboratory method of experimentally determining the optimal moisture content at which a given soil type will become most dense and achieve its maximum dry density.

Subgrade: the native material underneath a constructed road, pavement or railway track

Turbidity: Measurement of the amount of light absorbed by a bacterial suspension.

Unconfined compressive strength (UCS): the maximum axial compressive stress that a right-cylindrical sample of material can withstand under unconfined conditions

Urease producing bacteria: bacterial capable of producing urease enzymes that catalyzes the conversion of urea to ammonia and carbon dioxide

Ureolytic bacterium: bacteria capable of calcium carbonate production as precipitation occurs as a byproduct of common metabolic processes

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APPENDIX – RESEARCH RESULTS

WHAT WAS THE NEED?

Expansive soils typically undergo significant changes in volume with changing water content. These types of soils are widespread and annually cause billions of dollars in damages to various infrastructures around the world, especially to pavement infrastructure. Various ground improvement techniques like chemical stabilization, deep soil mixing and moisture barriers are employed to counteract the problems caused by these soils. However, these methods are either expensive or have negative effects on the environment. A more sustainable and economic alternative could be found in microbiological treatment of soils. Microbial Induced Calcite Precipitation (MICP) is an innovative process that could be used to improve the engineering properties of soil through calcite precipitation using urease-producing bacteria. While MICP was studies as a mitigation method to arrest liquefaction in sandy soils, no studies evaluated the possibility of using this method to treat expansive soils.

WHAT WAS OUR GOAL?

The main goal of this project was to evaluate the application of environment-friendly biological processes to stabilize expansive subgrades.

WHAT DID WE DO?

Microbial-induced calcite precipitation (MICP) is an innovative approach that uses soil bacteria to precipitate calcite and alter the behavior of the soil. Experience with MICP implementation has been primarily using bioaugmentation (introducing microorganisms into the soil), especially in case of sands where microbial populations are minimal. Bioaugmentation has several issues including the survivability of the bacteria in the new environment which results in uncertain treatment performance. However, in the case of clays, there is no need to introduce exogenous bacteria as clays are natural incubators for microorganisms. Hence, this research study used bio-stimulation to encourage indigenous bacteria to precipitate calcite and alter soil behavior.

The study tasks were accomplished in two stages: stage 1 involved of laboratory work while stage 2 focused on preliminary fieldwork. The objectives of the laboratory work were to study the role of soil type, clay content and bacterial populations on treatment effectiveness and develop a protocol for field implementation. Laboratory work involved development of a treatment solution delivery system and testing the effects of MICP in different types of artificial as well as natural soils. Four artificial soil mixes and four natural soils with varying clay contents and plasticity characteristics were tested in this research. The artificial mixes were prepared using medium-to-coarse sand (obtained from a local quarry) and bentonite mineral powder (obtained from a commercial source). The sand/bentonite ratio was 95/5, 90/10, 85/15, and 80/20 for AM-1, AM-2, AM-3, and AM-4 mixes respectively. The four natural soils were collected from different locations in Idaho and Montana that had different plasticity characteristics. The natural soils are denoted as MS (Marsing, ID), GF (Great Falls, MT), DC (Dry Creek, MT) and BR (Bad Route, MT) indicating the location from which they were obtained.

The field work was intended to explore the effectiveness of laboratory protocols in the field and help develop a system for future field applications. Experiments in field were done by injecting a bio-enrichment solution (mixture of urea, sodium acetate and solulys in water) to help the bacteria in the soil grow and a bio-cementation solution (mixture of calcium chloride, urea, sodium acetate and solulys in water) to induce precipitation of calcite. The composition of treatment solutions was identical to that of laboratory experiments. Injections were done at a depth of 3ft through two configurations of 2" (5 cm) diameter holes - one with a center-to-center distance of 16" (40.64 cm) between the injection points and the second with a center-to-center distance of 30" (76.2 cm). The injections were performed using a packer system to ensure the borehole was sealed during injection and the treatment solutions do not escape from the annular space between the injection pipe and the borehole. Five rounds of treatments were performed at one-week intervals starting with one round of enrichment solutions followed by four rounds of cementation solutions. Each treatment

consisted of pumping approximately 25 gallons (94.6 liters) of the treatment solution. Samples were collected from the bottom of borehole after each treatment to be tested for calcite content and free swell index. Figure i and Figure ii show the increase in calcite content and corresponding improvement in swell potential with time during the treatment cycles.
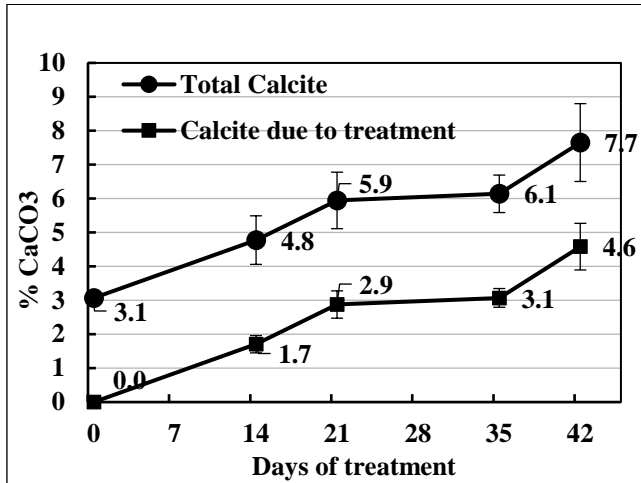

Figure i: Change in calcite content with treatment cycles (in field)
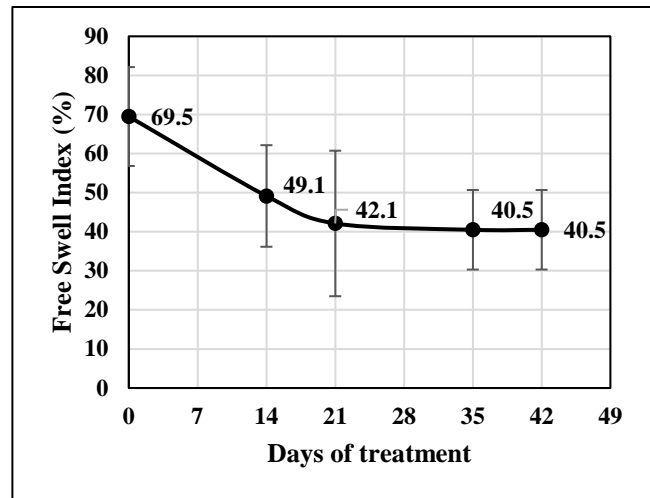

Figure ii: Change in free swell index with treatment cycle (in field)

WHAT WAS THE OUTCOME?

Overall, it was found from laboratory tests that, there was increased calcite precipitation with increasing clay content in natural soils. The impact of calcite precipitation on strength improvement and swell reduction was significant. Results convincingly showed that it is possible to achieve calcite precipitation in natural soils using native soil bacteria and alter the behavior of the soils. Field test results showed that calcite precipitation increased with treatments (up to 8% total) and the free swell index dropped from 114% to 29%. It is now evident that microbial induced calcite precipitation can be successfully replicated in the field through successive injections of enrichment and cementation solutions into the soil. Based on the preliminary field tests, a field protocol has been developed for future implementations in the field. Additional field tests are necessary for improving the system and realizing the full benefits of MICP.

WHAT WAS THE BENEFIT?

The payoff of this research project is considerable, as federal and state governments can realize significant cost savings and positive environmental impacts in both new pavement construction and repairs of distressed pavements in regions with expansive soils. The proposed treatment methodology will provide a sustainable alternative for expansive soil treatments. Tangible *benefits* include:

- Extended Life and Reduced Cost—Fewer incidences of cracking would result in lower infrastructure maintenance costs. In addition, you could apply the method without major reconstruction to existing pavements showing subgrade heaving distress.

- Improved Health and Environment—Federal and state agencies would gain a sustainable treatment alternative for expansive soil problems beneath transportation infrastructure.

- Wide Use—Outcomes may recommend the treatment method for other problem soils like soft clays and collapsible soils, thus avoiding sinkhole damage if detected early.

With further research and optimization of the injection technique with filed implementations, this environmentally friendly technique could be applied on existing highways that suffer distresses due to prevalent expansive soils. Tackling the existing subgrade, without excavation and complete removal of the pavement structure would be a cost-effective measure for treating existing road structures.