Evaluating the Applicability of Biostimulated Calcium Carbonate Precipitation to Stabilize Clayey Soils

Md Touhidul Islam
Gannett Fleming, Inc.

Bhaskar C.S. Chittoori
Boise State University

Malcolm Burbank
EnBiorganic Technologies

Evaluating the Applicability of Biostimulated Calcium Carbonate Precipitation to Stabilize Clayey Soils

Md Touhidul Islam
Geotechnical Designer
Gannett Fleming, Inc.
Marlton, NJ
tislam@gfnet.com

Bhaskar C.S. Chittoori
Assistant Professor
Dept. of Civil Engineering
Boise State University,
Boise, ID
bhaskarchittoori@boisestate.edu

Malcolm Burbank
Environmental Microbiologist
CDM Smith
Kellogg, ID
burbankm@cdmsmith.com

Abstract

Clayey soils with medium to high plasticity are prevalent in several parts of the world, causing billions of dollars in damage annually to various civil infrastructures. Several ground-improvement-techniques were employed to counteract this issue. However, these methods are impractical in certain situations and unsustainable in others due to their economic and environmental impacts. Microbial Induced Calcite Precipitation (MICP) could provide a more sustainable alternative. Researchers have successfully used MICP to alter specific geotechnical properties of the sands and silts. This research investigates the applicability of MICP via biostimulation to treat clayey soils with low to high plasticity. The goal is to determine the viability of this technique to alter the engineering behavior of clayey soils, especially given the low permeability of these soils. For this purpose, four soils were selected from four different locations in Idaho and Montana. The soils were selected such that their plasticity varied from low to high to study the effect of plasticity index on the effectiveness of MICP treatments. In addition to the four soils, three additional artificial mixes were studied to study the effect of clay content on MICP effectiveness. Both macro and micro scale studies were conducted on untreated and biostimulated soils to observe strength gain, swelling reduction, calcium carbonate precipitation. The results show that MICP via biostimulation would be a promising method to treat problematic clayey soils.

Keywords: expansive soils, clays, biostimulation, calcium carbonate precipitation, MICP, swelling

Introduction

Clayey soils with varying plasticity characteristics have been problematic to civil infrastructures for several decades. 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 (Jones and Holtz 1973; Jones and Jefferson 2012). 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 (Petry and Little 2002). The prevalence and annual damages caused by these soils have influenced researchers and practitioners to develop different stabilization measures to mitigate this issue.
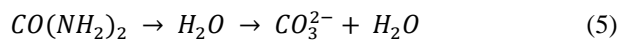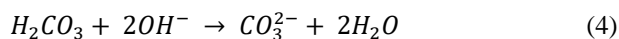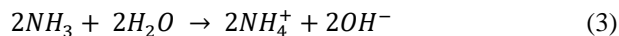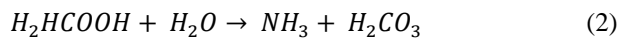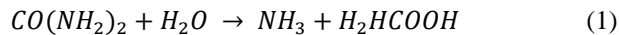
Chemical and mechanical stabilization techniques were implemented with different success rates to stabilize clayey soils. Cement, lime, fly ash, and granulated blast furnace slag have been used to treat clayey soils for decades (Jones 1958; Thompson 1970; Little 1999; Little 2000; Obuzor 2011). On the other hand, mechanical stabilization, i.e., installing water barriers or geomembranes could be a viable alternative for treating these soils (McDonald 1973; Steinberg 1981). However, those stabilization techniques and chemical stabilizers harm the environment and economy. The production of cement and lime is a prime source of greenhouse gases (UNEP 2010). UNEP (2010) 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. There is a distinct need to develop sustainable and eco-friendly solutions to mitigate the problems with high plastic clayey soils.

Microbial Induced Calcium Carbonate Precipitation (MICP) is an environmental-friendly bio-mediated soil improvement technology resulting from the interdisciplinary pathways of microbiology, geochemistry, and civil engineering. Researchers have shown that MICP is suitable for mitigating seismic-induced liquefaction, reducing permeability and compressibility, increasing unconfined compressive strength and shear strength (DeJong et al. 2006; Whiffin et al. 2007; Van Paassen 2009; Burbank et al. 2011; Martinez et al. 2013; Al Qabany and Soga 2013). In this research, an attempt was made to use this technique to treat clays with varying plasticity and improve their engineering behavior. There are two ways to apply this technology to soils: *bioaugmentation* and *biostimulation*. *Bioaugmentation* is a process where urease-producing bacteria are added (injected) to the soil, whereas biostimulation takes advantage of the indigenous bacteria already present in the soil and stimulates them to precipitate calcite. Past studies (Burbank et al. 2011; Burbank et al. 2012; Gomez et al. 2018; Tsesarsky et al. 2016) 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. For this purpose, eight soils were tested with varying plasticity characteristics and clay contents. Both macro and micro scale studies were conducted on untreated and biostimulated soils to observe changes in plasticity, strength, swelling, and mineralogical characteristics. The results from these studies and the ensuing conclusions are presented in this paper.

Background

The mechanism of calcium carbonate precipitation using urease producing bacteria consists of urea hydrolysis and calcium carbonate precipitation (Stocks-Fischer et al. 1999; Hammes and Verstraete 2002; Burbank et al. 2013). These bacteria hydrolyze 1 mole of urea ($CO(NH_2)_2$) into 1 mole of ammonia and 1 mole of carbamic acid (Equation 1). Carbamic acid decomposes into ammonia and carbonic acid (Equation 2). Ammonia then hydrolyzes into ammonium ion, which increases the pH of the system (Equation 3). The carbonic acid dissociates into dissolved inorganic carbonate (Equation 4). With the creation of nucleation sites and the addition of Ca^{2+} ion to this system, calcium carbonate crystals form on the cell wall (Equation 5 and Equation. 6). Chemical reactions associated with calcium carbonate precipitation are described here (modified Burne and Chen 2000)-

$$CO(NH_2)_2 + H_2O \rightarrow NH_3 + H_2HCOOH \qquad (1)$$

$$H_2HCOOH + H_2O \rightarrow NH_3 + H_2CO_3 \qquad (2)$$

$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^- \qquad (3)$$

$$H_2CO_3 + 2OH^- \rightarrow CO_3^{2-} + 2H_2O \qquad (4)$$

$$CO(NH_2)_2 \rightarrow H_2O \rightarrow CO_3^{2-} + H_2O \qquad (5)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \downarrow \qquad (6)$$

Applications of MICP

Several studies showed promise for MICP technique to alter the engineering behavior of sandy and silty soils (DeJong et al. 2010; Mortensen et al. 2011; Chu et al. 2012; Soon 2013). However, limited studies were found related to the implementation of MICP in clays, especially in expansive soils. The major hindrance of introducing MICP in clays is the geometric compatibility between soils and microbial community. The typical cell diameter of common soil bacteria ranges from 0.5 to 3 μm (Mitchell and Soga 2013). In another study, Rao and Revanasiddappa (2005) stated the pore sizes of soils ranges from 60 to 6 μm (macro pores), 6 to 0.01 μm (medium pores) and 0.01 to 0.002 μm (micro pores). On the basis of the cell diameter of soil bacteria and pore sizes of soils, Chittoori et al. (2016) conducted a Mercury Intrusion Porosimetry (MIP) test to observe the compaction effort on the pore size and pore volume on two expansive soils. The results showed that 30% and 50% of the pore volume is larger than 1.5 μm (average diameter of the soil bacteria) respectively at maximum dry density for those two expansive soils. This research indicated that the space required for bacterial mobilization is available through pores of the soils. Bing (2015) conducted biotreatment on

different forms of clay, i.e., kaolin, marine clay, and bentonite. They observed that strength increased around 150% and 400% for treated kaolin and treated marine clay, respectively. Bentonite with bacteria performed better than the untreated bentonite when the water content was reduced to 150%. Cheng and Shahin (2015) assessed three different MICP methods, including injection, premixing, and diffusion for clayey sands, to investigate the variation of strength and amount of calcium carbonate precipitation. They recommended injection method for soils having less than 5% clay content, though a 150% increase of strength was achieved in the case of premixing. Although the diffusion method increased the strength, the slow mass diffusion reduced the calcite at the end of the column. In other research, Cardoso et al. (2018) investigated the compressibility and pore clogging of the biocemented sand-kaolin mixture. They found that the osmotic consolidation effect might be a contributing factor for high compressibility along with the bacterial activity.

MICP Protocols

There are two strategies to apply MICP in soils, namely *bioaugmentation* and *biostimulation*. In *bioaugmentation*, exogenous bacteria are added to soil to encourage calcium carbonate precipitation. Researchers have used bioaugmentation on sandy or silty types of soils using urease producing bacteria for soil improvement (Whiffin et al. 2007; van Passen et al. 2010). To date, Neupane (2016) has investigated the use of bioaugmentation to treat low to moderate plasticity clays and found that it could be an alternative stabilizing method for mitigating soil swelling. In that study, three soils having low, medium, and high plasticity were investigated. Lime and MICP treatments were performed with different curing periods, treatment cycles, and bacterial populations. Two protocols were chosen to precipitate calcium carbonate. In one protocol, different concentrations of cultured bacteria (10^8 and 10^{10} microbes/gm) and substrate were mixed into the soil and cured for 7 days and then tested. In another protocol, different concentrations of cultured bacteria (10^8 and 10^{10} microbes/gm) were added in the soils, and the substrate solutions were injected through the soils for 1, 3 and 7 pore volumes. The results showed that this approach would work for low plastic soils, however, in the case of medium and high plastic soils showed mixed results and were inconclusive.

However, adding new bacteria can cause several problems, i.e., survivability of exogenous bacteria, uneven distribution, longer time needed for the permeation of bacteria, costly for the cultivation and special cautions required while mixing (DeJong et al. 2010; Tsesarsky et al. 2016). In the case of biostimulation, indigenous bacteria are stimulated to achieve calcium carbonate precipitation. In order to overcome the difficulties of bioaugmentation, researchers have been stimulating natural microbes for precipitating a large amount of calcite (Fujita et al. 2008; Burbank et al. 2011). Generally, the number of bacteria in natural soils is 10^6 to 10^{12} per gram of soil (Torsvik et al. 1990). Boquet et al. (1973) demonstrated that most soil bacteria could precipitate calcite via various mechanisms. Hence, in this study, the applicability of the biostimulation technique on clayey soils was investigated.

The research team chose treatment solutions (e.g., enrichment and cementation solution) to stimulate the indigenous bacteria for precipitating calcium carbonate. A Treatment Solution Delivery System (TSDS) was used to accelerate the treatment phase of clay. Neupane (2016) originally developed this device. This device was connected to pressurized cylinders to inject treatment solutions into low permeability soils. After injecting one pore volume of enrichment and one pore volume of cementation solutions on each of the soils, response measure tests that included Atterberg Limit test, Unconfined Compressive Strength, 1-D Swell test, Calcite determination test, and SEM test were conducted to observe the changes in these soils before and after MICP treatments.

Materials and Methods

Soil Types

Eight different soils were tested in this research out of which four were natural soils obtained from Idaho and Montana regions, and four were artificial mixes prepared from one of the natural soils. The natural soils were studied to understand the effect of varying plasticity characteristics and microbial communities on MICP effectiveness in altering the soil behavior while the artificial mixes were intended to isolate the effect of clay content on MICP effectiveness. In this paper, the natural soils are sometimes referred to as soils with different microbial communities, and the artificial mixes are referred to as soils with same microbial communities. No additional bacteria were added to these eight soils, and only the existing indigenous soil microbes were stimulated to precipitate calcite.

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 four artificial soils were prepared by adding different amounts of medium to fine sand (D_{60} = 0.68, D_{10} = 0.24 and C_u = 2.83) to MS soil in order to create soil mixes with varying clay contents while keeping the microbial community origins the same. These soils are denoted as C-40 (40% Clay Content), C-30 (30% Clay Content), C-20 (20% Clay Content) and C-10 (10% Clay Content). Sieve and Hydrometer Analysis (ASTM D422) along with Atterberg limits (ASTM D4318) tests were conducted on all eight soils to determine the soil gradation and classify the soils. 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. While the MS and GF are classified as A-7 soils and DC and BR are classified as A-6 and A-7-6 respectively as per the AASHTO classification system. All the artificial mixes were classified as low plastic soils (CL) according to USCS and, according to AASHTO, the C-40, C-30, C-20, and C-10 are classified as A-7, A-7-6, A-2-6, and A-2-6 respectively.

Evaluation Tests

The selected soils were subjected to additional geotechnical tests, including Standard Proctor Compaction test (ASTM D698), Unconfined Compressive Strength (UCS) test (ASTM D2166) and 1-D Swell test (ASTM D4546). These same tests were conducted on biostimulated soils to determine the effect of treatment. Table 1 and Table 2 present the baseline data for both natural and artificial soils, respectively. It should be noted from these tables that the Liquid Limit (LL) and Plasticity Index (PI) of all eight soils were in the range of low to very high swelling potential indicating expansive nature of these soils.

In addition to the macro-scale geotechnical tests, several micro-scale tests including Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray spectrometry (EDX) and Carbonate test were conducted before and after treatments on all eight soils. The SEM tests help visualize the precipitated calcite and qualitatively identify the presence of calcite in the soils. The carbonate tests help quantify the amount of calcite precipitated after treatments. The following sections present a brief description of the test procedures followed in this research.

Carbonate Analysis

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 hydrochloric acid (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 a 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.

Scanning Electron Microscopy

The purpose of doing Scanning Electron Microscopy (SEM) was to visualize the presence of calcium carbonate in the soil mass. This analysis was performed in Idaho Microfabrication Laboratory (IML) situated at Boise State University. With an accelerating voltage of 2 kV and current of 25 µA along with a T2 secondary electron detector, optimal quality images were developed for both untreated and biostimulated soil samples. The representative samples were carbon coated, forming a thin layer which reduces the charge interference of charged clay particles. The samples of both treated and untreated samples were carbon coated and placed inside the FEI Teneo FE-SEM to collect images of calcium carbonate. The Energy Dispersive X-ray (EDX) analysis was helpful in approximately quantifying the calcium, oxygen, and carbon, which indicate the presence of calcium carbonate.

Treatment Protocol

Biostimulation is a two-stage process where the first stage requires the bacteria to hydrolyze urea, and the second stage is to precipitate calcite. Hence, two solutions were chosen for biostimulation, and those are the enrichment and cementation solutions. The formula for those solutions was partly taken from other research where stimulation of indigenous bacteria proved for sands (Burbank et al. 2013). The ingredients of enrichment solution were 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 or ammonia from the hydrolyzed urea acted as a nitrogen source. The corn steep liquor supplies amino acids, vitamins, and minerals. The ingredients of cementation solution were sodium acetate (100 mM), urea (333 mM), corn steep liquor (0.5 g/L) and $CaCl_2$ (250 mM). In addition, with the chemicals used in enrichment solution, Calcium Chloride was added to this phase of treatment. When bacteria hydrolyze urea, dissolved inorganic carbon and ammonium are released into the microenvironment of the urease producing bacteria. With the presence of calcium ions, local supersaturation is introduced, and calcite forms on the bacterial cell wall and the bacteria cells are encapsulated by calcite.

To establish a proper treatment methodology for clayey soils using biostimulation, the research team followed a distinct protocol. A pictorial representation of protocol was shown in Fig.1. First, a specific soil type (either natural or artificial soils) was chosen to start the treatment phase. This soil was compacted at a maximum dry density and optimum moisture content using a Static Compactor to ensure uniform pore spaces throughout the soil specimens. Prepared soil specimens (71 mm x 142 mm) were kept inside the Treatment Solution Delivery System (TSDS), and this TSDS is a special device constructed to treat low permeable soils with desired pressure. A brief description of TSDS is given in a later section. TSDS was connected to pressure regulated nutrient reservoirs. Two reservoirs were chosen for the treatment process. One reservoir was filled with enrichment solution, and another reservoir was filled with cementation solution. After placing the soil specimen inside the TSDS, the enrichment solution was injected to get one pore volume through the sample. During the collection of pore volume for enrichment solution, pH was tested several times, and it increased gradually from 7 to above 9. The enrichment phase was considered complete when one pore volume of the effluent of enrichment solution and desired pH were achieved. Then, the chamber was emptied and refilled with cementation solution using another pressure-regulated reservoir. Again, pH was measured several times during the cementation phase. The treatment cycle was continued until one pore volume of cementation solution was collected as effluent. The treatment cycle was considered complete when one pore volume of enrichment followed by one pore volume of cementation solutions was collected through soil specimen. In the case of artificial soil samples, this was achieved in 2-14 days. The moisture content of the soils was measured before and after treatment and found that the moisture content of all the soils was close to their respective degrees of saturation after treatment. The treated soil specimens were first tested for UCS at saturation, and then oven dried and kept for conducting further testing.

Treatment Solution Delivery System

Neupane (2016) developed a "mini soil microcosm" set up to speed up the treatment process of expansive soils. Similar TSDS were used, but in addition to that system, two pressure regulated reservoirs were selected to inject enrichment and cementation solution separately. This device consists of a chamber made from a Schedule 80 clear Polyvinyl Chloride (PVC) tube that houses soil samples that are 71 mm (2.8 in.) in diameter and 142 mm (5.6 in.) in height. This device is capable of delivering treatment solutions at injection pressures as high as 137 kPa (20 psi). This chamber is sandwiched between two 50 mm thick PVC plates that are held together using threaded rods and screw caps (Fig.2). 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 that ensure the latex membrane is tightly in place and also restricts 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 treatment solution that eluted after traveling through the soil samples were collected in an effluent collector. This device is capable of driving treatment solutions through the soil sample at pressures ranging from 14 kPa to 137 kPa (2 psi to 20 psi). All the chambers were thoroughly checked for leaks and safety tested at a pressure of 137 kPa (20 psi).

Test Results and Discussion

This section presents the test results from all the evaluation studies performed in this research along with a discussion on results explaining the reasons behind the changes due to treatments. 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.

Plasticity Characteristics

Atterberg limits (liquid limit and plastic limit) were determined before and after treatments for all eight soils to observe the changes in plasticity characteristics. The biostimulated soils were oven dried and crushed before doing the Atterberg limits. The variations of Liquid Limit (LL) and Plasticity Index (PI) of untreated and biostimulated natural soils were shown in Fig. 3. It was observed that both LL and PI increased after treatments for all soils tested in this research. The increase in LL for MS, GF, BR, and DC soils was 25%, 9%, 5% and 7% respectively (Fig. 3a) while the increase in PI was observed to be 43%, 34%, 75%, and 47%, respectively Fig. 3b). Similar variations were noted for the artificial soil, and the results are presented in Fig. 4. Hence, regardless of microbial communities, clay contents, and plasticity characteristics, the research group observed an increase in both LL and PI for all soils after treatments.

Similar results were observed in earlier studies (Neupane 2016; Chittoori et al. 2018; Islam, 2018) and the increase in PI and LL was attributed to the presence of an extracellular polymeric substance (EPS). As an organic polymer, EPS contains polysaccharides, protein, and nucleic acids and holds 50% to 90% of a biofilm's total organic matter (Flemming et al. 2000). This organic compound can substantially alter the plasticity nature of soils. A study conducted by Mitchell and Soga (2013) showed that an increase in 1% organic content could increase the Atterberg limit by 10 to 20%. However, it should be noted that the increase in LL and PI of the soils due to the increase in organic content will not have any impact on the swell/shrink nature of the soils and the calcite bonding between soil aggregates will minimize any impact of organics on the deformation under stresses as was evidenced by the strength and swell characteristics data.

Strength Characteristics

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 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 Fig. 5 while those for the artificial mixes are presented in Fig. 6. In case of natural soils, the UCS-α increased by 66%, 10% and 51% (Fig. 5a) and the UCS-β increased by 24%, 32% and 22% for GF, BR, and DC soils, respectively (Fig. 5b). This increase in strength could be 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 (~25%) and hence can be considered as no change in UCS value, the increase of 300% in UCS-β is noteworthy. This distinct behavior compared to the rest of the soils could be attributed to the presence of high amounts of clay fraction in this soil (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 completely 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.

In the case of artificial mixes, it can be observed from Fig. 6a that the UCS-α increased by 2%, 9%, 6% and 11% and the UCS-β increased 96%, 3%, 4% and 38% for C-40, C-30, C-20 and C-10 soils respectively (Fig. 6b). Less improvement was observed in the case of UCS-α because of the breakage of the bonds. The research team dried and broke the biostimulated samples and prepared new UCS samples for determining UCS-α of biostimulated soils. During this intense process of sample preparation, the rigidity of the biostimulated soils would have broken and resulted in low strength. A considerable increase in strength was observed in the case of UCS-β. This increase in strength is attributed to the presence of calcite, which forms a bridge between the soil particles resulting in a stronger soil. It should be noted that although there was an increase in the UCS values the increased strength was still below the typical threshold UCS of 350 kPa (typical for pavement subgrade treatments) for three of four natural soils tested in this research (TxDOT 2005; U.S. Army TM 5-882-14/AFM 32-1019 1994). However, these strengths were attained after one round of the treatments the soil samples. The authors believe that further treatment cycles will certainly result in higher strengths that will be comparable to conventional treatment methods.

Swelling Characteristics

The 1-D swell tests were performed on untreated and biostimulated soils to study the effect of MICP treatments on the 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 Fig. 7. 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 (Fig. 7a), while the swell pressure decreased by 38%, 36%, 18% and 70% respectively (Fig. 7b). The 1-D swell strain and swell pressures of C-40, C-30, C-20, and C-10 soils are presented in Fig. 8. The 1-D swell strain decreased by 35%, 52%, 15% and 3% (Fig. 8a) and swell pressure decreased by 50%, 60%, 23% and 17% (Fig. 8b) for C-40, C-30, C-20 and C-10 soils respectively. This considerable decrease of swelling for these soils strongly suggests that MICP by biostimulation may be a viable alternative for treating expansive soils.

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 they were below the 1.5% of the generally accepted upper limit for 1-D swell strains (Snethen 1984) however, in the case of MS and GF soils the swell strains were higher than 1.5%. This does not mean that MICP will not work for these soils; these soils may need additional treatment cycles to bring the swelling strain below threshold levels. Additional testing is underway to test this hypothesis and will be presented in future publications.

Fig. 7b and Fig. 8b present the swell pressure data obtained before and after treatments for the natural and artificial soils, respectively. It can be noted from these figures that there was a considerable reduction in swell pressure for all soils tested here. The percentage reduction in case of natural soils ranged from 18 to 70% while the same for artificial mixes ranged from 17 to 60%. The highest reduction in case of natural soils was observed for BR soil while the same for artificial soils was observed for C-30 soil. In case of artificial mixes, the reduction in clay content directly correlated with the reduction in swell strain, i.e., soils with highest clay content had the highest reduction in swell pressure while those will low clay content showed a lower reduction in swell pressure. This indicates better MICP performance with high clay content due to the presence of higher bacterial populations. In the case of natural soils, such correlation was not observed as the origins of the microbial communities differed between these soils and hence, the effect of plasticity or clay content could not be isolated.

Role of Calcium Chloride

In order to ensure that the reduction in swelling observed in these soils is due to the microbial activity and the resulting calcite precipitation and not just the presence of calcium ion which has the ability to suppress swelling due to its larger atomic radius, additional testing was performed on MS soil with different treatment solutions. For these tests, untreated MS was placed inside a consolidometer to perform 1-D swell strain test. Three types of tests were performed, and each of these tests used different chemical solutions as inundating fluid in place of water to cause swelling. Test-A used 250 mM $CaCl_2$, Test-B used the cementation solution, while Test-C used both enrichment and cementation solutions (current MICP protocol). In the case of Test-A and Test-B, soil samples were inundated with their respective solutions for seven days. In the case of Test-C, the test ran for 14 days as there were two solutions involved in this test. The 1-D swell strains determined at the end of these tests were 5.8%, 3.5% and 1.8% for Test-A, Test-B, and Test-C, respectively. The same for samples inundated with deionized water was 17.5%. It is evident that the presence of calcium reduces the swell percentage considerably; however, the bacterial activity in the case of Test-B and Test-C was bringing the swell further down. Another observation from this testing is that the enrichment followed by cementation combination is the most effective of both MICP treatments tested here.

Carbonate Analysis

In order to quantify the carbonate precipitation, a Rapid Carbonate Analyzer was used to determine the amount of precipitated calcium carbonate. In the case of natural soils collected from different sources, their untreated soils contained calcium carbonate. The amount of calcium carbonate for MS soils was nearly zero, and this amount increased to 1.56% (w/w) after MICP treatments. Similarly, the untreated GF, BR, and DC soils had 1.41%, 5.46%, and 1.22% of calcium carbonate while the biostimulated samples had 2.14%, 6.19%, and 1.61%, respectively (Fig. 9a). On the other hand, the untreated artificial soils did not have any considerable amount of calcium carbonate. Fig. 9b shows that the untreated C-40, C-30, C-20, and C-10 soils had nearly zero amount of calcium carbonate content while the biostimulated soils had 0.88%, 0.78%, 0.72%, and 0.43% of calcium carbonate respectively. This increase in calcium carbonate precipitation with the increase of clay content indicates that the activity of soil bacteria present in the clay portion of the soil was essential for precipitating calcite.

SEM Analysis

The SEM and EDX analysis of MS soil are shown in Fig. 10. Fig 10a and Fig. 10c present the SEM image while Fig. 10b and Fig. 10d present the EDX peaks obtained at the corresponding locations. Representative SEM images for one untreated and the corresponding treated soil were presented to identify key features before and after treatments. Similar observations were made for other soils but nor presented here in this paper. It can be observed from Fig. 10b that considerable amounts of Oxygen, Carbon, Silicon, and Aluminum were noticed the EDX graph, but no significant calcium peak was noted, which indicates the absence of calcite in the soil mass before treatment. On the other hand, it is clear from Fig. 10c that calcite precipitated after the treatment and is bridging the soil grains. The EDX graph of biostimulated soils is shown in Fig. 10d which clearly shows the existence of calcium, oxygen, and carbon, which confirms the precipitation of calcium carbonate in the soil pores.

Summary and Conclusions

This research investigated the viability of indigenous bacteria in stabilizing clayey soils with varying plasticity. The research team envisaged the applicability of biostimulation technique using natural microbes present in clayey soils to precipitate calcite and alter clay behavior. Two sets of soils were tested to test this hypothesis, natural soils, and artificial soils. Natural soils helped study the application of MICP in soils with varying plasticity and microbial communities (based on the source from which they were collected) while the artificial soils helped study the impact of clay content on MICP effectiveness by keeping the origin of the microbial communities constant. Both the natural and artificial soils were subjected to treatments to induce calcium carbonate precipitation using the TSDS. Each soil was subjected to one treatment cycle, which included one round of enrichment solution followed by one round of cementation solution injected into the soil. Strength changes due to treatments were observed using UCS test while changes in swell characteristics were observed using 1-D swell strain tests. Calcite precipitation was observed quantitatively using rapid carbonate analyzer and qualitatively using SEM studies.

Major findings from this study are listed as follows:

1. Although there was an increase in the LL and PI of the treated soils, this increase did not adversely impact the swelling or strength behavior of the treated soils. The increase in LL and PI was attributed to the presence of EPS, which increases the organic content of the soil and thereby the LL and PI.

2. UCS values increased for all soils tested in this research except for MS soil tested at OMC and MDUW. While this increase in strength was below the threshold strength required for stabilized subgrade for some soils, it was evident that MICP using biostimulation can increase the strength of clayey soils. Additional treatment cycles may increase strength beyond threshold levels.

3. The increase in strength was attributed to the formation of calcium carbonate within the soil pore.

4. The swelling potential reduced for all soils tested in this research, which shows great promise for mitigating swelling soil problem. The reduction in 1-D swell strain after treatment ranged from 3% to 52% for the artificial mixes and 18% to 70% for natural soils. While this reduction is considerable, two of the natural soils (MS and BR) had 1-D swell strains above the allowable limit (1.5%). These soils could benefit from additional treatment cycles and bring the swell strain below threshold limits.

8

5. It was noted that considerable amounts of calcium carbonate precipitated after the treatments. In the case of artificial mixes, calcite precipitation increased with an increase in clay content. This indicates that MICP is more effective in case of soils containing higher clay contents due to the presence of higher bacterial populations within clay fraction. However, higher clay fraction will reduce permeability, making percolation of treatment solutions slower, thereby requiring a longer time for treatments as evidenced in this research.

6. The SEM and EDX analysis confirmed the presence of calcite inside the biostimulated natural and artificial soils.

7. The duration of each treatment cycle (one round of enrichment and one round of cementation) ranged from two to six weeks for natural soils depending on the clay content of the soil while the same for artificial soils range from two to fourteen days.

Based on these findings, it can be concluded that it is viable for MICP technique via biostimulation to alter the engineering properties of clayey soils with varying plasticity characteristics. However, further studies are needed for better understanding of this phenomenon and establish proper treatment methodologies to ensure consistent performance. This paper is a first step in understanding the potential of MICP as a treatment method for clayey soils.

Acknowledgments

References

Bing, L. (2015). "'Geotechnical porperties of Biocement treated sand and clay.'" School of Civil and Environmental Engineering, Nanyang Technological University.

Boquet E, Boronat A, R.-C. A. (1973). "Production of calcite (calcium carbonate) crystals by soil bacteria is a general phenomenon." *Nature*, 246, 527–529.

Burbank, M. B., Weaver, T. J., Green, T. L., Williams, B., and Crawford, R. L. (2011). "Precipitation of calcite by indigenous microorganisms to strengthen liquefiable soils." *Geomicrobiology Journal*.

Burbank, M., Weaver, T., Lewis, R., Williams, T., Williams, B., and Crawford, R. (2013). "Geotechnical Tests of Sands Following Bioinduced Calcite Precipitation Catalyzed by Indigenous Bacteria." *Journal of Geotechnical and Geoenvironmental Engineering*, 139(6), 928–936.

Burbank Weaver, T., Lewis, R., Williams, T., Williams, B., and Crawford, R., M., Burbank, M., Weaver, T., Lewis, R., Williams, T., Williams, B., and Crawford, R. (2012). "Geotechnical Tests of Sands Following Bio-Induced Calcite Precipitation Catalyzed by Indigenous Bacteria." *Journal of Geotechnical and Geoenvironmental Engineering*, 139(6), 928–936.

Burne, R. A. and Chen, Y. Y. (2000). "Bacterial ureases in infectious diseases." *Microbes and Infection*, 2, 533–542.

Cardoso, Rafaela; Pires, Ines; Duarte, S. O. D. and Monteiro, G. A. (2018). "Effect of clay's chemical interaction on biocementation." *Applied Clay Science*, 156, 96–103.

Cheng, L., and Shahin, M. A. (2015). "Assessment of different treatment methods by microbial-induced calcite precipitation for clayey soil improvement." *68th Canadian Geotechnical Conference*, Quebec, Canada.

Chittoori, B. C. S., Burbank, M., and Islam, M. T. (2018). "Evaluating the Effectiveness of Soil-Native Bacteria in Precipitating Calcite to Stabilize Expansive Soils." *International Foundations Congress and Equipment Expo.*, Orlando, Florida, 59–68.

Chittoori, B., Moghal, A. A. B., Pedarla, A., and Al-Mahbashi, A. M. (2016). "Effect of Density on the Pore Size and Pore Volume of Expansive Clays." *Geotechnical Special Publication*.

Chu, J., Stabnikov, V., and Ivanov, V. (2012). "Microbially Induced Calcium Carbonate Precipitation on Surface or in the Bulk of Soil:" *Geomicrobiology Journal*, 29(February), 544–549.

DeJong, J. T.. Mortensen, B. C., and Nelson, D. C. (2010). "'Bio-mediated Soil Improvement.'" *Ecological Engineering*, 197–210.

DeJong, J. T., Fritzges, M. B., and Nüsslein, K. (2006). "Microbially induced cementation to control sand response to undrained shear." *Journal of Geotechnical and Geoenvironmental Engineering*, 132(11), 1381–1392.

Flemming, Hans-Curt; Wingender, Jost; Griebe, Thomas; Mayer, C. (2000). *"Physico-Chemical Properties of Biofilms."*

Fujita, Y., Taylor, J. L., Gresham, T. L. T., Delwiche, M. E., Colwell, F. S., Mcling, T. L., Petzke, L. M., and Smith, R. W. (2008). "Stimulation of microbial urea hydrolysis in groundwater to enhance calcite precipitation." *Environmental Science and Technology*, 42(8), 3025–3032.

G.N. Obuzor, J.M. Kinuthia, R. B. R. (2011). "Enhancing the durability of flooded low-capacity soils by utilizing lime-activated ground granulated blastfurnace slag (GGBS)." *Eng. Geol.*, 123, 179–186.

Gomez, M. G., Graddy, C. M. R., DeJong, J. T., Nelson, D. C., and Tsesarsky, M. (2018). "Stimulation of native microorganisms for biocementation in samples recovered from field scale treatment depths." *Journal of Geotechnical and Geoenvironmental Engineering*.

Hammes, F., and Verstraete, W. (2002). "Key roles of pH and calcium metabolism in microbial carbonate precipitation." *Reviews in Environmental Science and Biotechnology*, 1(1), 3–7.

Islam, M. T. (2018). "Studying the Applicability of Biostimulated Calcite Precipitation in Stabilizing Expansive Soils." Boise State University.

Jones, C. W. (1958). (1958). "Stabilization of Expansive Clay with Hydrated Lime and with Portland cement." *Highway Research Bulletin 193, Highway Research Board,* 40–47.

Jones Jr, D. E., and Holts, W. G. (1973). "Expansive Soils - The Hidden Disaster." *American Society of Civil Engineers*, 43(8), 49–51.

Jones, L. D., and Jefferson, I. (2012). "Expansive Soils." *ICE manual of geotechnical engineering. Volume 1, geotechnical engineering principles, problematic soils and site investigation*, 413–441.

Little, D. N. (1999). "Evaluation of structural properties of lime stabilized soils and aggregates." *National Lime Association*, 1.

Little, D. N. (2000). *Evaluation of structural properties of lime stabilized soils and aggregates*.

Martinez, B., DeJong, J., Ginn, T., Montoya, B., Barkouki, T., Hunt, C., Tanyu, B., and Major, D. (2013). "Experimental Optimization of Microbial-Induced Carbonate Precipitation for Soil Improvement." *Journal of Geotechnical and Geoenvironmental Engineering*, American Society of Civil Engineers, 139(4), 587–598.

McDonald, E. B. (1973). *'"Experimental moisture barrier and waterproof surface."'*

Mitchell, J.K., and Soga, K. (2013). *Fundamentals of soil behavior*. John Wiley & Sons, Inc. New York.

Mortensen, B. M., Haber, M. J., Dejong, J. T., Caslake, L. F., and Nelson, D. C. (2011). "Effects of environmental factors on microbial induced calcium carbonate precipitation." *Journal of Applied Microbiology*, 111(2), 338–349.

Neupane, S. (2016). "Evaluating the Suitability of Microbial Induced Calcite Precipitation Technique for Stabilizing Expansive Soils." Boise State University.

Petry, T. M., and Little, D. N. (2002). "Review of Stabilization of Clays and Expansive Soils in Pavements and Lightly Loaded Structures—History, Practice, and Future." *Journal of Materials in Civil Engineering*, 14(6), 447–460.

Al Qabany, A., and Soga, K. (2013). "Effect of chemical treatment used in MICP on engineering properties of cemented soils." *Geotechnique*, 63(4).

S M, Rao and Revanasiddappa, K. (2005). *"Role of micro fabric in matricsuction of residual soils."*

Snethen, D. R. (1984). "Evaluation of experdient methods for identification and classification of prentially expansive soils." *Proceedings of the Fifth International Conference on Expansive Soils*, Adelaide, Australia, 22–26.

SOON, N. W. (2013). "Improvements in Engineering Properties of Tropical Residual Soil By Microbially-Induced Calcite Precipitation." (July), 1–162.

Steinberg, M. L. (1981). "Deep vertical fabric moisture barriers under swelling soils." *Transportation Research Board*, 87–94.

Stocks-Fischer, S., Galinat, J. K., and Bang, S. S. (1999). "Microbiological precipitation of CaCO3." *Soil Biology and Biochemistry*, 31(11), 1563–1571.

Thompson, M. R. (1970). *Soil stabilization of pavement systems—State of the art.*

Torsvik, V., Goksoyr, J., and Daae, F. L. (1990). "High Diversity in DNA of Soil Bacteria." *Applied and Environmental Microbiology*, 56(3), 782–787.

Tsesarsky, M., Gat, D., and Ronen, Z. (2016). "Biological aspects of microbial-induced calcite precipitation." *Environmental Geotechnics*, ICE Publishing, 5(2), 69–78.

TxDOT. (2005). "Guidelines for Treatment of Sulfate-Rich Soils and Bases in Pavement Structures." *Texas Department of Transportation*.

U.S. Army TM 5-882-14/AFM 32-1019. (1994). *Soil Stabilization for Pavements. Technical Manual No. 5-822-14; Air Force Manual No. 32-1019, Joint Departments of the Army and the Air Force, USA*.

UNEP. (2010). *Greening Cement Production has a Big Role to Play in Reducing Greenhouse Gas Emissions*.

Van Paassen, L. a. (2009). "'Biogrout, ground improvement by microbially induced carbonate precipitation.'" Department of Biotechnology, Delft University of Technology, the Netherlands.

Van Paassen, L. A., Ghose, R., van der Linden, T. J. M., van der Star, W. R. L., and van Loosdrecht, M. C. M. (2010). "Quantifying Biomediated Ground Improvement by Ureolysis: Large-Scale Biogrout Experiment." *Journal of Geotechnical and Geoenvironmental Engineering*, 136(12), 1721–1728.

Van Paassen, L. A., Whiffin, V. S., and Harkes, M. P. (2007). "Microbial carbonate precipitation as a soil improvement technique." *Geomicrobiology Journal*, 24(5), 417–423.