STUDYING THE APPLICABILITY OF BIOSTIMULATED CALCITE PRECIPITATION IN STABILIZING EXPANSIVE SOILS

by

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DEDICATION

To all the travelers who never returned home

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ABSTRACT

Of the four types of soils, clays are often associated with issues related to low bearing capacity, high compressibility, swelling and shrinking nature. For example, expansive soils swell and shrink with moisture ingress and digress and are prevalent in several parts of the world causing billions of dollars in damages annually to various civil infrastructures. Several ground improvement techniques such as chemical stabilization, deep soil mixing, moisture barriers, and others were employed to counteract these soils. However, these methods are impractical in certain situations and unsustainable in others due to their economic and environmental impacts. Microbiological treatment of soils could provide a more sustainable alternative. Microbial Induced Calcite Precipitation (MICP) is one such process where urease-producing bacteria can precipitate insoluble calcite in the presence of urea and calcium chloride. Researchers have successfully used MICP to alter specific geotechnical properties of the sands and silts and improve the overall behavior of soils. In this research an attempt is made to use this technique on clays and improve their engineering behavior. There are two ways to apply this technology to soils, and those are bioaugmentation and biostimulation. Bioaugmentation is a process where urease-producing bacteria are injected into the soil, whereas biostimulation takes advantage of the indigenous bacteria already present in the soil and stimulates them to precipitate calcite. Past studies 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 selected out of which four soils were artificially made from a natural soil to have similar microbial communities with varying clay content, while the remaining four soils are naturally occurring soils from different locations and had dissimilar microbial communities. Both macro and micro scale studies were conducted on untreated and biostimulated soils to observe changes in plasticity, strength, swelling and mineralogical characteristics. A considerable amount of strength gain, swelling reduction, and calcium carbonate precipitation was observed in this study. It was noted that calcite precipitation via biostimulation could be applied to clayey soils and alter their engineering behavior. It was also observed that the soils were able to precipitate calcite regardless of the origin of microbial communities.

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CHAPTER ONE: INTRODUCTION AND BACKGROUND

1.1 Statement of Problem

Clays are often associated with issues related to low bearing capacity, high compressibility, and swelling and shrinking nature. Most common types of problematic clays are soft clays and expansive clays. This research focused on expansive clays and non-expansive clays with low bearing capacity. Expansive soils (or clays) show large amounts of contraction and expansion with the fluctuation of moisture content (Nelson, J. D. & Miller, 1992). These soils cover most of the region of the world including the United States. These soils are so widespread that it would not be a feasible solution to avoid this type of phenomena. Moreover, the damage to lightly loaded structures built on these soils is more than any other natural disasters such as earthquakes and floods (Jones Jr and Holts 1973). The annual cost of damage for these soils was estimated by several researchers. The estimated cost increased from $2.2 billion/year in 1973 (Jones Jr and Holts 1973) to $15 billion/year in 2012 in the USA (Jones and Jefferson, 2012).

Soil stabilization techniques have been implemented to mitigate expansive soil issues for several decades. Soil stabilization refers to the modification of physical and engineering characteristics of problematic soil to achieve desired strength and workability. Both chemical and mechanical soil stabilization techniques have been implemented to find a sound solution for these clays. Chemical stabilization is the most commonly used technique for these soils. There are a plethora of chemical stabilizers that were used over the years including traditional stabilizers such as lime, Portland cement,

fly ash, and nontraditional stabilizers such as ammonium chloride, sulfonated oils, along with byproduct stabilizers such as kiln dust. Of all these stabilizers lime is the most used, and its use dates back over five decades (Jones 1958). The pozzolanic reaction of lime-stabilized clay, its strength gains, and applicability in the pavement industry have been understood through various research works (Thompson 1970; Little 1999). The Portland Cement Association (1970) described the use of cement materials to alter the properties of highly plastic clay (Little et al. 2000). The combination of lime and Class F fly ash (Little 2000), lime and granulated blast furnace slag (Obuzor 2011) have been used for clay stabilization. Moreover, other chemical agents, e.g., acids or alkalines (Carroll and Starkey 1971) and electro-osmosis or potassium (O'Bannon et al. 1976) are available to stabilize expansive soils.

In the case of mechanical stabilization, the main goal is to limit the infiltration of water as well as increase the strength to hold the pressure applied by the superstructure. In a recent study, Islam (2017) showed that the active zone of expansive soils could be as deep as 11 ft from the pavement surface and installation of moisture barriers would not be a feasible solution in those situations. Moreover, Steinberg (1981**)** investigated the potential use of geomembranes in controlling the behavior of expansive clay. Later, Tamim (2017) compared the performance of geocells, geogrids and hybrid geosynthetic reinforced system (HGRS) in a large box test and observed that the differential heave reduced from 31% to 54%.

The commonly available stabilization techniques and chemical stabilizers have an adverse effect on the environment and economy. The formation of ettringite due to the presence of calcium-based stabilizers e.g. lime, Portland cement and fly ash can cause

swelling and distresses of infrastructures (Little, D. N., and Petry 1992). In addition, the production of cement and lime is a prime source of greenhouse gases (UNEP 2010). As per UNEP (2010), one ton of cement and lime production could release 1 and 1.2 ton of CO2 into the environment, respectively. That report also concluded that around 7-8% of CO2 emissions result from only cement production each year. Besides, the increase in pH due to the addition of lime is affecting both flora and fauna of nature. Mechanical stabilization could have been a reasonable alternative; however, these techniques consume more energy with little economic benefit. Hence, researchers are in search of a sustainable alternative to overcome these drawbacks. Hence, Microbial Induced Calcium Carbonate Precipitation (MICP) is an eco-friendly and sustainable alternative where microbes play a major role to strengthen soils by precipitating calcium carbonate. The MICP is suitable for mitigating seismic-induced liquefaction, reducing permeability and compressibility, and 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). MICP has been implemented on sandy and silty type of 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 on clay or expansive soils. In this study, the applicability of biostimulation technique on expansive clay soils was investigated based on plasticity, strength, swelling and microstructural point of view.

1.2 Background

The mechanism of calcium carbonate precipitation consists of urea hydrolysis and calcium carbonate precipitation (Stocks-Fischer et al. 1999; Hammes and Verstraete

2002; Burbank et al. 2013). Urease-producing bacteria hydrolyze 1 mole of urea ($CO(NH_2)_2$) into 1 mole of ammonia and 1 mole of carbamic acid (Equation 1). Then, carbamic acid decomposes into ammonia and carbonic acid (Equation 2). Ammonia hydrolyzes into ammonium ion, which increases the pH of the system (Equation 3). Carbonic acid dissociates into dissolved inorganic carbonate (Equation. 4). With the creation of nucleation sites and the addition of Ca^{2+} ions to this medium, 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)-

$$\boldsymbol{CO(NH_2)_2 + H_2O \rightarrow NH_3 + H_2HCOOH} \quad \textbf{(1)}$$

$$\boldsymbol{H_2HCOOH + H_2O \rightarrow NH_3 + H_2CO_3} \quad \textbf{(2)}$$

$$\boldsymbol{2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^-} \quad \textbf{(3)}$$

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

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

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

There are two strategies to apply MICP on soils: bioaugmentation and biostimulation. In bioaugmentation, exogenous bacteria are added to soil to encourage calcium carbonate precipitation. Researchers have used bioaugmentation on sandy or silty type of soils using urease producing bacteria for soil improvement (Whiffin et al. 2007; van Passen et al. 2010). However, adding new bacteria can cause several problems, i.e., survivability of exogenous bacteria, uneven distribution, and longer time needed for the permeation of bacteria which is costly for the cultivation and special cautions required while mixing (DeJong et al. 2010; Tsesarsky et al. 2016). In case of the biostimulation, indigenous bacteria are stimulated to achieve calcium carbonate

precipitation. Generally, the number of bacteria per gram of natural soils is 106 to 1012 (Torsvik et al. 1990). Boquet et al. (1973) demonstrated that almost all soil bacteria could precipitate calcite. In order to overcome the difficulties of bioaugmentation, researchers have been stimulating natural microbes to precipitate large amount of calcite (Fujita et al. 2008; Burbank et al. 2011). Later, Neupane (2016) investigated the use of bioaugmentation in expansive clays and found that it could be an alternative solution for mitigating soil swelling.

1.3 Research Objectives and Tasks

The research hypothesis of this thesis is that indigenous urease producing bacteria can be stimulated using substrate solutions to precipitate calcite which assists in stabilizing expansive soils. To validate this hypothesis, several research objectives were considered and listed here-

1) To study the effect of biostimulation on clayey soils having similar and dissimilar microbial communities

2) To study the effect of biostimulation on varying clay content

3) To study the effect of biostimulated on clayey soil's plasticity characteristics

4) To study the effect of biostimulated on clayey soil's strength characteristics

5) To study the effect of biostimulated on clayey soil's swelling characteristics

6) To study the effect of biostimulated on clayey soil's mineralogical characteristics

A pictorial representation of research work is shown here-

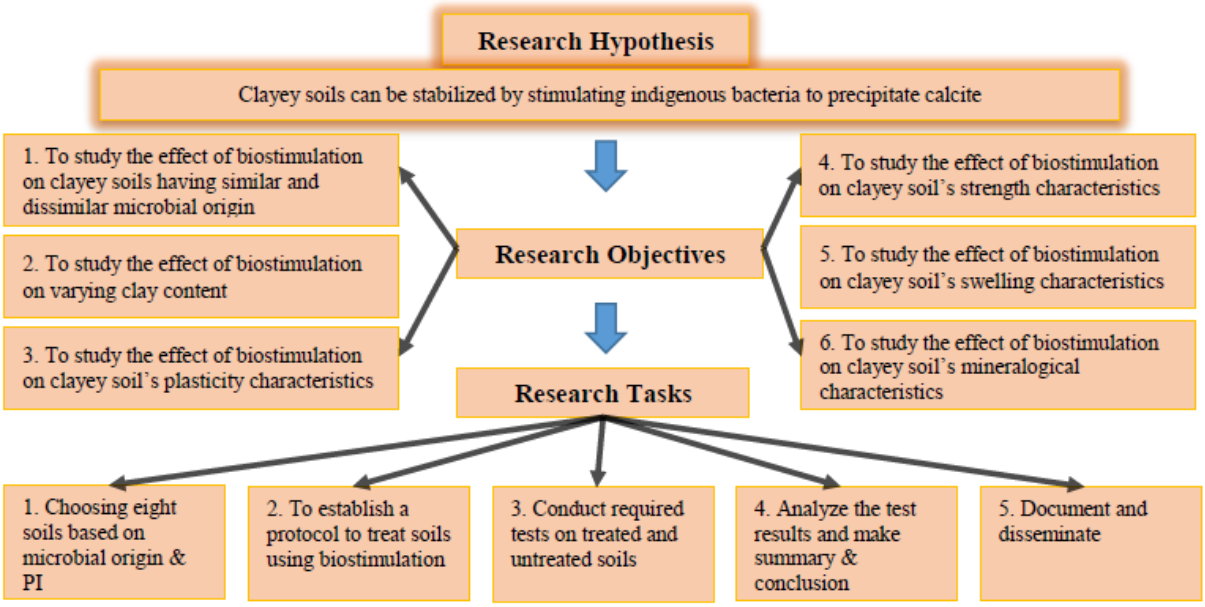

Figure 1-1: Pictorial representation of research work

The research tasks to accomplish these research objectives are given here-

a) Four expansive natural soils and four artificial soils were selected to study the effect of biostimulation on expansive soils having similar and different microbial origin. Four natural soils were chosen to observe the effect of biostimulation regardless of the origin of soils. These four natural soils were collected from four different locations situated in Idaho and Montana. On the other hand, the four artificial soils were prepared by adding sands with one natural soil to establish four artificial mixes of varying plasticity.

b) A protocol was established to treat all these eight soils. Treatment Solution Delivery System (TSDS) was incorporated to facilitate the treatment phase as these soils have low permeability and it would take a longer period to complete the treatment cycles.

c) The Atterberg Limits test, compaction tests, unconfined compressive strength test and 1-D swell test were conducted on both untreated and biostimulated soils to understand the effect of biostimulation on clayey soil's plasticity, strength, and swelling characteristics.

d) The carbonate determination test, X-ray diffraction test and scanning electron microscopy tests were conducted on untreated and biostimulated soils to understand the effect of biostimulated soils on mineralogical characteristics.

1.4 Organization of the thesis

This thesis consists of an overall introduction in Chapter 1 and two manuscripts; where manuscripts are inter-related to each other and serve a common purpose. In both manuscripts, the applicability of biostimulation technique is investigated to stabilize the expansive soils by precipitating calcium carbonate. Manuscript one explains the

effectiveness of this technique on two soils from the same microbial origin and different plasticity characteristics. This manuscript was published in the International Foundation Congress and Equipment Expo Conference (IFCEE 2018) in Orlando, Florida. Manuscript two is a continuation of manuscript one where four natural soils and four artificial soils were chosen to study biostimulation. This manuscript was submitted to the ASCE Journal of Materials in Civil Engineering.

CHAPTER TWO – EVALUATING THE EFFECTIVENESS OF SOIL-NATIVE BACTERIA IN PRECIPITATING CALCITE TO STABILIZE EXPANSIVE SOILS

Abstract

The use of chemical additives to stabilize expansive soils is a common practice. However, the environmental concerns associated with greenhouse gas generation during the production of these chemicals has launched engineers in search of sustainable stabilization alternatives. Microbial Induced Calcite Precipitation (MICP) is a bio-cementation technique that could be a potential solution to this problem. Typically, MICP is achieved via bio-augmentation; however, bio-stimulation was argued to be a more realistic alternative due to its field implementation potential. Hence, in this research study, two expansive soils with varying plasticity characteristics were examined to understand the potential of MICP in treating expansive soils. These two soils were subjected to MICP treatments using enrichment and cementation solutions. The treatment effectiveness was studied via response measures such as Atterberg limits, unconfined compressive strengths, one-dimensional swell test and Calcium Carbonate precipitation. The results indicate that MICP has potential in stabilizing expansive soils and further research is warranted to explore this idea.

2.1 Introduction

Clayey soils in general present major geotechnical challenges to engineering and construction firms at significant costs. Engineering properties of clays span extreme

ranges, exhibiting high shear strengths when dry, to being very soft under wet conditions. Expansive clays have been a major concern since they swell and shrink as moisture fluctuates. As a result, structures built on expansive soils tend to undergo moderate to severe cracking problems (Mackenzie & Mitchell 1966; A. J. Puppala, E. Wattanasanticharoen 2003). Lightly loaded structures such as one or two-story residential and industrial structures and pavements have experienced severe damage (Petry and Little 2002), often associated with substantive repair and mitigation costs. In their study of U.S. construction, (Jones and Holtz 1973) show losses associated with the repairs of damaged structures constructed on expansive soils as close to $9 billion per year.

Researchers have developed several methods to resolve all these construction problems resulting from the expansive soil. Petry and Little (2002) present a historical perspective on expansive soil treatment dating back to the late 1950s. In their work, several stabilization methods including mechanical compaction, chemical stabilization, pre-wetting and moisture barriers, lime injections, and deep soil mixing were described. Altering the physicochemical behavior of these soils by mixing with chemicals such as lime and cement is a widely-used approach both in the United States and around the world (Sherwood 1993). However, doing so raises environmental concerns because of: (1) greenhouse gases generated to produce these chemicals; and (2) negative impacts on plant growth that come from elevated pH levels in the soils after treatment. The elevated pH levels (often >12.4) become a major problem where soil erosion is a concern and plant growth is necessary to protect soils against erosion. Environmentally safe techniques such as pre-wetting and moisture barriers are only possible for small confined spaces and are not suitable for larger construction projects such as highways and railways

which spread for miles especially in the case of high swelling soils where the active zone can extend several meters into the ground.

Therefore, an environmentally-friendly alternative that is sustainable and cost-effective is needed. Turning soils into a cement-like material utilizing bacteria known as biocementation is one such method that can be a viable alternative to treat expansive soils. The most successful biocementation process to date is microbial induced calcite precipitation (MICP) using *Sporosarcina pasteurii*. In this method, microorganism hydrolyzes urea and facilitates the formation of calcium carbonate (or calcite) in the presence of calcium source (Al Qabany & Soga 2013). MICP had successful implication on sandy soil according to the previous studies (Chu et al. 2012; DeJong et al. 2006). It has become a subject of research in recent years (Chu et al. 2012; DeMuynck, DeBelie & Verstraete 2010).

Despite advances in the understanding of MICP and a few field trials, the necessity of cultivation and injection of bacterial strain hinders this technology to become a cost-effective approach. From the environmental perspective, uncertainty regarding the ecological consequences of introducing non-native bacterial culture into natural soil ecosystem has become a challenge. Therefore, the role of indigenous bacteria in the bio-cementation process must be considered to determine the feasibility of MICP as a field-scale implementation (Gomez et al. 2015). Biostimulation is the process of modification of environmental conditions such as substrates, nutrients, electron acceptors to improve indigenous microorganism with desirable metabolic capabilities (Snoeyenbos-West, Nevin, Anderson, & Lovley 2000).

Previous results proved that soil improvement through the bio-stimulation process has the potential to improve soil properties in situ for sandy and silty soils (Burbank et al. 2011). In this research study, an attempt is made to broaden the horizons of this technique by applications into expansive soil treatment. Laboratory experiments were performed where indigenous microbes in expansive natural soils were stimulated to hydrolyze urea in the presence of divalent calcium ions and thereby to cause the precipitation of calcite within the pores of the soil. This paper presents the details of this study and the findings thereof.

2.2 Background

Microorganisms that are capable of hydrolyzing urea to carbon dioxide and ammonia are common in soils (Burbank et al. 2011; Lloyd & Sheaffe, 1973) showed that 17-30% of microorganisms from cultivable aerophilic, microaerophilic and anaerobic microorganisms are capable of hydrolyzing urea. 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: $Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3$. The generation of NH_4^+ increases local pH (~8.5), and importantly further increases the rate of calcium carbonate precipitation (Hammes and Verstraete 2002). Microbial-induced calcite creates a bridge between soil grains which cements soil grains together (DeJong et al. 2006).

There are two approaches to apply MICP: bio-stimulation and bio-augmentation. In bio-stimulation, indigenous bacteria are stimulated with a nutrient and carbon source to increase in number and calcite precipitation (Burbank et al. 2011) depending on the availability of calcifying bacteria and also on spatial distribution. In the case of bio-

augmentation, exogenous bacteria are provided to the soil system. Augmented culture to survive and work effectively in a new environment is difficult because of the presence of native microorganism which affects their survival rate and metabolic potential (Wenderoth et al. 2003). Several research studies have injected solutions containing a model ureolytic bacterium, *Sporosarcina pasteurii*, into soil followed by passing nutrient solution which induces the calcite precipitation. Problems such as the uneven distribution of bacteria and clogging near the inlet due to calcite precipitation were reported in the case of bio-augmentation (Stocks-Fischer et al. 1999). Also, it was observed that the survivability of exogenous microorganisms, after introduction into a new environment tend to decline rapidly and rarely propagate (van Veen et al. 1997). In one study, it was shown that a bacterium strain which was isolated from a coastal marsh in Louisiana and grown in the laboratory could be reintroduced to their environment but failed to survive into another similar coastal marsh environment (LaRock and Donovan, 2001).

On the other hand, in bio-stimulation elimination of non-native bacterial cultivation and injection into the soil can be avoided. (Burbank et al., 2011) discussed the feasibility of biostimulation of MICP treatment based on the soil sample collected from Snake River through laboratory and field testing. Also, in one-dimensional centimeter scale column experiments, calcite precipitation through bio-stimulation was possible in a variety of granular soils from the depositional environment (Gomez et al. 2015). There are investigations needed to address the possibilities and limitation related to the biostimulation process. Further, MICP through bio-stimulation to stabilize expansive soil is still a hypothesis. This research is an initial step to check the feasibility of this hypothesis and to understand the challenges associated with stabilizing expansive soil.

2.3 Materials and Methods

To evaluate the effectiveness of MICP in mitigating expansive soil swelling, two natural soils with varying plasticity characteristics were selected. Both soils were obtained from Marsing, Idaho along highway US-95 that runs north-south along the Idaho/Oregon border. The soils are denoted as S1 and S2. Soil S1 has a liquid limit of 111 and a plasticity index of 70.6 while soil S2 has an LL of 62 and PI of 40.7. Both soils are considered to have high swelling potential. The soils were obtained in their natural form without much disturbance to the microorganism population. The soils were first tested for various geotechnical engineering properties such as maximum dry unit weight (MDUW) and optimum moisture content (OMC); unconfined compression strength (UCS) along with one-dimensional (1-D) swell strain and swell pressure as per the corresponding ASTM standards provided in Table 2.1.

Table 2.1: Baseline data for the two natural soils tested in this research

Soil Type	LL	PI	MDUW (kN/m^3)	OMC (%)	UCS (kPa)	1-D Swell Strain (%)	Swell Pressure (kPa)
	(ASTM D4318)		(ASTM D698)		(ASTM D2166)	(ASTM D4546)	
S-1	111	71	10.95	32.6	156.36	17.9	287
S-2	62	41	13.98	28.5	76.49	9.14	179

Note: LL-Liquid limit; PL-Plasticity Index; MDUW-Maximum Dry Unit Weight; OMC-Optimum Moisture Content; UCS-Unconfined Compression Strength

The soil samples were subjected to MICP using the bio-stimulation process. The bio-stimulation process requires that the ureolytic bacteria present in the soil be stimulated by providing the necessary nutrients and ensure urea hydrolysis. Once the bacteria start to hydrolyze urea calcium is introduced into the soil system so that calcium carbonate is precipitated. The solution containing the nutrients and urea is called an *enrichment solution* while the solution with the calcium source is termed a *cementation solution*. Since expansive clays have very low permeability gravity feeding these solutions into the soil microcosm is very time taking. Hence a new device is developed that can deliver the treatment solutions at a faster pace.

2.4 Development of Treatment Solution Delivery System (TSDS)

The TSDS was designed and developed to deliver treatment solutions to the microorganism in soil samples at different pressures. Trial runs were performed for ensuring no leakage while doing the final test run. Four chambers have been constructed where two sources for enrichment solutions and cementation solutions have been separately connected. Solutions were able to provide a specified flow pressure.

In this setup, a schedule 80 clear Poly Vinyl Chloride (PVC) chamber houses the soil sample on a 5 cm thick PVC base pedestal. Latex membranes wrap around the soil sample to protect it from unwanted surface erosion and soil samples with latex membranes were shown in Figure 1. Both the top cap and the bottom pedestal had grooves that are capable of holding O-rings that hold the latex membrane tightly in place and also restrict water from percolating through the gap between soil sample and membrane. Holes in the top cap allow water and treatment solution to flow through them. The bottom pedestal was glued to the base plate and includes holes with a puddle arrangement to collect effluent from the sample. Once the soil sample was ready, we placed the PVC chamber to the base plate. We selected a scheduled PVC clear tube to accommodate threaded connections. Soil samples in the treatment delivery system were shown below in Figure 2-1. After adjusting all the connections, the chamber is usually filled with a treatment solution through a pipe arrangement from a pressure-regulated water reservoir above the base plate.

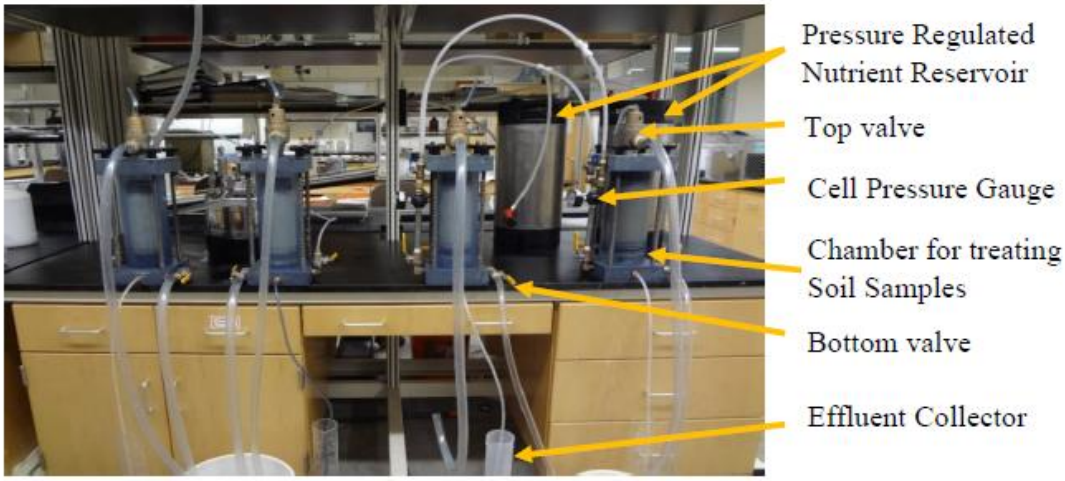

Figure 2-1: Soil samples in TSDS

2.5 Treatment Solutions

As discussed earlier, two types of treatment solutions were used in this research. The enrichment solution consisted of 100 mM of Sodium Acetate, 333 mM of Urea, 0.5 g/L of Corn Steep Liquor (CSL). The cementation solution consisted of 100 mM of Sodium Acetate, 333 mM of Urea, 0.5 g/L of Corn Steep Liquor (CSL) along with 250 mM of Calcium Chloride. Corn steep liquor consists of amino acids, vitamins, and minerals necessary for microorganism survival. Hence, it is congenial to grow bacteria, it was provided in both the enrichment solution and the cementation solution. The enrichment solution stimulates the growth of bacteria which uses acetate as a carbon source and urea or ammonia as a nitrogen source. The increase in the pH results from the production of ammonia from urea hydrolysis which creates an environment that is favorable for bacteria. When the microbe population becomes more ureolytic, more hydrolysis happens and more calcite is precipitated (Burbank et al., 2011).

2.6 Test Protocols

The S-1 and S-2 soil samples were prepared using their respective OMC and MDUW. Static compaction was used to compact the specimens in order to ensure continuous pore connectivity within the sample which will ease the flow of water. The prepared soil samples were wrapped using latex membranes and were placed inside the PVC chamber. The chamber is then closed and the enrichment solution is allowed into the chamber. Using the top and bottom valves it is ensured that there are no air bubbles at the top of the chamber. After checking all the connections, the enrichment solution was allowed to pass through the soil specimen under 20 psi pressure. It was decided to collect one pore volume of the effluent after which the effluent is tested for a pH. When the pH

reached 8.0 or higher the enrichment solution is stopped and cementation solution was started to initiate the precipitation of calcite within the soil mass. The pH of 8~9 was achieved throughout the processes of enrichment and cementation.

2.7 Result and Discussion

Several geotechnical tests including Atterberg limits, Unconfined Compression Strength, and 1-D Swell tests were conducted after the treatment process was complete to evaluate the plasticity, strength and swelling behavior of expansive soil. The amount of calcium carbonate present in the soil before and after treatments was also determined. Table 2.2 presents a summary of these test results and the following sections discuss these data.

Table 2.2: Treated test results of two natural soils tested in this research

Soil Type	LL	PI	UCS after Treatment (kPa)	UCS before Treatment* (kPa)	1-D Swell Strain (%)	Swell Pressure (kPa)
S-1	139	101	77.22	17.47	13.13	191
S-2	76	57	42.12	21.54	5.96	95

2.7.1 Atterberg Limits

Figure 2-2 presents the variation of LL and PI for both soils before and after treatments. It can be observed that the liquid limit for S1 and S2 soil increased after treatment. The LL increased by 28% and 14% for S-1 and S-2 while the PI increased by 29.5% and 16.1%. Similar results were observed by Neupane (2016). Possible reasons for this increase could be the presence of extracellular polymer substance (EPS) secreted by microbes during the formation of biofilm. EPS can work as a sponge which can absorb water from the environment. In an EPS matrix surface water can be attracted by osmotic and capillary forces (Or et al. 2007).

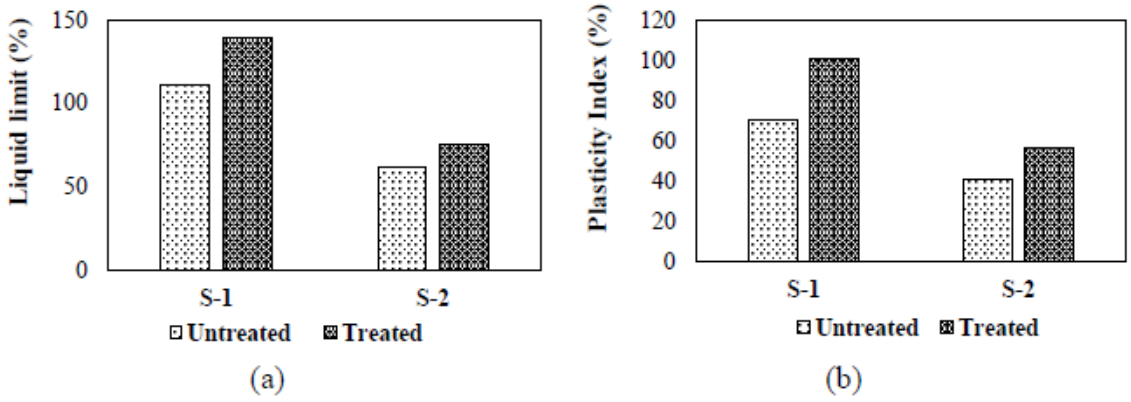

Figure 2-2: Comparison of test results of treated soil with untreated soil (a) Liquid Limit and (b) Plastic Limit

2.7.2 Unconfined Compressive Strength

Figure 2-3a presents the UCS test results obtained before and after MICP treatments for both soils S1 and S2. The UCS values shown for treated soils were tested immediately after one pore volume of cementation solution was collected. Hence the moisture content at which these samples were tested were different from the control soil samples which were tested at OMC. In order to be able to compare the UCS values before and after treatments control soil samples were re-compacted at the same moisture content at which the treated soils were tested and UCS values were determined. After treatment, the moisture content for S1 and S2 soils was determined to be 70% and 59% respectively. Comparing these values, UCS increased by 77% and 49% for S-1 and S-2 respectively.

Figure 2-3b presents the variation in initial tangent modulus for both soils before and after treatments. This modulus is obtained from the stress-strain curves generated during the UCS testing. It can be noted that the stiffness of the treated samples increased with treatment and this could be due to the higher stiffness of the calcium precipitated.

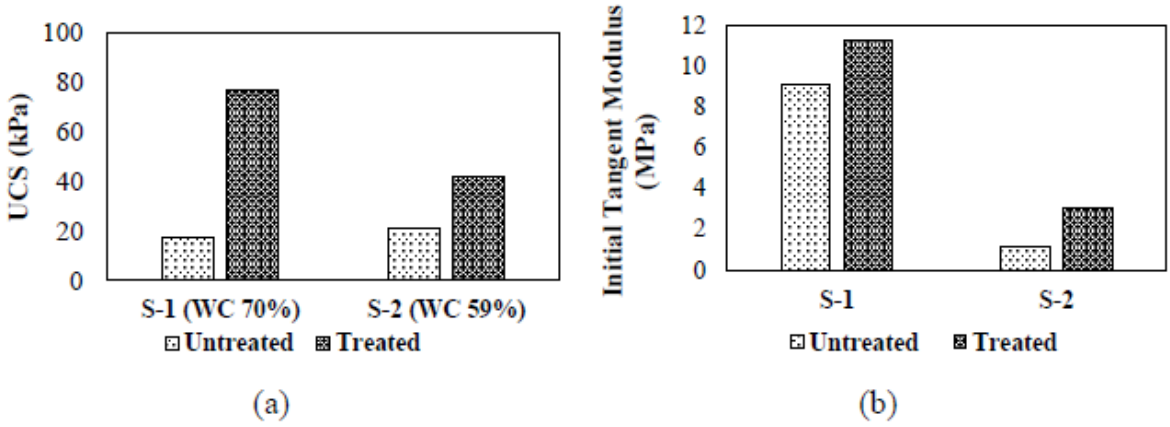

Figure 2-3: Comparison of test results of treated soil with untreated soil (a) UCS and (b) Initial Tangent Modulus

2.7.3 One-Dimensional Swell Strain and Swell Pressure

1-D Swells tests were performed on treated soils on re-compacted oven-dried samples. Similar swelling and loading sequences to control soil samples were followed. Test results presented in Figure 2-4a show that the swell strain decreased by 27% and 35% for soils S1 and S2 respectively. Similarly, the swell pressures were also observed to decrease by 33% and 47% for S1 and S2 soils respectively (Figure 2-4b). This reduced swell strain and stress could be due to the precipitation of calcite which binds soil particles. Hence, this study shows that MICP could be used for expansive soil treatments and further studies are underway to establish threshold levels where MICP could be effectively used in expansive soil treatments.

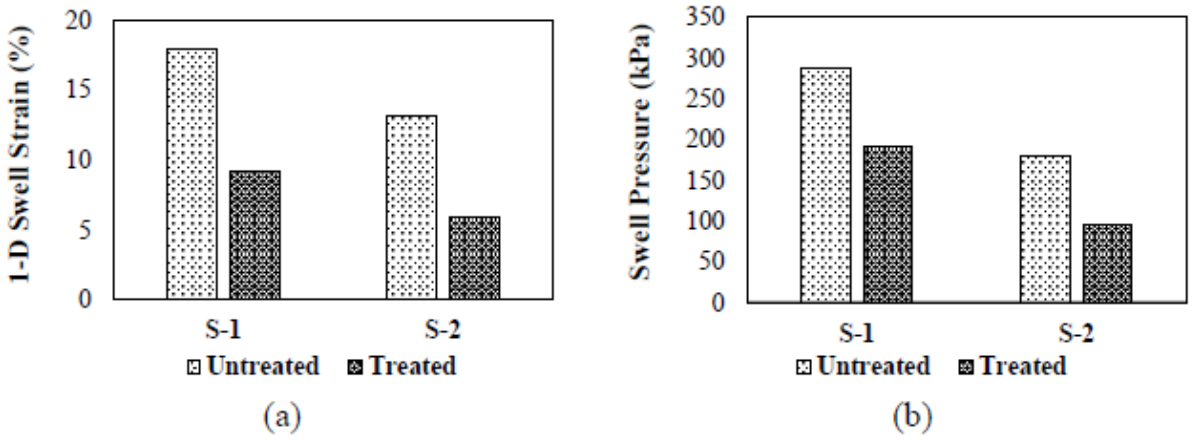

Figure 2-4: Comparison of test results of treated soil with untreated soil (a) 1-D Swell Strain (b) Swell Pressure

2.7.4 Calcium Carbonate Content

In addition to the UCS and 1-D Swell tests percentage calcium carbonate was also determined on untreated and treated soil samples. Precipitated calcium carbonate was detected using Rapid Carbonate Analyzer. Test results show that the control soil samples did not contain any calcium carbonate while the treated soils contained 1.56 % and 0.88% of calcium carbonate (by dry weight of the soil) for S-1 and S-2 soils respectively as shown in Figure 2-5a. This amount of precipitation was obtained after one MICP treatment and resulted in strength increase and swell reduction. The incorporation of more treatment cycles could increase more calcite precipitation. The challenge right now is the permeability of the soil samples. Due to the precipitation of calcite and other microbial activity within the pore spaces of the soil sample the permeability is further reducing which means that the treatments could take longer. The permeability changes before and after treatments are presented in Figure 2-5b.

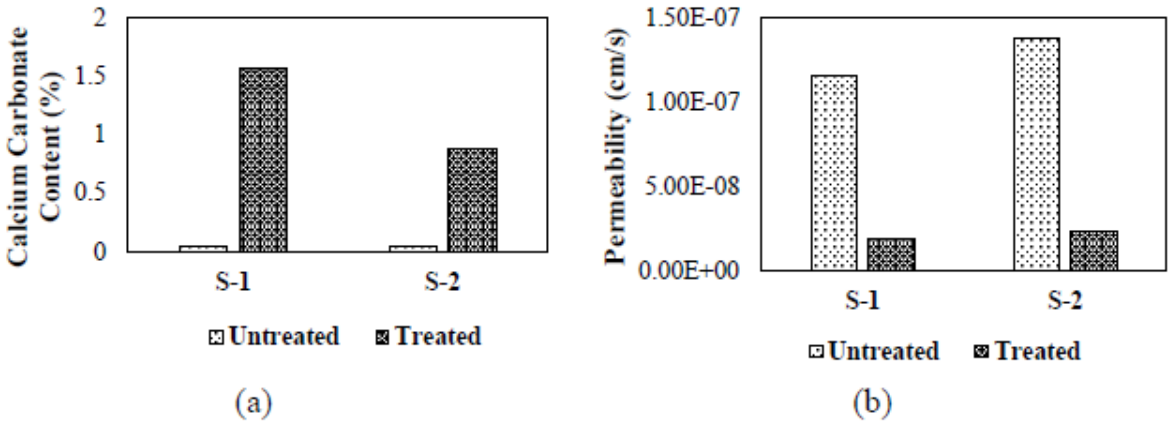

Figure 2-5: Comparison of test results of treated soil with untreated soil (a) Calcium Carbonate Content (b) Permeability

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CHAPTER THREE – APPLICATION OF BIOSTIMULATED CALCIUM CARBONATE PRECIPITATION TO STABILIZE EXPANSIVE SOILS

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. Various 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. Its application to treat clays, especially expansive clays, is novel in this research. Hence, this research investigates the applicability of MICP via biostimulation to treat expansive soils. For this purpose, eight soils were selected out of which, four soils were collected from four different locations representing dissimilar microbial communities while the remaining four soils had similar microbial communities. Both macro and micro scale studies were conducted on untreated and biostimulated soils to observe strength gain, swelling reduction, and calcium carbonate precipitation. The results show that MICP via biostimulation would be a promising method to treat problematic clayey soils.

3.1 Introduction and Background

Clayey soils especially expansive soils have been problematic to civil infrastructures for several decades. Estimated annual costs related to 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 expansive nature of these soils is due to the clay mineral montmorillonite, which expands upon the addition of water, and contracts upon the removal of water. These volumetric changes due to moisture variation cause damages to lightly loaded structures such as pavements, retaining walls, and residential houses. These damages are usually in the form of pavement heaving, uplifting of the foundation, failures of slopes and retaining walls and overall instability of the structures. 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 expansive soils. Cement, lime, fly ash, and granulated blast furnace slag have been used to treat expansive 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 type of soils (McDonald 1973; Steinberg 1981). However, those stabilization techniques and chemical stabilizers have an adverse effect on the environment and economy. The production of cement and lime is a prime source of greenhouse gases (UNEP 2010). This report (UNEP 2010) mentioned that one ton of cement and lime production could cause 1 and 1.2 tons of CO_2, respectively. That report also concluded that around 7-8% of CO_2 emissions result from only cement production each year. Conversely, for every metric ton of urea hydrolyzed during MICP, 733 kg of

CO_2 is sequestered in soil and mineralized as calcite. Besides, the increase in pH due to lime treatment can affect both flora and fauna of nature. From these points, we must strive to develop sustainable and eco-friendly solutions to mitigate the problems of expansive soils.

Microbial Induced Calcium Carbonate Precipitation (MICP) is an environmental-friendly and 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, and 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).

3.2 Application of MICP

MICP has been implemented on sandy and silty type 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 on clays or expansive soils. The major hindrance of introducing MICP in clay is the geometric compatibility between soils and microbial communities. 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 (macropores), 6 to 0.01 μm (medium pores) and 0.01 to 0.002 μm (micropores). 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 the pores of 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 the injection method for soils having less than 5% clay content, though a 150% increase in 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.

There are two strategies to apply MICP on 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). However, adding new bacteria can cause several problems, i.e., survivability of exogenous bacteria, uneven distribution, and 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. 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. In order to overcome the difficulties of bioaugmentation, researchers have been stimulating natural microbes for precipitating large amounts of calcite (Fujita et al. 2008; Burbank et al. 2011). To date, Neupane (2016) only has investigated the use of bioaugmentation in expansive clays and found that it could be an alternative solution for mitigating soil swelling. The author chose three soils having low, medium and high plasticity. Lime and MICP treatments were performed with different curing periods, treatment cycles and bacterial population. 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 added to soil and cured for 7 days. In another protocol, different concentrations of cultured bacteria (10^8 and 10^{10} microbes/gm) were added in the soils and the substrate was injected through the soils at 1, 3 and 7 pore volume. Treated and untreated soils were tested for strength as well as swelling data and showed promising results for the applicability of MICP in clay.

In this study, the applicability of the biostimulation technique on natural expansive clay soils was investigated based on plasticity, strength, swelling and microstructural point of view. Eight soils were chosen from different locations of Idaho and Montana. Those soils were divided into two broad categories, i.e., different microbial origin (four natural soils) and same microbial origin (four artificial soils). No additional bacteria were added to these eight soils and only the existing indigenous soil microbes

were present in the tested soils for the biostimulation experiments. To prepare artificial soils having the same microbial communities, commercially available sands was added to one natural soil resulting in four artificial mixes having different clay contents. This initiative was required to understand how soils behave with increasing clay content using the biostimulation technique. On the other hand, four natural soils were chosen to have different microbial communities based on their source of origin. This research work was initiated to gauge the behavior of clayey soils regardless of the microbial origin. The research team chose treatment solutions (e.g., enrichment and cementation solution) to stimulate the indigenous bacteria for precipitating calcium carbonate in those soil mixes. A Treatment Solution Delivery System (TSDS) was installed to accelerate the treatment phase of clay. This device is connected with pressurized cylinders to inject treatment solutions for low permeability soils. After injecting one pore volume of enrichment and one pore volume of cementation solution from each type of natural and artificial soils, response measure tests that included Atterberg Limit test, Unconfined Compressive Strength, 1-D Swell test, Calcite determination test, XRD, and SEM test were conducted to observe the changes in expansive soils before and after MICP treatment.

3.3 Materials and Methods

3.3.1 Soil Types

To understand the applicability of biostimulation on clayey soils, eight soils were chosen where four natural soils had different microbial origin while the four artificial soils had a same microbial origin. The test soils were collected from Idaho (ID) and Montana (MT). The natural soils are denoted as MS (Marsing, ID), GF (Great Falls, MT), DC (Dry Creek, MT) and BR (Bad Route, MT). The four artificial soils were prepared by

adding a certain amount of medium to fine sand (D_{60} = 0.68, D_{10} = 0.24 and C_u = 2.83) to MS soils in order to create a soil mix with predetermined clay content. 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). Soil classification was determined according to USCS and AASHTO. Sieve and Hydrometer Analysis (ASTM D422) were conducted to determine the soil gradation for all 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. The MS and GF are classified as A-7 soils and DC and BR are classified as A-6 and A-7-6 respectively. Again, 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, A-2-6 respectively.

3.3.2 Macro scale studies

The selected soils were subjected to several geotechnical tests including Atterberg Limits test (ASTM D4318), Standard Proctor Compaction test (ASTM D698), Unconfined Compressive Strength test (ASTM D2166) and 1-D Swell test (ASTM D4546) for determining the baseline data for artificial and natural mixes to compare with bio-stimulated soils. Table 3.1 and Table 3.2 represent the baseline data for 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 soils. This guideline was taken from the research of Chen (1988). No significant correlations were found between the four natural soils with regard to MDUW, OMC, UCS, and swelling. This could be due to the presence of different clay mineralogy in those soils.

For artificial soils, the maximum dry unit weight ranged from 13.98 to 16.65 kN/m^3 and the OMC ranged from 28.5 to 16.5 % with the decrease in clay content. A considerable increase in maximum dry unit weight and a decrease in optimum moisture content with the decrease of clay content were observed here. The same correlation was found in the case of UCS values of artificial soils. The increase of clay particles from C-10 soils to C-40 soils contributed the gradual increase of unconfined compressive strength in those soils. The inert bonding of finer particles might be another reason for this gradual improvement of strength. Besides, the 1-D swell strain ranged from 9.14 to 0.03% and the swell pressure ranged from 9 to 191 kPa with the decrease of clay content in artificial soils. As clay has a different mineralogical structure, which causes swelling, it is concluded that the higher the clay content, the higher the swelling strains and swell pressure for artificial soils.

Table 3.1: Establishing baseline data for natural soils

Soil Type	LL	PI	MDUW (kN/m^3)	OMC (%)	UCS (kPa)	1-D Swell Strain (%)	Swell Pressure (kPa)
	(ASTM D4318)		(ASTM D698)		(ASTM D2166)	(ASTM D4546)	
MS	111	71	10.95	32.6	156.36	17.90	287
GF	103	62	12.84	36.7	159.26	10.27	210
DC	37	17	17.25	16.9	166.37	1.15	40
BR	42	16	16.57	19.8	370.18	1.38	50

Note: LL-Liquid limit; PL-Plasticity Index; MDUW-Maximum Dry Unit Weight; OMC-Optimum Moisture Content; UCS-Unconfined Compression Strength

Table 3.2: Establishing baseline data for artificial soils

Soil Type	LL	PI	MDUW (kN/m^3)	OMC (%)	UCS (kPa)	1-D Swell Strain (%)	Swell Pressure (kPa)
	(ASTM D4318)		(ASTM D698)		(ASTM D2166)	(ASTM D4546)	
C-40	62	41	13.98	28.5	142.71	9.14	191
C-30	43	23	15.63	21.5	115.12	2.58	70
C-20	36	16	16.10	18.7	99.03	0.47	22
C-10	27	12	16.65	16.5	76.73	0.03	9

Note: LL-Liquid limit; PL-Plasticity Index; MDUW-Maximum Dry Unit Weight; OMC-Optimum Moisture Content; UCS-Unconfined Compressive Strength

3.3.3 Micro Scale Studies

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray spectrometry (EDX) are useful tools to take images and qualitative analysis on the atomic scale. To determine the microstructure of both artificial and natural soils before and after treatment, X-ray diffraction (XRD) test and Scanning Electron Microscopy (SEM) test were conducted. In addition to these two tests, the carbonate content determination test (ASTM D4373) was also performed on treated and untreated soils to estimate the amount of calcite precipitation.

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 a 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.

Although, the quantitative analysis of carbonate was performed using this device, the presence of calcium carbonate could not be confirmed with this test alone. The resulted CO_2 pressure could be from magnesium carbonate or other forms of carbonate present in the soil. In order to confirm the presence of calcium carbonate in the soils, XRD tests were performed on all soils. It is a quantitative analysis used to analyze the microstructure of the soils. XRD test can easily identify the precipitated calcium crystals. In this research, the XRD test was performed using Cu-Kα radiation. The range of 2θ was in between 2° to 80° at 2 sec step time and 0.02 step-size. The soil samples were crushed into finer particles and placed on a slit. Usually, 40 kV and 30 mA settings were chosen for doing XRD tests. The output data of the XRD test was analyzed and compared using the standard calcite phase (PDF 00-002-0629) collected from the Powder Diffraction File (PDF) database of International Center for Diffraction Data (ICDD) and the previous research (Burbank et al. 2013).

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 the Idaho Microfabrication Laboratory (IML) situated at Boise State University. With an accelerating voltage of 2 kV and current of 25 µA with T2 secondary electron detectors optimal quality images were used in 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.

3.3.4 Treatment Process

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 the enrichment solution were sodium acetate (100 mM), urea (333 mM) and corn steep liquor (0.5 g/L). The purpose of using the enrichment solution was to stimulate the growth of bacteria where acetate acted as a carbon source and urea or ammonia acted as a nitrogen source. The corn steep liquor supplies amino acids, vitamins, and minerals. The ingredients of the 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 the 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 expansive soils using biostimulation, the research team followed a distinct protocol. A pictorial representation of the protocol was shown in Figure 3-1. First, a specific soil type (either natural or artificial soils) was chosen to start the treatment phase. This soil was compacted at the maximum dry density and optimum moisture content using a Static Compactor to ensure uniform pore spaces throughout the soil specimens. Prepared soil specimens (3’’x 6’’) were kept inside the Treatment Solution Delivery System (TSDS) and this TSDS is a special device constructed to treat highly permeable soils with the 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. So, overall completion of the treatment cycle was considered complete when one pore volume of enrichment followed by one pore volume of cementation solution was collected through the soil specimen. One pore volume was targeted for a longer period of treatment as low permeable clay soils could take 4-6

weeks for one round of the treatment cycle. Treated soil specimens were dried and kept for conducting other tests.

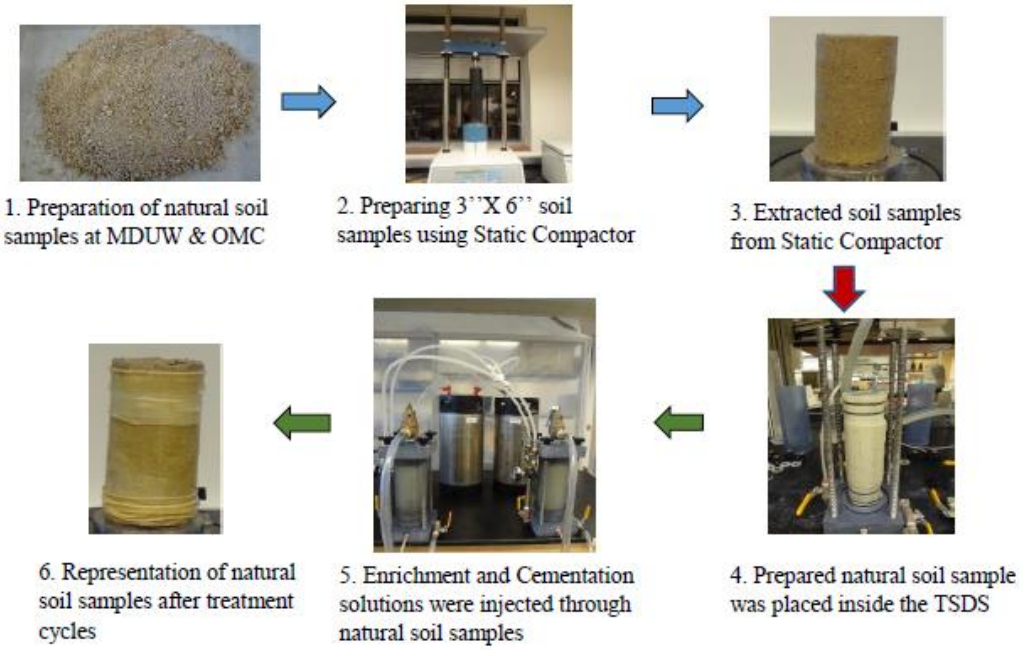


Figure 3-1: Pictorial representation of biostimulated treatment process of artificial and natural soils

3.3.5 Treatment Solution Delivery System (TSDS)

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 PVC tube that houses soil samples that are 2.8 in. (71 mm) in diameter and 5.6 in. (142 mm) in 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 (

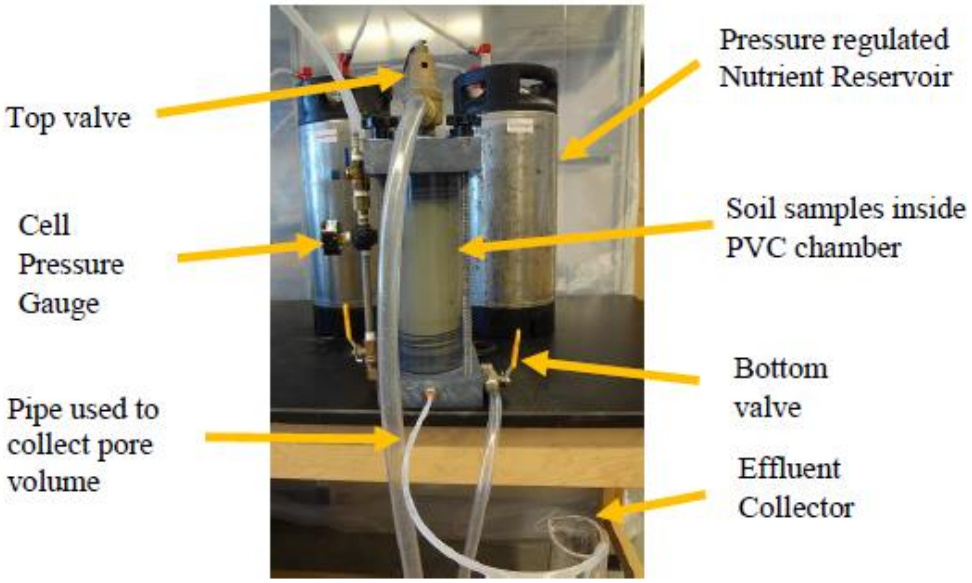


Figure 3-2). Inside the PVC chamber, the soil sample rests on a bottom pedestal and is covered using a top cap. Latex membranes were used to wrap around the soil sample as well as the pedestal and top cap to protect it from unwanted surface erosion. Both top cap and bottom pedestal have grooves to accommodate 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 2 psi to 20 psi (14 kPa to 137 kPa). All the chambers were thoroughly checked for leaks and safety tested at a pressure of 20 psi (137 kPa).

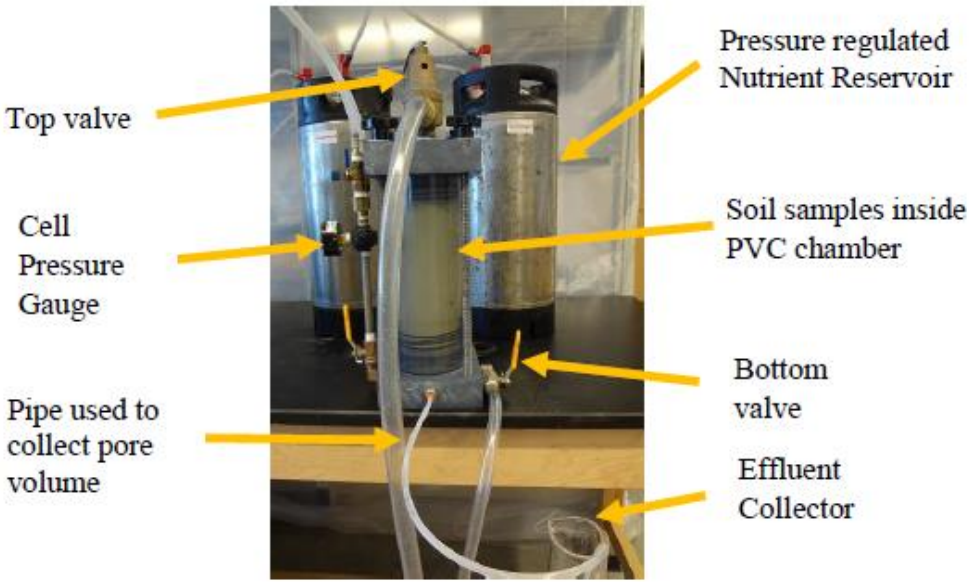


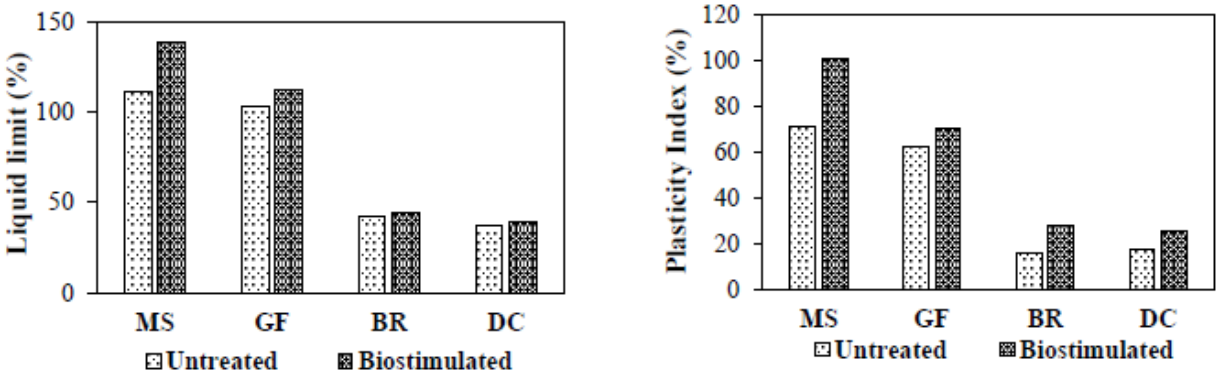
Figure 3-2: Treatment Solution Delivery System (TSDS)

3.4 Discussions

3.4.1 Plasticity Characteristics

The variation of Liquid Limit (LL) and Plasticity Index (PI) of untreated and biostimulated natural soils were shown in Figure 3-3. The increase of LL and PI were observed for all four natural soils. The increase of LL of MS, GF, BR, and DC was 25%, 9%, 5% and 7% respectively (Figure 3-3a). The increase of PI was obtained 43%, 34%,

75% and 47% for MS, GF, DC, and BR respectively (Figure 3-3b). On the other hand, the variation of LL and PI of untreated and biostimulated artificial soils having the same

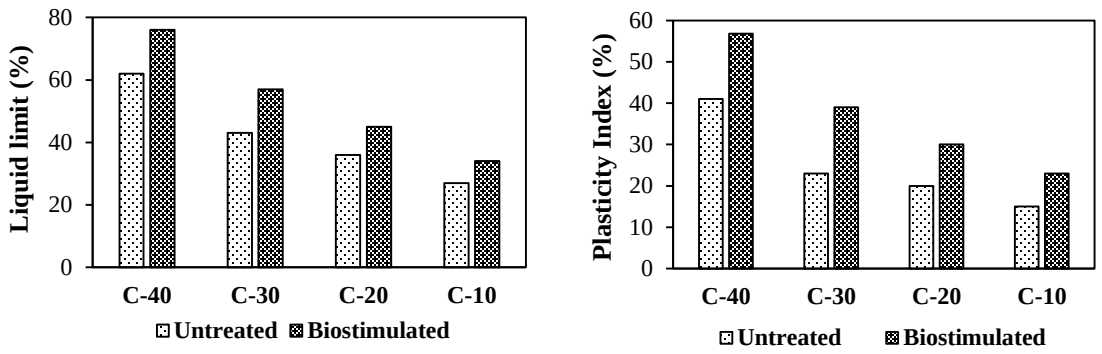


(a) (b)

microbial origin were shown in Figure 3-4. The similar patterns, i.e., increasing LL and

Figure 3-3: Variation of plasticity characteristics of untreated and biostimulated natural soils

PI were observed in the case of artificial soils. The LL increased 23%, 33%, 25%, and 26% for C-40, C-30, C-20 and C-10 soils respectively (Figure 3-4a). Again, the increase of PI was observed at 39% , 70%, 50%, and 53% for respective C-40 to C-10 soils (Figure 3-4b). Hence, regardless of microbial origin, the research group has seen the increase of LL and PI for all soils.



(a) (b)

Figure 3-4: Variation of plasticity characteristics of untreated and biostimulated artificial soils

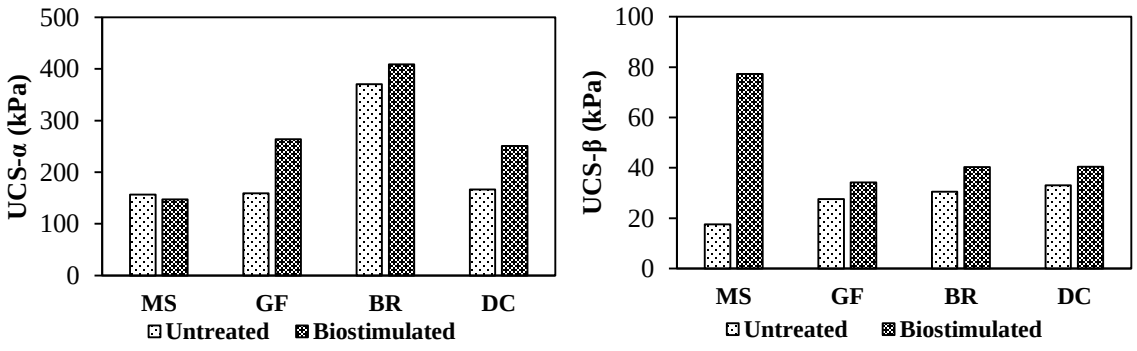
Similar results were observed by previous researchers (Neupane, 2016; Chittoori et al. 2018). When calcium ions are added as a form of calcium salts to the solution, it reduces the diffuse double layer. As a result, the formation of flocculated/aggregated fabric releases free water from trapping inter-pellets. However, a combination of urease producing bacteria and chemicals can produce calcium carbonate and stranded bonding. The water might be entrapped in between the calcium carbonate and clay layer. This entrapped water could be the reason for increasing LL and PI in microbial treated clay types of soils (Bing 2015). Another viable option could be 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%. In this study, the EPS was not quantified as quantifying EPS was out of this research scope.

Therefore, the entrapped water within the clay pallets and the presence of EPS could be the reasons for increasing LL and PI for all eight soils.

3.4.2 Strength Characteristics

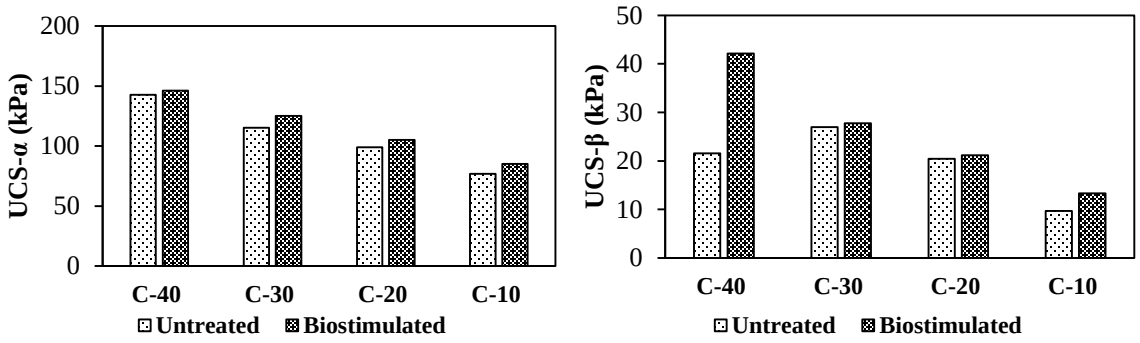
The comparisons of strength for both untreated and biostimulated clayey soils were determined by considering the two types of unconfined compressive strength (UCS) values. These two types are UCS-α and UCS-β. The samples for UCS-α were prepared at optimum moisture content and maximum dry density for both untreated and biostimulated soils. On the other hand, the UCS-β of biostimulated soils were determined by running the UCS test of the sample immediately after the completion of the treatment. The moisture content used for testing UCS-β of biostimulated soils were used to determine the UCS-β of untreated samples. The results of UCS-α and UCS-β for soils having the different microbial communities are presented in Figure 3-5. Again, the results of UCS-α and UCS-β for soils having the same microbial communities are presented in Figure 3-6. In case of natural soils, the UCS-α was increased by 66%, 10% and 51% (Figure 3-5a) and the UCS-β were increased by 24%, 32% and 22% for GF, BR, and DC respectively (Figure 3-5b). The reasons for the appreciable increase in strength is likely due to the presence of calcium carbonate (calcite) that binds the soil particles. A small decrease in UCS-α and a large increase in UCS-β were observed for MS soils. The variation of moisture content in both the cases could be the reasons for variation of unconfined compressive strength. After the treatment process, the accumulation of entrapped water and the formation of calcium carbonate that has higher specific gravity might be the reasons behind an increase of UCS-β of biostimulated MS soils and decrease of UCS-α of biostimulated MS soils. Besides, the cation exchange capacity of

untreated and biostimulated MS soils as well as the formation of a biofilm could be another reason for this strength variation. The other three soils have shown considerable results that might be their characteristics were not substantially changed from untreated to biostimulated phase. In Figure 3-6a, the UCS-α was increased 2%, 9%, 6% and 11% and the UCS-β was increased 96%, 3%, 4% and 38% for C-40, C-30, C-20 and C-10 soils respectively (Figure 3-6b). 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 could be the presence of calcite which forms a bridge between the soil particles resulting into stronger soil mass. However, these strengths were attained by collecting one pore volume of the treatment solution. The increase in pore volume could increase the strength gradually. Hence, more pore volumes were not collected due to the short span of time.



(a) (b)

Figure 3-5: Variation of strength characteristics of treated and biostimulated natural soils



(a) (b)

Figure 3-6: Variation of strength characteristics of treated and biostimulated artificial soils

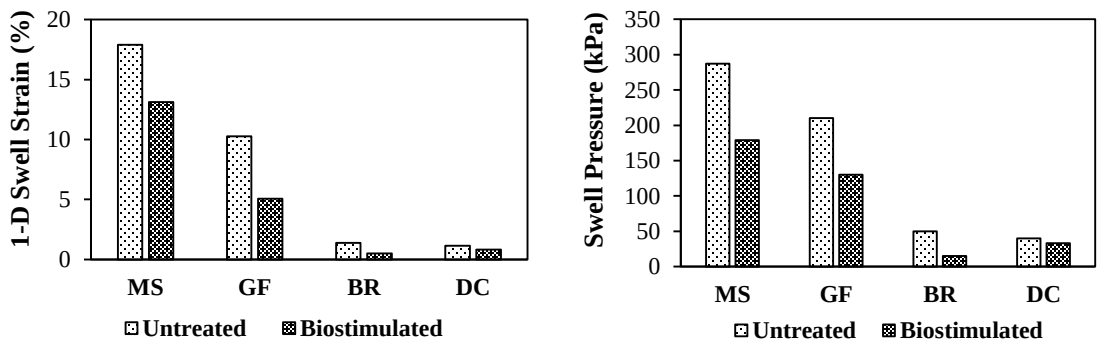
3.4.3 Swelling Characteristics

As all the soils are expansive clay, it can cause a significant amount of swelling with the addition of water. There are different clay minerals, e.g., illite, kaolinite, and montmorillonite based on their structural formation. Among them, montmorillonite is predominant and prone to swelling. Due to isomorphic substitution and diffusive double layer, clay shows swelling phenomena. In this research, the main objective was to reduce this swelling using the biostimulation technique to mitigate the heaving or road distresses to some extent.

The 1-D swell tests were performed on untreated and biostimulated soils. All untreated and biostimulated samples were dried, remolded, prepared at MDUW and OMC and placed inside the consolidometer to determine the 1-D swell strain and swell pressure. In Figure 3-7 the results of 1-D swell strain and swell pressure of MS, GF, BR, and DC soils were presented. The untreated MS soils 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-

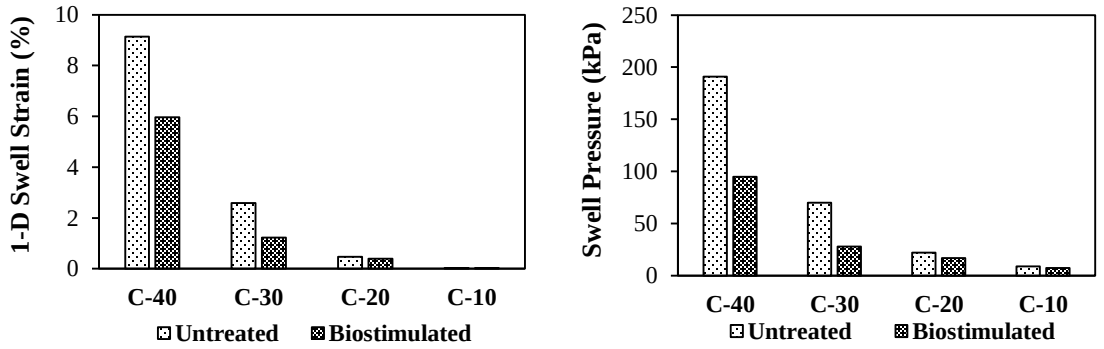
D swell strain and swell pressure were decreased for biostimulated natural soils. For MS, GF, BR, and DC soils, the 1-D Swell strain decreased by 27%, 51%, 28%, and 64% (Figure 3-7a) and the swell pressure decreased by 38%, 36%, 18%, and 70% respectively (Figure 3-7b) . The 1-D swell strain and swell pressures of C-40, C-30, C-20, and C-10 soils were included in Figure 3-8. The 1-D swell strain decreased 35%, 52%, 15%, and 3% (Figure 3-8a) and swell pressure decreased 50%, 60%, 23%, and 17% for C-40, C-30, C-20, and C-10 soils respectively (Figure 3-8b). This considerable decrease of swelling for expansive soils strongly suggests that MICP by biostimulation may be a viable alternative for field applications.

The formation of calcium carbonate might reduce the diffusive double layer, and the biofilm could create a barrier between the charged clay particles and water molecules. All those reasons ended up forming soils with less swelling potential. The percentage decrease was appreciable, but the overall decrease in swelling strain was not satisfactory. The 1-D swell strain of MS soils decrease from 17.9 % to 13.13 %, similarly for GF soils, it decreased from 10.27 to 5.06 %, it decreased 1.15 to 0.83 % for DC and from 1.38 to 0.5 % for BR soils. Similar circumstances were noticed in the case of artificial soils. The percentage change of swelling is still considered on the higher side, but more treatment cycles can reduce the swelling and improve the serviceability of the roadway.



(a) (b)

Figure 3-7: Variation of swelling of untreated and biostimulated natural soils



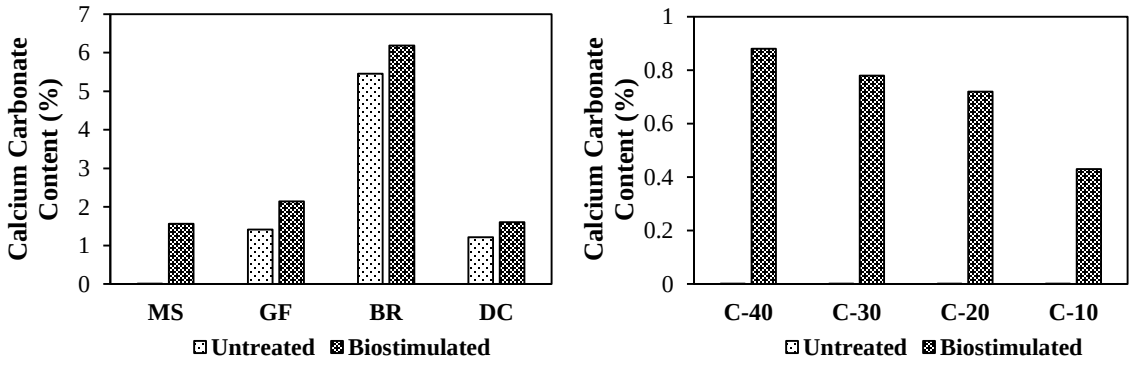
(a) (b)

Figure 3-8: Variation of swelling of untreated and biostimulated artificial soils

3.4.4 Carbonate Analysis

In order to quantify the carbonate precipitation, a Rapid Carbonate Analyzer was used to determine the amount of precipitated calcium carbonate. After the completion of the treatment phase, the biostimulated soil samples were oven dried and crushed into smaller particles passing #40 sieve in order to get as finer particles as possible. The comparison of calcium carbonate precipitation was initiated by conducting calcium

carbonate content determination tests on both the untreated and biostimulated eight soils. The amount of calcium carbonate was determined by the dry weight of soils. In 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, but the soils collected from Montana had a significant amount of calcium carbonate. The untreated GF soils had 1.413% (w/w) of calcium carbonate, but the biostimulated GF had 2.144% (w/w) of calcium carbonate (Figure 3-9a). In the same figure, the percentage increase of calcium carbonate was 52 %, 13 %, and 32 % for GF, BR, and DC soils respectively. No definite correlation was made as the microbial communities of these soils were different. On the other hand, the untreated artificial soils did not have any considerable amount of calcium carbonate. The four artificial soils were prepared by adding sand to MS soils, which did not have a significant amount of calcium carbonate. This is one of the reasons to choose MS soils for preparing artificial mixes. In Figure 3-9b, it is shown that the untreated C-40, C-30, C-20, and C-10 soils had nearly zero amount of calcium carbonate content but after the biostimulation, those soils had 0.88% (w/w), 0.78% (w/w), 0.72% (w/w), and 0.43% (w/w) of calcium carbonate respectively. This increase of calcium carbonate precipitation with the increase of clay content indicates that the activity of soil bacteria was increased with the increase of natural clay soils that had soil bacteria for precipitating calcite. Although the untreated natural soils had calcite, the major findings of this research were to precipitate appreciable amount of calcite in the biostimulated natural clay soils. Besides, even larger amounts of calcite precipitation could be achieved with more treatments.

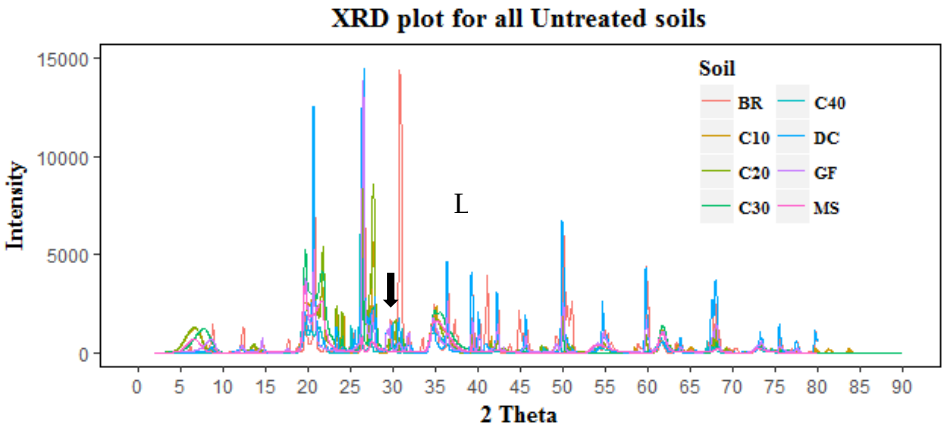


(a) Natural Soils (b) Artificial Soils

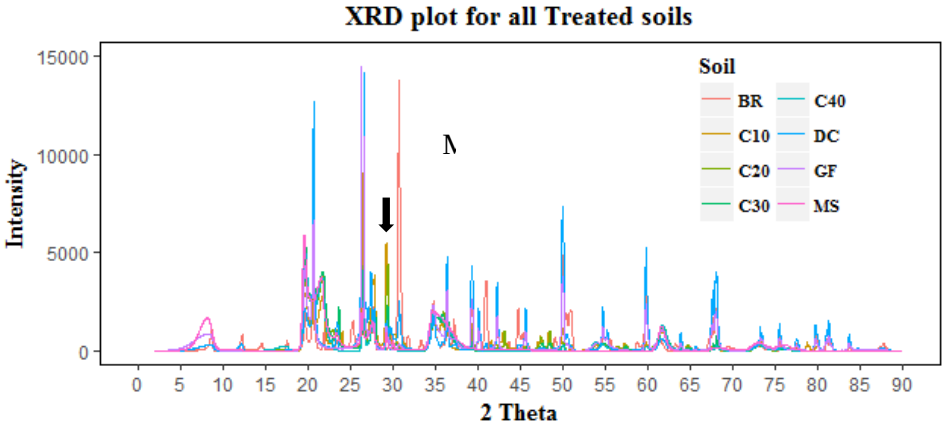
Figure 3-9: Comparison of precipitated calcite of untreated and biostimulated soils

3.4.5 Microstructural Analysis

The untreated and biostimulated eight soils are presented in Figure 3-10. All eight soils were dried and powdered for preparing the representative samples of XRD test. The diffraction peaks of quartz, feldspar, kaolinite, illite, and montmorillonite were found in this figure. According to ICDD, the 2θ of pure calcium carbonate is usually found at 29°. Due to the presence of calcium carbonate in the natural and artificial soils, a small peak of calcite was observed for untreated soils (Figure 3-10a). A considerable pick was found at that 2θ of calcium carbonate in the biostimulated soils indicating the presence of calcite (Figure 3-10b).



(a) Untreated natural and artificial soils



(b) Biostimulated natural and artificial soils

Figure 3-10: XRD test analysis

The SEM and EDX analysis are shown in Figure 3-11. A representative SEM image of untreated soils is shown in Figure 3-11. A representative SEM image of untreated soils is shown in Figure 3-11a. No significant binding was observed in the untreated specimens. It was also observed from the EDX graph that no calcium peak was noticed which indicated the absence of calcite in the soil mass (Figure 3-11b). For artificial soils, the grains of sand and clay particles could be seen but not any noticeable cohesiveness was observed. From the EDX analysis of untreated soil samples (Figure

3-11b), a considerable amount of Oxygen, Carbon, Silicon, and Aluminum were noticed, but an insignificant amount of calcium peak was observed. On the other hand, it is clear from the Figure 3-11c that the distinct calcite formed a stronger bridge in between the soil grains. Calcium carbonate-linked the soil grains in a way that soil particles looked like a crystal composition embedded to each other. The EDX graph of biostimulated soils was shown in Figure 3-11d. The EDX analysis of treated soils is showing the existence of calcium, oxygen, and carbon. It confirms the precipitated calcium carbonate in the soils. There is a thin coating of calcite was also observed in both the biostimulated natural and artificial soil samples.

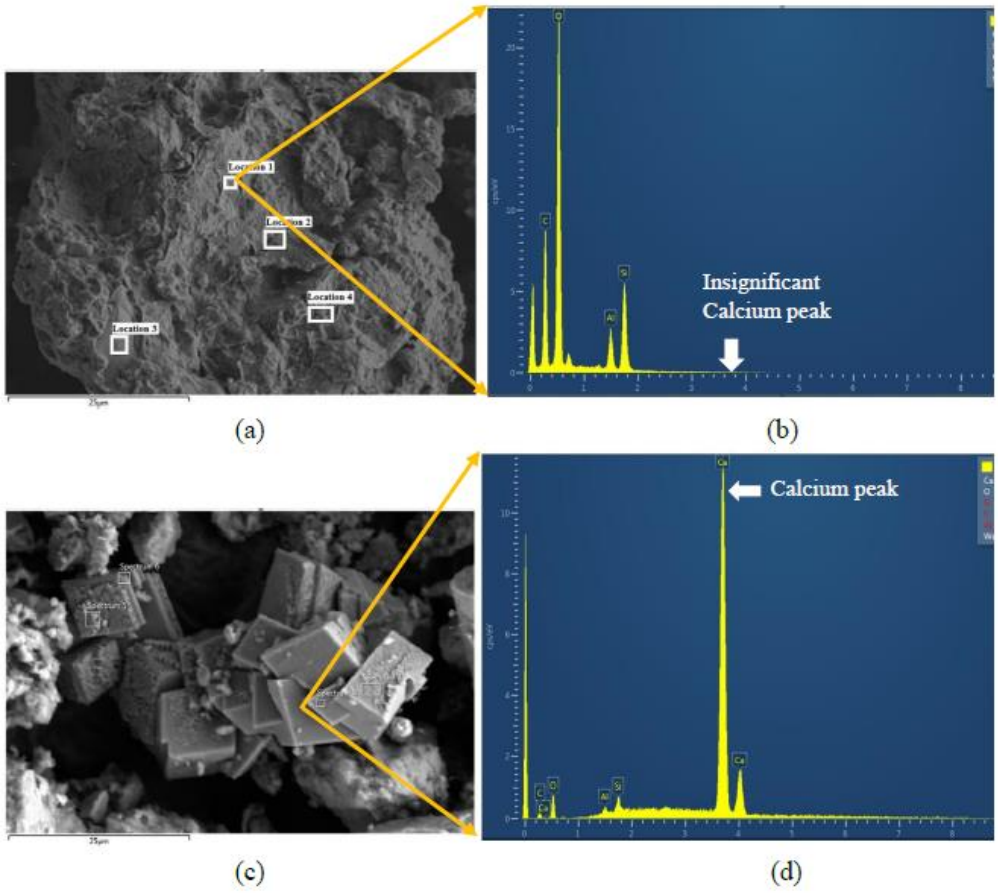


Figure 3-11: SEM and EDX analysis of untreated and biostimulated clayey soils

3.4.6 Other Observations

There are several factors that affect the efficiency of MICP, e.g., bacteria type, bacterial cell concentration, pH, temperature and urea and Ca^{2+} concentration (Anbu et al. 2016). The research team investigated all those factors. The bacteria type and bacterial cell concentration were not specified rather than the urease activity of all eight soils were determined. This test indicated the ability of soil bacteria to hydrolyze urea. In the case of pH, Stocks-Fischer et al. (1999) showed that the urease enzyme activity for *Sporosarcina pasteurii*, the optimal pH for the enzyme is around 8, but the range of pH from 6.0 to 10.0 could be considered as an active period of calcium carbonate precipitation. This range of pH was targeted as an indicator of calcite precipitation. In this research, pH was determined from 7.4 to 9.8 for all the soils during the treatment process. The research team delivered specific concentrations of urea (333 mM) and calcium concentration (250 mM) throughout the biostimulation phase. Those concentrations were proved optimum for other research where a large amount of calcite was precipitated for the sandy type of soils by biostimulating indigenous ureolytic microbes (Burbank et al. 2013). Another factor, e.g., the temperature could be a determining factor for calcium carbonate precipitation. Research showed that if the temperature increased from 35°C to 55°C, the reduction of enzyme activity was 47% for *S. pasteurii* (Dhami et al. 2014). To overcome this factor, a constant temperature (22°C) was ensured during the biostimulation of natural and artificial soils. The permeability decreased one order of magnitude for all biostimulated soils. As an example, permeability decreased in the order of 10^{-8} from 10^{-7} for MS soils. This low permeability is good for soils, but it could lower the possibility of further treatment cycles. The research team targeted for collecting one pore volume of

enrichment solution and one pore volume of cementation solution because of this low permeability of expansive soils. Soils with high clay content took almost 4-6 weeks to finish one round of treatment cycle.

3.5 Acknowledgments

The authors acknowledge the support provided by the IDEA program of the National Cooperative Highway Research program. Special thanks are due to Dr. Inam Jawed for his continued support throughout the course of this research. Thanks are also due to John Arambarri and Keith Nottingham of the Idaho Transportation Department (District 3) for their help with sample collection and delivery. The research team would like to thank the personnel from the Idaho Microfabrication Laboratory located at Boise State University to provide the facility of doing XRD and SEM test. The SuRGE laboratory in the Department of Civil Engineering at Boise State University assisted the research team in various aspects and our gratitude towards them would be unparalleled.

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SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

4.1 Summary and Conclusions

This research investigated the viability of indigenous bacteria in stabilizing expansive soils. Without adding any laboratory-grown bacteria, the research team envisaged the applicability of biostimulation techniques on natural microbes present in expansive soils to precipitate calcite. Hence, both the natural and artificial soils were selected to induce calcium carbonate precipitation regardless of the microbial origin. The TSDS was used, and one round of enrichment solution followed by one round of cementation solution was injected to treat the soils. The UCS test on treated soils was run immediately after the completion of the treatment. Later, the UCS value of both untreated and treated soils were determined at OMC, and results were compared. The strength and swelling test data showed that the implementation of the biostimulation technique could be a promising tool to reduce swelling in distressed prone areas. A considerable amount of carbonate was precipitated, and the qualitative analysis using XRD and SEM showed the presence of calcium carbonate in these soils. Here, the research focused on the suitability of biostimulation on soils having different or similar microbial communities. Hence, in order to get more improved characteristics of soils used in this study, further research needs to be continued to find the optimum rounds of treatment solution for a certain soil.

Major findings from this study are listed as follow:

1. The research team witnessed the change of plasticity, strength gain, swelling reduction and calcium carbonate formation of both soils either having similar or different microbial origin. In addition, the artificial soils were prepared for two purposes. One was to observe the efficacy of soils with varying clay content and the other one was to keep the microbial communities. The test data showed a promising result for implementation of MICP by biostimulation in expansive soils at the field level.

2. The LL and PI were increased for all eight soils regardless of the microbial communities. Both the precipitated calcium carbonate and the clay particles could entrap water that might be a viable option for increasing those plasticity parameters.

3. A considerable increase in strength was found in almost all types of treated soils. The bonding of calcium carbonate with the presence of finer and coarser particles could contribute to this strength increase.

4. The swelling potential was reduced for all types of treated soils. The formation of calcium carbonate might reduce the diffusive double layer, and the biofilm could create a barrier between the charged clay particles and water molecules. However, the swell strain was not reduced like with other chemical stabilizers, but more treatment cycles might reduce the swelling and save millions of dollars of damage every year.

5. This increase of calcium carbonate precipitation with the increase of clay content indicates that the activity of soil bacteria was increased with the increase of natural clay soils that had soil bacteria for precipitating calcite. Although the untreated natural soils had calcite, the major findings of this research were to precipitate appreciable amount of calcite in the biostimulated natural clay soils. In addition, a larger

amount of calcite precipitation could be achieved if more treatment cycles were performed.

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

4.2 Recommendations for Future Research

There are several research scopes that could be considered for furthering the biostimulated treatment process of clay. Some of the future research recommendations are enumerated as follows:

1. The biostimulation was performed on all eight soils for one pore volume of the treatment solution. The collection of more than one pore volume of effluent from these soils might improve the strength and reduce the swelling. Besides, optimum numbers of pore volume for achieving highest strength or reduced swelling could be investigated for each soil.

2. The urease activity test could be performed on these soils to know the capability of soils to hydrolyze urea resulting into ammonium release. This information could be a helpful tool to establish a correlation between natural soils.

3. The 1-D Swell test of biostimulated soil was conducted in the same way as untreated soils. In this thorough process of pulverizing and recompaction, the calcite bonds could be broken and showed less swelling. A suitable alternative might be explored to determine the more realistic 1-D swell strain of biostimulated soils.

4. The research team chose MS soil for preparing artificial mixes. The other soils especially GF soil could be used to prepare artificial mixes to observe the change of plasticity, strength and swelling with varying clay content.

5. The ingredients of treatment solutions could be altered to observe the change of different biotreatments on clayey soils.

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