STUDYING THE USE OF MICROBIAL INDUCED CALCITE PRECIPITATION AS A SHALLOW STABILIZATION ALTERNATIVE TO TREAT EXPANSIVE SOILS

by

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The following individuals read and discussed the thesis submitted by student Tasria Rahman, and they evaluated her presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

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DEDICATION

To my late father, Molla Safiar Rahman

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ABSTRACT

Expansive soils usually recognized as swell-shrink soils have been a problem for civil infrastructure for a long time. It has been a very common practice to use chemical stabilizers including cement and lime to stabilize expansive soils, especially for lightly loaded structures. However, due to the the detrimental effects of these stabilizers on the environment and several occurrences of premature failures after stabilizing with chemical additives, engineers are in search of sustainable stabilization alternatives. Microbial Induced Calcite Precipitation (MICP) is a promising process, which can improve the properties of expansive soil through calcite precipitation. Previous research has shown promise for the use of MICP in mitigating swelling distresses in expansive soils. There are generally two approaches to apply MICP: Bioaugmentation and Biostimulation. In this research, biostimulation was applied by mixing enrichment and cementation solutions with soils in an effort to develop a new alternative to shallow chemical stabilization. Three soils were selected with varying plasticity for this purpose. Soils were treated by mixing with enrichment and cementation solutions. Enrichment solutions were first added and were allowed to stimulate bacteria for different time periods, termed *mellowing periods*. At the end of each mellowing period cementation solutions were added to facilitate calcite precipitation. Two protocols were studied for this shallow mixing method of MICP application. In protocol-1, soils were mixed with enrichment solutions at optimum moisture content (OMC) and allowed to stimulate for mellowing periods of 1, 2, 3, and 4 days. Protocol-2 was similar to protocol-1 except for the the

initial amount of enrichment solution which was 95% of maximum dry unit weight on the wet-side of standard proctor curve in place of OMC. At the end of each mellowing period, the enrichment solution lost during this time was replaced with cementation solution to reach OMC and soil samples were compacted to untreated maximum dry unit weight. Treatment effectiveness was evaluated with Unconfined Compression Strength test and calcite test. The results indicated that protocol-1 performed better than protocol-2 which indicated that adding higher amounts of enrichment solutions was not beneficial for calcite precipitation and improvement of strength. Following this finding, protocol-2 was discontinued and protocol-1 was chosen for further testing. Five different mellowing periods, three different curing periods and two types of cementation solutions were studied by following protocol-1. Improved test results were observed with the lower concentration of calcium chloride used in the cementation solution. Also, medium to high plastic soils showed improvement in evaluation tests with respect to strength gain, swell reduction, and calcite precipitation. Unconfined Compressive Strength (UCS) value after treatments ranged from 45 to 267 kPa, calcite values ranged from 0% to 1.36% and the Free Swell Indices ranged from 8% to 266%. The maximum change in UCS (284%) was observed for medium plasticity soil C-30.

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LL-Liquid limit;

PL-Plasticity Index;

MDUW-Maximum Dry Unit Weight;

OMC-Optimum Moisture Content;

UCS-Unconfined Compression Strength

CHAPTER ONE: INTRODUCTION AND BACKGROUND

Problem and Possible Solution

Expansive soils are associated with several issues including low bearing capacity, high compressibility along with swelling and shrinking with moisture ingress and digress. Expansive soils exhibit large amounts of contraction and expansion with a change in moisture content (Nelson and Miller 1992). These soils are so widely distributed all over the world that it would not be a feasible solution to avoid constructing on them. Also, the damage to lightly loaded structures built on these soils is more than any other natural disasters including earthquakes and floods (Jones Jr and Holts 1973). Overall, the cost due to damage from expansive soils in the US annually increased from $2.2 billion in 1973 (Jones Jr and Holts 1973) to $15 billion in 2012 (Jones and Jefferson, 2012).

Various soil stabilization methods are being used to mitigate expansive soil issues for several decades. Soil stabilization can be defined as a modification of physical and engineering characteristics of problematic soils to achieve desired strength and workability (Petry and Little 2002). Both chemical and mechanical soil stabilization techniques have been implemented to stabilize expansive soils. Chemical stabilization is the most common technique for these soils. Over the years, several types of chemical stabilizers are used all over the world including traditional stabilizers such as lime, Portland cement, fly ash, and nontraditional stabilizers such as ammonium chloride and sulfonated oils (Petry and Little 2002). However, in some cases, some chemical stabilizers (calcium-based) can have an adverse effect in the presence of soluble sulfates

which results in the formation of Ettringite which can cause swelling related distresses on civil infrastructure (Petry and Little 2002). Also, 7-8% of the total CO_2 emissions result from cement production each year (UNEP 2010). In comparison with chemical stabilization, mechanical stabilization methods consume more energy with little economic benefit (Hasan et al. 2016). Islam (2017) showed that the active zone of expansive soils could be 3.35 m from the pavement surface and in those circumstances, shallow stabilization would not be an effective solution.

A possible alternative to chemical stabilization of expansive soils could be Microbial Induced Calcite Precipitation (MICP). MICP is an eco-friendly method to strengthen soils by precipitating calcium carbonate within soil pores with the help of microbes. In the past, MICP was used to mitigate seismic-induced liquefaction, reduce permeability and compressibility, and increase unconfined compressive strength (DeJong et al. 2006; Whiffin et al. 2007). Most of the of research studies on MICP have been conducted on sandy and silty soils (DeJong et al. 2010; Chu et al. 2012; Soon et al. 2013; Mortensen et al. 2011). In this research study, to apply MICP, biostimulation method has been applied where microbes present in the soil were stimulated to precipitate calcite. An alternative biostimulation approach has been investigated by studying three soils with varying plasticity with mixing substrate solutions into the soil which was similar to lime or cement stabilization.

MICP Background

The mechanism of MICP consists of urea hydrolysis followed by calcium carbonate precipitation (Stocks-Fischer et al. 1999; Hammes and Verstraete 2002). In this process, bacteria hydrolyze 1 mole of urea ($CO(NH_2)_2$) into 1 mole of ammonia and 1

mole of carbamic acid (see Equation 1). Carbamic acid decomposes into ammonia and carbonic acid as shown in Equation 2. Ammonia then hydrolyzes into ammonium ion, which increases the pH of the system (Equation 3) followed by carbonic acid dissociation into dissolved inorganic carbonate (Equation 4). With the addition of Ca^{2+} ions to this medium, calcium carbonate crystals form on the cell wall as shown in Equations 5 and 6 (Burne and Chen 2000).

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \quad (1)$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \quad (2)$$

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

$$H_2CO_3 \rightarrow HCO_3^- + H^+ \quad (4)$$

$$HCO_3^- + H^+ + 2OH^- \rightarrow CO_3^{2-} + 2H_2O \quad (5)$$

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

Mainly four factors affect MICP process: calcium ion concentration, dissolved inorganic carbon (DIC) concentration, pH, and availability of nucleation sites (Hammes and Verstraete 2002). In addition to this, the ability to metabolize, grow and reproduce affects the survivability of microbe (Rebata-Landa 2006). The factors are also termed as ‘limiting growth factors’.

Microbial growth, metabolic activity, and cell-surface charge are dependent on the change in pH (Rebata-Landa 2006). The ammonia produced with urea hydrolysis is the reason for increasing the pH of the medium. Stocks-Fischer et al. (1999) stated that the urease activity increased mostly from pH 6.0 to 8.0. Urease activity reached highest at pH 8.0 and decreased with higher pH although there was some urease activity noted at pH 9.0 for Sporosarcina Pasteurii. However, if there is sufficient chemical reagent, the rate of urea hydrolysis has a direct relationship with the bacterial cell concentration. More bacteria produce more urease per unit volume to start the urea hydrolysis. Stocks-Fischer

et al. (1999) observed that the bacteria cell can serve as a nucleation site for calcite to precipitate. Lian et al. (2006) identified from SEM images that the nucleation of calcite takes place at bacteria cell walls. High salinity can cause inhibition and stop microbial activity (Rivadeneyra et al. 1998). The salinity of cementation fluid is dependent on calcium salt. Microbial activity can be obstructed by high salinity which can limit the urease production from ureolytic bacteria (Nemati et al. 2005).

Applications of MICP

Calcite precipitation in MICP process bridges adjacent soil particles, cementing soil particles together (DeJong et al. 2006; Whiffin et al. 2007). The precipitation of calcite reduces the permeability and compressibility while increasing soil strength (DeJong et al. 2010). Calcite mineralization is the result of a by-product of microbial metabolic activity including photosynthesis, urea hydrolysis, sulfate reduction, and iron reduction (v. Knorre and Krumbein 2000).

MICP has several applications in diverse fields including increase in concrete strength and durability (De Muynck et al. 2008), soil strength (Van der Ruyt and van der Zon 2009; Lu et al. 2010) , sand impermeability (Nemati et al. 2005), brick durability (Sarda et al. 2009).

There are very few studies found that were related to the application of MICP on expansive soil. The geometric compatibility between soils and microbial communities is one of the main obstacles to introduce MICP in clay. The range of cell diameter soil bacteria present in soil is from.5 to 3 μm (Mitchell and Soga 2005). Chittoori et al. (2016) performed a Mercury Intrusion Porosimetry (MIP) test to observe the pore size and pore volume on two expansive soils after compaction. It was found that 30% to 50% of the

pore volume was larger than 1.5 μm at a maximum dry density which is the average diameter of soil bacteria. So, space is available through the pore spaces for bacterial mobilization. There were some studies regarding biotreatment on expansive soils. Bing (2014) conducted biotreatment on kaolin, marine clay, and bentonite and observed strength increased by around 150% and 400% for treated kaolin and treated marine clay, respectively. Cheng and Shahin (2015) attempted three different MICP methods including injection, premixing, and diffusion for clayey sands to investigate the variation of strength and amount of calcium carbonate precipitation. Soils having 5% clay content worked best in injection method. Cardoso et al. (2018) investigated the compressibility and pore clogging of the biocemented sand-kaolin mixture and found that the osmotic consolidation effect might be a contributing factor for high compressibility along with the bacterial activity.

MICP Methods

There are two methods to apply MICP: *Bioaugmentation* and *Biostimulation*. In *bioaugmentation*, exogenous bacteria are introduced into the soil to precipitate calcite. Most of the research studies have applied bioaugmentation method on silty and sandy soil (Whiffin et al. 2007; van Paassen et al. 2010; Soon et al. 2013; DeJong et al. 2010; Mortensen et al. 2011). Bioaugmentation process had a successful implementation in the improvement of concrete strength and durability (De Muynck et al. 2008); mitigation of sand liquefaction (Montoya et al. 2012); and sand impermeability (Nemati and Voordouw 2003). Chittoori and Neupane (2018) studied the application of bioaugmentation to mitigate expansive soil swelling. They studied two different protocols on three selected soils having low, medium and high plasticity characteristics. Different concentrations of bacteria and substrate were mixed with soil and cured for 7 days in one

protocol. In other protocol, different concentrations of bacteria were mixed into the soil and compacted; substrate solutions were injected into the compacted sample. It was reported that low to medium plastic soils can be effectively treated using MICP via bioaugmentation. However, in this method augmented exogeneous bacteria has to adjust to the new environment and compete with native microorganisms, which can definitely affect the survival rate and metabolic potential of the augmented bacteria (Wenderoth et al. 2003). It was observed that the survivability of exogenous microorganisms in a new environment, tend to decline rapidly and rarely propagate (van Veen et al. 1997). Also, the uneven distribution of bacteria and clogging near the inlet were other issues associated with this method (Stocks-Fischer et al. 1999). The requirement of injecting nonnative bacterial strains into soil has restricted the technology from becoming an economical method (Gomez et al. 2018).

On the other hand, *biostimulation* uses indigenous bacteria for calcite precipitation (Burbank et al. 2011) and this method is becoming a popular method of application for MICP. This approach does not require expensive non-native monoclonal bacterial cultivation and injection into natural soil ecosystems which have made it economically and environmentally beneficial. These ureolytic microbes are more resilient than the injected microbes which resulted in a uniform distribution of calcite and sustained enzymatic capabilities (Gomez et al. 2018). Usually, the microbe population is 10^6 to 10^{12} per gram in soil (Torsvik et al. 1990; Boquet et al. 1973). A study by Boquet et al. (1973) showed a likelihood that most bacteria can precipitate calcite. With the biostimulation process, it is possible to increase their number in a variety of soils (Burbank et al. 2011). It was first demonstrated by Burbank et al. (2011) that native

ureolytic microorganisms can hydrolyze urea and induce calcite precipitation with saturated and unsaturated soil from Snake River and media was added to promote biostimulation. Gomez et al. (2014) also demonstrated field test for calcite precipitation in granular soils, using one-dimensional column specimens which resulted in significant improvement of geotechnical properties, including unconfined compressive strength and permeability. Chittoori et al. (2018) initiated a treatment to treat natural expansive soils through an injection system. A significant reduction in swelling strain and increase in unconfined strength after one treatment cycle were observed. In this research study, biostimulation method has been applied through a mixing protocol. This research study is an initial step to establish an alternative treatment protocol for stabilizing shallow expansive soils. Hence, in this study, an MICP approach has been investigated by studying three soils with varying plasticity with mixing substrate solutions into the soil. Enrichment solution was mixed with the soil to stimulate the bacteria and allowed to escape moisture from the mix. This period is term as “mellowing period”. Then, cementation solution which contain calcium chloride was added with the amount of lost moisture for calcite precipitation. Samples were cured under controlled humidity and temperature. These periods are termed as “curing periods”.

Research Objectives

The overarching research hypothesis of this thesis is that *indigenous urease producing bacteria can be stimulated to precipitate calcite by shallow mixing substrate solutions* as in the case of lime or cement stabilization. To test this research hypothesis the following research objectives were met:

1. Develop a shallow mixing protocol for the application of MICP in expansive soils
2. Optimize the protocol by studying different mellowing and curing periods
3. Study the effect of calcium chloride concentration in cementation solution to optimize calcite precipitation
4. Study the effect of soil type on MICP effectiveness in these soils

A pictorial representation of research work is shown here-

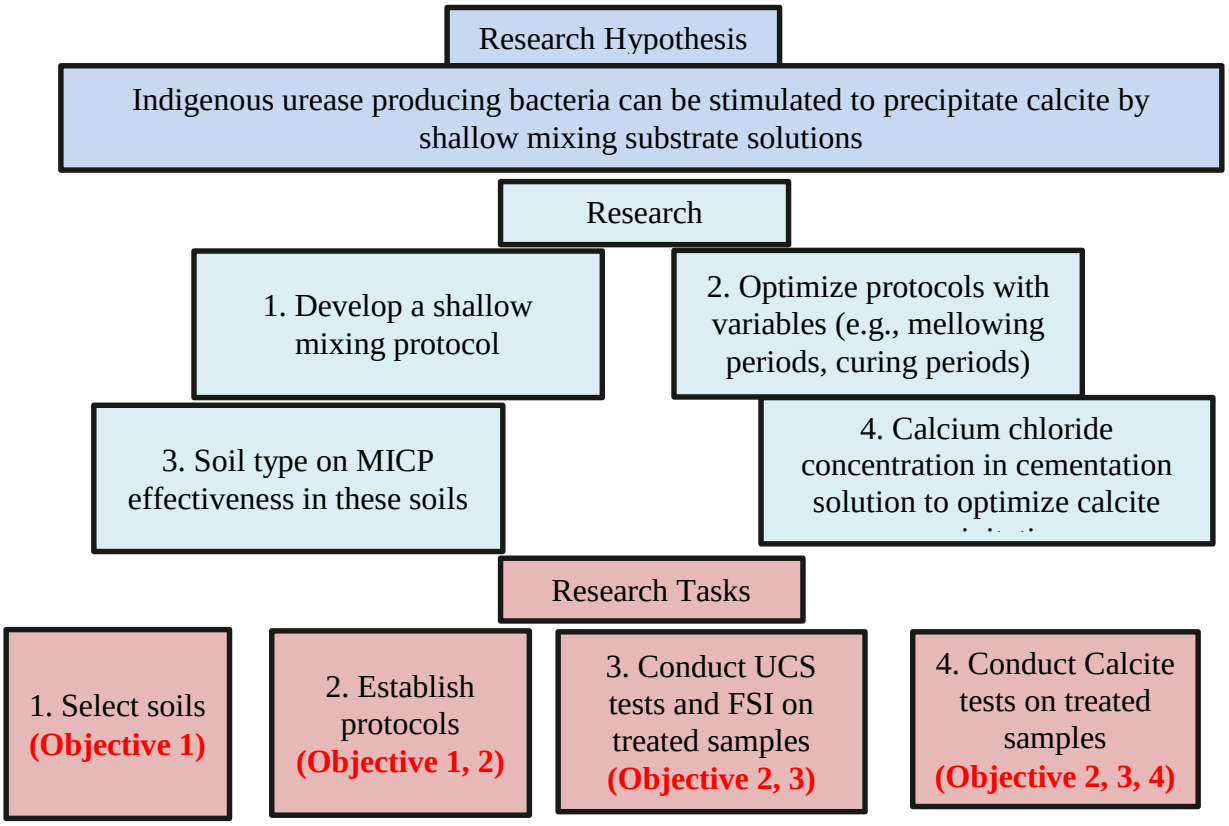

Figure 0.1: Pictorial representation of research objectives and tasks

Research Tasks

a) *Select soils* - Three soils with varying plasticity characteristics were selected to study the effect of biostimulation on expansive soils. Baseline data was generated

by conducting tests including Atterberg Limits test, compaction tests, Unconfined Compression Strength test, and 1-D swell tests

b) *Establish protocols* - Two protocols were studied to treat all three soils. Soils were treated with enrichment solution and then with cementation solution.
c) *Conduct UCS tests and FSI on treated samples* - Unconfined Compression test, Free Swell Index test was conducted on biostimulated soils to understand the effect of biostimulation on clayey soil's strength, and swelling characteristics.
d) *Conduct Calcite tests on treated samples* - carbonate determination tests were conducted on untreated and biostimulated soils to understand the effect of biostimulated soils on mineralogical characteristics.

Organization of the Thesis

This thesis consists of an overall introduction (Chapter 1) and two manuscripts; where manuscripts are related to one another to serve a common purpose. In both manuscripts, the applicability of alternative application method biostimulation technique is investigated to stabilize the expansive soils by precipitating calcium carbonate.

Chapter two presents manuscript one. In chapter two that examined three expansive soils with varying plasticity and mineralogical characteristics. Two protocols for shallow mixing were studied. In Protocol-1, soil samples were mixed with enrichment solutions at optimum moisture content and allowed to mellow for 1, 2, 3, and 4 days. In Protocol-2, soil samples were mixed with enrichment solutions at moisture content corresponding to 95% of maximum dry unit weight on the wet-side of a standard Proctor curve. Unconfined compression strength and calcium carbonate precipitation tests were used to evaluate the strength improvements after treatments. The results show promise for this method as an alternative to current shallow stabilization methods. This manuscript was accepted for the Geo-Congress 2019, the Eighth International Conference on Case Histories in Geotechnical Engineering.

Chapter three presents manuscript 2 which is a continuation of manuscript one where three soils were studied with an intent to optimize protocol 1 by studying five different mellowing periods, three different curing periods and two types of cementation solutions. Treatment effectiveness was evaluated using UCS, Calcium Carbonate concentration, and Free Swelling Index tests. Better results were observed in the case of lower concentration of calcium chloride used in the cementation solution. This paper will be submitted to ASCE Journal of Materials in Civil Engineering.

Chapter four presents a summary and findings from both manuscripts.

CHAPTER TWO: EVALUATING SHALLOW MIXING PROTOCOLS AS APPLICATION METHODS FOR MICROBIAL INDUCED CALCITE PRECIPITATION TARGETING EXPANSIVE SOIL TREATMENT

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Abstract

Expansive soils, also known as swell-shrink soils, undergo substantial volumetric changes due to moisture fluctuations from seasonal variations. These volumetric changes cause millions of dollars in damages annually. Microbial Induced Calcite Precipitation (MICP) is a promising soil improvement technique, which uses urease producing bacteria to precipitate calcium carbonate. In this study, a stabilization alternative for expansive soils was studied using MICP. Specifically, indigenous bacteria were stimulated by mixing enrichment and cementation solutions with expansive natural soils to precipitate calcium carbonate and make soil stronger and less expansive. This study examined three expansive soils with varying plasticity and mineralogical characteristics. Two protocols for shallow mixing were studied. In Protocol-1, soil samples were mixed with enrichment solutions at optimum moisture content and allowed to mellow for 1, 2, 3, and 4 days. In

Protocol-2, soil samples were mixed with enrichment solutions at moisture content corresponding to 95% of maximum dry unit weight on the wet-side of a standard Proctor curve. Moisture was allowed to escape from the mix during the mellowing period under both protocols. Following the mellowing periods, the lost moisture is replaced with cementation solution to reach optimum moisture content, and the soil sample was compacted to its maximum dry unit weight. Unconfined compression strength test was used to evaluate the strength improvements due to treatments. The treatment effectiveness was also evaluated with measurements of calcium carbonate precipitation. The results show promise for this method as an alternative to current shallow stabilization methods. An increase in the mellowing period for low and medium plastic soils was determined to be beneficial. The current results also showed that the presence of higher amounts of enrichment solution and addition of less cementation solution is not advantageous for this procedure based on the performance of Protocol-2.

Keywords: MICP, expansive soils, soil stabilization, biostimulation, calcite precipitation

Introduction and Background

Expansive soils a tend to swell when moisture is increased and shrink when moisture is decreased (Nelson and Miller 1992). High plasticity clays, overconsolidated clays rich with montmorillonite clay minerals, and highly weathered shales are some examples of expansive soils (Puppala and Pedarla 2017). Expansive soils are generally found in regions with arid or semi-arid climate conditions (Hussein 2001). Forty-eight of the fifty states in the USA have expansive soils presence (Chen 1988). These soils cause severe damage to lightly loaded structures such as pavements and residential structures,

resulting in billions of dollars spent on maintenance and repair costs (Puppala et al. 2006). The estimated annual cost of damage to structures built on expansive soils in the USA increased from $2.2 billion/year (Jones Jr and Holts 1973) to $15 billion/year in 2012 (Jones and Jefferson 2012).

To combat the expansive soil problem, researchers over the years have developed a variety of methods. Petry and Little (2002) discussed several of these stabilization methods, including mechanical compaction, chemical stabilization, pre-wetting, moisture barriers, lime injections, and deep soil mixing. There are several application methods to stabilize expansive soils chemically. These can be broadly classified as (1) shallow stabilization, (2) deep soil mixing, and (3) injection. Subgrade stabilization under roadways generally uses shallow stabilization method (Puppala and Pedarla 2017). Unfortunately, even after shallow stabilization, sometimes subgrades tend to fail. This can be attributed to (a) loss of stabilizer over time, or (b) ineffective stabilizer selection. In addition to this possible ineffectiveness, traditional stabilization techniques may be harmful environmentally – mainly when using additives such as lime or Portland cement. These additives may leach into the environment and increase adjacent soil pH, and they are known to generate high carbon emissions, which may contribute to climate change. For all these reasons, it would be beneficial if a more environment friendly method is available to stabilize expansive soils. One such innovative alternative uses microorganisms, either naturally present in the subsurface soils or augmented, to precipitate calcium carbonate and improve the engineering properties of soils (DeJong et al. 2006a). This method is known as Microbial Induced Calcite Precipitation (MICP).

In MICP, ureolytic bacteria such as *Sporosarcina pasteurii* catalyze the hydrolysis of urea to produce ammonium and carbonate ions (Eq. 1). With the addition of Ca^{2+} ion, calcium carbonate crystals form on the cell wall of the bacteria (Burne and Chen 2000).

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \quad (1)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \quad (2)$$

The microbially induced calcium carbonate bridges adjacent soil particles and increases the shear strength and stiffness of soil and decreases permeability (van Paassen et al. 2010; Cheng and Cord-Ruwisch 2014)**.** The primary factors affecting calcite precipitation are calcium ion concentration, dissolved inorganic carbon concentration, pH and availability of nucleation sites (Hammes and Verstraete 2002). There are two methods to apply MICP: bioaugmentation and biostimulation.

In bioaugmentation, exogenous bacteria are added to soil to precipitate calcite. The applications of this process have shown promising results in diverse fields including, improvement of concrete strength and durability (De Muynck et al. 2008); mitigation of sand liquefaction (Montoya et al. 2012); and sand impermeability (Nemati and Voordouw 2003). Mostly, researchers have applied bioaugmentation on sandy and silty type soil using urease producing bacteria (Whiffin et al. 2007; van Paassen et al. 2010). (Chittoori and Neupane 2018) studied the application of bioaugmentation to mitigate expansive soil swelling and noted that low to medium plastic soils can be effectively treated using MICP via bioaugmentation. However, bioaugmentation may not be effective in all cases as it is dependent on the augmented bacteria to adjust to the new environment and compete with native microorganisms, which affect the survival rate and

metabolic potential of the augmented bacteria (Wenderoth et al. 2003). Van Veen et al. (1997) observed that the survivability of exogenous microorganisms after introducing into a new environment, tend to decline rapidly and rarely propagate. Another issue with bioaugmentation is the uneven distribution of bacteria and clogging near the inlet were observed in this method (Stocks-Fischer et al. 1999). The need for injecting nonnative bacterial strains into soil has limited the technology from becoming a cost-effective approach (Gomez et al. 2018).

In case of the biostimulation, indigenous bacteria are used to achieve calcite precipitation (Burbank et al. 2011). This method has essential economic and environmental benefits through the elimination of expensive non-native monoclonal bacterial cultivation and injection into natural soil ecosystems. These natural ureolytic microbes are more resilient in their native environment than the injected strains which result in uniform distribution of calcite and sustained enzymatic capabilities (Gomez et al. 2018). Usually, the number of bacteria per gram of natural soils is 10^6 to 10^{12} (Boquet et al. 1973; Torsvik et al. 1990). Boquet et al. (1973) showed that all soil bacteria could precipitate calcite. Also, it is possible to increase their number in a variety of soils through biostimulation with calcite precipitation (Burbank et al. 2012). Burbank et al. (2011) first demonstrated the ability of native ureolytic microorganisms to hydrolyze urea and induce calcite precipitation in liquid media using ureolytic strains obtained from the Eastern Snake River. Gomez et al. (2014) demonstrated the ability of stimulation techniques to enable calcite precipitation in granular soils, using one-dimensional column specimens which resulted in significant improvement of geotechnical properties, including unconfined compressive strength and permeability. Chittoori et al. (2018)

evaluated the effectiveness of the biostimulation approach to treating natural expansive soils using an injection system. They reported a significant reduction in swelling strain and increased in unconfined strength after one treatment cycle. Chittoori et al. (2018) study is an initial step in establishing an alternative treatment protocol for expansive soils. Biostimulation using the ureolytic bacteria present in the soil is becoming a preferred method of application for MICP.

In order to stimulate the ureolytic bacteria present in the soil and precipitate calcite, substrate solutions must pass through the soil. In the case of clayey soils, percolating or flushing under gravity is not practical due to the low permeability of these soils. Hence, injecting under high pressures is a viable alternative. This approach was studied by Chittoori et al. (2018), who found that calcite precipitation is possible by injecting treatment solutions at high pressures into expansive soils. However, in the case of shallow treatment methods for pavement applications, injecting at high pressures could be counterproductive, as higher pressures can fracture the soil or heave pavement. Hence, in this study, an MICP application method is investigated by mixing substrate solutions into the soil similar to lime or cement stabilization. Two different protocols were studied on three different soils to evaluate their feasibility in precipitating calcium carbonate and increasing the strength of the soil. This paper presents the results obtained from this study.

Materials and Methods

Three soils with varying plasticity characteristics were studied to evaluate the effectiveness of MICP in mitigating expansive soil swelling studied. One of the three soils was a naturally occurring expansive soil obtained from Marsing, Idaho along

highway US-95 near milepost 16.0. This soil was denoted as C-70. The 70 indicates the percentage of clay present in the soil. C-70 soil had a liquid limit of 111 and a plasticity index of 71 which classifies it as a high swelling soil. The remaining two soils were alterations of the C-70 soil to minimize the clay content and correspondingly the swelling capabilities. The clay content in the soil was minimized by adding different percentages of medium to fine sand bringing the clay content of the artificial soils to 40% and 30% and denoted as C-40 and C-30, respectively. All three soils were tested for various geotechnical engineering properties including Atterberg limits, maximum dry unit weight (MDUW) and optimum moisture content (OMC), specific gravity, 1-D swell strain, swell pressure, and unconfined compression strength (UCS).

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

Soil Type	LL (%)	PI (%)	Specific Gravity	MDUW (kN/m^3)	OMC (%)	UCS (kPa)	1-D Swell Strain (%)	Swell Pressure (kPa)
	ASTM D4318		ASTM D854	ASTM D698		ASTM D2166	ASTM D4546	
C-70	111	71	2.53	11.04	32.6	155.1	17.9	287
C-40	62	41	2.66	13.98	28.5	88.2	9.14	179
C-30	43	19	2.6	15.65	21.5	69.6	2.58	70

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

Treatment Solutions

Two types of treatment solutions were used in this research to achieve biomineralization: enrichment solution and cementation solution. Enrichment solutions contained both carbon and nitrogen sources along with other necessary nutrients to facilitate bacterial growth. As recommended by Burbank et al. (2011), the enrichment solutions consisted of 100 mM of Sodium Acetate, 333 mM of Urea and 0.5 g/L of Corn Steep Liquor (CSL). Corn steep liquor consisted of amino acids, vitamins, and minerals

and was provided in both enrichment solution and cementation solution (Burbank et al. 2011) and is necessary for microorganism survival. This cementation solution differed from the enrichment solution only by the calcium presence which facilitated calcium carbonate precipitation. Consequently, 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.

Treatment Protocols

Two protocols were studied to achieve calcite precipitation by mixing the enrichment and cementation solutions into the soil. In Protocol-1, soil samples were mixed with the same volume of enrichment solutions corresponding to an optimum moisture content from the Standard Proctor test. After mixing, the samples were allowed to hydrolyze urea for different periods of time (1, 2, 3, and 4 days). These periods were called mellowing periods, as per the shallow stabilization jargon which identifies the period between mixing and sample compaction (for curing) in chemical stabilization protocols. During the mellowing period, the samples are left on the countertop to allow moisture loss. After completion of the certain mellowing period, the amount of enrichment solution lost was replaced with cementation solution to bring the overall moisture of the sample equal to the optimum moisture content. The soil was compacted into a UCS sample of dimensions 7.1 cm in diameter and 14.2 cm in height. After preparing the sample, the UCS test was performed on the compacted samples as per ASTM D2166. After performing the UCS test, a small portion of the tested soil sample was taken to measure the calcium carbonate content according to ASTM D4373. As per ASTM D 4373, a simple portable device was used to carry out this gasometric method of

carbonate content determination. This device consisted of a reaction cylinder which contained a small cup filled with 1M hydrochloric acid (HCl) and a pressure gauge. Initially, the soil samples were poured into the reaction cylinder, and 20 ml of HCl was placed inside the chamber in the small cup provided. The reaction cylinder was closed tight, and the small cup was tilted to initiate the reaction between the HCl and soil sample. Due to this reaction carbon dioxide was released and pressurized the cylinder. This pressure was recorded using the pressure gauge located on the device. **Figure 2.1** presents a photographic representation of the treatment procedure.

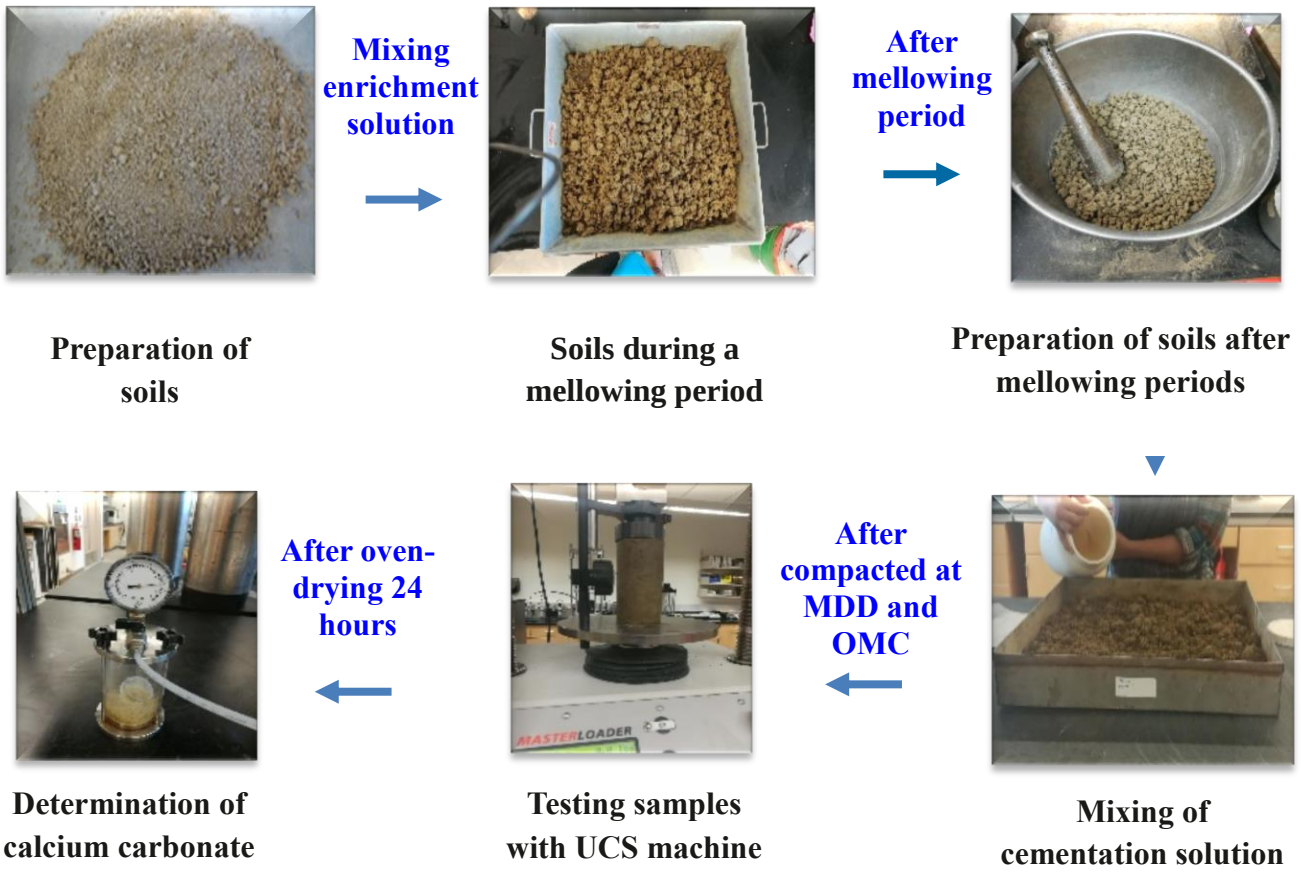

Figure 2.1: Photographic representation of a typical treatment process

In these two protocols, samples prepared for the UCS test were not cured. UCS tests were performed on the samples immediately after preparation. The procedure for Protocol-2 was identical to Protocol-1 except for the initial volume of enrichment solution which corresponded to the moisture content at 95% of MDUW on the wet-side of the standard Proctor curve in place of OMC.

Results and Discussions

Both UCS and calcite content tests were performed on treated and untreated soils to evaluate strength changes and calcite precipitation after treatments. **Table 2.2** and **Table 2.3** present a summary of these results for Protocol-1 and Protocol-2. Both protocols involved the same number of mellowing periods ranging from one to four days. The mellowing periods are denoted as 'MP'. MP-1 denotes a mellowing time of one day, MP-2 denotes a mellowing time of two days and so on.

Table 2.2: Summary of UCS and Calcite tests data for Protocol-1

	UCS (kPa)				Calcite Concentration (%)			
Soil Type	**MP-1**	**MP-2**	**MP-3**	**MP-4**	**MP-1**	**MP-2**	**MP-3**	**MP-4**
C-30	97.3	111.5	139.0	174.8	0.71	0.9	1.01	1.12
C-40	96.4	103.1	123.5	167.0	0.73	0.83	0.9	1.1
C-70	183.6	228.7	141.7	127.7	0.99	1.08	0	0

Table 2.3: Summary of UCS and Calcite tests data for Protocol-2

	UCS (kPa)				Calcite Concentration (%)			
Soil Type	**MP-1**	**MP-2**	**MP-3**	**MP-4**	**MP-1**	**MP-2**	**MP-3**	**MP-4**
C-30	76.1	100.2	120.5	158.4	0.34	0.78	0.95	1.04
C-40	91.0	97.0	112.9	142.6	0.53	0.73	0.85	0.99
C-70	154.9	124.1	114.3	109.4	0.95	0	0	0

Unconfined Compression Test

Figure 2 presents UCS variation with mellowing periods for all three soils. **Figure 2.2**(a) presents the UCS results for Protocol-1 while **Figure 2.2**(b) presents the same for Protocol-2. Both **Figure 2.2**(a) and **Figure 2.2**(b) showed untreated UCS values for all

three soils. Please note that the untreated C-70 soil showed highest UCS value of 155 kPa and C-30 showed the lowest value of 69.6 kPa. Although C-30 soil had higher sand content, the strength was lower due to the unconfined nature of the test.

It can be observed from **Figure 2.2**(a) that for both C-30 and C-40 soils, the increase in the mellowing period appears to increase UCS. This could be due to the formation of calcium carbonate in the void spaces between particles which bonds particles and increases strength. However, for C-70 soil the UCS increased after MP-1 and MP-2 and reduced for MP-3 and MP-4. This reduction in strength after three and four mellowing periods for C-70 soil could be due to bacteria becoming dormant after two days of mellowing and forming pores which may not have resulted in calcite precipitation. Although cementation solutions contained nutrients for bacteria, since the UCS test was conducted immediately after mixing there was not sufficient time to hydrolyze urea and precipitate calcite. Further testing is underway to confirm this hypothesis.

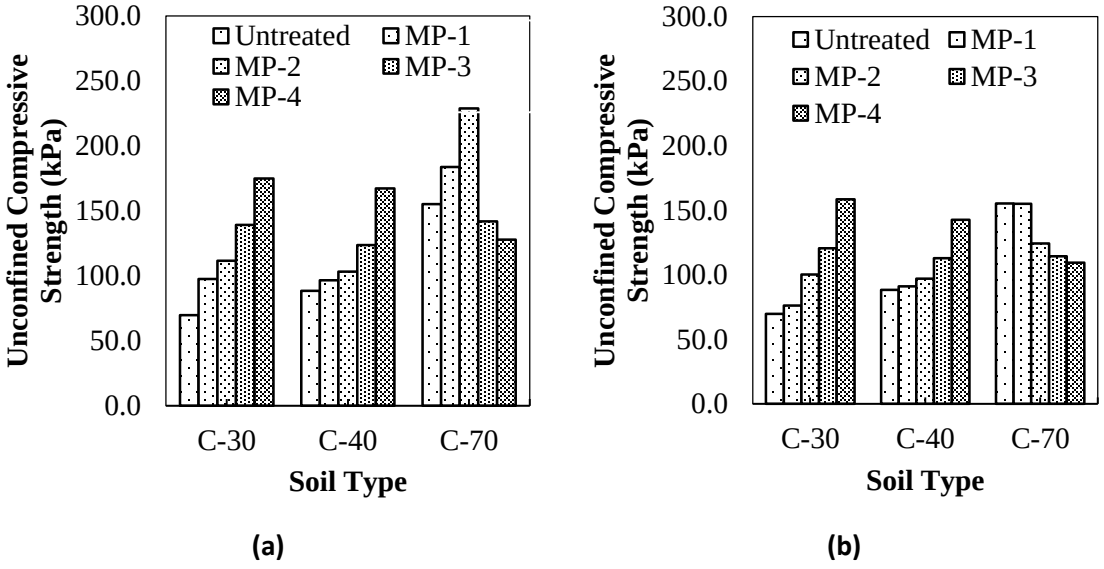

Figure 2.2: Variation of UCS values with mellowing periods for both protocols (a) Protocol-1 (b) Protocol-2

Figure 2.3 shows the percentage change in UCS of all three soils between the protocols. This percentage change is compared with the untreated strength of the soil. It can be noted from **Figure 2.3**(a) and **Figure 2.3**(b) that Protocol-1 performed slightly better than Protocol-2. Nevertheless, both protocols increased the UCS values. In the case of C-70 soil, from **Figure 2.3**(c), it can be noticed that Protocol-2 did not perform well for any of the mellowing periods. This could be due to the addition of less cementation solution after mellowing periods to bring up the moisture content up to OMC. Currently, testing is underway to extend the mellowing periods beyond four days.

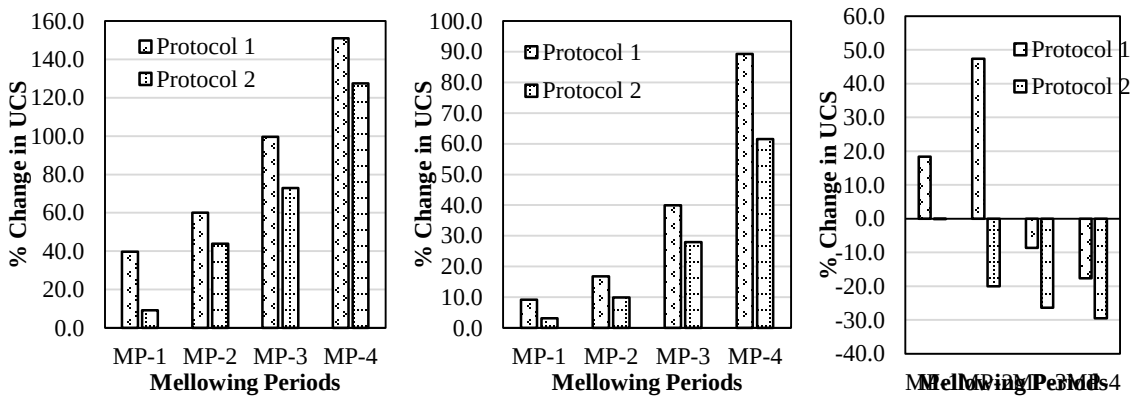

Figure 2.3: Comparison of the percentage change in UCS between protocols for all three soil types (a) C-30 Soil, (b) C-40 Soil, (c) C-70 Soil

Calcium Carbonate Test

All soil samples were tested for the presence of calcium carbonate before and after treatments to confirm that the increase in strength observed was related to calcite precipitation. Visual inspection of the sample after UCS tests showed that that precipitated calcite was uniform across the sample which implied that bacteria and substrate solution were present uniformly in the soil sample. **Figure 2.4** presents the calcite concentration for both protocols. It should be noted here that the calcite reported is

a percentage of the dry weight of soil. It can be observed from **Figure 2.4**(a) that calcite precipitation increased with the increase in the mellowing period for C-30 and C-40 soils for Protocol-1. However, for C-70 soil calcite precipitation increased for MP-1 and MP-2 but was absent in MP-3 and MP-4 samples. This is corroborating the UCS observations, and the reasons for this could be similar to the ones explained in the UCS section of this paper. In case of Protocol-2, as presented in **Figure 2.4**(b), calcite precipitation was evident in both C-30 and C-40 soils, but C-70 soil did not have any calcite precipitation after MP-1. Further testing is underway to measure urease activity of these soils to understand why calcite is not precipitating after for MP-2, MP-3, and MP-4 cases. These results will be discussed in future publications.

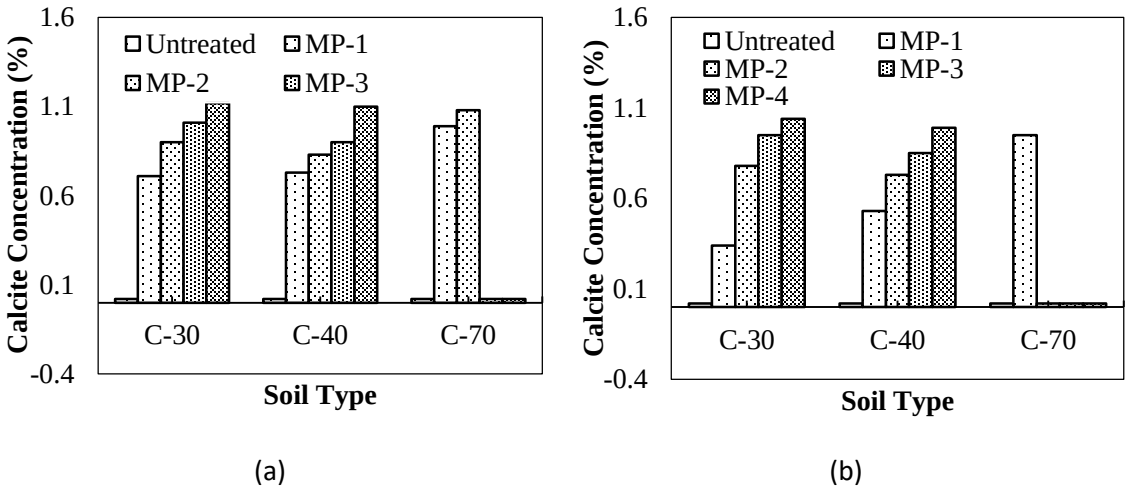

Figure 2.4: Variation of calcite concentration with mellowing periods for both protocols (a) Protocol-1 (b) Protocol-2

Summary and Findings

Experiments were conducted to study the effectiveness of shallow mixing protocols to apply MICP technique to stabilize expansive soils. Two protocols were applied to three soils with varying plasticity characteristics, and their performance was measured using UCS and Calcite precipitation tests. Protocol-1 performed slightly better

than Protocol-2 for all three soils. C-30 and C-40 soils showed improvement in strength with increase in mellowing periods. This improvement in strength was correlated to calcite precipitation in these soils. However, for C-70 soil, the UCS increased for one and two days of mellowing but decreased for three and four days of mellowing. The current results showed that the presence of higher amounts of enrichment solution and addition of less cementation solution is not advantageous for this procedure based on the performance of Protocol-2. Also, in addition to this, an increase in mellowing periods for low and medium plastic soils (C-30 and C-40) was beneficial. However, the mellowing period beyond two days was not beneficial for high plastic soil (C-70). This could be due to bacteria becoming dormant after two days of mellowing in soils with high plasticity due to the hydrophilic nature of these soils. Further testing is underway to measure urease activity of these soils to understand why calcite is not precipitating after for MP-2, MP-3, and MP-4 cases. These results will be discussed in future publications.

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CHAPTER THREE: OPTIMIZING THE USE OF SHALLOW MIXING METHODS TO STIMULATE INDEGENOUS BACTERIA TO PRECIPITATE CALCITE AND ALTER EXPANSIVE SOIL BEHAVIOR

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Abstract

Expansive soils generally recognized as swell-shrink soils have been a problem for civil infrastructure from a long time. The use of chemical stabilizers including cement and lime to stabilize expansive soils especially for lightly loaded structures has been a common practice. However, due to detrimental effects on the environment and several occurrences of premature failures after stabilizing with chemical additives, engineers are in search of sustainable stabilization alternatives. Microbial Induced Calcite Precipitation (MICP) is a promising biocementation process which can improve the properties of expansive soil through calcite precipitation. Past research has shown promise for the use of MICP in mitigating swelling distressed from expansive soils. There are mainly two approaches to apply MICP: Bioaugmentation and Biostimulation. Both bioaugmentation and biostimulation were attempted in the past by injecting treatment solutions into the soil with mixed success. In this research, biostimulation was attempted by mixing enrichment and cementation solutions with soils in an effort to develop a new alternative

to shallow chemical stabilization. For this purpose, three soils with varying clay contents and plasticity characteristics were selected. Soils were treated by mixing with enrichment solution and were allowed to mellow and stimulate bacteria. During the mellowing period, moisture was allowed to escape from soil and the lost moisture was replaced with cementation solution at the end of the mellowing period. Following the addition of cementation solution, soil samples were compacted at the maximum dry density and optimum moisture content and were cured at 100% humidity. Five different mellowing periods, three different curing periods and two types of cementation solutions were studied to optimize the method. Treatment effectiveness was evaluated using Unconfined Compression tests, Calcium Carbonate tests, and Free Swelling Index tests. Improved test results were observed with a lower concentration of calcium chloride used in the cementation solution. The best improvement was observed at two days of mellowing, seven days of curing.

Keywords: MICP, expansive soils, soil stabilization, biostimulation, calcite precipitation

Introduction

Clays are often associated with low bearing capacity, high compressibility, along with swelling and shrinkage behavior. These phenomena are caused by a change in moisture. The change in moisture could be due to seasonal or climatic variations and evapotranspiration of vegetation. The change in swelling pressure can contribute to lifting of structure in the vertical direction, and shrinkage causes differential settlement under the foundation (Jones and Jefferson 2012). Volumetric changes owing to moisture variation cause damage to the lightly loaded structures including pavements, retaining

walls, and residential houses. The most common problematic clays are soft clays and expansive clays. Expansive soils swell and shrink with the fluctuation of moisture content (Nelson and Miller 1992). The reason behind the expansive behavior of soil is the presence of heaving mineral known as montmorillonite which has an expanding lattice. Some factors influencing this behavior are soil composition, dry density, soil fabric, confinement and permeability (Nelson and Miller 1992). Due to this problem, damage to a lightly loaded structure built on these soils is more than any other natural disaster such as earthquakes and flood (Jones Jr and Holts 1973). The annual cost of damage due to this type of soil increased from $2.2 billion/year in 1973 (Jones Jr and Holts 1973) to $ 15 billion/year in 2012 (Jones and Jefferson 2012).

The implementation of soil stabilization technique to mitigate this problem has been an issue for a few decades. Soil stabilization can be defined as the modification of physical and engineering characteristics of problematic soils to attain sufficient strength and workability. Petry and Little (2002) discussed several stabilization methods including mechanical compaction, chemical stabilization, pre-wetting, moisture barriers, lime injections, and deep soil mixing. To alter the physicochemical behavior of expansive soil, additives including lime and cement are the most widely used approaches in the United States and around the world (Sherwood 1993). However, there were some environmental concerns associated with these methods including the generation of greenhouse gases and adverse impact on the plants due to elevated pH levels. The production of cement and lime is one of the main sources of greenhouse gases. Cement is used in concrete, and concrete is used in building structures including buildings, roads, foundations, and bridges. It is a common belief that concrete is the second most consumed substance after

water (WBCSD, 2009). Cement is produced by heating limestone along with other clay minerals in a kiln at 1400ºc. The product from the kiln is mixed with gypsum to form cement. Manufacturing of cement is highly energy and emissions intensive because it requires 60-130 kg of fuel and 110 kWh of electricity leading to the emissions of around 900 kg CO_2 (Factsheets 2009) to produce a ton of cement. With the increase in cement production at the rate of 2.5% annually, and is expected to rise from 2.55 billion tons in 2006 to 3.7-4.4 billion by the year 2050 (WBCSD, 2009). Other greenhouse gases are also related to the production of cement. Also, heating of limestone in a kiln directly contributes to the emission of CO_2. Another issue with chemical stabilization is related to the longevity of chemical stabilization. Subgrade failures were observed due to loss of stabilizer over time due to water table fluctuation and rainfall infiltration. Therefore, it was important to identify an alternative stabilization method which can be both environmentally friendly, long-lasting and cost-effective.

Microbial Induced Calcite Precipitation (MICP) is an environment-friendly technique which could be an alternative to the conventional stabilization methods. In recent years, the use of MICP technique is gaining attention as a versatile and green method of soil improvement. Biostimulation is a type of MICP process where indigenous microbes are stimulated to precipitate calcite. However, to stimulate the ureolytic bacteria present in the soil, substrate solutions should pass through the soil and reach the microbes. But it is very difficult to use a percolating or flushing system under gravity to pass the substrate solutions in clayey soils due to their low permeability. Hence, Chittoori et al. (2018) studied biostimulation in clayey soils by injecting substrate solutions under high pressures. In that study, it was found that calcite precipitation was possible by

injecting treatment solutions at high pressures. However, for shallow treatments including pavement applications, injecting at high pressures could be counterproductive, because higher pressures can fracture the soil or heave pavement.

Also, precipitate calcite can lower the porosity and permeability which in the result, can reduce the infiltration rate (Cheng and Shahin 2015). In addition, clogging at the injection location could be of concern as well. Hence, in this study, a new MICP application approach was evaluated by mixing substrate solutions with soil similar to mixing lime or cement in case of chemical stabilization. In this approach, soil samples were first mixed with enrichment solutions to stimulate the bacteria followed by cementation solutions to precipitate calcite. The protocol consists of mixing the enrichment solutions at optimum moisture content and allowing them to stimulate bacteria for different time periods termed mellowing periods. During the mellowing period's samples were left on the countertop and moisture loss (enrichment solution loss) was allowed. At the end of the mellowing period, the lost moisture was replaced with cementation solutions that contain calcium chloride and the soil sample was compacted at OMC and maximum dry unit weight (MDUW). The compacted samples were sealed and cured for different time periods at 100% humidity conditions. Three soils with varying plasticity and clay characteristics were used to evaluate the approach. Five different mellowing periods and three different curing periods were evaluated to arrive at optimum time periods for each step. Two types of cementation solutions whose calcium chloride concentrations varied were also studied to study the effect of cementation solutions on the treatments. Performance of treatments was evaluated using unconfined compression

strength, free swell index, and percentage calcite precipitated. The results obtained from these studies are presented in this paper.

Background

MICP process is based on the comprehension of microbiology, geochemistry and geotechnical engineering (DeJong et al. 2010). In this process, the alkalinity or pH of the system increases, which affects the calcite precipitation (v. Knorre and Krumbein 2000). Bacteria are very dominant soil inhabitant and there are 10^6-10^{12} bacterial cells in a gram of soil (Torsvik et al. 1990). *S. pasteurii* species from Bacillus group, an alkalophilic soil bacterium, has high urease enzyme activity (DeJong et al. 2006b) and is a commonly used in MICP laboratory research.

In this process, ureolytic bacteria hydrolyzes urea to produce ammonium and carbonate ions (Eq. 1). After the addition of Ca^{2+} ion, calcium carbonate crystals (Eq. 2) are precipitated on the cell wall of the bacteria (Burne and Chen 2000).

$$CO(NH_2)_2 + 2H_2O \longrightarrow 2NH_4^+ + CO_3^{2-} \quad (1)$$

$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3 \quad (2)$$

Mainly four factors affect the MICP process: calcium ion concentration, dissolved inorganic carbon (DIC) concentration, pH, and availability of nucleation sites (Hammes and Verstraete 2002). In addition to this, the ability to metabolize, grow and reproduce affects the survivability of microbe (Rebata-Landa 2006). The factors are also termed as ‘limiting growth factors’.

Microbial growth, metabolic activity, and cell-surface charge are dependent on the change in pH (Rebata-Landa 2006). The ammonia produced with urea hydrolysis is the reason for increasing the pH of the medium. Stocks-Fischer et al. (1999) stated that

the urease activity increased mostly from pH 6.0 to 8.0. Urease activity reached highest at pH 8.0 and decreased with higher pH although there was some urease activity noted at pH 9.0. However, if there is sufficient chemical reagent, the rate of urea hydrolysis has a direct relationship with the bacterial cell concentration. More bacteria produce more urease per unit volume to start the urea hydrolysis. Stocks-Fischer et al. (1999) observed that the bacteria cell can serve as a nucleation site for calcite to precipitate. Lian et al. (2006) identified from SEM images that the nucleation of calcite takes place at bacteria cell walls. High salinity can cause inhibition and stop microbial activity (Rivadeneyra et al. 1998). The salinity of cementation fluid is dependent on calcium salt. Microbial activity can be obstructed by high salinity which can limit the urease production from ureolytic bacteria (Nemati et al. 2005).

Applications of MICP

Calcite precipitation in MICP process bridges adjacent soil particles, cementing soil particles together (DeJong et al. 2006; Whiffin et al. 2007). The precipitation of calcite reduces the permeability and compressibility while increasing soil strength (DeJong et al. 2010). Calcite mineralization is the result of a by-product of microbial metabolic activity including photosynthesis, urea hydrolysis, sulfate reduction, and iron reduction (v. Knorre and Krumbein 2000).

MICP has several applications in diverse fields including increase in concrete strength and durability (De Muynck et al. 2008), mitigation of sand liquefaction (Montoya et al. 2012); sand impermeability (Nemati and Voordouw 2003), soil strength (Van der Ruyt and van der Zon 2009; Lu et al. 2010) , sand impermeability (Nemati et al. 2005), brick durability (Sarda et al. 2009).

There are very few studies found that were related to the application of MICP on expansive soil. The geometric compatibility between soils and microbial communities is one of the main obstacles to introduce MICP in clay. The range of cell diameter soil bacteria present in soil is from.5 to 3 μm (Mitchell and Soga 2005). Chittoori et al. (2016) performed a Mercury Intrusion Porosimetry (MIP) test to observe the pore size and pore volume on two expansive soils after compaction. It was found that 30% to 50% of the pore volume was larger than 1.5 μm at a maximum dry density which is the average diameter of soil bacteria. So, space is available through the pore spaces for bacterial mobilization. There were some studies regarding biotreatment on expansive soils. Bing (2014) conducted biotreatment on kaolin, marine clay, and bentonite and observed strength increased by around 150% and 400% for treated kaolin and treated marine clay, respectively. Cheng and Shahin (2015) attempted three different MICP methods including injection, premixing, and diffusion for clayey sands to investigate the variation of strength and amount of calcium carbonate precipitation. Soils having 5% clay content worked best in injection method. Cardoso et al. (2018) investigated the compressibility and pore clogging of the biocemented sand-kaolin mixture and found that the osmotic consolidation effect might be a contributing factor for high compressibility along with the bacterial activity.

MICP Methods

There are two methods to apply MICP: *Bioaugmentation* and *Biostimulation*. In *bioaugmentation*, exogenous bacteria were introduced into the soil to precipitate calcite. Most of the research studies have applied bioaugmentation method on silty and sandy soil (Whiffin et al. 2007; van Paassen et al. 2010; Soon et al. 2013; DeJong et al. 2010;

Mortensen et al. 2011). Bioaugmentation process had a successful implementation in the improvement of concrete strength and durability (De Muynck et al. 2008); mitigation of sand liquefaction (Montoya et al. 2012); and sand impermeability (Nemati and Voordouw 2003).

Chittoori and Neupane (2018) studied the application of bioaugmentation to mitigate expansive soil swelling. They studied two different protocols on three selected soils having low, medium and high plasticity characteristics. Different concentrations of bacteria and substrate were mixed with soil and cured for 7 days in one protocol. In other protocol, different concentrations of bacteria were mixed into the soil and compacted; substrate solutions were injected into the compacted sample. It was reported that low to medium plastic soils can be effectively treated using MICP via bioaugmentation. However, in this method augmented exogeneous bacteria has to adjust to the new environment and compete with native microorganisms, which can definitely affect the survival rate and metabolic potential of the augmented bacteria (Wenderoth et al. 2003). It was observed that the survivability of exogenous microorganisms in a new environment, tend to decline rapidly and rarely propagate (van Veen et al. 1997). Also, the uneven distribution of bacteria and clogging near the inlet were other issues associated with this method (Stocks-Fischer et al. 1999). The requirement of injecting nonnative bacterial strains into soil has restricted the technology from becoming an economical method (Gomez et al. 2018).

On the other hand, *biostimulation* uses indigenous bacteria for calcite precipitation (Burbank et al. 2011) and this method is becoming a popular method of application for MICP. This approach does not require expensive non-native monoclonal

bacterial cultivation and injection into natural soil ecosystems which have made it economically and environmentally beneficial. These ureolytic microbes are more resilient than the injected microbes which resulted in a uniform distribution of calcite and sustained enzymatic capabilities (Gomez et al. 2018). Usually, the microbe population is 10^6 to 10^{12} per gram in soil (Torsvik et al. 1990; Boquet et al. 1973). It was proven by Boquet et al. (1973) that all soil bacteria could precipitate calcite. With biostimulation process, it is possible to increase their number in a variety of soils (Burbank et al. 2011). It was first demonstrated by Burbank et al. (2011) that native ureolytic microorganisms can hydrolyze urea and induce calcite precipitation in liquid media using ureolytic strains obtained from the Eastern Snake River. Gomez et al. (2014) also demonstrated field test for calcite precipitation in granular soils, using one-dimensional column specimens which resulted in significant improvement of geotechnical properties, including unconfined compressive strength and permeability. Chittoori et al. (2018) initiated a treatment to treat natural expansive soils through an injection system. A significant reduction in swelling strain and increase in unconfined strength after one treatment cycle were observed. In this research study, the biostimulation method has been applied through a mixing protocol. This research study is an initial step to establish an alternative treatment protocol for stabilizing shallow expansive soils.

Materials

Soils

Three soils with varying plasticity were chosen to evaluate the proposed method of MICP application. Out of the three soils, one soil is a naturally occurring expansive soil while the other two soil were prepared by mixing different percentages of the natural

soil and a medium fine sand (D_{60} = 0.68 mm, D_{10} = 0.24 mm and C_u = 2.83). This was done to study the role of clay content and plasticity characteristics on this method. The natural soil was collected along US 95 highway close to Marsing, Idaho. This soil contained about 70% clay and is denoted as C-70. This clay content is adjusted to be 30 and 40% by adding the sand and these soils are denoted as C-30 and C-40, respectively. All three soils were tested for various geotechnical engineering properties including Atterberg limits, maximum dry density. It can be observed from **Table 3.1** that the MDUW ranged from 11.04 to 15.65 kN/m^3 and the OMC ranged from 32.6% to 21.5% with the decrease in clay content. A significant increase in maximum dry unit weight and a decrease in optimum moisture content with the decrease of clay content were observed here. Also, the increase of clay particles from C-30 soils to C-70 soils contributed to the gradual increase of unconfined compressive strength in those soils**.** The gradual improvement of strength could be due to the inner bonding of fine particles. Besides, the 1-D swell strain ranged from 17.9 %to 2.58% and the swell pressure ranged from 287 kPa to 70 kPa with the decrease of clay content. Also, Liquid Limit and Plastic Limit decreased from 111 to 43 and 71 to 10 respectively with the decrease in clay content. It can be summarized that here, with the decrease in the finer particle, LL decreased, PI decreased, swell strain and swell pressure decreased.

Table 3.1: Baseline data for all three soils

Soil Type	**LL (%)**	**PI (%)**	**Specific Gravity**	**MDUW (kN/m^3)**	**OMC (%)**	**UCS (kPa)**	**1-D Swell Strain (%)**	**Swell Pressure (kPa)**	**Free Swell Index (%)**
	ASTM D4318		**ASTM D854**	**ASTM D698**		**ASTM D2166**	**ASTM D4546**		
C-70	111	71	2.53	11.04	32.6	155.1	17.9	287	108
C-40	62	41	2.66	13.98	28.5	88.2	9.14	179	123
C-30	43	19	2.6	15.65	21.5	69.6	2.58	70	162

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

Treatment Solutions

Soil treatments to stimulate bacteria for calcite precipitation consisted of enrichment and cementation solutions. Enrichment solutions contained both carbon source as acetate and nitrogen source in the form of urea. The composition of enrichment solutions was 100 mM of Sodium Acetate, 333 mM of Urea and 0.5 g/L of Corn Steep Liquor (CSL). Corn steep liquor is consisted of amino acids, vitamins, and minerals and used to stimulate the initial activity within the soil profile (Burbank et al. 2011). The enrichment solution stimulates the growth of bacteria that which use Sodium Acetate as a carbon source and urea or ammonia as a nitrogen source. The presence of urea works as a nitrogen source and the increase in the pH as a result of the presence of ammonium from urea hydrolysis creates an environment for bacteria that they can survive in a high-pH environment and use urea or ammonia as a nitrogen source. When microbe being ureolytic, the rate of hydrolysis increases, which in result increases the rate of precipitation (Burbank et al. 2011).

Cementation solution contained all of the enrichment solutions with the addition of the calcium chloride. In this research study, two types of cementation solutions were used. In cementation solution1, the concentration of calcium chloride is 250 mM and in another cementation composition, the concentration of calcium chloride is 500 mM. Two concentration of calcium chloride has been used to observe the effect of the variation of the amount of calcium chloride in calcite precipitation.

Table 3.2: Chemical compositions in substrate solution

Solution Type	Chemical Name (Formula)	Concentration	Remarks
Enrichment Solutions	Sodium Acetate	100 mM	Carbon source
	Urea	333 mM	Nitrogen source
	Corn Steep Liquor	0.5 g/L	Nutrient source
Cementation Solutions	Sodium Acetate	100 mM	Carbon source
	Urea	333 mM	Nitrogen source
	Corn Steep Liquor	0.5 g/L	Nutrient
	Calcium Chloride	250 mM (CS-1) 500 mM (CS-2)	Cementation source

Methods

Treatment Protocol

The treatment consisted of mixing soil with a volume of enrichment solutions corresponding to an optimum moisture content from the Standard Proctor test. After mixing, the samples were allowed to hydrolyze urea for different periods of time (1, 2, 3, 4 and 7 days). These periods were called mellowing periods to follow the shallow stabilization jargon. In chemical stabilization protocols, mellowing period is the time between mixing (soil with chemicals and water) and sample compaction (for curing). Unlike shallow stabilization protocols, moisture loss was permitted during this time in this research. The lost moisture was replaced with the cementation solution. After mixing with the cementation solutions and bring the moisture back to OMC, the soil samples were compacted into a cylinder of dimensions, 7.1 cm diameter, and 14.2 cm height. After that, samples were cured with controlled humidity and temperature for 0, 3 and 7 days or curing time. The curing periods are denoted as ‘CP’. The mellowing periods are denoted as ‘MP’. Figure 3.1 presents a pictorial representation of the treatment protocol.

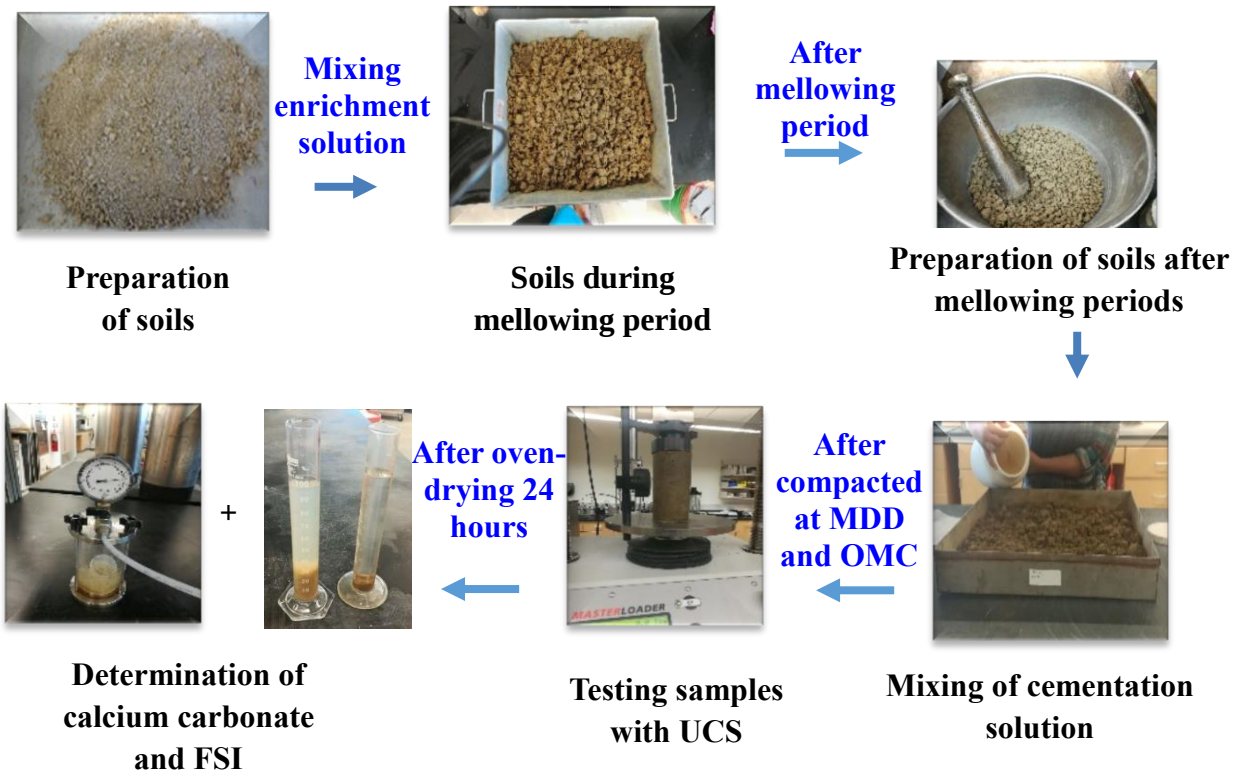


Figure 3.1: Pictorial description of the treatment protocol

Evaluation Tests

To evaluate the effect of bio stimulated MICP, three evaluation tests including Unconfined Compression Test (UCS), Calcium Carbonate Test and Free Swelling Index (FSI) test. The following sections briefly describe the procedures followed to conduct these tests.

Unconfined Compression Strength (UCS)

The purpose of this test is to determine the compressive strength of the soil. Unconfined Compression test is an unconsolidated undrained test where the lateral confining pressure is equal to zero. To perform an unconfined compression test, the 71 mm by 142 mm sample was extruded from the sampler. A cylindrical sample of soil had the length-to-diameter ratio was on the order of two. The soil sample was placed in a

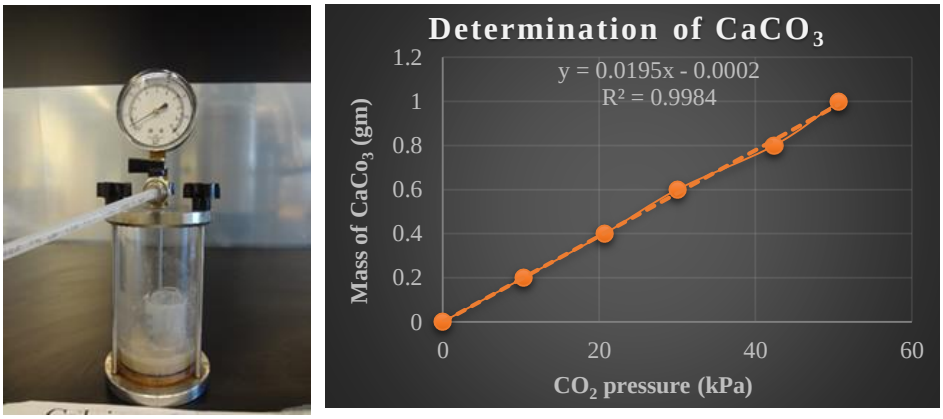
loading frame on a metal plate. The equipment used for this test was shown in **Figure 3.2**. The load was gradually increased to shear the sample, and readings were taken periodically of the force applied to the sample and the resulting deformation. The loading was continued until the soil developed an obvious shearing plane or the deformations became excessive. The measured data were used to determine the strength of the soil specimen and the stress-strain characteristics. The maximum load per unit area was defined as the unconfined compressive strength, q_u.

Figure 3.2: UCS testing machine used in this research

Calcite Test

After UCS tests, the samples were oven dried and used to measure the carbonate content in soils according to ASTM D4373. A simple portable device was used to carry out this gasometric method. This device consisted of a reaction cylinder, a cup filled with hydrochloric acid (HCl) and pressure gauge (**Figure 3.3**a)). Initially, the soil samples were poured into the reaction cylinder and a small cup with HCl was put inside the chamber. The reaction cylinder was closed tightly, and the small cup was tilted to create a reaction between the HCl and soil samples which released carbon-di-oxide and

pressurized the chamber. The pressure inside the chamber was recorded using a pressure gauge mounted on the chamber. This pressure is related to the amount of carbonate present in the soil using a calibration curve (See **Figure 3.3** b) prepared with known amounts of reagent grade $CaCO_3$. It was assumed that the carbonate present in the soil was calcium carbonate or calcite, especially after treatments.



a) b)

Figure 3.3: Equipment for calcium carbonate test (left) and Calibration Chart

Free Swell Index (FSI)

The free swell index is a simple experimental procedure performed to estimate a given soil's expansion potential (Holtz and Gibbs 1956). It is defined as the increase in the volume of a soil without any external constraints after submergence in water. In this test, two representative oven-dried soil samples (passing # 40 sieve) weighing 10 grams each were poured in to two graduated cylinders of 100 ml capacity with the help of a funnel. One cylinder was filled with distilled water while the other was filled with kerosene up to 100 ml mark. Entrapped air was removed by mild shaking and stirring with a glass rod. Soil samples are allowed to attain equilibrium state of volume without

any further change in the volume of the soils in 24 hours. In the end, the final volume of soil samples in both cylinders are recorded in **Figure 3.4**.

Figure 3.4: Free Swelling Index Test

Results

UCS, calcite content and FSI tests were performed on treated and untreated soils to evaluate strength changes, calcite precipitation, and swell changes respectively. Each of these tests is discussed in the following subsections. The data corresponding to the tests is presented first in a summary table followed by a discussion on the test results with the help of plots.

UCS Test Results and Discussion

The UCS values for the untreated C-30, C-40, and C-70 soils were 69, 88, and 155 kPa. **Table 3.3** presents the UCS values for all three soils tested in this study. UCS data for each of the mellowing and curing periods for both cementation compositions can be observed in Table 2. It can be observed from this table that the UCS values ranged from 63 kPa to 267 kPa with different curing and mellowing periods for CS-1 while those for CS-2 ranged from 45 to 182 kPa.

The reason behind the increase in strength could be the longer curing period with controlled humidity which was beneficial for bacteria for reproduction and production of calcite. Also, it could be due to sufficient pore size between soil grains which lead to sufficient calcite precipitation resulting in high UCS.

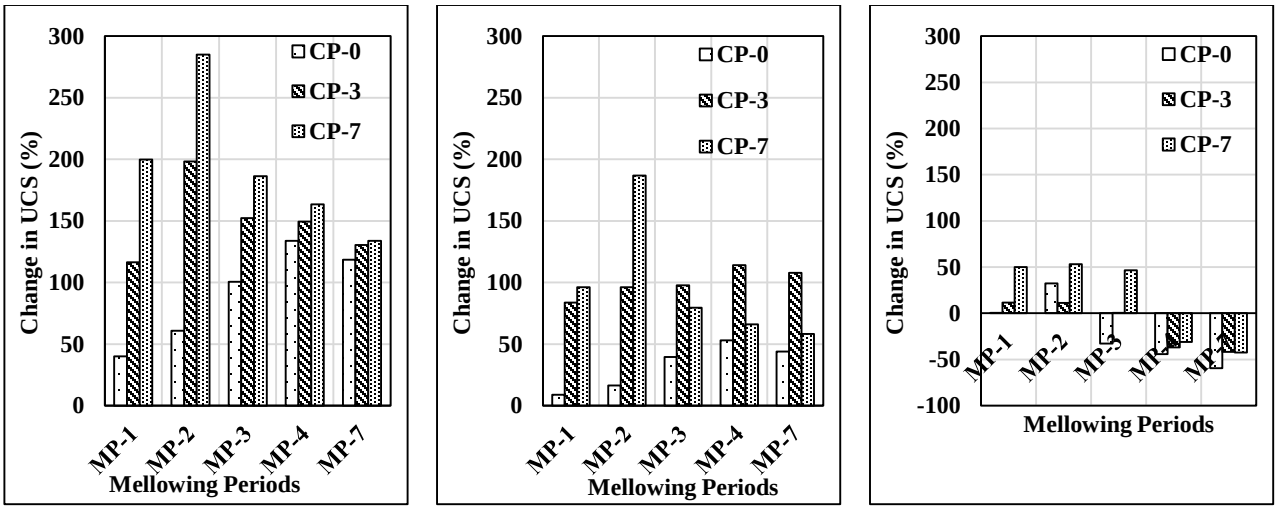
Table 3.3: Summary of treated UCS samples

UCS (kPa)											
Soil Type	**Curing Period**	**CS-1**					**CS-2**				
		MP-1	**MP-2**	**MP-3**	**MP-4**	**MP-7**	**MP-1**	**MP-2**	**MP-3**	**MP-4**	**MP-7**
C-30	**CP-0**	97.1	111.6	139.2	162.2	151.6	79.2	102.4	106.8	121.8	116.7
	CP-3	150.1	206.9	175.0	173.0	159.7	139.8	161.5	135.4	111.9	107.7
	CP-7	207.8	266.9	198.5	182.6	162.2	151.7	167.2	153.1	125.8	101.8
C-40	**CP-0**	96.3	103.0	123.4	135.4	127.3	90.5	95.6	100.7	53.3	45.2
	CP-3	162.4	173.5	174.9	189.3	183.8	65.1	102.2	141.4	120.2	96.3
	CP-7	173.5	253.6	158.7	146.8	139.8	58.5	99.2	143.9	113.3	70.2
C-70	**CP-0**	155.5	205.4	104.3	86.5	63.2	143.1	138.9	135.3	115.9	98.9
	CP-3	173.0	172.4	155.9	98.0	90.1	150.0	141.9	125.7	115.1	92.9
	CP-7	232.8	237.1	227.0	106.8	89.3	182.3	135.7	118.5	109.2	91.8

Effect of Mellowing and Curing Periods

Figure 3.5 presents the percentage change in UCS for different mellowing and curing periods for all three soils treated with CS-1. The percentage change is determined using the untreated UCS of the corresponding soil. It can be observed from **Figure 3.5** (a) that the UCS values for C-30 soil were increasing with mellowing periods, MP-1 through MP-4 for CP-0 curing samples. The UCS value dropped for MP-7 for CP-0 curing. A similar trend was observed for CP-3 and CP-7 curing periods after just two days of mellowing. It can also be observed that CP-7 at MP-2 gave the maximum increase (284%) in UCS. A similar trend was observed for the UCS with C-40 soil. Maximum

increase (186%) in UCS value can be observed with CP-7 and MP-2. Also, the C-70 soil was increased with mellowing periods, MP-1 and Mp-2 and dropped after that for all curing periods, CP-0, 3, 7. Maximum increase (186%) in UCS value can be observed with CP-7 and MP-2. It could be due to hydrophilic nature of the C-70 soil having finer particles, the loss of enrichment solution would be faster and as a result, bacteria could have been dormant after two mellowing days which followed the decrease in UCS. Overall, the best treatment periods for all soil was mellowing period, MP-2 in combination with curing period 7. It was noted that an increase in the mellowing period beyond two days was not beneficial for any soils. In case of mellowing periods beyond two-days the bacteria may have become dormant as the enrichment solutions are drying out. Upon the addition of cementation solutions after the mellowing period completion, the cementation solution is probably taking the role of enrichment as the bacteria may have sporulated due to insufficient nutrients beyond two days. Since calcium is present in the cementation solutions it may be shunting bacteria growth as was observed in earlier research (Burbank et al. 2011; Nemati et al. 2005).



a) **b)** **c)**

Figure 3.5: Effect of mellowing periods and curing periods on UCS with cementation composition 1 a) C-30 b) C-40 c) C-70

Effect of Type of Cementation Solution

The improvement of UCS was increased most for Mellowing Periods, MP-1, MP-2 and MP-3 with curing period 7. From **Figure 3.6**, the effect of cementation compositions was observed with different soils with these mellowing periods with curing period 7. The increase for C-30 soil has been observed from Figure Figure 3.6(a) with MP-1, MP-2 and dropped for MP-3 for CS-1. Also, the same trend was observed with for CS-2. From Figure 6(b), the increase in UCS for C-40 soil has been observed with MP-1, MP-2 and dropped for MP-7 for CS-1. In contrast, UCS has been dropped with MP-1 but increased with MP-2 and MP-3 for CS-2. In Figure 6(c), the increase in UCS for C-70 soil was following the same trend similar to C-30 and C-40 soil for CS-1. On the contrary, UCS has been increased with MP-1 but dropped with MP-2 and MP-3 for CS-2. Also, it can be observed from **Figure 3.6** that, the strength of the tested samples were also dependent on the concentration of calcium chloride of cementation solution used in the treatment. The increase in UCS was higher for CS-1 than CS-2 for different

mellowing periods. This could be attributed to the crystal morphology of the precipitated calcium carbonate and due to the formation of a less stable form of calcium carbonate named as vaterite (Al Qabany et al. 2012). Also, the presence of calcium could cause inhibition impact on the microbe, using more conc. of calcium chloride in the cementation phase is decreasing bacteria growth at that stage and as a result having less calcite precipitation.

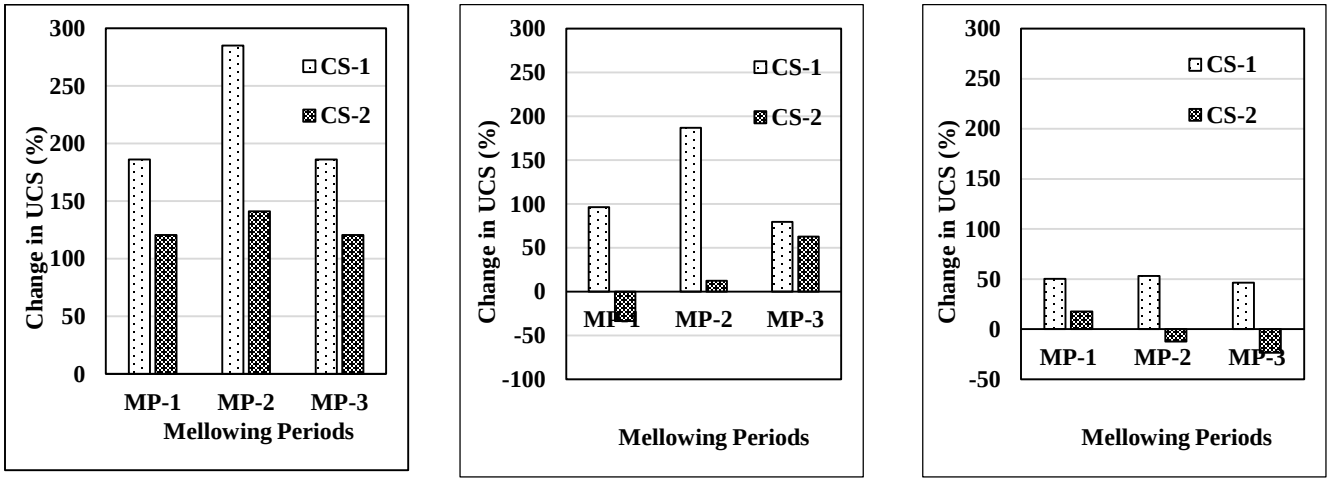
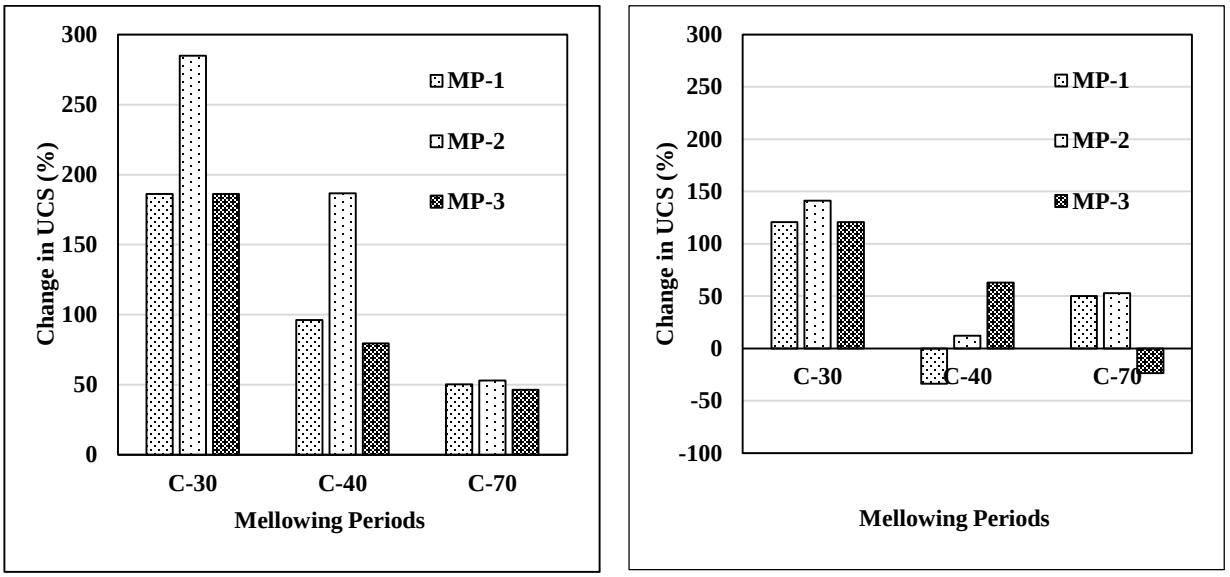


Figure 3.6: Effect of variation of cementation compositions with curing period 7 a) C-30 b) C-40 C) C-70

Effect of Soil Type

From **Figure 3.7**, the effect of soil types was observed with different cementation compositions with mellowing periods MP-1, MP-2, and MP-3 with curing period 7. From **Figure 3.7**(a) UCS was decreased with MP-1 with CS-1 for C-30, C-40, and C-70 respectively. Also, the same trend was observed with MP-2 and MP-3. From Figure 3.7(b), The UCS increased for MP-1 and dropped and again increased with CS-2 for C-30, C-40, and C-70 respectively. The same trend was observed with MP-2. In contrast, the percentage change in UCS showed a decreasing trend with MP-3. C-30 soil had maximum UCS increase and C-70 soil had minimum UCS decrease which can be

attributed to the pore size distribution of these soils. As, the C-30 soil had more pores which could lead to more calcite precipitation and eventually, resulted in high UCS strength. C-70 soil showed the opposite trend having fewer pores.



a) **b)**

Figure 3.7: Effect of soil type on UCS change with different mellowing periods a) Cementation composition 1 (CS-1) b) Cementation composition 2 (CS-2)

Calcite Test Results and Discussions

The calcite values for the untreated C-30, C-40, and C-70 soils was 0%. **Table 3.4** presents the calcite values for all three soils tested in this study. Calcite data for each of the mellowing and curing periods for both cementation solutions compositions can be observed in this **Table 3.4**. It can be observed from this table that the calcite values ranged from 0% to 1.36% with different curing and mellowing periods for CS-1 while those for CS-2 ranged from .0 % to .88%.

The reason behind the increase in calcite could be the longer the curing period which was beneficial for bacteria for reproduction and production of calcite. Also, it

could be due to sufficient pore size between soil grains which lead to sufficient calcite precipitation with controlled humidity.

Table 3.4: Calcite test results of treated samples

Soil	Curing Period	CS-0					CS-2				
		MP-1	MP-2	MP-3	MP-4	MP-7	MP-1	MP-2	MP-3	MP-4	MP-7
C-30	CP-0	0.72	0.91	1.01	1.13	0.82	0.49	0.58	0.58	0.68	0.62
	CP-3	0.78	1.13	0.88	0.93	0.88	0.78	0.88	0.78	0.58	0.58
	CP-7	1.13	1.17	1.01	0.88	0.86	0.88	0.97	0.82	0.68	0.58
C-40	CP-0	0.72	0.82	0.91	1.10	0.58	0.39	0.58	0.58	0.19	0.00
	CP-3	0.78	0.84	0.88	0.97	1.07	0.49	0.58	0.78	0.68	0.39
	CP-7	0.88	1.36	0.86	0.82	0.88	0.19	0.68	0.82	0.82	0.39
C-70	CP-0	0.99	1.08	0.00	0.00	0.00	0.19	0.19	0.00	0.00	0.00
	CP-3	0.88	0.78	0.84	0.00	0.00	0.19	0.19	0.00	0.00	0.00
	CP-7	1.21	1.27	1.17	0.00	0.00	0.39	0.00	0.00	0.00	0.00

Effect of Mellowing and Curing Periods

Figure 3.8 presents the percentage change in calcite for different mellowing and curing periods for all three soils treated with CS-1 and CS-2. The percentage change was determined using the untreated calcite of the corresponding soil. It can be observed from **Figure 3.8**(a) that the calcite values for C-30 soil were increasing with mellowing periods, MP-1 through MP-4 for CP-0 curing samples. The calcite values dropped for MP-7 for CP-0 curing. This could be due to the long wait period between the addition of enrichment solutions and cementation solutions (7 days) during which time the bacteria

may have become dormant due to lack of nutrient supply. However, bacterial activity tests were not run to confirm this hypothesis. A similar trend was observed for CP-3 and CP-7 curing periods after just two days of mellowing. It can also be observed that CP-7 at MP-2 gave the maximum increase 1.17% in calcite. A similar trend was observed for the calcite with C-40 soil. Maximum increase 1.36% in calcite values can be observed with CP-7 and MP-2. Also, the C-70 soil was increased with mellowing periods, MP-1 and Mp-2 and dropped after that for all curing periods, CP-0, 3, 7. Maximum increase 1.27% in calcite value can be observed with CP-7 and MP-2. It could be due to hydrophilic nature of the C-70 soil having finer particles, the loss of enrichment solution would be faster and as a result, bacteria could be dormant after two mellowing days which followed the decrease in calcite precipitation. Overall, the best treatment periods for all soil was mellowing period, MP-2 with in combination with curing period, CP- 7.

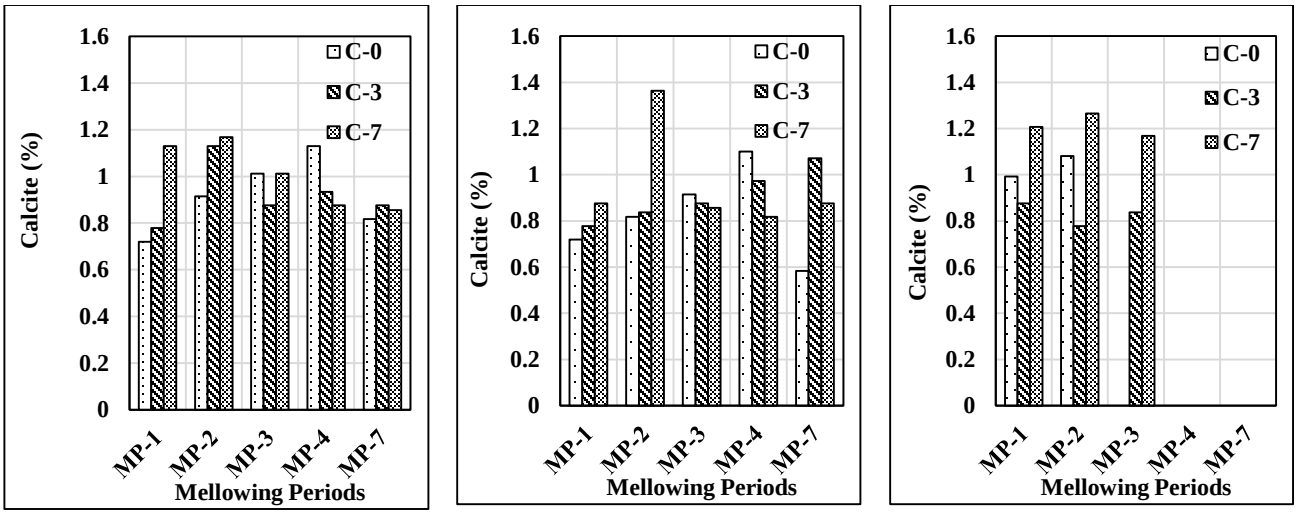


Figure 3.8: Effect of mellowing periods and curing periods on calcite precipitation with cementation composition 1 a) C-30 b) C-40 c) C-70

Effect of Type of Cementation Solution

The improvement of calcite was increased most for Mellowing Periods, MP-1, MP-2 and MP-3 with curing period 7. From **Figure 3.9** the effect of cementation compositions was observed with different soils with these mellowing periods with curing period 7. The increase for C-30 soil has been observed from **Figure 3.9**(a) with MP-1, MP-2 and dropped for MP-3 for CS-1. Also, the same trend was observed with for CS-2. From Figure 3.9(b), the increase in calcite for C-40 soil has been observed with MP-1, MP-2 and dropped for MP-7 for CS-1. In contrast, calcite has been increased with MP-1, MP-2, and MP-3 for CS-2. In **Figure 3.9**(c), the increase in calcite for C-70 soil was following the same trend similar to C-30 and C-40 soil for CS-1. On the contrary, calcite has been increased with MP-1 but dropped with MP-2 and MP-3 for CS-2. Also, it can be observed that **Figure 3.9** that, the calcite of tested samples was also dependent on the concentration of calcium chloride of cementation solution used in the treatment. The

increase in calcite was higher for CS-1 than CS-2 for different mellowing periods. This could be attributed to the crystal morphology of the precipitated calcium carbonate and due to the formation of a less stable form of calcium carbonate named as vaterite (Al Qabany et al. 2012) and inhibition impact on the microbe leading to less calcite.

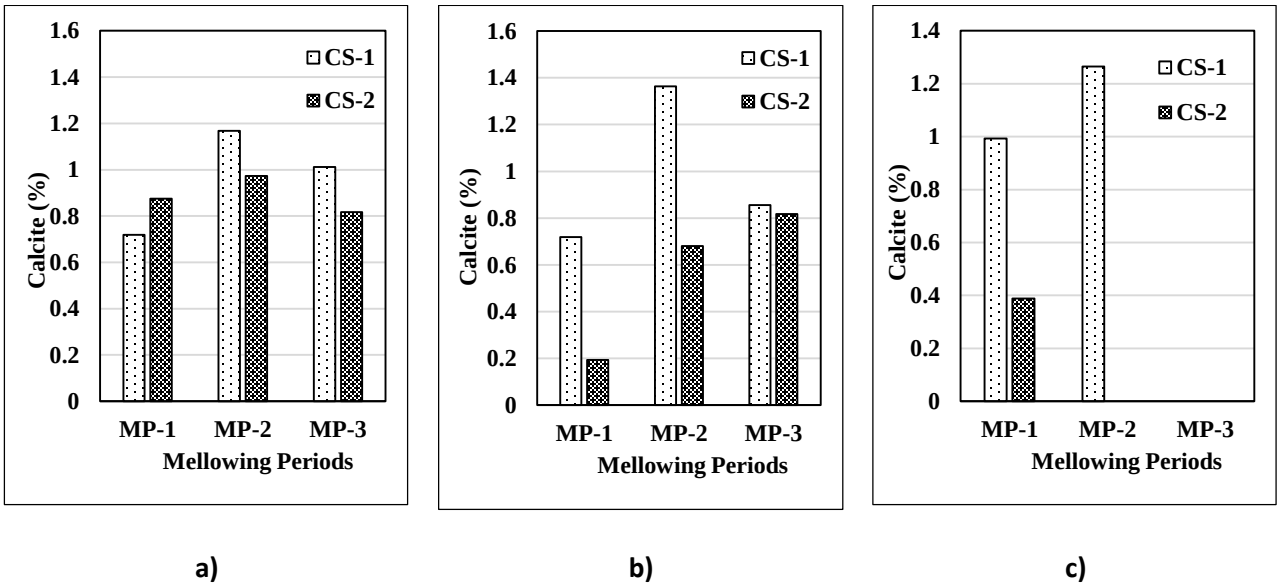
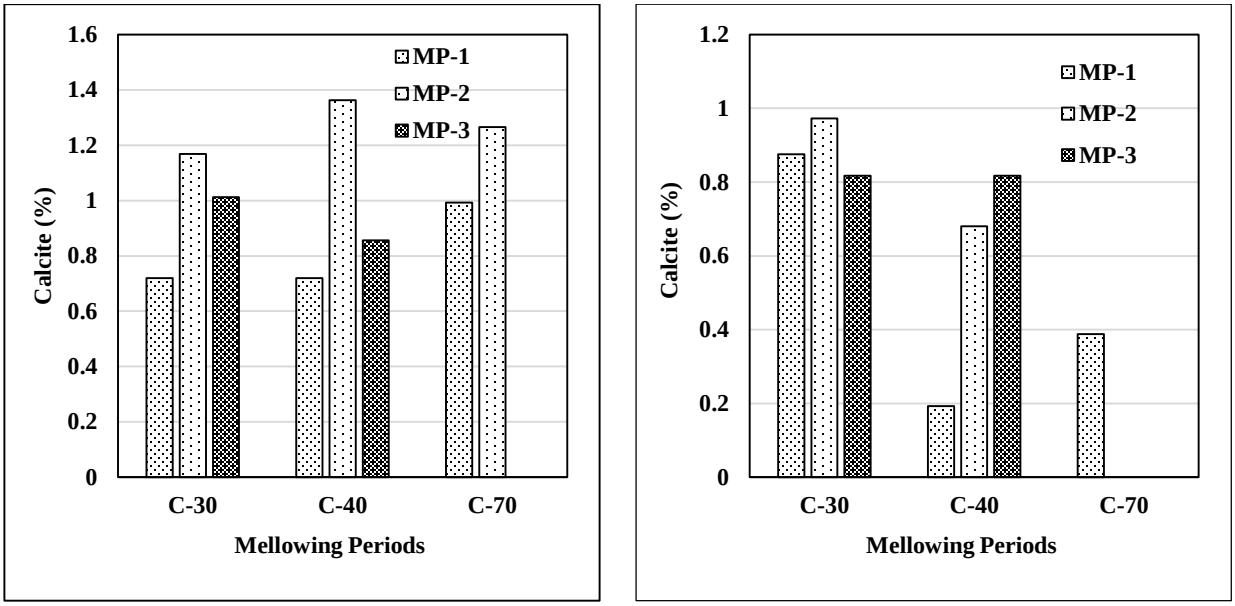


Figure 3.9: Effect of variation of cementation compositions on calcite precipitation with curing period 7 a) C-30 b) C-40 C) C-70

Effect of Soil Type

From **Figure 3.10**, the effect of soil types was observed with different cementation compositions with mellowing periods MP-1, MP-2, and MP-3 with curing period 7. From **Figure 3.10**(a) calcite was increased with MP-1 with CS-1 for C-30, C-40, and C-70 soil respectively. Calcite increased and decreased with mellowing periods MP-2 for C-30, C-40, and C-70 soil respectively. Calcite gradually decreased with MP-7 for C-30, C40, and C-70 soil respectively. From 10 (b), calcite gradually decreased with all mellowing periods, MP-1, MP-2 and MP-3 for C-30, C-40 and C-70 soil respectively.

The maximum calcite precipitation was observed with C-40 (1.36%), C-30 (1.17%), and C-70 (1.27%) respectively with MP-2 and CP-7.



a) b)

Figure 3.10: Effect of soil type on calcite change with different mellowing periods a) Cementation composition 1 (CS-1) b) Cementation composition 2 (CS-2)

FSI Test Results and Discussions

The FSI values for the untreated C-30, C-40 and C-70 soils was108%, 123%, and 162%. **Table 3.5** presents the decrease in FSI (%) for all three soils tested in this study. FSI data for each of the mellowing and curing periods for both cementation solutions compositions can be observed in this **Table 3.5**. It can be observed from this table that the FSI values ranged from with different curing and mellowing periods for CS-1 from 8% to 190% while those for CS-2 ranged from 33% to 266%. C-70 soil has been maximum FSI 190% and 266% with CS-1 and CS-2 due to expanding lattice of Montmorillonite.

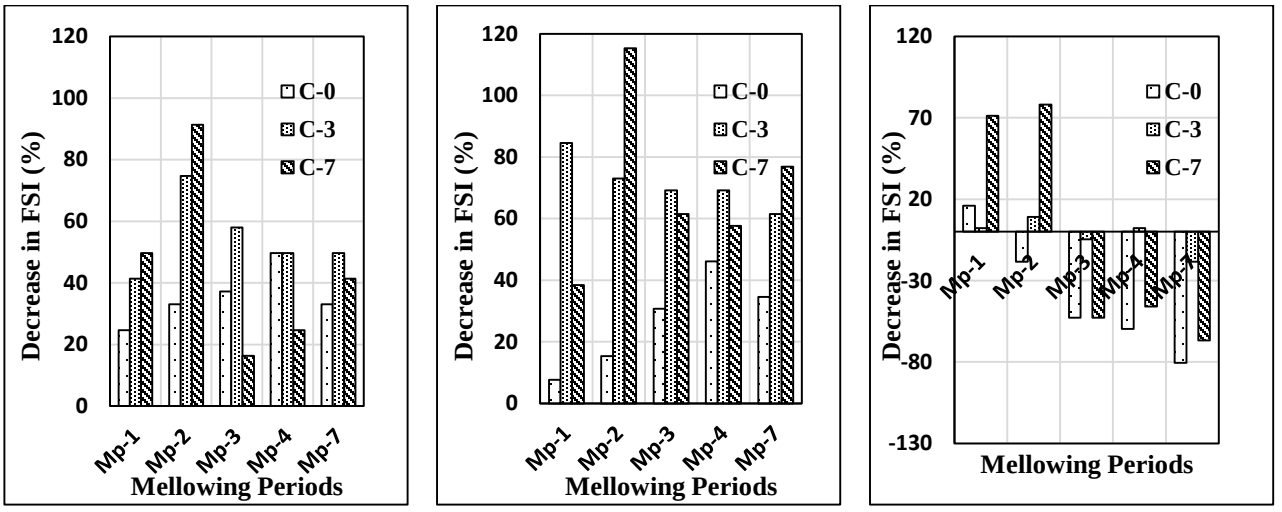
Table 3.5: FSI (%) test results for treated soil samples

Soil	Curing Periods	CS-1					CS-2				
		MP-1	MP-2	MP-3	MP-4	MP-7	MP-1	MP-2	MP-3	MP-4	MP-7
C-30	CP-0	83	75	71	58	75	108	79	67	50	75
	CP-3	67	33	50	58	58	75	33	58	75	83
	CP-7	58	17	92	83	67	58	88	83	83	75
C-40	CP-0	115	108	92	77	88	92	108	100	146	162
	CP-3	38	50	54	54	62	185	162	62	58	123
	CP-7	85	8	62	65	46	162	146	62	69	85
C-70	CP-0	107	141	176	183	203	52	183	203	128	93
	CP-3	121	114	128	121	141	93	114	128	169	266
	CP-7	52	45	176	169	190	45	114	134	148	162

Effect of Mellowing Periods and Curing Periods

Figure 3.11 presents the percentage change in FSI for different mellowing and curing periods for all three soils treated with CS-1 and CS-2. The percentage change was determined using the subtraction from untreated FSI to treated FSI of the corresponding soil. It can be observed from **Figure 3.11**(a) that the FSI for C-30 soil were increasing with mellowing periods, MP-1 through MP-4 for CP-0 curing samples. The FSI values dropped for MP-7 for CP-0 curing. A similar trend was observed for CP-3 and CP-7 curing periods after just two days of mellowing. It can also be observed that CP-7 at MP-2 gave the maximum decrease 91 %. A similar trend was observed r with C-40 soil. Maximum increase 115% in FSI values can be observed with CP-7 and MP-2. Also, the C-70 soil was decreased with mellowing periods, MP-1 and MP-2 and increased after that

for all curing periods, CP-0, 3, 7. Maximum decrease 78% can be observed with CP-7 and MP-2. The increase of FSI could be the due formation of EPS and other organic content. Overall, the best treatment periods for all soil was mellowing period, MP-2 with in combination with curing period, CP- 7.



a) **b)** **c)**

Figure 3.11: Effect of mellowing periods and curing periods on the decrease in FSI with cementation composition 1 a) C-30 b) C-40 c) C-70

Effect of Type of Cementation Solution

From **Figure 3.12** the effect of cementation compositions was observed with different soils with these mellowing periods with curing period 7. The decrease for C-30 soil has been observed from **Figure 3.12**(a) with MP-1, MP-2 and increased for MP-4 for CS-1. With CS-2, it increased and decreased with mellowing periods, MP-1, MP-2, and MP-4 respectively. From **Figure 3.12**(b), the decrease in FSI for C-40 soil has been observed with MP-1, MP-2 and increased for MP-7 for CS-1. In contrast, FSI has been increased for MP-1 and MP-2 but decreased for CS-2. In Figure 3.12(c), the decrease in FSI for C-70 soil was following the same trend similar to C-30 and C-40 soil for CS-1. On the contrary, FSI was decreased with MP-1 but increased with MP-2 and MP-3 for

CS-2. Also, it can be observed that Figure 3.12 that, the decrease in FSI in tested samples were also dependent on the concentration of calcium chloride of cementation solution used in the treatment. It could be due to the formation of temporary calcite formation which could lead to less decrease in FSI.

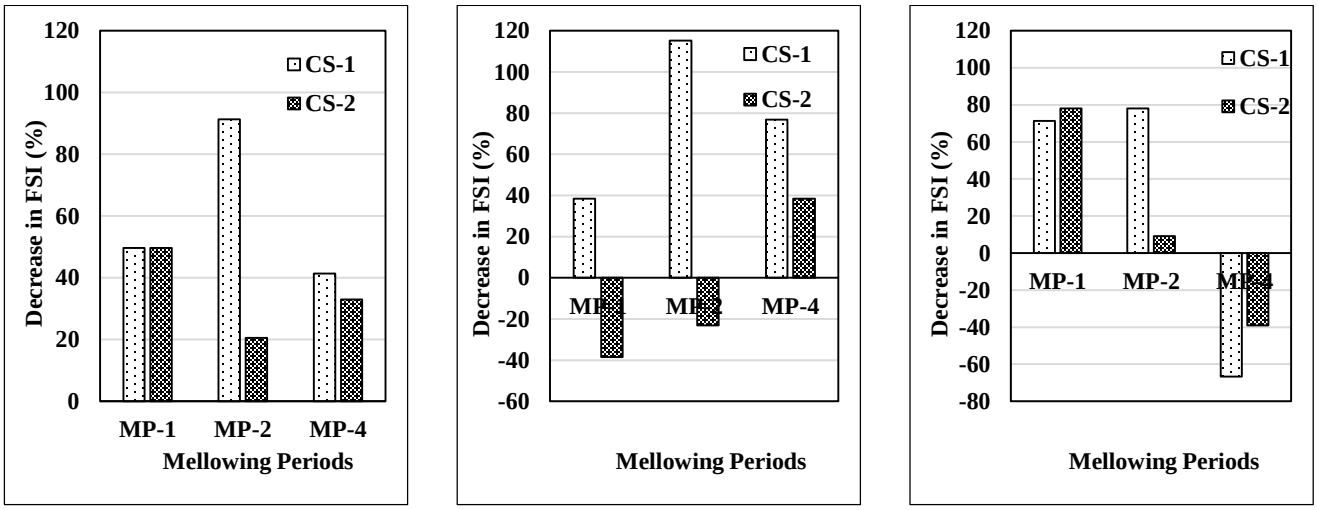
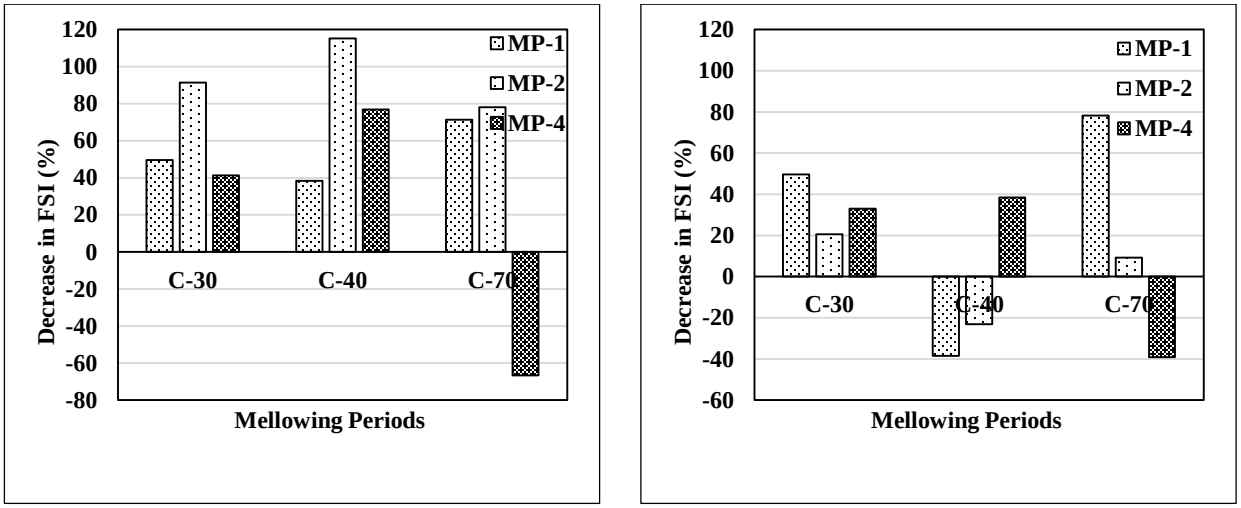


Figure 3.12: Effect of variation of cementation compositions in a decrease of FSI (%) with curing period 7 a) C-30 b) C-40 C) C-70

Effect of Soil Type

From **Figure 3.13**, the effect of soil types was observed on FSI with different cementation compositions with mellowing periods MP-1, MP-2, and MP-4 with curing period 7. From **Figure 3.13**(a) FSI decreased with MP-1 with CS-1 for C-30, C-40, and C-70 soil respectively. FSI decreased with C-30 and C-40 and increased C-70 soil. The same trend was observed with MP-4 for all of the soils. **Figure 3.13** (b), FSI decreased with C-30 soil but increased with C-40 soil and again decreased with C-70 soil with MP-1 and CS-2. The same trend was observed with MP-2 samples. With MP-4, FSI decreased with C-30 and C-40 soil but increased with C-70 soil. FSI decreased mostly

with C-40 and C-30 soil which could be due to calcite precipitation leading to decrease in FSI and less with C-70 soil due to less calcite precipitation.



a) **b)**

Figure 3.13: Effect of soil type on calcite change with different mellowing periods a) Cementation composition 1 (CS-1) b) Cementation composition 2 (CS-2)

Summary and Conclusions

A new method of biostimulated MICP application was attempted in this research. Experiments were conducted to study the effectiveness of mixing protocols to stimulate indigenous bacteria to stabilize expansive soils. Three soils with varying plasticity characteristics were studied, and their performance was evaluated using UCS, Calcite precipitation and FSI tests. It was observed that the improvement in strength was proportional to calcite precipitation in these soils. Also, free swelling index test results were inversely proportional with calcite precipitation and UCS. Findings from this research study are summarized as follows:

1. High plasticity soil (C-70) had the highest swelling potential among three soils possibly due to having high amounts of expanding lattice Montmorillonite.

2. There was an overall improvement in UCS, calcite and FSI values for CS-1 in comparison with CS-2, which could be due to the inhibition effect on microbial activity which can limit the urease production from ureolytic bacteria (Nemati et al. 2005) with the higher concentration of calcium chloride. Also, Al Qabany and Soga (2013) observed lower concentration of $CaCl_2$ led to more homogeneous $CaCO_3$ crystal formation at the particle contact points which contributed to the strength improvement with minimum soil disturbance and permeability reduction.
3. It was observed that medium plastic soil C-30 and high plastic C-40 soils showed an overall improvement in strength than very high plastic C-70 soil.
4. It has been observed that mellowing period 2 and curing period 7 were optimal treatment periods and worked best for all of the soils.
5. It was noted that an increase in the mellowing period beyond two days was not beneficial for any soils.

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CHAPTER FOUR: SUMMARY AND FINDINGS

Summary

In this research, biostimulation was applied by mixing enrichment and cementation solutions with soils in an effort to develop a new alternative to shallow chemical stabilization. Three soils were selected with varying plasticity for this purpose. Soils were treated by mixing with enrichment and cementation solutions. Enrichment solutions were first added and were allowed to stimulate bacteria for different time periods, termed mellowing periods. At the end of each mellowing period cementation solutions were added to facilitate calcite precipitation. Two protocols were studied for this shallow mixing method of MICP application. In protocol-1, soils were mixed with enrichment solutions at optimum moisture content (OMC) and allowed to stimulate for mellowing periods of 1, 2, 3, and 4 days. Protocol-2 was similar to protocol-1 excpet for the the initial amount of enrichment solution which was 95% of maximum dry unit weight on the wet-side of standard proctor curve in place of OMC. At the end of each mellowing period, the enrichment solution lost during this time was replaced with cementation solution to reach OMC and soil samples were compacted to untreated maximum dry unit weight. Treatment effectiveness was evaluated with Unconfined Compression Strength test and calcite test. The results indicated that protocol-1 performed better than protocol-2 which indicated that adding higher amounts of enrichment solutions was not beneficial for calcite precipitation and improvement of strength. Following this finding, protocol-2 was discontinued and protocol-1 was chosen

for further testing. Five different mellowing periods, three different curing periods and two types of cementation solutions were studied by following protocol-1.

Findings

Major findings from this research study are as follows:

1. Protocol-1 performed better than Protocol-2 for all three soils which indicate that the presence of higher amounts of enrichment solution and addition of less cementation solution is not advantageous for this procedure based on the performance of Protocol-2.
2. There was an overall improvement in UCS, calcite and FSI values for CS-1 in comparison with CS-2, which could be due to the inhibition effect on microbial activity which can limit the urease production from ureolytic bacteria (Nemati et al. 2005) with the higher concentration of calcium chloride. Also, Al Qabany and Soga (2013) observed lower concentration of $CaCl_2$ led to more homogeneous $CaCO_3$ crystal formation at the particle contact points which contributed to the strength improvement with minimum soil disturbance and permeability reduction.
3. It was observed that medium plastic soil C-30 and high plastic C-40 soils showed an overall improvement in strength than very high plastic C-70 soil.
4. It has been observed that mellowing period 2 and curing period 7 were optimal treatment periods and worked best for all of the soils.
5. It was noted that an increase in the mellowing period beyond two days was not beneficial for any soils.

Recommendations for Future Research

Based on the knowledge gained from this research and the need for further understanding of this application method, the following recommendations were made for future research:

Role of Urease activity: The reduction in strength and calcite precipitation after two days of mellowing period were hypothesized to be related with bacterial activity. This hypothesis could be tested by determining the urease activity at each mellowing period. One protocol of urease activity has been developed on the basis of urea hydrolysis which involves the production of ammonium and carbonate ion. The production of ionic species from non-ionic substrates creates an increase in the conductivity in solution. With more urea hydrolysis, ion concentration increases and also, electrical conductivity increases which is proportional to the concentration of active urease (Whiffin 2004). The actual conductivity (mS/min) is the conductivity multiplied by the dilution factor. The actual conductivity variation rate can be converted to urea hydrolysis rate (mM urea hydrolyzed/min) with the basis of a correlation that 1 mS/min corresponds to a hydrolysis activity of 11 mM urea/min (van Paassen 2009).

Steps involved in this protocol are as follows:

1. Homogenize 0.5g soil sample in 10 mL of 50 mM Sodium Acetate, pH 5 for 2 minutes to remove carbonated from soils before analysis.
2. Take 1 mL for soil background control
3. Take 1.5 mL soil solution to mix with 13.5ml 1.67 M urea solution and incubate at 37℃ for 2 hours
4. Measure the electrical conductivity at soil-urea solution

5. Take 1 ml of soil urea solution
6. Centrifuge the soil-urea solution at 8000 × g for 1 minute and collect the supernatant.
7. Centrifuge soil background control tube at 8000 × g for 1 minute
8. Measure the OD of soil-urea solution with respect to soil background control.

Mineralogical and microstructural changes: In this test macro scale testing was only used for treatment evaluation. Microscale studies such as X-Ray Diffraction and Scanning Electron Microscopy studies would give insights into mineralogical and microstructural changes within the soil samples

Role of Extracellular Polymeric Substances (EPS): Biofilm formation and production of EPS in MICP process can impact on soil behavior. The effect of EPS is needed to identify its impact on swelling properties and other physical properties of soil.

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