EVALUATING THE SUITABILITY OF MICROBIAL INDUCED CALCITE PRECIPITATION TECHNIQUE FOR STABILIZING EXPANSIVE SOILS

by

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The following individuals read and discussed the thesis submitted by student Sikha Neupane, 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 grandfather and grandmother, Gorakh Bahadur Singh and Indira Singh.

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ABSTRACT

Expansive soils, also known as swell-shrink soils have been a problem for civil infrastructures including roads and foundations from ancient times. The use of chemical additives such as cement and lime to stabilize expansive soils is a common practice among geotechnical engineers especially for lightly loaded structures. However, several occurrences of subgrade failures were observed after stabilizing with chemical additives hence engineers are in search of sustainable stabilization alternatives. Microbial Induced Calcite Precipitation (MICP) is gaining attention as an environmental friendly soil improvement technique. Several researchers have successfully tested its feasibility in mitigating liquefaction induced problems in sandy soils. This research focuses on evaluating its effectiveness in stabilizing expansive soils. For this purpose, three natural expansive soils with high and low plasticity properties were subjected to MICP treatments. Two methods of MICP treatments were followed in this research. The first method was bio-augmentation. In this method the soil samples were first augmented with bacterium *Sporosarcina pasteurii* and then treated with calcium chloride and urea (substrates) and cured for seven days. In the second method bio-augmentation was followed by stimulation using a nutrient delivery system which was developed to treat microbes with substrates. Variables such as soil types, microbial concentrations and number of pore volumes of substrate injected were studied in this research. Geotechnical testing including Atterberg limits, unconfined compressive strength (UCS) and one-

Dimensional (1-D) swell test along with specific surface area were performed to evaluate the efficacy of MICP treatments.

The results indicated that MICP treatments could be a viable alternative for expansive soils treatments. Although the improvement in UCS values after both types of MICP treatments were notable, the strength gain was considerably lower than lime treated soils. However, 1-D strain reduction was on par with lime stabilized soils. It was also observed that MICP treatments do not result in significant clay mineralogy changes.

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LIST OF ABBREVIATIONS

ASTM	American Society for Testing and Materials
BCM	Biologically Controlled Mineralization
BIM	Biologically Induced Mineralization
CFU	Colony Formation Unit
CPT	Cone Penetration Test
DIC	Dissolved Inorganic Carbon
MICP	Microbial Induced Calcite Precipitation
MDD	Maximum Dry Density
MDUW	Maximum Dry Unit Weight
OD	Optical Density
OMC	Optimum Moisture Content
UCS	Unconfined Compressive Strength
EPS	Extracellular Polymer Substance
LB	Luria Broth

CHAPTER ONE: INTRODUCTION

Background

The need for stabilizing soils becomes necessary mainly because of two reasons: i) weak or inconsistent soil properties and ii) need for urbanization especially in areas with problematic soils such as expansive or high plasticity clays. These highly plastic soils cause heaving on the ground surface (volume change) with change in moisture content. The change in moisture content could be due to seasonal or climatic variations and evapo-transpiration of vegetation. Structures built on expansive soils tend to undergo moderate to severe cracking problems (Mitchell, 1986; Nelson & Miller, 1992). In particular, lightly loaded structures such as one or two story residential and industrial structures and pavements often experience severe damage (Petry & Little, 2002) associated with substantive repair and mitigation costs. Snethen, Townsend, Johnson, Patrick & Vedros (1975) in their report stated that expansive soils are so widely distributed in United States that altering the highway routes to avoid the expansive soils was virtually impossible.

It is believed that the demand for new and sustainable soil stabilization techniques, continues to grow with more than 40,000 soil stabilization projects being carried out worldwide with total costs exceeding US$ 6 billion/year (DeJong, Mortensen, Martinez & Nelson, 2010). The artificial cementation of soil particles due to soil stabilization is often achieved through the use of chemical stabilizers via shallow/deep mixing or injecting chemical grouts that can permeate through soils (Ismail, Joer, Sim & Randolph, 2002).

Physical properties of soil can be modified by the use of mechanical compaction and/or compaction grouting while chemical properties of soil can be modified by the use of chemical stabilizers such as Portland cement, lime and fly ash. Mechanical compaction is recommended for sandy soils and is effective or economical to a depth less than 10 m (Ivanonv & Chu, 2008). Chemical stabilization is typically recommended for expansive soils (Petry & Little, 2002). Environmentally safe techniques such as pre-wetting and moisture barriers are only possible for small confined spaces, and are not suitable for larger construction projects such as highways and railways which spread for miles. As mentioned above, artificial cementation techniques are not always feasible and environmentally friendly. However, reduction in the use of artificial cementation techniques can be practiced by substituting with environmental friendly techniques or materials. One such method of soil stabilization technique is, Microbial Induced Calcite Precipitation (MICP). This technique employs microbes as a primary factor for stabilization. Successful implementation of MICP will have its application in a wide variety of civil engineering fields such as stability of retaining walls, embankments and dams, controlling soil erosion, stabilizing cohesionless soils to facilitate the stability of underground constructions, increasing bearing capacity of shallow and piled foundation and reducing the liquefaction potential of soils (Kucharski, Cord-Ruwisch, Whiffin & Al-thawadi, 2012; Ivanov & Chu, 2008).

MICP Applications

Microbes are often responsible for the chemical cementation of soils in nature due to the precipitation of cementing materials into the voids of soils and rocks (Ivanov & Chu, 2008). Microbes are able to precipitate cementing materials such as calcium, magnesium, iron,

manganese and aluminum which are crystallized to form carbonates, silicates, phosphates, sulfides and hydroxides (DeJong, Fritzges & Nüsslein, 2006). The prime role of microbes in the precipitation of minerals is their ability to create an alkaline environment through various physiological activities (Douglas & Beveridge, 1998). Calcium carbonate (calcite) precipitation is observed to be a general mineral precipitation process in the microbial world under ambient environment (Bang, Galinat & Ramakrishnan, 2001).

Soil stabilization via MICP is one of several applications of bio-remediated processes. MICP can be used for the elimination of soluble calcium from wastewater generated by industries (Hammes et al., 2003). The high calcium concentration in water can clog pipes and malfunction reactors. Hammes et al. (2003) concluded that soluble calcium was precipitated in the form of calcite by the use of bacteria as an alternative for chemical precipitation of calcite. Thus making MICP as an alternative and environmental friendly technique for the removal of calcium from industrial waste water. Ramachandran, Ramakrishnan & Bang (2001) studied the effect of MICP on the compressive strength of Portaland Cement by mixing urease enzyme producing bacteria, *Sporosarcina. pasteurii* with cement mortar. For this purpose, they prepared 5 cm cube molds containing cement and bacteria, and were cured for 28 days in urea/calcium solution. The cube was tested for compressive strength. It was reported that the strength increased by 24% compared to untreated cube. Urease producing bacteria have been used in the oil industry to reduce the permeability of the surface and subsurface media thus reducing the flow of the fluid and enhancing the recovery of oil from reservoirs and limiting the spread of the contaminants from a spill site. This process is called mineral plugging. The increase in pH due to the formation of ammonia as a byproduct during the breakdown of urea in the presence of

urease enzyme as a catalyst, this increase in pH provides a favorable condition for the precipitation of calcite in the presence of calcium ions. (Kucharski, Cord-Ruwisch, Whiffin & Al-thawadi, 2012).

MICP technique is considered to be a better and more environmentally friendly alternative to the conventional technologies. However, more investigations are needed to properly understand the possibilities and limitations. Further, its application in effectively stabilizing expansive soils is still a hypothesis and this research is an initial step in evaluating this hypothesis and understanding the applicability of MICP technique to stabilize expansive soils.

Research Objective

The main objective of this thesis is to evaluate the effectiveness of MICP in stabilizing expansive soils. For this purpose, three naturally occurring expansive soils with varying plasticity characteristics were studied. These soils were subjected to one conventional chemical treatment (in this case quick lime) and two methods of MICP treatments (Bio-augmentation and Bio-augmentation+Stimulation) and their performance was compared with one another and to untreated soil. The performance was measured by monitoring the plasticity characteristics, swelling potential and unconfined compressive strength of these soils with various treatments. Variables such as soil type, bacterial population during augmentation, along with the number of treatment cycles were studied in this research.

The main hypothesis in this research is that under the suitable condition ureolytic bacteria such as *Sporosarcina pasteurii* are able to hydrolyze urea into ammonium ions and carbonate ions, when ammonium ions are formed, the pH of the system increases and

the carbonate ions in presence of calcium ions, react together to form calcium carbonate. The calcium carbonate may precipitate into the voids of soil samples and bind soil particles together to increase the strength and reducing the expansive nature of clayey soils.

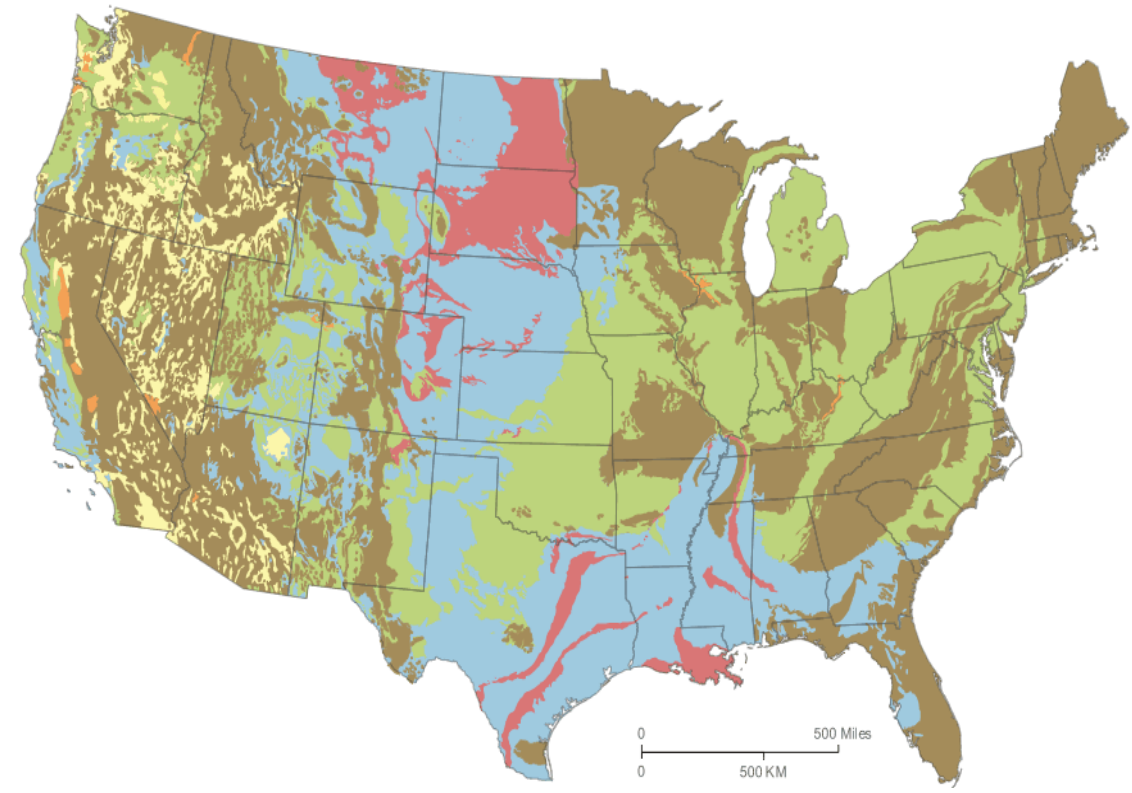
Organization

Chapter 2 of this thesis presents findings from an extensive literature on expansive soils and their properties and clay mineralogy, and the available stabilization techniques. The environment required for the growth of bacteria is also reviewed. The use of bacteria in geotechnical applications are also addressed in this chapter. Chapter 3 addresses the materials and methods used in this research. Basic and advanced testing are adopted to analyze the effectiveness of MICP over conventional chemical treatment of expansive soil. Chapter 4 mainly concentrates on the results and discussion of the research, supported by data and graphs. Chapter 5 presents the summary and recommendations made as a result of this research.

CHAPTER TWO: LITERATURE REVIEW

Expansive Soil

Expansive soils swell and shrink with changes in moisture content (Nelson & Miller, 1992; Hardcastle, 2003). This volume change behavior is the reason for foundation issues in lightly loaded structures such as residential buildings and pavement infrastructure. The reason for this behavior is the presence of the heaving mineral known as montmorillonite that has an expanding lattice. This clay mineral expands when it is exposed to water. Soils rich with this mineral can be found in many places all over the world; especially in the arid and semi-arid regions (El Arabi, 2002). In the United States, expansive soils range from the west coast to east coast. Figure 1 shows the location of expansive soils in various parts of the United States. In this Figure the purple and blue colors represent problem zones, with purple representing more severe conditions compared to blue.



Over 50 percent of these areas are underlain by soils with abundant clays of high swelling potential.

Less than 50 percent of these areas are underlain by soils with clays of high swelling potential.

Over 50 percent of these areas are underlain by soils with abundant clays of slight to moderate swelling potential.

Less than 50 percent of these areas are underlain by soils with abundant clays of slight to moderate swelling potential.

These areas are underlain by soils with little to no clays with swelling potential.

Data insufficient to indicate the clay content or the swelling potential of soils.

The map above is based upon "Swelling Clays Map of the Conterminous United States" by W. Olive, A. Chleborad, C. Frahme, J. Shlocker, R. Schneider and R. Schuster. It was published in 1989 as Map I-1940 in the USGS Miscellaneous Investigations Series.
Land areas were assigned to map soil categories based upon the type of bedrock that exists beneath them as shown on a geologic map. In most areas, where soils are produced "in situ", this method of assignment was reasonable. However, some areas are underlain by soils which have been transported by wind, water or ice. The map soil categories would not apply for these locations.

Figure 1. Map of Expansive Soils in the USA (Source: http://geology.com/articles/soil/expansive-soils-map-900.gif)

The damage caused by the expansive soils to structures built on them is immense. A study sponsored by the National Science Foundation (NSF) reported that the damage to structures caused by expansive soils, particularly to light buildings and pavements, is more than any other natural disaster, including earthquakes and floods (Jones & Holtz, 1973). Through a detailed review of expansive soils, Gromko (1974) estimated that the annual cost of damage from these soils in the United States alone is $2.3 billion. Petry & Armstrong (1989) noted that it was more economical to perform initial stabilization of these soils before/during construction of the overlying structures rather than performing remedial treatments later on with existing structures around.

According to Wiseman, Komornik & Greenstein (1985), the following factors govern the severity of the problem when expansive soils are encountered: 1) soil type that exhibits considerable volume changes associated with changes of moisture content; 2) climatic conditions such as extended wet or dry seasons; 3) changes in moisture content (climatic, man-made or vegetation); and 4) presence of lightly loaded structures that are very sensitive to differential movement.

Expansive soils can be identified by using index tests such as plasticity index, shrinkage limit or free swell percentage. Table 1 lists these commonly used tests along with typical ranges corresponding to problematic soil behavior.

Table 1. Expansive Soils Identification (From Wiseman et al., 1985)

Index Test	Usually No Problems	Almost Always Problematic
Plasticity	< 20	> 32
Shrinkage Limit	> 13	< 10
Free Swell (%) (as per Holtz and Gibbs, 1965)	< 50	> 100

Many cities, roads and structures are built over soils rich in montmorillonite. Snethen, Townsend, Johnson, Patrick & Vedros (1975) observed that expansive soils are so widely distributed in the United States that altering highway routes to avoid expansive soils was impossible. As a result, annual damage costs associated with expansive soils have been estimated to be several billions. Table 2 lists the annual cost of damage to structures caused by expansive soils in different parts of the world. Note that the costs for United Kingdom (UK), France, and China have been reported in their respective currencies.

Table 2. The annual cost of damage to structures constructed on/with expansive soils for regions of the world. (Source: Vanapalli and Adem, 2013)

Region	Cost of damage/year	Reference
USA	$13 billion	Puppala & Cerato (2009)
United Kingdom (UK)	£400 million	Driscoll & Crilly (2000)
France	€3.3 billion	Johnson (1973)
Saudi Arabia	$300 million	Ruwaih (1987)
China	¥100 million	Ng et al. (2003)
Victoria, Australia	$150 million	Osman et al. (2005)

The shrinking-swelling behavior can cause severe damage to supporting structures (Jones & Jefferson, 2012). Swelling pressure of expansive soil contributes to heaving or lifting of structures in vertical directions while shrinkage can cause differential settlement beneath foundation (Jones & Jefferson, 2012). During rainfall water migrates underneath the edges of foundation of the structure, soils around and beneath the edges of foundation of structure start to swell, pushing up edges of the foundation. The edges of the structure suffer from cracks leading to the failure of the structure. This condition is known as *end lift*. Over the period of time as water migrates underneath the center of the structure, center lift of the structure occurs, causing further damage to the structure. This condition is known as *center lift*. End lift and center lift from swell can be expected for lightly loaded structures, including residential buildings and pavements constructed on expansive soils.

Factors Influencing the Expansive Behavior of Soils

Some of the factors influencing the expansive behavior of soils are: soil composition, dry density, soil fabric, confinement and permeability (Nelson & Miller, 1992). These intrinsic properties contribute to swelling and shrinkage with change in water content in the ambient environment, and can be used to determine the behavioral characteristics of expansive soils. Brief discussions on these factors have been presented in the following subsections.

Soil Fabric

Soil fabric is defined as the arrangement of particles, particles groups, and pore spaces in a soil. Soil fabric influences its expansive characteristics (Mitchell & Soga, 2013). Clays tend to exhibit higher swelling potential when flocculated; however, the swelling potential reduces when particle arrangements are altered to disperse upon

compaction (Snethen, Townsend, Johnson, Patrick & Vedros, 1975; Nelson & Miller, 1992). The vertical movement of the soil that is experienced as heave at the surface is dependent on soil fabric and anisotropy. For soils with few fissures the vertical movement could be equal to the total volumetric movement while the same for heavily fissured isotropic soils could be one-third of the total volumetric movement (Army Manual, 1983). Also, Du, Li & Hayashi (1999) demonstrated the difference between the swelling behavior of undistrubed and remolded soil samples. Remolding supresses the structural strength and the strong connections between soil particles that were a result of long and complicated natural events. Due to this, remolded soil samples swell freely compared to undisturbed soils.

Surcharge Loads

Using surcharge or external load can reduce the amount of swelling by balancing inter-particle repulsive forces. Confining pressure has a significant influence on the swelling potential of clays. Greater the confining pressure, smaller will be the deformation. Overburden pressures exerted due to lightly loaded structures such as pavements are too small to counter excessive swelling pressures applied by underlying expansive soils (Snethen, Townsend, Johnson, Patrick & Vedros, 1975). Hence, placement of a non-swelling layers in the form of slabs, heavy bases can help in countering the effects of expansive soils.

Permeability

It can be referred to as a function of initial moisture content, dry density and soil fabric. Permeability is high for low moisture content and dry density and decreases to some

constant value at optimum moisture content (OMC) because of greater particle contact at OMC (Snethen, Townsend, Johnson, Patrick & Vedros, 1975; Nelson & Miller, 1992).

Clay Mineral

The magnitude of swelling is a function of the amount and type of clay mineral present in the soil (Snethen, Townsend, Johnson, Patrick & Vedros, 1975). Clay mineral types that are commonly responsible for volume changes are: smectite, vermiculites and some mixed layers of these minerals. Montmorillonite which falls in smectite group of minerals is highly expansive in nature. Kaolinite on the other hand, is significantly less expansive in nature, but can cause volume change when mineral particle sizes are less than few tenths of micron (Nelson & Miller, 1992). On the other hand, mineralogical composition along with environment are responsible for swelling potential of the soil (Snethen, Townsend, Johnson, Patrick & Vedros, 1975). Higher the dry density, larger is swelling potential of the soil. This is mainly due to closer particle spacing corresponding to soils compacted to higher densities which results in greater particle contact, and thereby leads to significant volume changes (Snethen, Townsend, Johnson, Patrick & Vedros, 1975; Nelson & Miller, 1992; Chen, 1975).

Clay Mineralogy

The amount and type of clay present in the expansive soil influences the engineering properties, such as plasticity, shrinking-swelling potential, hydraulic conductivity, compressibility, and internal angle of friction (Mitchell & Soga, 2013). Clay minerals are hydrous aluminosilicates in nature with variable amounts of iron, magnesium, alkali metals, alkaline earths and other cations. These minerals are arranged in sheets which are made up of planes of cations. These sheets may be tetrahedral or octahedral in nature

and are arranged in layers. Tetrahedral sheets consist of a central cation such as Si^{4+} surrounded by four O^{2-} whereas octahedral sheets consist of a central cation such as Al^{3+} or Mg^{2+} in octahedral structure with O^{2-} or OH^- (Mitchell & Soga, 2013).

If layers consist of one tetrahedral and one octahedral sheet, then the mineral is described as 1:1 and mineral from Kaolin group falls under this category. As shown in Figure 2, a sheet of tetrahedral is combined with octagonal OH^- which is then shared with an Al_2O_3 octahedral sheet. Kaolin has little substitution of other elements but Al^{3+} may be substituted by Fe^{3+} and some Al^{3+} may be possibly substituted by Si^{4+}. Thus little substitution of cations in Kaolin layer makes the charge on the Kaolin minimal (Murray, 1999).

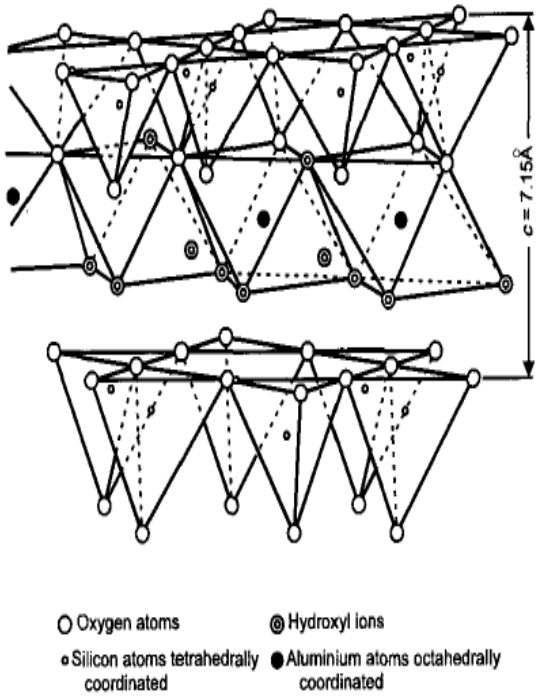


Figure 2. Schematic of the structures of Kaolin (Murray, 1999)

If layers involve two tetrahedral sheets and one octahedral sheet then the mineral is described as 2:1, minerals from smectite group falls under this category (Figure 3). Some

2:1 clay minerals have interlayer sites occupied by cations between successive 2:1 unit. These interlayer sites of cations are often responsible for hydration. Considerable substitution of cations takes place in smectite group, usually between Fe^{3+} and Mg^{2+} for Al^{3+} which can create a charge deficiency within the layer. Further charge imbalance is created with substitution of Al^{3+} for Si^{4+} in tetrahedral sheet (Murray, 1999). Thus attracting dipole molecules like water causing hydration within the interlayer of two 2:1 unit. This is the reason why clay minerals from smectite group swell more than the clay minerals from kaolin group.

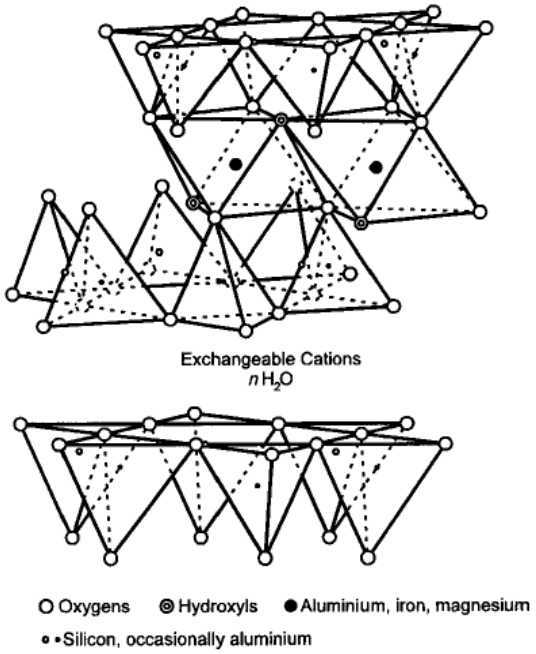


Figure 3. Schematic diagram of the structures of smectite (Murray, 1999)

Chemical Stabilization of Expansive Soils

Over the years, researchers have developed a variety of methods to address heaving and shrinking problems in expansive soils. These methods include mechanically compacting the soil, using chemicals to alter the physicochemical behavior of soils, and designing resilient foundations to withstand volume changes. Petry & Little (2002)

presented a historical perspective on expansive soil treatment dating back to the late 1950s. In their work, they described several stabilization methods including mechanical compaction, chemical stabilization, pre-wetting and moisture barriers, lime injections, and deep soil mixing. However, only use of chemical stabilizers is discussed in this section. Chemical stabilizers being the most widely and popularly used stabilization techniques are very effective nevertheless possess many drawbacks.

Soil stabilization is a process of modifying geotechnical properties in order to achieve better and improved quality of soil to prevent structural damage. There are traditional and nontraditional stabilizers. Use of nontraditional stabilizers are associated with uncertainties because these stabilizers are not supported through research studies. This is one of the main reason why traditional stabilizers such as cement, lime and fly ash are preferred over nontraditional stabilizers (Petry & Little, 2002). Traditional stabilizers such as lime, cement and fly ash modify soil chemically in the presence of water. These stabilizers strengthen soils mainly by two reactions, cation exchange and pozzolanic reaction. Rapid physical chemical reactions occur resulting in the exchange of cations between soil and stabilizer which is followed by flocculation whereas it takes longer time for pozzolanic reactions to occur. During pozzolanic reaction calcium aluminate hydrates and calcium silicate hydrates are formed in the form of gel. These gels help to reduce the permeability of the soil and increase its strength (Chittoori et al., 2011). Pozzolanic reaction is time dependent and mainly depends on temperature, calcium quantity, pH value and the percentage of reactive silica and alumina in the soil (Eades & Grim, 1960).

Factors affecting the quality of traditional stabilizers are moisture and degree of pulverization. First, during pavement construction, soils are often compacted at dry side of

optimum which results in the inadequate chemical reaction between stabilizers and soil due to lack of water. Inadequate moisture in soils makes chemical stabilizers less effective. Second the degree of pulverization required is below the standard. In order to initiate the pozzolanic reaction lime must react with soil particles intimately, larger the particle, the longer the process will take (Petry & Little, 2002). Longer the time required for pozzolanic reactions to occur longer time will it take to impart strength.

Usage of lime and cement can also be counterproductive in case of sulfate rich soils. Release of alumina from lime and cement can react with sulfate in the soil to form an expansive mineral called ettringite. The amount of heaving caused by ettringite usually depends on the rate and amount of release of alumina into the solution. The heaving caused by formation of ettringite is non-reversible process and will create the distress on the surface of the pavement (Mitchell & Dermatas, 1992).

Use of chemical stabilizers also raises environmental concerns because of: (1) greenhouse gases generated to produce these chemicals; and (2) negative impacts on plant growth that come from elevated pH levels in soils after treatment. Cement is the common ingredient used in concrete. Concrete are used in building structures such as buildings, roads, foundation and bridges. It is believed that concrete is the second most consumed substance after water (WBCSD, 2009). Cement is prepared by heating limestone along with other clay minerals in a kiln at 1400^{o}c. The product obtained from kiln is grounded and mixed with gypsum to form cement. Manufacturing of cement is highly energy and emissions intensive because producing a ton of cement requires 60-130 kg of fuel and 110 kWh of electricity leading to the emissions of around 900 kgCO_2/t (GNCS factsheets, 2012). The production of cement is also responsible for the release of greenhouse gases:

heating of limestone in kiln directly contributes to the emission of CO_2 while use of fossil fuels to heat the kiln indirectly contributes to the emission of CO_2. Cement production is increasing annually at the rate of 2.5% and is expected to rise from 2.55 billion tons in 2006 to 3.7-4.4 billion by the year of 2050 (WBCSD, 2009).

On the other hand, cement and lime elevate pH levels (often >12.4) of soil when mixed together and this can become a major problem where soil erosion is a concern and plant growth is necessary to protect soils against erosion. There has also been subgrade failure even after the stabilization with chemical stabilizers due to loss of stabilizer over time. Loss of stabilizers may be due to the external factors such as water table fluctuation, rainfall infiltration etc. The soil after losing stabilizer becomes ineffective and does not perform according to the designed standards and exhibits premature failures (McCallister & Petry, 1992). Therefore, it becomes important to identify an alternative stabilization method that is both environmental friendly and cost effective at the same time.

'Green' Stabilization Alternative

In recent years, use of Microbial Induced Calcite Precipitation (MICP) technique to alter the engineering properties, is gaining attention as a versatile and green method of soil improvement (Ivanov & Chu, 2008). MICP utilizes the comprehension of microbiology, geochemistry and geotechnical engineering to improve soil properties (Dejong, Mortensen, Martinez & Nelson, 2010). When a soil is treated using MICP technique, microbial induced calcite bridges adjacent soil particles, cementing soil particles together (Burbank et al., 2013; Burbank, Weaver, Williams & Crawford, 2012; Whiffin, van Paassen & Harkes, 2007; DeJong, Fritzges & Nüsslein, 2006;). The precipitation of calcite between particle-particle also helps in reducing the permeability, compressibility

and increasing soil strength (DeJong, Mortensen, Martinez & Nelson, 2010). MICP can be achieved in two ways:

1. Bio-stimulation- This method involves the modification of the environmental condition by stimulating the indigenous bacteria present in the soil. This is done by introducing various nutrients into the soil.
2. Bio-augmentation- This method involves the introduction of the required microbes along with nutrients required to stimulate the microbes into the soil.

Bio-stimulation is normally favored over bio-augmentation, as stimulating native microbes that are accustomed to the environment is likely to be more stable than artificially introducing bacteria into new environment (Burbank et al., 2013). However, the main challenge exists in the uniform treatment of microbes within the site and the time associated with stimulation and growth. To overcome these challenges, researchers often prefer bio-augmentation (DeJong et al., 2013). However, introduction of exogenous bacteria are always not successful because of complex communal relationship of microbes including competition, predation, and parasitism. Therefore, bio-augmentation of exogenous bacteria followed by stimulation is more practical and reliable. In this process, microbes are first mixed within the soil, followed by the treatment of nutrients and substrates such as urea and calcium chloride.

Microbially Induced Carbonate Precipitation (MICP) by Urea Hydrolysis

Microbes although being the smallest forms of life, collectively contribute to the total biomass greater than plants and animals (Hogan, 2014). These microbes are responsible for carrying out the essential chemical reactions needed for the higher organisms in the ecosystem (Madigan, Clark, Stahl & Martinko, 2010). Microbes influence

the geological processes in the soil. They are responsible for the change in soil and rock properties and also influence the geochemical processes in the soil (Rebata, 2007). Biogeochemical process can lead to a significant geotechnical consequence over reasonably short period of time (Ehrlich in Geomicrobiology, 1996). Microbes are able to produce wide range of minerals. It has been observed that microbes from saline, freshwater and soil habitats are responsible for the formation of marine calcareous skeletons, carbonate sediments and soil carbonate deposits respectively (Achal, Mukherjee & Reddy, 2010).

The biotic precipitation of minerals by microbes are either biotically controlled or biotically induced. When microbes have some control over the location, size and composition, as in case of mineral formations such as skeletons and shells, this process is said to be biotically controlled (Frankel & Bazylinski, 2003). If microbes synthesize minerals as a result of microbial activity and have no control over the mineralization then this process is biotically induced (Frankel & Bazylinski, 2003).

Calcite mineralization can occur as a by-product of microbial metabolic activity such as photosynthesis, urea hydrolysis, sulfate reduction and iron reduction. During these different metabolic processes, the alkalinity or pH of the system increases, favoring the calcite precipitation (Knorre & Krumbein, 2000). It is believed that bacteria are dominant soil inhabitants. There are 10^6-10^{12} bacterial cells in a gram of soil (Torsvik, Goksøyr & Daae, 1990). *S. pasteurii* (previously known as *Bacillus pasteurii*) species of Bacillus group, a common alkalophilic soil bacterium have high urease enzyme activity (Dejong, Fritzges & Nüsslein, 2006). *S. pasteurii* use urea as an energy source which hydrolyzes $CO(NH_2)_2$ (urea) into NH_3 and H_2CO_3 (Equations 1 and 2).

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

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

NH_3 and H_2CO_3 equilibrate in water to form HCO_3^-, NH_4^+ and OH^- (Equations 3 and 4).

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

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

It is during this stage the pH of system increases and shifts the HCO_3^- equilibrium to form CO_3^{2-} (Equation 5 and 6). The CO_3^{2-} produced will precipitate calcite ($CaCO_3$) in the presence of Ca^{2+} (Dejong, Fritzges & Nüsslein, 2006).

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

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

The calcite precipitation is influenced mainly by four factors: calcium ion concentration, dissolved inorganic carbon (DIC) concentration, pH and availability of nucleation sites (Hammes & Verstraete, 2002). However, the survivability of microbial cells depends on the ability to metabolize, grow and reproduce (Rebata, 2007). The factors that affect the microbial growth are termed as ‘limiting growth factors’.

Limiting Factors for Bacterial Growth

Bacteria are unicellular microorganisms that can thrive in diverse environments. Bacteria are capable of growth, reproduction, movement and metabolism. They require carbon and energy source and abiotic factors such as temperature, water potential, pH, light, osmotic pressure and, redox to survive. Any of these factors can act as the limiting

factor influencing the survivability, metabolism activity, growth and reproduction of the microorganisms (Rebata, 2007). These abiotic factors are explained below.

Temperature

Microbes can survive in extreme conditions from -60°c in Antartica to temperature greater than 150°c in hydrothermal vent. There are many microbes which can exist only in certain temperature limits such as psychrophile exist at low temperatures (10°c), thermophile lives in temperature around 40°c, hyperthermophiles exist at temperature greater than 60°c (Figure 4). This explains that microbes are adapted to the wide range of temperature in the environment (Kirchman, 2012; Madigan, Clark, Stahl & Martinko, 2010). Temperature plays a vital role in the microbial activity.

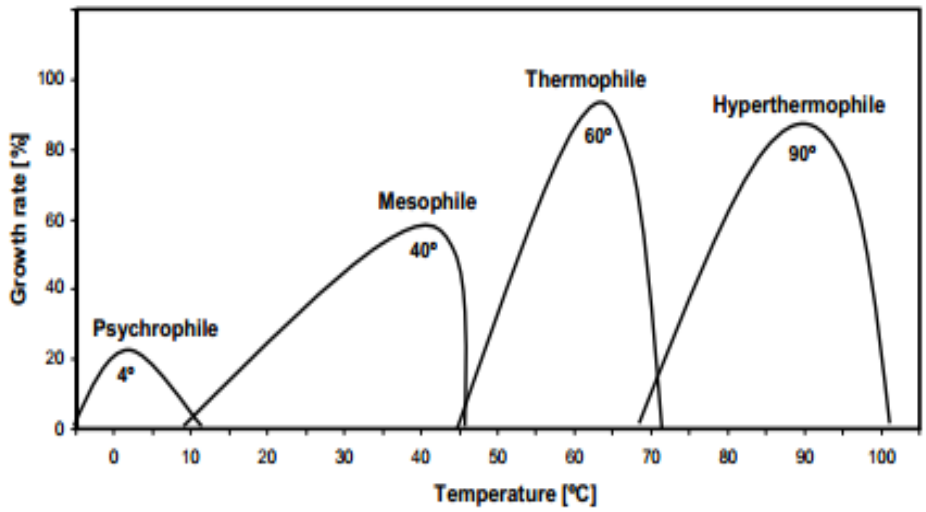


Figure 4. Temperature and growth rate in different temperature classes of microorganisms (Madigan et al. 2010)

There is a specific relation between growth rate and metabolic rate with the temperature. As the temperature increases to optimal temperature (i.e.10°c for psychrophile, 30°c for mesophile and so on) the growth rate and metabolic also increases but with increase or decrease of temperature above and below optimal temperature

respectively, microbes need more energy to function properly so the metabolic rate decreases with change in temperature from the optimal temperature. Most bacteria found in soils are mesophilic in nature with optimal temperature of 25°C to 35°C (Alexander, 1961).

Water Potential

Some microbes exist without water by going in to resting stage called spores but none can survive without water. Water potential is the measure of force required to move water. This force is the combination of osmotic pressure, gravity, surface tension and pressure. The lower the water potential, lesser is the availability of water. Water potential has the logarithmic relationship with microbial metabolism similar to temperature and microbial metabolism. Microbial activity becomes water limited at water potential of -4000 kPa (Kirchman, 2012).

Osmotic pressure and water potential can alter the growth of the microbial community from free swimming to sessile mode. Sessile condition is when the microbes create a biofilms made from polysaccharides. This biofilm acts as a medium to collect nutrients, control redox potential, pH. This biofilm also protects microbes from certain type of predators.

pH

pH has similar effect to temperature. pH homeostatis is maintained by expending cellular energy. Microbes can live in wide range of pH. Acidophilic microbes tend to grow in water with pH of 1-3 while alkaliphiles tend to grow in water with pH of 9-11. The pH also plays a critical role in the chemical state of several compounds and elements. The adsorption of essential nutrients such as phosphate and nitrate to soil and sediment is

regulated by the pH (Kirchman, 2012; Madigan, Clark, Stahl & Martinko, 2010). Microbial growth, metabolic activity and cell-surface charge are also effected by change in pH of the surroundings (Rebata, 2007).

Light

Light is the main source of energy for phototrophic microbes. Energy is produced by synthesizing organic carbon by fixing carbon dioxide. Light can damage DNA and if let unrepaired this may cause mutations of the microbe. Many aerobic microbes are rich in enzymes which can prevent from the damage of the light (Kirchman, 2012).

Osmotic Pressure

Osmotic pressure is defined as the solute available in the solution. Higher the solute, higher is the osmotic pressure. Water has a tendency to diffuse from higher osmotic pressure to lower osmotic pressure. If the extracellular of the microbe has high osmotic pressure than intracellular, the microbe will lose water and get dehydrated. So in order to maintain the osmotic pressure within the environment, the microbe will either gain electron from the environment or expand extra energy to move water in to the cell.

Redox Potential

Redox potential is a way to calculate the tendency of a chemical species to accept electron and get reduced. This affects the microbial community growth by controlling the respiratory potential. Aerobic microbes are found in oxygen rich environment where as anaerobic microbes are found in low oxygen environment.

pH and redox potential can affect the solubility and type of minerals in the environment. Most of the minerals acts as the source of energy for microbes which make their living on the surface of the minerals.

Applications of MICP

van Passen, Ghose, van der Linden, van der Star & van Loosdrecht (2010) performed a large scale experiment to determine the feasibility of biogrouting using *S. pasteurii*. A concrete container (8.0 m x 5.6m x2.5m) was compacted to an average dry density of 1560 kg/m^3. After 16 days of extensive treatment with 96 m^3 of solution containing $CaCl_2$ (1M) and urea (1M) about 40 m^3 of cemented sand body was excavated. The cemented sand body was cored and tested for Unconfined Compressive Strength (UCS) and the value ranged from 0.7-12.4 MPa. However, the result was not satisfactory because the calcite precipitation appeared to be heterogeneously distributed throughout the cemented sand. The heterogeneity in the specimen could be explained as space in soil matrix where calcite precipitates mainly depended on the distribution of bacteria and its bacterial activity. But this activity is very complex to assess in terms of time and space.

Burbank et al. (2013) showed that the stimulation of indigenous bacteria was possible to precipitate calcite by performing cone penetration test (CPT) before and after the test to compare the strength. CPT results shown in Figure 5 for untreated, 5 treatments and 6.5 treatments. The CPT value was relatively high from 20-30 cm for 5 treatments and 6 treatments as compared to untreated. After 6.5 treatments the tip resistance increased from 32 cm depth and after 46 cm the cone could not be pushed further resulting in heavy cementation. This proved that the indigenous ureolytic bacteria can be stimulated to precipitate calcite in the soil matrix. Hence it is well explained that the MICP technique is possible and feasible in sandy soils. However, the timeframe for calcite precipitation depends on the frequency and concentration of substrate (urea and calcium chloride) being flushed (DeJong, Fritzges & Nüsslein, 2006). Conversely, the degree of cementation can

also be regulated by controlling the concentration and number of substrate flow within the soil sample.

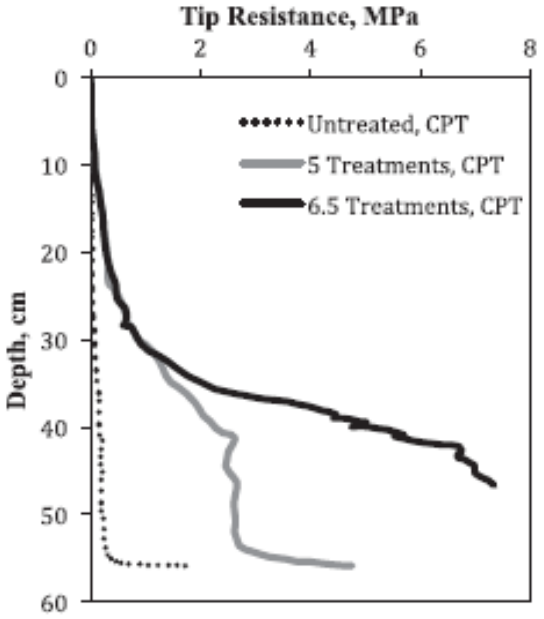


Figure 5. PT values for untreated, 5 treatments and 6.5 treatments (Burbank et al., 2013)

Ng, Lee & Hii (2012), performed MICP on residual soil having liquid limit 58% and plastic limit 44.3 %. The test was mainly performed in order to determine the shear strength of the MICP treated soil sample. In this study *Bacillus megaterium* was cultured in nutrient broth at a temperature of 37°C. The cementation fluid contained 3 g nutrient broth, 10 g NH_4Cl and 2.12 g $NaHCO_3$ per liter of deionized water along with cementing reagents (urea and calcium chloride each having concentration of 0.25 M). The residual soil was then compacted in three different densities, i.e. 85% of maximum dry density (MDD), 90% of MDD and 95% of MDD where the MDD value was reported to be 1563 kg/m^3. The MICP was then performed by injecting one pore volume of cementation fluid at an interval of 6 hours for 7 times during 48 hours of treatment duration. These treatments

were constant for all the soil specimens. These soil specimens were then tested for shear strength. The shear strength was carried out by performing UCS test. The shear strength results were quite satisfactory for the MICP treated residual soil for all densities. It was observed that the strength improvement ratios increased with increasing MDD i.e. 1.41, 2.59 and 2.64 for specimens of 0.85MDD, 0.9MDD and 0.95MDD respectively.

In 2014, Sadjadi, Nikooee & Habibagahi, performed 1-D swell test on MICP treated soil sample. Soil sample was composed of 70% fine sand, 15% Kaolinite and 15% Sodium Bentonite. Plasticity index for this soil sample was reported to be 18.5%. *Bacillus sphaericus* as urease enzyme producing microbe was selected to precipitate Calcite.

In Test 1, the immediate compaction of the sample reduced the available space and voids for microbes to move freely. In Test 2 and Test 3, enough space was available for free movement of microbes. However, in Test 3, the precipitated calcite was distributed due to the compaction which may result in the reduction of the improvement. These tests result clearly show the effectiveness of MICP in fine grained soil. However further researches are needed to be performed on different types of soils to further explore the efficacy of MICP. It also becomes necessary to understand long term behavior of MICP application in geotechnical engineering.

Definitions of Terms Related to Microbiology

As this thesis is interdisciplinary in nature and mostly read by geotechnical engineers who may not have background in microbiology, this section describes some of the terms related to microbiological applications used in this thesis.

Autoclave- Autoclave is a heating device that is used to kill microorganisms. This device uses steam under pressure to kill endospores that require typically temperatures higher than 100ºC.

Endospores- Endospores are produced by certain species of bacteria. Endospores enable bacteria to resist extremely harsh weathers, temperature, chemicals and radiation. Bacteria becomes dormant after the formation of endospores. All *Bacillus* species including the one used in this research (*Sporosarcina pasteurii*) produce these endospores and are capable to resist harsh environments.

Colony Formation Unit (CFU) - Some cells are able to divide and form offspring by binary fission. These types of cells are known as viable cells. Counting the number of viable cells will help in determining the concentration of microbes present in a solution. Colony Formation Unit (CFU) is typically used to count these viable cells. In this research, CFU method is used to determine the concertation of bacteria present in the solution. More details about the CFU and how to determine this is presented in chapter 3.

Serial Dilution – Serial dilution is a stepwise process for obtaining dilute solution. The dilution factor in each step results in a geometric progression of the concentration of a solution. For instance, to obtain a serial dilution of 1:10, 1 ml of culture is introduced into the 9 ml of nutrient broth solution. This will give a dilution of 1:10 in a 10 ml of diluted solution (1 ml of culture and 9 ml of nutrient broth).

Optical Density (OD) - Optical density (OD) is a measure to estimate the growth of cells in a culture. OD is the ability of a bacterial specimen present in a culture to absorb or block the passage of light. In other words, OD of a sample can be the indicator of turbidity. OD is measured in a spectrophotometer.

Extracellular Polymer Substance (EPS) - Extracellular polymer substances (EPSs) are natural polymers secreted by microorganisms into the surrounding. EPSs are mostly made up of polysaccharides and protein. EPSs are produced during the microbial settlements in the environment and are important components in biofilm formation. These substances made up 50% to 90% of a biofilm's total organic matter.

CHAPTER THREE: MATERIALS AND METHODS

This chapter deals with the materials and methods used in this research to achieve the research objectives. The variables studied in this research were, soil type, microbial concentration/population, and curing periods. Three different properties were measured to study the variation of MICP and lime treatments on three different soils and these properties were compared with the properties of soil treated with lime. In order to see the effects of MICP in geotechnical properties of these natural soils such as plasticity, strength and swelling, these soils were tested for Atterberg limits, Unconfined Compression Strength (UCS), and one dimensional swell percentage.

Materials

The materials used in this research are discussed under four broad categories including: soil types, bacterial strains and growth media, substrate solutions, and lime additive.

Soil Sample

Three different soil samples were used throughout this research. Both soil samples are naturally occurring soils obtained along US-95 between Milepost 16.0 to 18.0 near Marsing, Idaho. These soils range from low PI to high PI. These soils are designated as S1 (low plasticity), S2 (medium plasticity) and S3 (high plasticity). According to the Unified Soil Classification System, both of these soils are designated to be CH soils.

Bacterial Strain

The bacterial strain used this research was obtained from the previous research conducted by Dr. Malcolm Burbank and others at University of Idaho. The bacterial strain was exogenous in nature. Figure 6 shows a picture of *S. pasteurii* plated on LB plate that was used in this research. The growth media used to grow the microorganisms was primarily Luria Broth (LB).

Figure 6. *S. pasteurii* plated on Luria Broth plate

Lime

Lime stabilization was used as control to verify the effectiveness of MICP treatments on soils. Lime was chosen as the control as it is a very commonly used stabilizer for arresting expansive soil heaves especially for lightly loaded structures like pavements and residential buildings. Commercially available laboratory grade lime was used in this research. The percentage of lime required for each of the soil type was determined using Eades & Grimm (1960) procedure discussed in later sections.

Substrate Solutions

Commercially available urea and calcium chloride were used in this research. The concentration of urea and calcium chloride was 333 mM and 250 mM respectively. The concentration of substrate was established from previous research conducted on sand through MICP technique.

Soil and Microbial Characteristics

In this section the various experimental procedures used to establish the soil and microbial characteristics are discussed. Tests such as gradation, Atterberg limits, moisture-density characteristics, Eades and Grimm pH tests were conducted on all control soils while tests to determine Colony Formation Unit (CFU) that establish the microbial concentration were performed on the *S. pasteurii.*

Gradation Test (ASTM-D 6913-04)

The gradation test helps to determine the particle size distribution of a given soil sample. The gradation test was performed on all three untreated soil samples according to the ASTM-D 6913-04.

Atterberg Limits (ASTM-D4318)

Atterberg limit tests are performed to determine the behavior and consistency of fine grained soil samples. The behavior and consistency is based on the water content. Liquid limit is defined as the water content at which fine grained soils changes from plastic to liquid state where as plastic limit is the water content where the fine grained soils changes from semi-solid to plastic state. Typical liquid limit and plastic limit apparatus are shown in Figure 7 and 8 respectively.

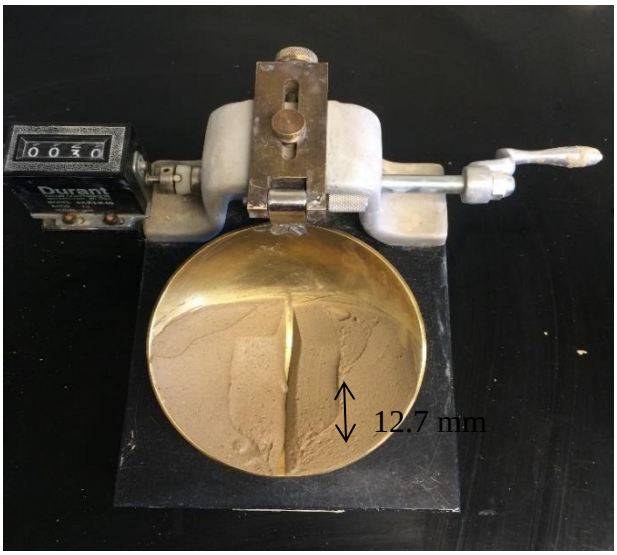


Figure 7 Liquid limit test.

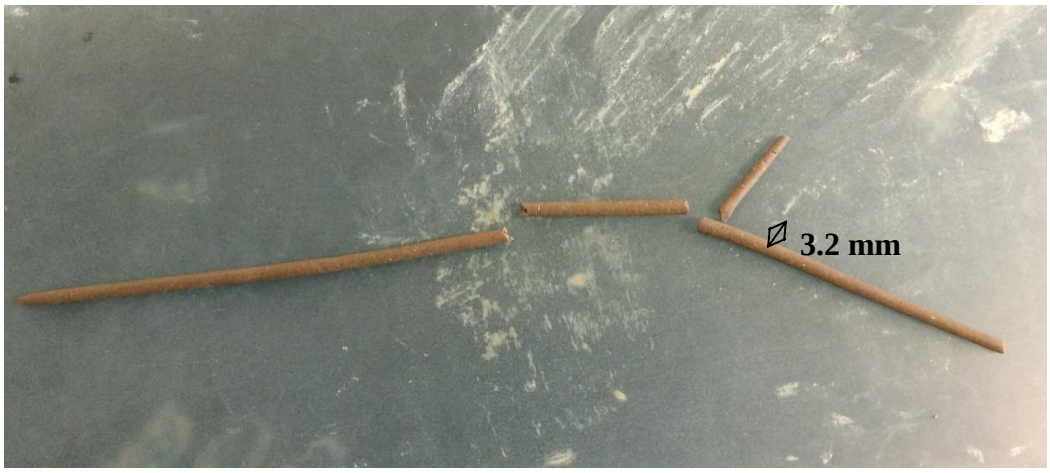


Figure 8. Plastic limit test.

Moisture-Density Characteristics (ASTM- D 698)

Standard Proctor compaction test was performed to determine the moisture-density characteristics of a soil. The optimum moisture content (OMC) is the moisture content at which soil will have its maximum dry unit weight (MDUW). Proctor compaction test was performed on all three untreated soils samples.

Eades and Grim pH test (ASTM- 6276)

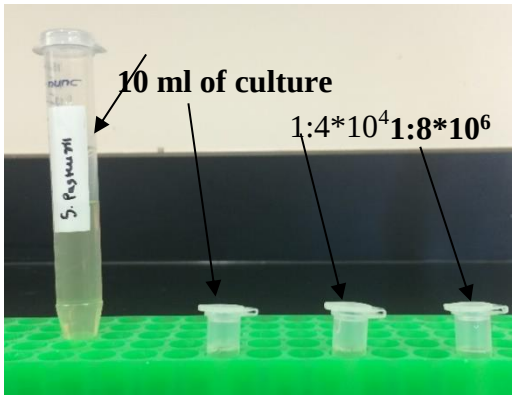
Eades and Grim pH test was performed to determine the percentage of lime required for lime stabilization of a given soil. In this test each 25 g of a given soil sample are treated with 2%, 3%, 4%, 5%, 6%, 7% and 8% of lime by weight. These soils are then tested for pH. The percentage of lime that results in a pH of 12.3 is considered optimum lime percentage for lime stabilization of a given soil sample. Eades and Grim pH test was performed on all three soil samples to establish the minimum lime required to stabilize these soils. This lime percentage was used in the lime treatment method.

Microbial Concentration

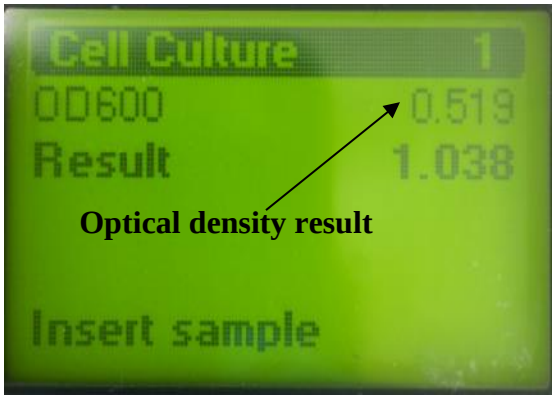
Two different microbial concentration tests were used in this research to determine the effect of microbial concentration in evaluating the effect of MICP in expansive soils. In order to maintain the consistency of microbial concentration throughout the research, colony formation unit (CFU) method was adopted to determine the concentration of microbes in a given solution. This method is viable for cells that are able to divide and produce offspring. For this purpose, *S. pasteurii* was cultured in Luria broth (LB), incubated for 48 hours at room temperature. After 48 hours of inoculation, the optical density (OD) of these cultured microbes was measured. OD is the method of determining concentration of microbes in a sample by measuring the turbidity of the sample at certain wavelength, usually 600 nm. These cultured microbes were then serially diluted in various ratios such as 1:200, 1:40000, 1:8000000. After serial dilution, 100 μL of the serial diluted media was taken and then plated in a LB plate (LB plate was prepared by mixing 10 g of LB and 6 g of agar in 400 ml of distilled water. The media after autoclaving was poured into the petri dish. The media solidifies after few hours due to the presence of agar.) After

48 hours of plating, the number of colonies were counted (Figure 9). The CFU/ml for each serial dilution is given as per Equation (7).

$$\frac{\text{CFU}}{\text{ml}} = \frac{\text{No.of colonies counted} * \text{dilution factor}}{\text{Volume of culture}} \quad (7)$$



(a)



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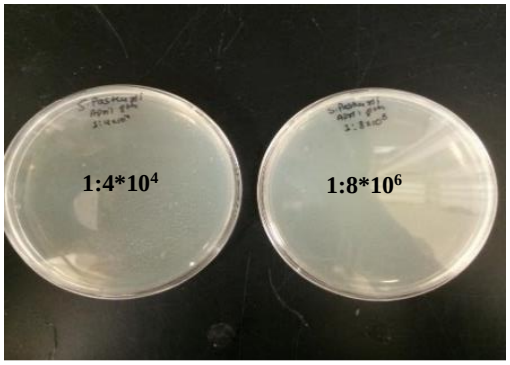

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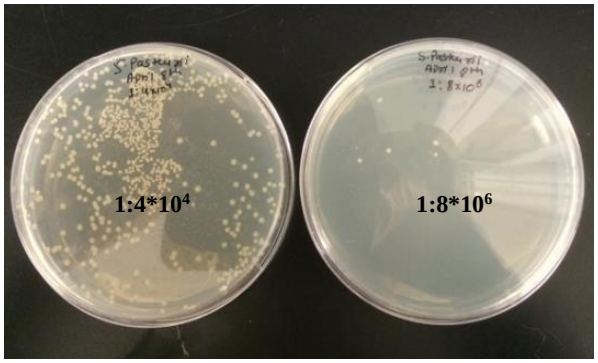


(d)

Figure 9. (a*) S. pasteurii* culture in 10 ml of LB growth media and serial dilution of the culture (b) optical density measurement (c) plating of 100 µL of the serial diluted media (d) colony formation of *S. pasteurii* after 48 hours.

Two different microbe concentrations were used in this research. Here M1 and M2 stand for microbial concentration of 10^8 microbes/gm and 10^{10} microbes/gm respectively.

Treatment Methods

In order to compare the effectiveness of MICP, two different methods were adopted. The first method was bio-augmentation where microbes along with substrates were mixed into the soil samples and compacted at maximum dry density and optimum moisture content. This process creates a soil mass that has known amount of *S. pasteurii* added into the soil samples. The second method was bio-augmentation followed by stimulation in which the microbes are stimulated by using substrate solution at different treatment cycles. In addition to comparing the performance of the treatments with untreated soils, a conventional treatment method in the form of lime stabilization was also conducted on these soils. This section of the thesis describe the methods followed for each of the treatment methods along with the various test procedures used to measure performance.

Bio-augmentation

In this method microbes were mixed in the soil sample along with substrates. The mixed sample was then compacted at MDUWD and OMC. The compacted sample was then cured for seven days before being tested for UCS, Atterberg limits and 1-D swell tests. Curing was done under controlled moisture and humidity chamber for all bio-augmented and lime treated samples. Bio-augmentation was performed to replicate the lime treatment where lime is directly mixed with soil and water and compacted at MDUW and OMC. The lime treatment is performed in-situ and to understand the efficacy of bio-augmentation in in-situ soil, this treatment was adopted.

Bio-augmentation Followed by Stimulation

Bio-augmentation alone resulted in unsatisfactory results. This may be due to the dormancy of microbes with no moisture and oxygen within the microbial environment.

When microbes become dormant, all the metabolic activities slow down. During this period, microbes become unable to produce any urease enzymes to hydrolyze urea in the system and as a result no calcite can be precipitated. In this bio-augmentation followed by stimulation method soil samples were prepared as in the case of bio-augmentation method. The samples instead of being cured at constant temperature and humidity, were placed in a nutrient delivery system. Using this system, substrate solutions were passed through the soil samples and the effluent was collected. One pore volume (1 PV), three pore volumes (3 PV) and seven pore volumes (7 PV) of effluent was collected. One pore volume here represents the volume of voids of a given sample, compacted at MDUW and OMC. Collection of effluent is termed as treatment cycles in this research. After collecting respective pore volumes, samples were then tested for UCS, Atterberg limits, 1-D swell tests and Specific surface area (SSA).

Nutrient Delivery System

In order to stimulate the bacteria mixed into the soil, substrate solution consisting of urea and $CaCl_2$ solution need to be passed through the soil sample. As the permeability of these soils is very low ($< 10^{-6}$ cm/sec) gravity feeding was not feasible in the available time frame. Hence, for this purpose a nutrient solution delivery system was developed as shown in Figure 10. In this set up the chamber was made up of schedule 40 PVC tube, 9.4 cm diameter. The plates shown in Figure 10, were also made up of PVC, 15.24 cm x 15.24 cm in dimension. This chamber can hold pressures as high as 138 kPa. This chamber had two inlets and two outlets. One inlet was connected to the reservoir containing substrate solution. The purpose of reservoir was to fill the chamber with substrate solutions. The other inlet was connected to a pressurized container which also contained substrate

solution. This pressurized container was used to pressurize the chamber to the desired pressure. The substrate in the pressurized container was pushed into the chamber under pressure which percolated through the sample. One of the outlets was used to drain the chamber while the other outlet was used to collect the effluent. The soil sample having dimensions 7.62 cm x 15.24 cm was placed between the top cap and the base pedestal and was wrapped around by the latex membrane in order to protect the sample from being washed away during the test. The top cap and base pedestal were facilitated with grooves in order to hold O-rings. The O-rings hold the membrane in the place and also prevented water from entering inside the sample. The top cap and bottom pedestal had holes in them through which solutions passed through the soil sample and was collected through the outlet respectively.

In order to maintain uniform retention period throughout the research, the pressure was maintained, such that one pore volume of effluent was collected in 24 hours. The soil samples were treated until 1, 3 and 7 pore volumes of effluent were collected. These were termed as treatment cycles, and denoted as 1PV, 3PV and 7PV representing 1, 3 and 7 pore volumes of effluent collection respectively.

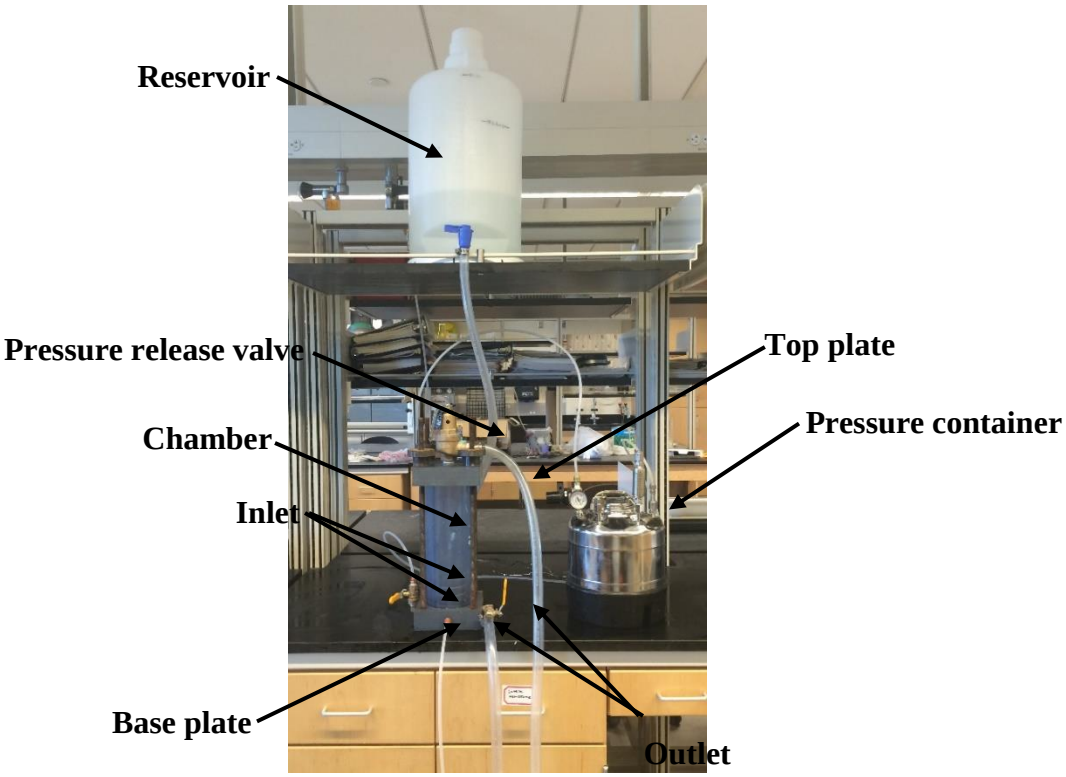


Figure 10. Nutrient delivery system

Lime Treatment

For lime treated soils, samples were prepared by mixing lime and water and compacted at MDUW and OMC according to the ASTM-D 5102 but the OMC and MDUW of lime treated samples were not determined. The lime treated samples were compacted according to the MDUW and OMC of untreated soil samples. This was mainly done to maintain the compaction conditions constant for all treatments including MICP treated samples. In order to make the comparison with bio-augmentation, lime treated soil samples were prepared and cured for seven days. Lime treated soil samples were also prepared and cured for one day, three days and seven days and then placed in nutrient delivery system. The nutrient delivery system contained deionized water through the sample. This was done primarily to saturate lime treated samples before testing in order to compare the results with bio-augmentation followed by stimulation where the samples were tested after

saturation. After collecting one pore volume (when saturation was assumed to be complete), samples were tested for UCS, Atterberg limits and 1-D swell tests.

Soil Sample Preparation

Natural soil contains 10^6 of microbes in one gram of soil with (Torsvik, Goksøyr & Daae, 1990). The exogenous microbes introduced into the soil may face problems such as uneven distribution, predation and competition from microbes already present in the soil (Burbank et al. 2013). In order to ensure the survivability of microbes introduced into the soil samples, S1 and S2 soil samples were autoclaved. Soil samples before and after autoclaving were inoculated in LB growth media for 24 hours. The inoculation was then plated in LB agar plate to observe the microbial population before and after the autoclaving. It was observed that all the microbes present in the soil samples were not fully sterilized after autoclaving as observed in Figure 11. This may be due to the endospores present in soil samples. Microbes produce endospores to survive in unfavorable conditions. Endospores are dormant, tough and non-reproductive structure produced by microbes. As a result, soils were not autoclaved. Soils not being autoclave also gives a picture of field treatment in the future. An autoclave is a pressure chamber that is used for sterilization of apparatus and other materials such as medical equipment, glass bottles, growth media and many more at high temperature.

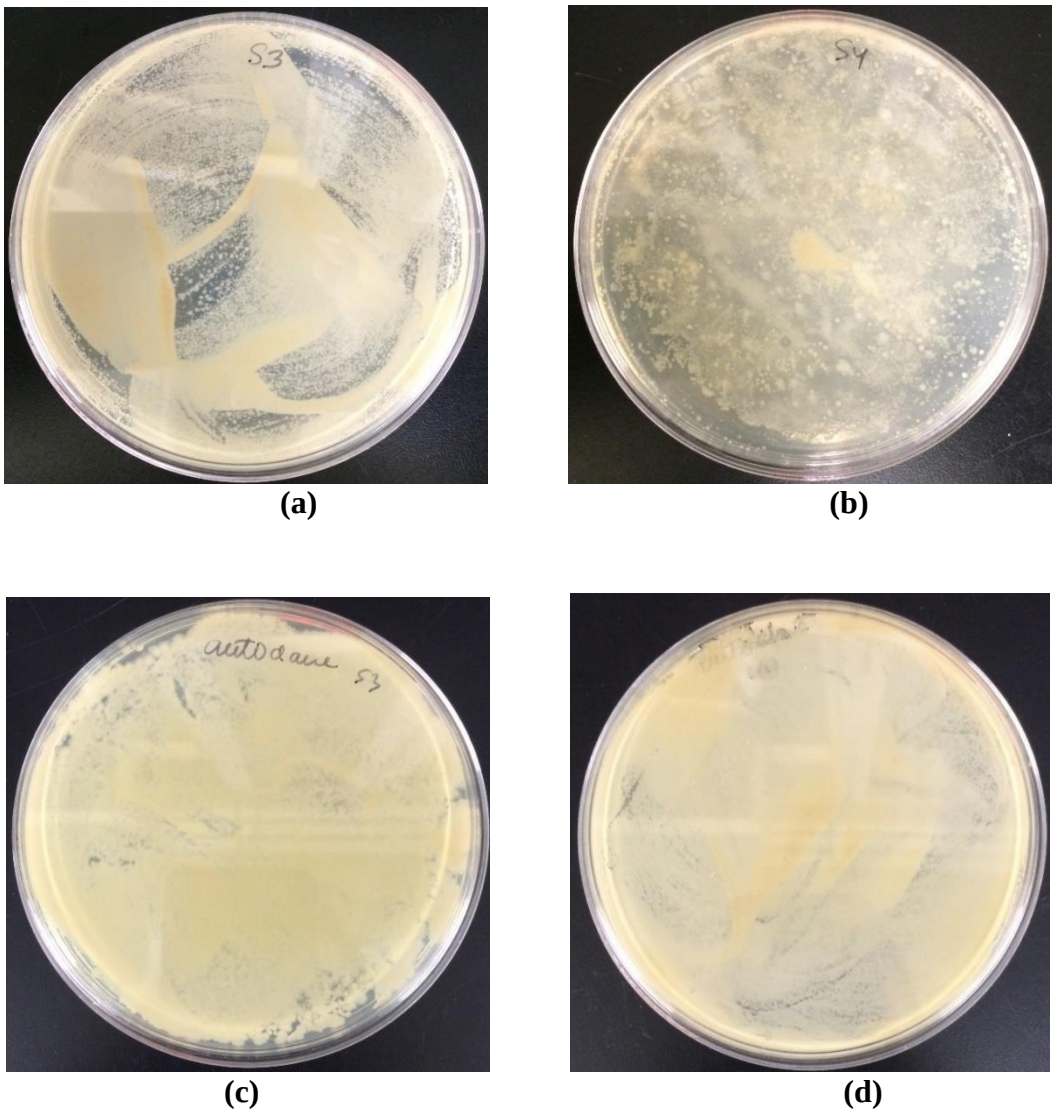
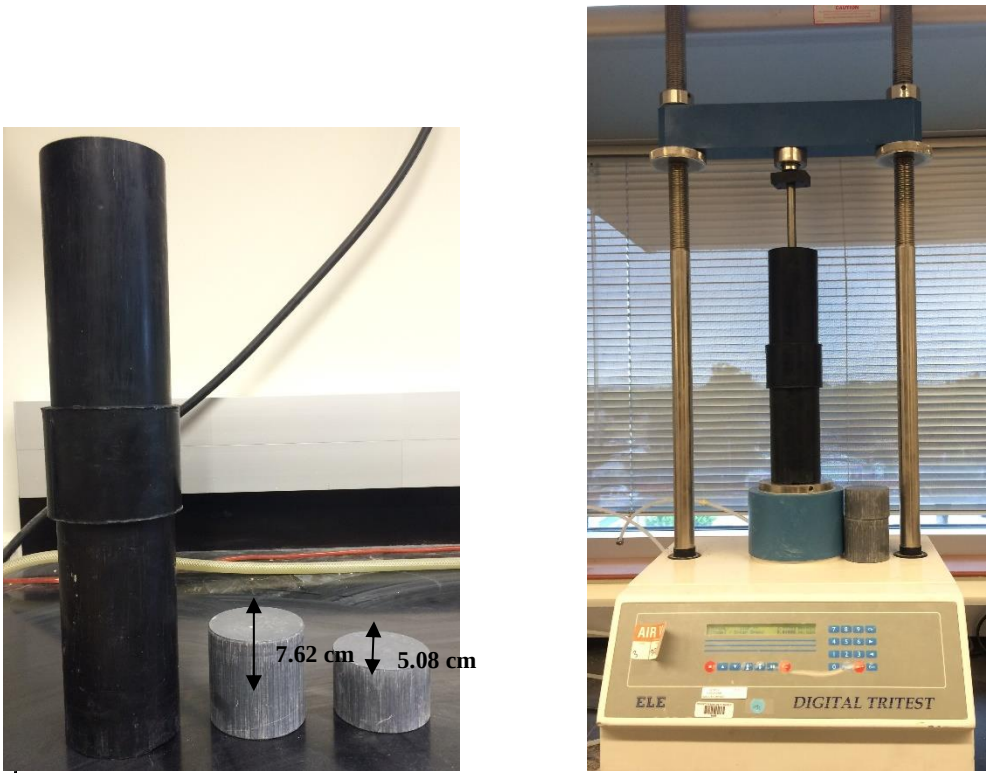


Figure 11. (a) S1 before autoclaving (b) S2 before autoclaving (c) S1 after autoclaving and (d) S2 after autoclaving

All the samples were prepared by compacting at MDUW and OMC. Soil samples were mixed with media containing microbial population and substrates in a 40.64 cm hollow tube with 7.62 cm diameter. The hollow tube was closed by 5.08 cm and 7.62 cm blocks. The tube was then placed under static compactor. The tube was first compacted from 5.08 cm block end and then inverted and compacted from 7.62 cm block end. The reason behind inverting the tube is to ensure homogenous compaction throughout the soil

samples. Conventional compaction was not carried out so as to avoid layers with in the soil samples. Layers in the soil samples may create disconnection within the pore paths. Figure 12 shows the sample preparation method adopted in this research.



(a) **(b)**

Figure 12. (a) Tube and blocks used for sample preparation (b) static compactor used for sample compaction

Performance Measuring Experiments

Tests such as Unconfined Compressive Strength (UCS), 1-Dimensional (1-D) swell test and specific surface area (SSA) are performed to determine the efficacy of MICP in expansive soils. The UCS test determines the compressive strength, 1-D swell test determines the change in swell percentage and SSA helps to determine the percentage of montmorillonite before and after the treatment. These treatments are the performance

indicator of MICP in soil samples. Apart from above mentioned test, Atterberg limits are also performed to determine the plasticity characteristics of treated soil samples.

Unconfined Compressive Strength (UCS) (ASTM- D2166)

UCS test is a quick test to obtain the shear strength of fine grained soils. The soil samples were compacted at MDUW and OMC for both samples treated with MICP and lime. The UCS test for lime treated soil samples, were prepared according to the ASTM-D5102. For bio-augmented soil samples, samples were cured for 7 days and directly tested for UCS whereas for samples that were bio-augmented and stimulated, these tests were conducted after collecting one pore volume, three pore volumes and seven pore volumes of effluent through the soil samples. After performing UCS tests, these samples were further tested for Atterberg and 1-D tests. Typical UCS testing setup used in this research is shown in Figure 13.

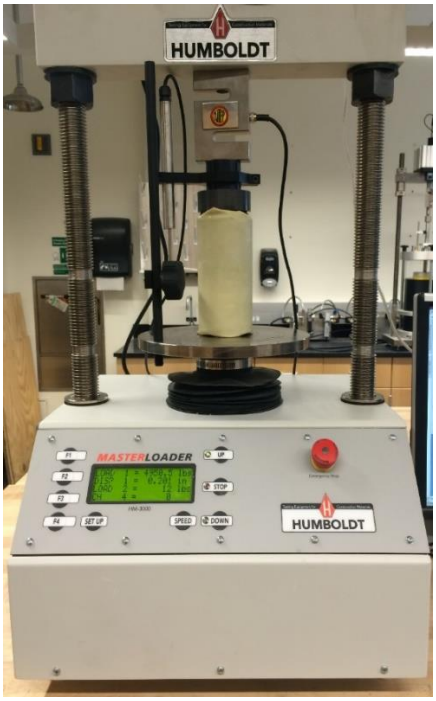


Figure 13. UCS test setup used in this research

One-Dimensional (1-D) Swell Tests (ASTM-D4546)

The soil samples obtained from UCS tests as explained above were oven dried for 24 hours. After oven drying, soil samples were re-compacted to MDUW and OMC. These samples were trimmed to a diameter of 6.35 cm and thickness of 2.54 cm with the help of oedometer ring. 1-D swell test was performed according to the ASTM-D4546, method A. During the test only swell percentage was determined and not the swell pressure. 1-D swell test setup used in this research is shown in Figure 14.

Figure 14. 1-Dimensional swell test

Specific Surface Area (SSA) and Cation Exchange Capacity (CEC)

Specific surface area or SSA of a soil sample is the total surface area contained in a unit mass of soil. This property of the soil is primarily dependent on the particle size of the soil. Soils with smaller particle size have higher specific surface areas. The most commonly used method is the adsorption of ethylene glycol monoethyl ether (EGME) (Carter, Mortland & Kemper, 1986). This involves saturating prepared soil specimens, equilibrating them in vacuum over a calcium chloride – EGME ($CaCl_2$-EGME) solvate, and weighing to find the point when equilibrium is reached. Specific surface is then

determined from the mass of retained EGME in comparison to the amount retained by pure montmorillonite clay, which is assumed to have a surface area of 810 m^2/g (Carter, Mortland & Kemper, 1986). The detail procedural steps of SSA is shown in Figure 15 and typical SSA test carried out in lab is shown in Figure 16.

CEC of a soil can be defined as the capacity or the ability of the soil to exchange free cations that are available in the exchange locations. Cation exchange capacity (CEC) can be used to determine the mineral composition of the soil specimen with a high CEC value indicating a high amount of expansiveness due to the presence of the clay mineral montmorillonite.

In order to determine the percentage of montmorillonite present in the soil sample, the equation (Equation 8) introduced by Yukselen & Kaya (2006) is used to determine the CEC value of soil samples. The percentage of montmorillonite was obtained by using Equation 9 developed by Chittoori (2008).

$$CEC = -0.33*LL+0.4*SSA+8.8 \quad (8)$$

Where,

LL = Liquid limit

CEC = Cation Exchange Capacity

SSA = Specific Surface Area

$$\%M=-2.87+0.08*SSA+0.26*CEC \quad (9)$$

Where,

%M= Percentage by weight of the mineral montmorillonite in the fines fraction of the soil.

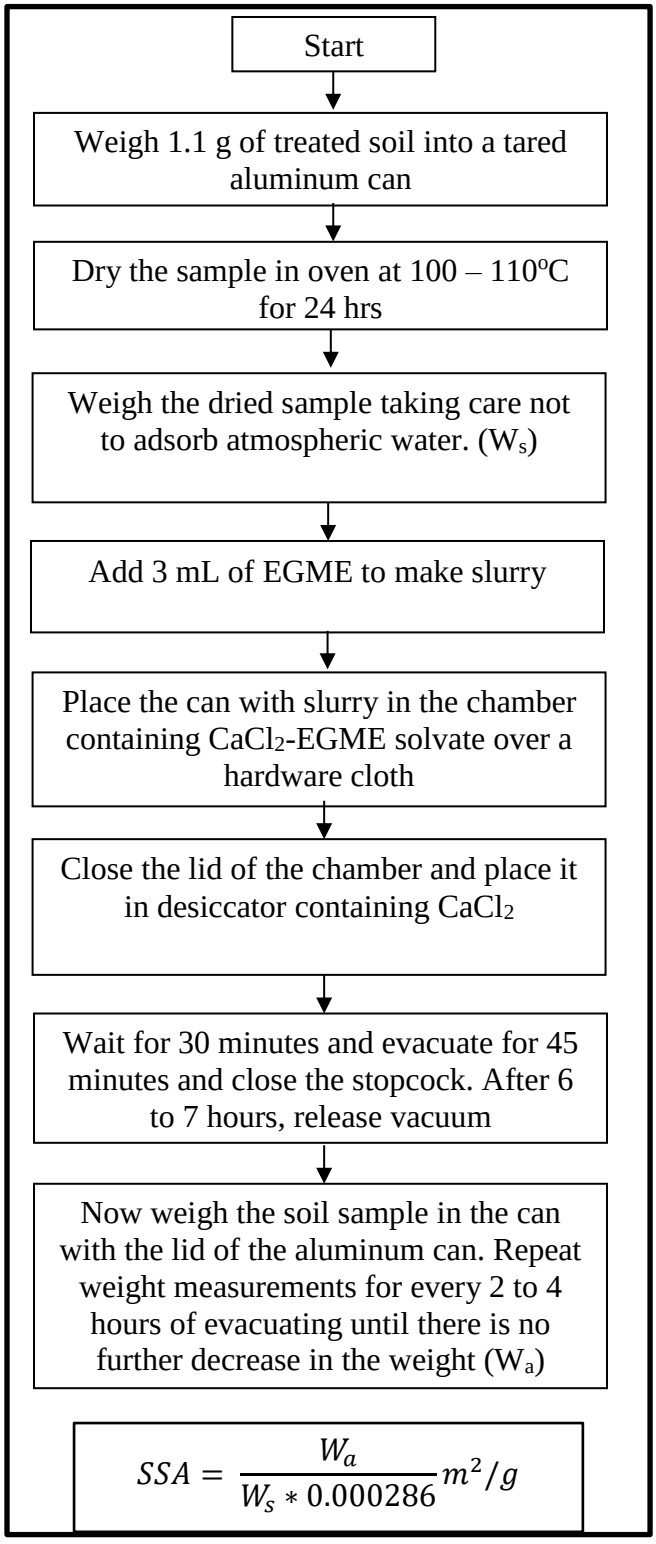


Figure 15. Detail procedural steps to determine Specific Surface Area.

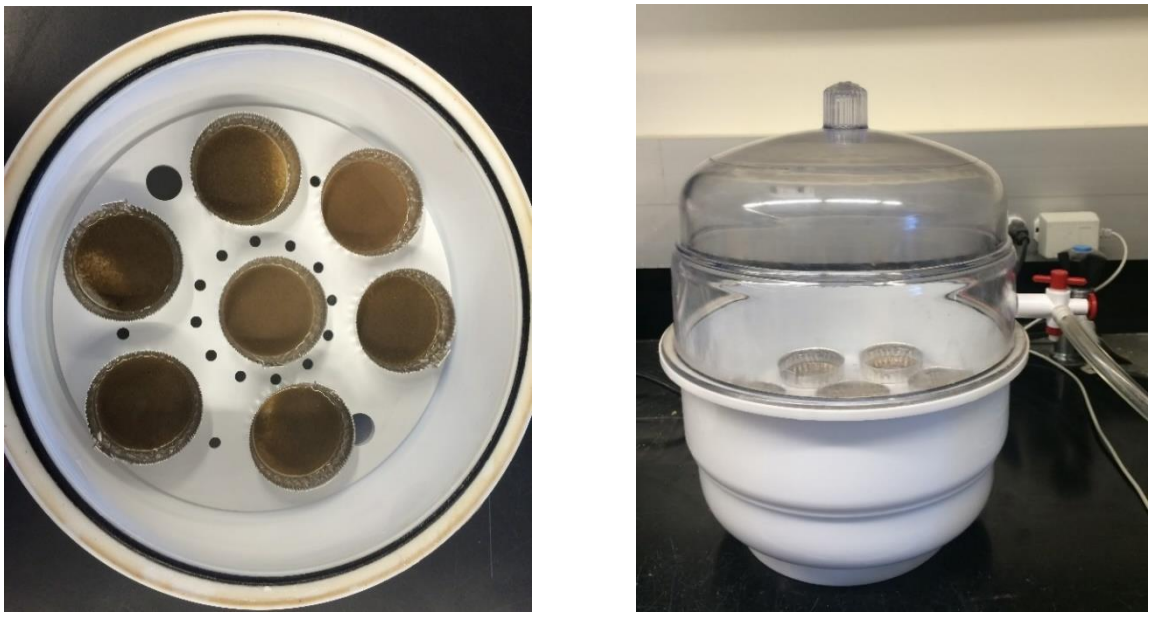


Figure 16. Specific surface area test

Here the main purpose of performing SSA test is to determine the percentage of montmorillonite before and after the treatment. Change in percentage of montmorillonite indicates the change in mineralogy of clay particles in soil samples.

The engineering properties of all three natural soils, S1, S2 and S3 are represented in Table 3. Figure 17 below presents the schematic of the experimental program followed in this research.

Summary

In order to achieve the objective of this research, 210 tests were carried out on three different soil samples with three different treatments. Seven days, bio-augmented sample tests were compared with seven days cured lime treated samples. The bio-augmented sample followed by stimulation samples for one pore volume (1 PV), three pore volumes (3 PV) and seven pore volumes (7 PV) were compared with one day, three days and seven days cured and then followed by collecting one pore volume by placing them in a nutrient delivery system.

Table 3. Engineering properties of natural soil samples

Properties		S1	S2	S3
Atterberg Limit	Liquid limit	54	58	115
	Plastic limit	39	31	53
	Plasticity index	15	27	62
MDUW (kN/m^3)		13.64	11.9	12.02
OMC (%)		30	38.5	34
UCS (kPa)	Saturated	24.5	21.56	28.56
	Unsaturated (w/c=100%)	58.85	179.3	239.5
% finer than 0.075 mm		70	86.4	74
1-D swell percentage		2.83	8.55	8.85
% of lime		2	2	2
SSA (m^2/g)		309	359	449
CEC (meq/100g)		150.18	171.39	226.49
% of Montmorillonite		60.89	70.38	91.97

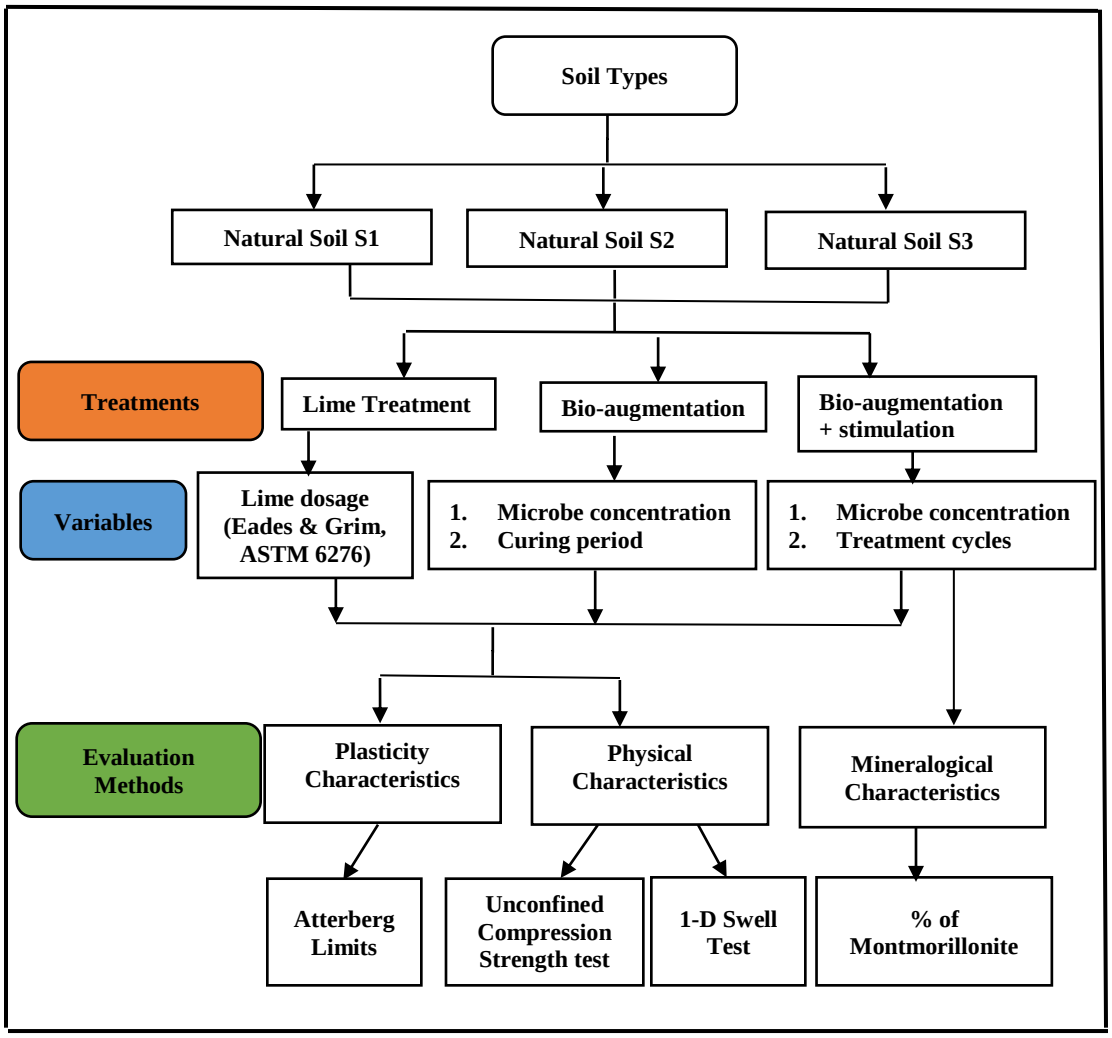


Figure 17. Schematic of materials and methods used in this research

CHAPTER FOUR: RESULTS AND DISCUSSION

This chapter discusses the results of various laboratory tests conducted as a part of this research. For each type of test, a brief summary of the results is presented followed by a discussion on the results followed by additional analysis using that test data.

Bio-augmentation

Bio-augmentation was carried out by mixing microbes (M1 only) along with substrates. The sample was then compacted at MDUW and OMC and left it cured for seven days. The outcomes obtained from the test were not satisfactory and so it was concluded that increase in microbial concentration was unnecessary. In this section all the test results obtained from bio-augmentation are presented.

UCS Values

The UCS values for seven days cured bio-augmented samples are presented in Table 4 for all the three soil samples. It can be observed from this table that the UCS value increased from 58.8 kPa to 88.0 kPa for S1 soil sample with increase of 49.5%, UCS value decreased by 30.6% for S2 soil and by 39.4 % for S3 soil. In case of lime treated soils, the UCS value increased for S1, S2 and S3 soil samples.

Table 4. Summary of UCS values of bio-augmented and lime treated soil samples

Soil Type	Untreated	Bio-augmented	Lime treated
	UCS (kPa)	UCS (kPa) (%)	UCS (kPa) (%)
S1	58.8	88.0 (49.5)	1095.8 (578.3)
S2	179.3	124.5 (-30.6)	454.0 (153.2)
S3	239.5	145.2 (-39.4)	657 (174.3)

Note-: 1. Numbers in brackets are change in UCS values compared to the untreated soils. 2. Negative values indicate decrease in strength and vice versa.

Figure 18 shows the UCS values for three different soil samples. These soil samples were bio-augmented for seven days and then tested for UCS. The UCS values decreased for S2 and S3 soil samples. This could have been due to several reasons. Firstly, the presence of microorganisms in the soil sample could affect the strength of the soil as the soil composition is changing. However, if the microbes precipitate calcite the sample could have increased its strength but in this case due to inadequate substrate present in the sample calcite precipitation may not have taken place. As microbes require moisture to survive and there is no additional moisture other than molding moisture content available for the microbes, they may have been dormant and inactive during the seven day curing period and did not precipitate calcite. In addition, soil samples S2 and S3 have high fines content (86.4% and 74% respectively, passing through sieve#. 200), this may have made the mobility of microbes less possible. Pore size distribution and the proportion of pore filled with water plays an important role in the contact between microbes and soil particles (Chenu & Stotzky, 2002).

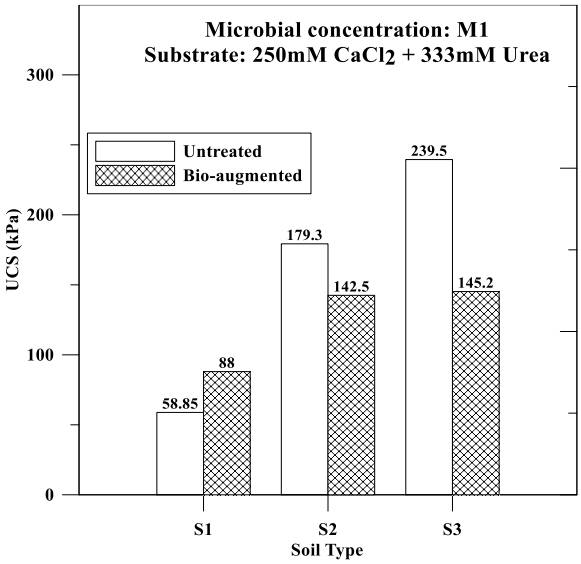


Figure 18. UCS values of untreated and bio-augmented soil samples

Atterberg Limits Test Results

The Atterberg limits test results for seven days cured bio-augmented samples are presented in Table 5. The liquid limit increased for S1 and S2 but decreased for S3. The liquid limit increased from 54% to 58% for S1 which is an increment of 7%. Similarly, the plasticity index increased by 58.2% for S1 from 15% to 24%. 48.3% of increment in liquid limit was observed for S2 from 58% to 86% and plasticity index increased by 29.6% from 27% to 35%. However, the liquid limit decreased from 115% to 96% by 16.5% also plasticity index decreased by 19.4% for S3 from 62% to 50%. The lime treated soil samples behaved as non-plastic.

Table 5. Summary of Atterberg limits test results of bio-augmented and lime treated soil samples

Soil Type	Untreated		Bio- augmented		Lime treated	
	Liquid Limit (%)	**Plasticity Index (%)**	**Liquid Limit (%)**	**Plasticity Index (%)**	**Liquid Limit (%)**	**Plasticity Index (%)**
S1	54	15	58 (7.0)	24 (58.2)	Np	Np
S2	58	27	86 (48.3)	35 (29.6)	Np	Np
S3	115	62	96 (-16.5)	50 (-19.4)	Np	Np

Note-: 1. Numbers in brackets are change in Atterberg limits test results compared to the untreated soils. 2. Negative values indicate decrease in strength and vice versa. 3. Np stands for non-plastic.

Figure 19 presents the liquid limit and plasticity index variation between untreated and bio-augmented soil samples for all three soils tested here. The liquid limit and PI increased for S1 and S2, this indicates that the bio-augmentation in natural soils did not perform well. However, the liquid limit and plasticity index of S3 sample tend to decrease. The increase in liquid limit and PI values in case of S1 and S2 soils could be attributed to the presence of higher organic material (in the form of EPS). Increase in organic carbon content by 1 % can result in increase in Atterberg limit by 10 to 20% (Mitchell & Soga, 2013). The reduction in case of S3 soil which has very high untreated liquid limit indicates a lack of bioactivity. However, the reduction in LL and PI could be due to the cation exchange between the cations present in clay particles and calcium ions present in the calcium chloride. The cation replacement may occur in clay minerals due if the valency of cations is higher than the cations in minerals (Mitchell & Soga, 2013).

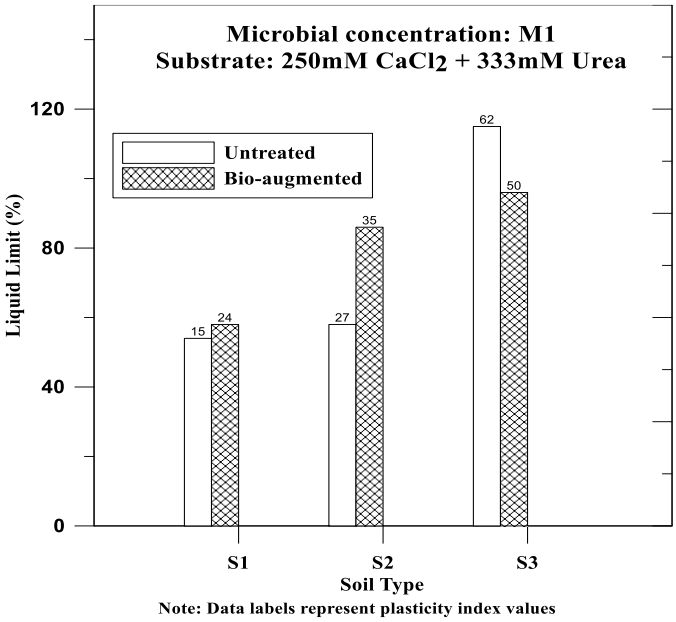


Figure 19. Atterberg limits test results of untreated and bio-augmented soil samples

1-D Swell Test Results

The 1-D swell strain for seven days cured bio-augmented samples are presented in Table 6. The swell strain was compared with untreated and treated for all soil samples. For S1 sample decreased by 11% from 2.83% to 2.52% whereas the swell strain value increased for S2 by 24.8% from 8.55% to 10.67% and decreased for S3 by 44.1 % from 8.85 % to 4.95% for S3 when bio-augmented. The 1-D swell also decreased for lime treated soil. For S1 sample 1-D swell decreased by 47.9% from 2.83% to 1.47%. However, for lime treated S2 sample the 1-D swell was observed to be 7.95% i.e. decrease in swell percent by 7.02%, compared to untreated S2 sample. It was also observed that for S3 sample treated with bio-augmentation had lower 1-D swell value than compared to lime treated but the overall swell reduced for lime treated sample from 8.85% to 7.1% which is a reduction of about 20%.

Table 6. Summary of 1-D swell strain of bio-augmented and lime treated soil samples

Soil Type	Untreated	Bio-augmented	Lime treated
	1-D swell strain	1-D swell strain (%)	1-D swell strain (%)
S1	2.83	2.52 (-11)	1.47 (-47.9)
S2	8.55	10.67 (24.8)	7.95 (-7.02)
S3	8.85	4.95 (-44.1)	7.1 (-19.8)

Note-: 1. Numbers in brackets are change in 1-D swell strain compared to the untreated soils. 2. Negative values indicate decrease in strength and vice versa.

From Figure 20, it is evident that bio-augmentation was effective for S1 sample with low plasticity. However due to the production of EPS by bacteria, the plasticity increased for S2 sample and the increase in plasticity also may have increased the swell percentage. S3 sample exhibited a decrease in plasticity and so do the 1-D swell test.

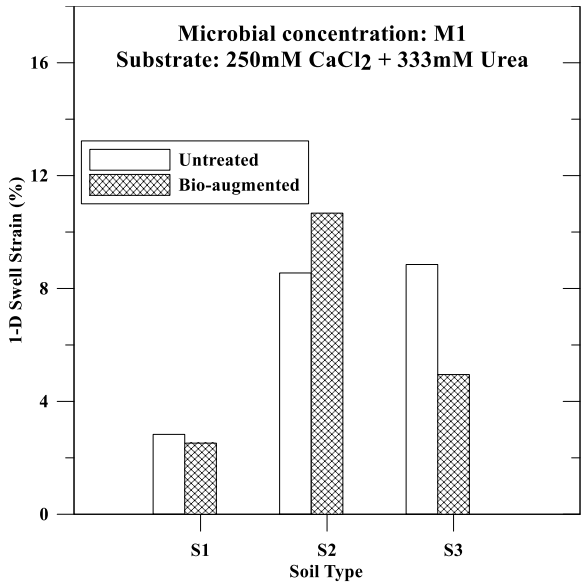


Figure 20. 1-D swell strain of untreated and bio-augmented soil samples

Bio-augmentation Followed by Stimulation

Bio-augmented samples were followed by stimulation by placing samples inside the nutrient delivery system. The curing period was replaced by number of pore volumes of effluent collected. In this method, soil samples were tested after collecting 1 PV, 3 PV and 7 PV of effluent from the samples. Samples were tested for UCS, Atterberg limits, 1-D swell test and SSA properties. In this section all the test results obtained from bio-augmentation followed by stimulation are presented.

UCS Test Results

Bio-augmentation followed by stimulation was carried out with two different microbial concentrations (M1 and M2) and three different treatment cycles, 1 PV, 3 PV and 7 PV. The UCS values are presented in Table 7 for all the three soil samples treated with M1 for all three pore volumes. For S1 soil, the UCS value gradually increased from 25.8 kPa to 54.2 kPa i.e. by 121% of untreated soil strength after 7 PV. The UCS value also increased for S2 by 36.4% after 7 PV. However, the treatment did not have similar effect on the strength of S3 soil. There was a slight increase in UCS value from 28.6 kPa to 32.2 kPa after 7 PV treatment which is increase in UCS value of 12.6%.

The UCS values are presented in Table 8 for all the three soil samples treated with M2 for all three pore volumes. Increase in UCS values was observed with M2 treatment. It was observed that the UCS increased for S1 soil samples after 7 PV with UCS value of 32.8 kPa. The increase in percentage of UCS for S1 after 7 PV was observed to be 34.2 %. There was gradual increase in UCS value for S2 from 1 PV to 7 PV with total increase of UCS value of 33.3 % for S2 after7 PV from 21.6 kPa to 28.8 kPa, whereas little or no change in UCS value was observed in case of S3.

Table 7. Summary of UCS values of bio-augmented followed by stimulation with microbial concentration of M1 after three treatment cycles

Soil Type	Untreated	Bio-augmented followed by stimulation		
		UCS (kPa)	UCS (kPa)	UCS (kPa)
	UCS (kPa)	1 PV (%)	3PV (%)	7 PV (%)
S1	24.5	25.8 (5.3)	33.6 (37.1)	54.2 (121.2)
S2	21.6	23 (6.7)	31.5 (46.1)	29.4 (36.4)
S3	28.6	26.9 (-5.7)	27.6 (-3.3)	32.2 (12.6)

Note-: 1. Numbers in brackets are change in UCS values compared to the untreated soils. 2. Negative values indicate decrease in strength and vice versa.

Table 8 Summary of UCS test results of bio-augmented followed by stimulation with microbial concentration of M2 after three treatment cycles

Soil Type	Untreated	Bio-augmented followed by stimulation		
		UCS (kPa)	UCS (kPa)	UCS (kPa)
	UCS (kPa)	1 PV (%)	3PV (%)	7 PV (%)
S1	24.5	18.4 (-24.8)	35.8 (46.1)	32.8 (34.2)
S2	21.6	21.2 (-1.7)	26.6 (23.4)	28.8 (33.3)
S3	28.6	29.1 (1.9)	29.8 (4.3)	27.5 (-3.7)

Note-: 1. Numbers in brackets are change in UCS values compared to the untreated soils. 2. Negative values indicate decrease in strength and vice versa.

From Figure 21 (a) it is evident that with the increase in number of pore volumes, the strength also increases. That is the microbes in the soil require incubation period to produce urease enzyme required to hydrolyze urea. Incubation period helps bacteria to grow and reproduce in to the liquid media (Burbank et al. 2013). It is also important to have uniform spatial distribution of microbes in the space. The uniform distribution of microbes also depends on variables such as injected microbe concentration, pore volumes

injected, injection rate and retention period. It is also observed that the MICP technique whether bio-augmented or bio-stimulated is favored in low plasticity index soil such as S1. As in the both cases the UCS value increased by 49% and 121 % respectively as compared with the untreated S1. The UCS value also increased for S2 with increase in number of pore volumes when treated with both concentrations for bio-augmentation followed by stimulation. However, very little or no increase in UCS value was noticed in case of S3 for either protocol. Further testing is required to understand little or no increase in UCS value.

From Figure 21(b) it can be observed that increase in microbial concentration did not increase the UCS value. Ramachandran, Ramakrishnan & Bang (2001) investigated that higher concentration of bacteria had no improvement in strength. This study suggested that slower rates of calcite formation was more prominent in imparting higher strength than high rates.

Effect of Initial Microbial Concentration

Comparison was made between the M1 and M2 microbial concentration for each pore volume in Figure 22. It was observed that the number of pore volumes did increase the strength of soil samples. However, the increase in microbial concentration did not increase the strength of these samples. The factors that influence the precipitation of calcite are mainly the concentration of Ca^{2+} and CO_3^{2-}, pH of the system and the nucleation site. Bacterial cell surface acts as nucleation site for the precipitation of the calcite. The solubility product (K_{sp}) of calcite is very low i.e 3.3 x 10^{-9} mol. L^{-1} at 25ºC and for precipitation of calcite supersaturation of Ca^{2+} and CO_3^{2-} must exist. Since calcite has very low K_{sp}, supersaturation can be achieved by simply mixing Ca^{2+} and CO_3^{2-} together in moderate concentrations. However, when reaction takes place rapidly, the crystals formed

are very small and powder like with little or no cementation strength (Whiffin, 2004). In order to have large crystal precipitation over an extended period of time with higher cementation strength, the supersaturating product concentration should remain low. The supersaturation of CO_3^{2-} is also influenced by the pH of the system. pH can be regulated by the dissociation of urea into NH_4^+ (equations 1 through 3 from Chapter 2). CO_3^{2-} concentration remains very low below pH 8 as shown in Figure 23. Thus the size of crystal can be increased or decreased by decreasing or increasing the pH of the system (Whiffin, 2004).

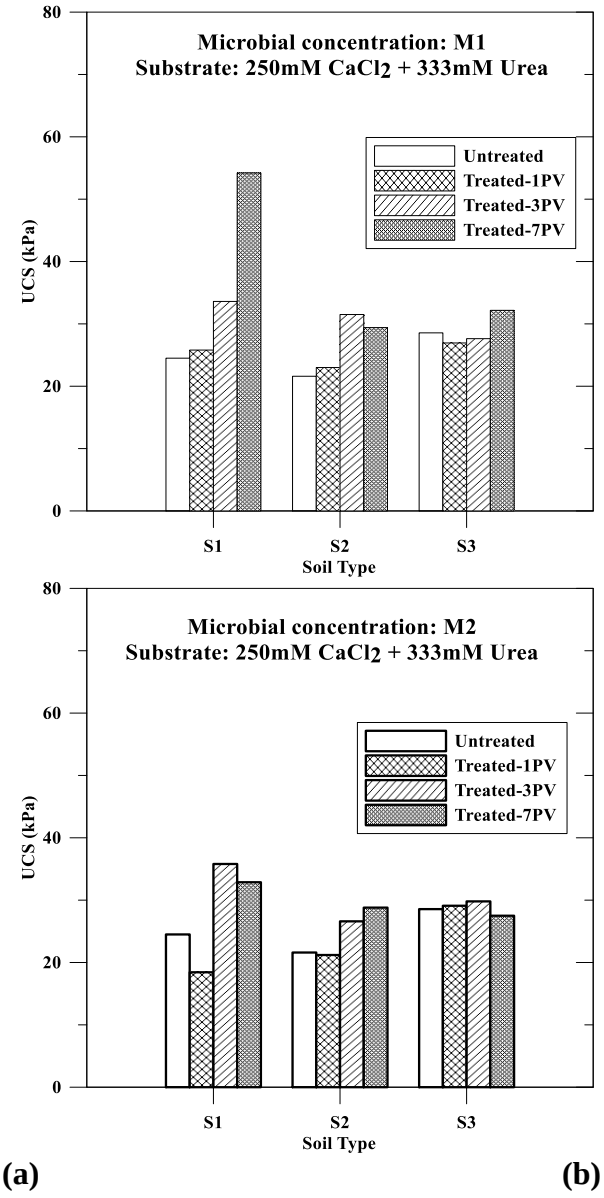


Figure 21. UCS values of untreated and bio-augmentation followed by stimulation soil samples with (a) M1 and (b) M2 microbial concentration after three treatment cycles

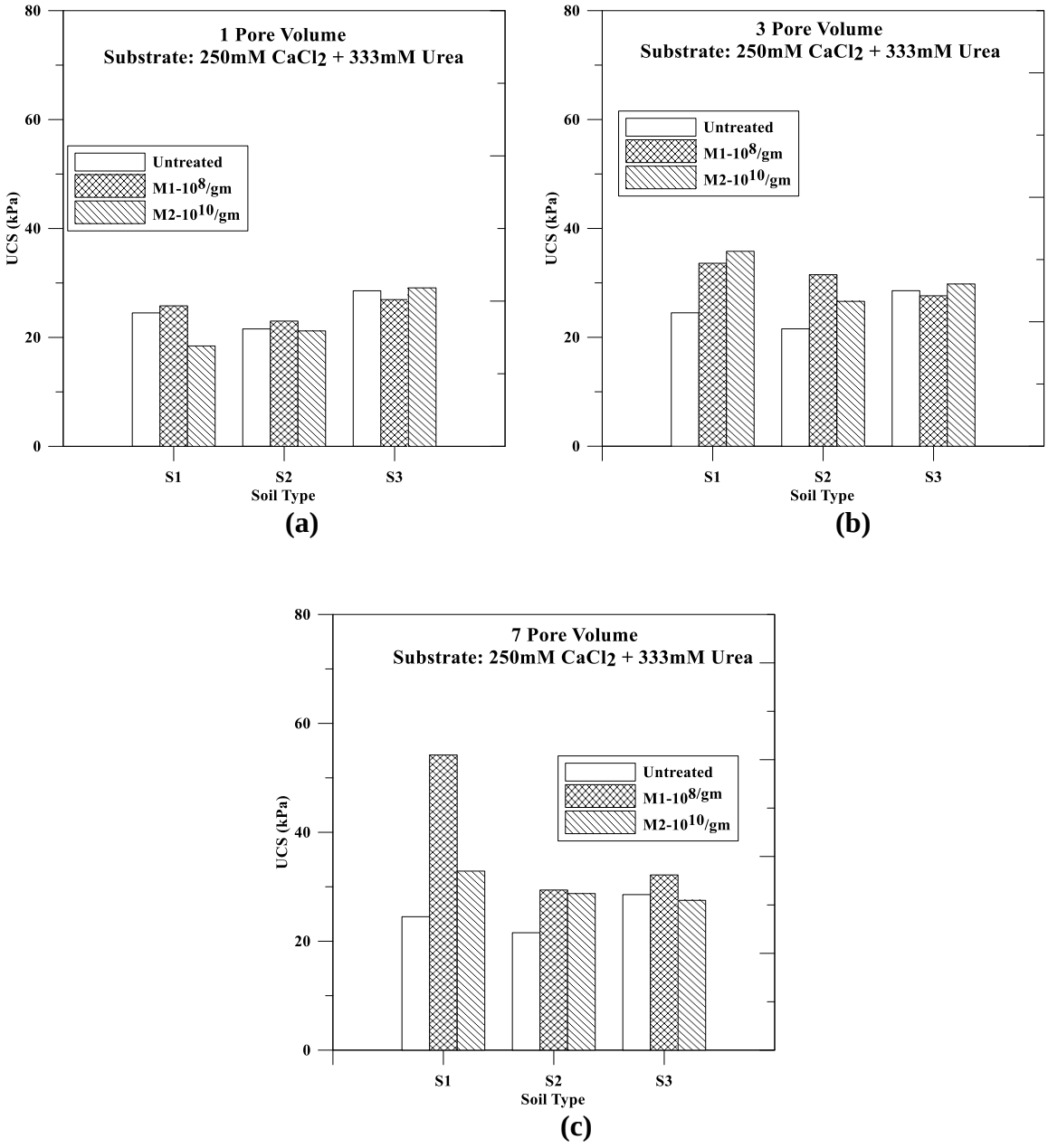


Figure 22. UCS values for 1 PV, 3 PV and 7 PV respectively with M1 and M2 microbial concentration

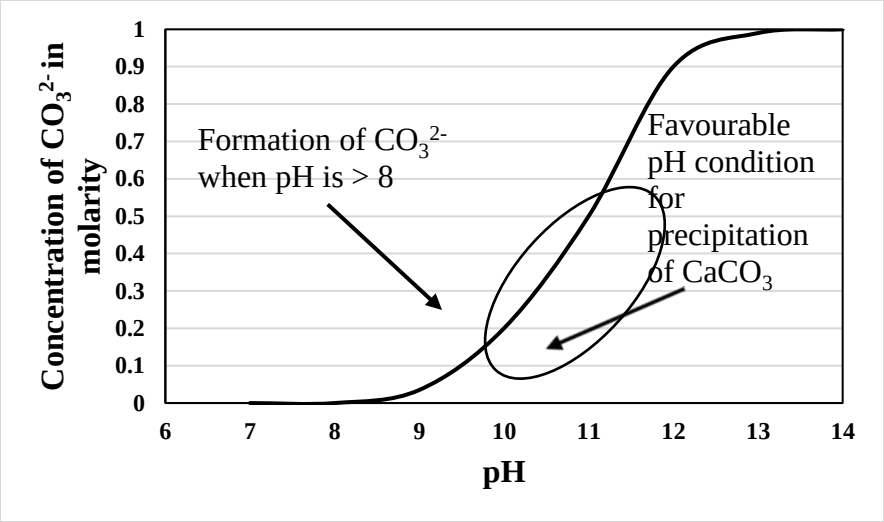


Figure 23. Dependence of CO_3^{2-} dissociation on pH (Modified from: Daniel C. Harris Quantitative Chemical Analysis 6th Edition)

The UCS value of lime treated soil samples followed by one cycle of wetting is shown in Table 9. It was observed that even after once cycle of wetting, the UCS value for all three samples were high compared to MICP treatments.

Table 9. Summary of UCS values of lime treated for one treatment cycles with three different curing periods

Soil Type	Untreated	Lime treated		
	UCS (kPa)	UCS (kPa) 1 day (%)	UCS (kPa) 3 days (%)	UCS (kPa) 7 days (%)
S1	24.5	365.4 (1391.4)	384.6 (1470.0)	580.3 (2268.5)
S2	21.6	241.1 (1018.5)	268.5 (1145.4)	406.7 (1786.3)
S3	28.6	204.6 (616.5)	216.9 (659.5)	270.4 (846.7)

Note-: 1. Numbers in brackets are change in UCS values compared to the untreated soils. 2. Negative values indicate decrease in strength and vice versa.

Percentage of Montmorillonite Results

The percentage of montmorillonite by weight in the fines fraction was determined with the help of equation (9) from Chapter 3. Table 10 presents the percentage of montmorillonite present in the soil before and after the treatment for M1 concentration. It was observed that the % of montmorillonite in S1 soil untreated was 60.89%. The maximum increase in percentage of montmorillonite was observed to be 4 % whereas the decrease in percentage of montmorillonite was observed to be 4.7 %. For S2 soil samples the maximum increase in percentage of montmorillonite was observed to be 1.6 % whereas the decrease in percentage of montmorillonite was observed to be 5.3 %. However, for S3 soil samples the percentage of montmorillonite decreased by 7 % approximately.

Table 10. Summary of percentage of montmorillonite (% of MM) by weight in the fines fraction of bio-augmented followed by stimulation with microbial concentration of M1 after three treatment cycles

Soil Type	Untreated	Bio-augmentation followed by stimulation		
	% of MM	% of MM	% of MM	% of MM
		1 PV (%)	3PV (%)	7 PV (%)
S1	60.9	62.4 (2.5)	63.3 (4.0)	58.1 (-4.7)
S2	70.4	71.5 (1.6)	74.1 (5.3)	66.6 (-5.3)
S3	91.9	85.5 (-7.1)	84.9 (-7.7)	84.8 (-7.8)

Note-: 1. Numbers in brackets are change in percentage of montmorillonite (% of MM) by weight in the fines fraction compared to the untreated soils. 2. Negative values indicate decrease in strength and vice versa.

Table 11 represents the percentage of montmorillonite present in the soil before and after the treatment for M2 concentration with three different treatments. The percentage of montmorillonite decreased for S1 soil samples after 1 PV and 7 PV by 5 % and 3.7 %

respectively whereas it remained constant after 3 PV. The percentage of montmorillonite remained constant for S2 soil samples treated after 1 PV and 3 PV but 3.7% increase in percentage of montmorillonite was noticed after 7 PV. However, 10.7% and 4.6% decrease in percentage of montmorillonite was observed in case of S3 soil samples after 3 PV and 7 PV respectively. No change in percentage of montmorillonite was observed for S3 soil samples after 1 PV.

Table 11. Summary of percentage of montmorillonite by weight in the fines fraction of bio-augmented followed by stimulation with microbial concentration of M2 after three treatment cycles

Soil Type	Untreated	Bio-augmented followed by stimulation		
	% of MM	% of MM	% of MM	% of MM
		1 PV	3PV	7 PV
S1	60.89	57.82 (-5.0)	60.45 (-0.7)	58.65 (-3.7)
S2	70.38	70.04 (-0.5)	71.37 (1.4)	67.84 (-3.6)
S3	91.97	91.39 (-0.6)	82.11 (-10.7)	87.70 (-4.6)

Note-: 1. Numbers in brackets are change in percentage of montmorillonite (% of MM) by weight in the fines fraction compared to the untreated soils. 2. Negative values indicate decrease in strength and vice versa.

Percentage of montmorillonite was determined to understand the change in mineralogy of soil samples. However, it was observed that the change in percentage of montmorillonite before and after the treatment was very small as shown in Figure 24. Hence it can be said that MICP technique has no effect on the mineralogy of clay minerals unlike chemical stabilizers.

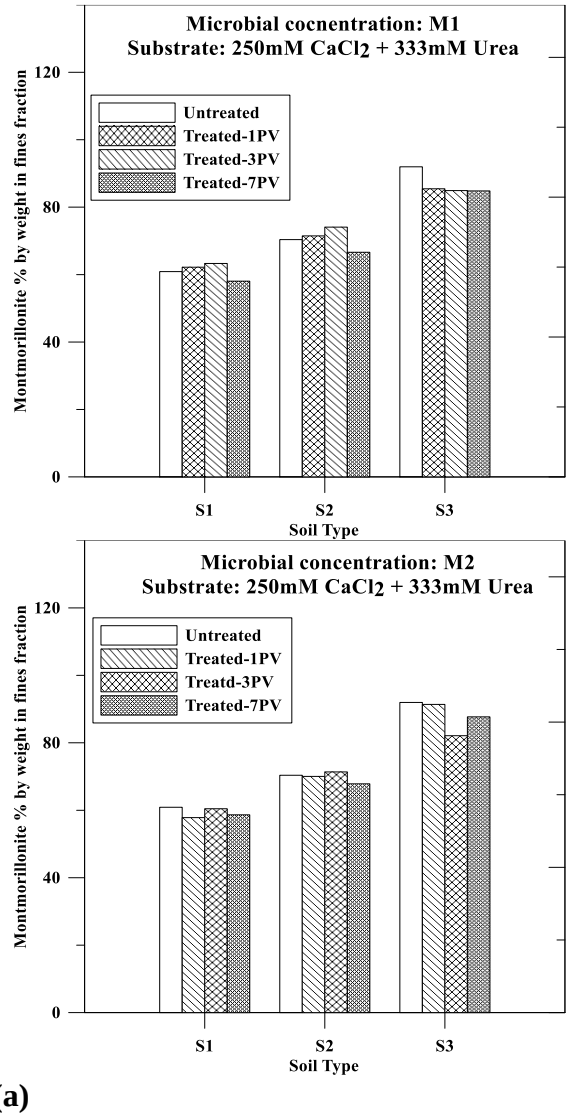


Figure 24. Montmorillonite percentage by weight in fines fraction of untreated and bio-augmentation followed by stimulation soil samples with (a) M1 and (b) M2 microbial concentration after three treatment cycles

Atterberg Limits Test Results

Table 12 presents a summary of Atterberg limits test results for all soil samples treated with microbial concentration of M1 after three treatment cycles. It was observed that there was no change in liquid limit for S1 soil samples. The maximum increase in S1 soil samples liquid limit was after 1 PV treatment, the liquid limit increased from 54% to 57.5 % with the increase of 6.5 % and plasticity index increased by 45 % i.e. from 15% to

22 %. For S1 soil samples after 7 PV, the change in liquid limit was 1.9% i.e. increase in liquid limit from 54 % to 55 %. Similarly, the plasticity index increased by 56.1 %. The liquid limits and plasticity indices increased for S2 samples in all three treatment cycles. The liquid limit increased from 58 % to 84 % after 1 PV which is 44.8 % increment. The plasticity index also increased from 27 % to 42 % by the total increment of 55.6 %. The liquid limit at plasticity index for S2 soil samples after 7 PV were 82% and 39% respectively. However, decrease in liquid limits and plasticity indices was observed for S3 soil samples for all three treatment cycles. The lowest liquid limit was observed after 1 PV and 7 PV with liquid limit changing from 115 % to 93.5 % and plasticity index changing from 47 % and 46 % respectively. The liquid limit decreased by 18.7 % and plasticity index decreased by 25% for both the treatments.

Table 12. Summary of Atterberg limits test results of bio-augmented followed by stimulation with microbial concentration of M1 after three treatment cycles

Soil Type	Untreated		Bio-augmented followed by stimulation					
	Liquid Limit (%)	Plasticity Index (%)	Liquid Limit (%) 1 PV	Plasticity Index (%) 1 PV	Liquid Limit (%) 3 PV	Plasticity Index (%) 3 PV	Liquid Limit (%) 7 PV	Plasticity Index (%) 7 PV
S1	54	15	57.5 (6.5)	22 (45.0)	56.5 (4.6)	19 (25.2)	55 (1.9)	24 (56.1)
S2	58	27	84 (44.8)	42 (55.6)	80 (37.9)	38 (40.7)	82 (41.4)	39 (44.4)
S3	115	62	93.5 (-18.7)	47 (-24.2)	99 (-13.9)	52 (-16.1)	93.5 (-18.7)	46 (-25.8)

Note-: 1. Numbers in brackets are change in Atterberg limits test results compared to the untreated soils. 2. Negative values indicate decrease in strength and vice versa.

Summary of Atterberg limits test results for all three samples with microbial concentration of M2 after three treatment cycles are presented in Table 13. It can be observed from this table that, for S1 sample similar results were obtained as in case of microbial concentration of M1. The liquid limits did not change significantly, the

maximum change in liquid limit was observed after 1 PV treatment with increase in liquid limit from 54.0% to 57.5% with total increment of 6.5%. Similarly, the plasticity index also increased by 25.2%. The least liquid limit was observed after 7 PV treatment, the liquid limit increased by 3.7% and the plasticity index increased by 33.3%. The liquid limit also increased for S2 after three treatment cycles. The change in liquid limit after 7 PV was observed to be 74% and plasticity index was observed to be 34% for S2 soil samples. However, the liquid limit and plasticity index tend to decrease for S3. The liquid ranged from 93% to 95.5% and plasticity indices ranged from 46% to 48%.

Table 13. Summary of Atterberg limits test results of bio-augmented followed by stimulation with microbial concentration of M2 after three treatment cycles

Soil Type	Untreated		Bio-augmented followed by stimulation					
	Liquid Limit (%)	Plasticity Index (%)	Liquid Limit (%) 1 PV	Plasticity Index (%) 1 PV	Liquid Limit (%) 3 PV	Plasticity Index (%) 3 PV	Liquid Limit (%) 7 PV	Plasticity Index (%) 7 PV
S1	54	15	57.5 (6.5)	19 (25.2)	57 (5.6)	20 (33.3)	56 (3.7)	20 (33.3)
S2	58	27	77.5 (33.6)	36 (33.3)	73 (25.9)	34 (25.9)	74 (27.6)	34 (25.9)
S3	115	62	93.5 (-18.7)	46 (-25.8)	93 (-19.1)	47 (-24.2)	95.5 (-17.0)	48 (-22.6)

Note-: 1. Numbers in brackets are change in Atterberg limits test results compared to the untreated soils. 2. Negative values indicate decrease in strength and vice versa.

Lime treated soil samples became non-plastic in nature due to the change in mineralogy.

From Figure 25, it is evident that there is little change in liquid limit and the plasticity index values for S1 soil. This is supported by the fact that there was no change in mineral montmorillonite percentage. The liquid limit and plasticity increased for S2 in both the cases for M1 and M2. However, there was no change in montmorillonite

percentage. One of the reasons for increase in liquid limit may be due to the formation of extracellular polymer substance (EPS) which is secreted by microbes during the formation of biofilms. These EPSs constitute 0.1 to 1.5 % of the soil organic matter (Or, Phutane & Dechesne, 2007). One of the main characteristics of EPS is to act as sponge which can considerably absorb water from the environment. Water can be attracted to EPS matrix surface by osmotic and capillary forces which results in the swelling of the matrix. EPS matrix can absorb water more than 15 to 20 gram of water per gram of EPS (Or, Phutane & Dechesne, 2007).

However, no change in UCS strength was observed in case of S3. This may be due to little or no microbial activity within the soil samples. As soil samples were not autoclaved microbes introduced within the soil may be the victim of predation. However, the reason for reduction of liquid limit and plasticity indices could not be explained in this research. Further investigations into the type of microbial activity, which might explain this behavior in this soil are recommended.

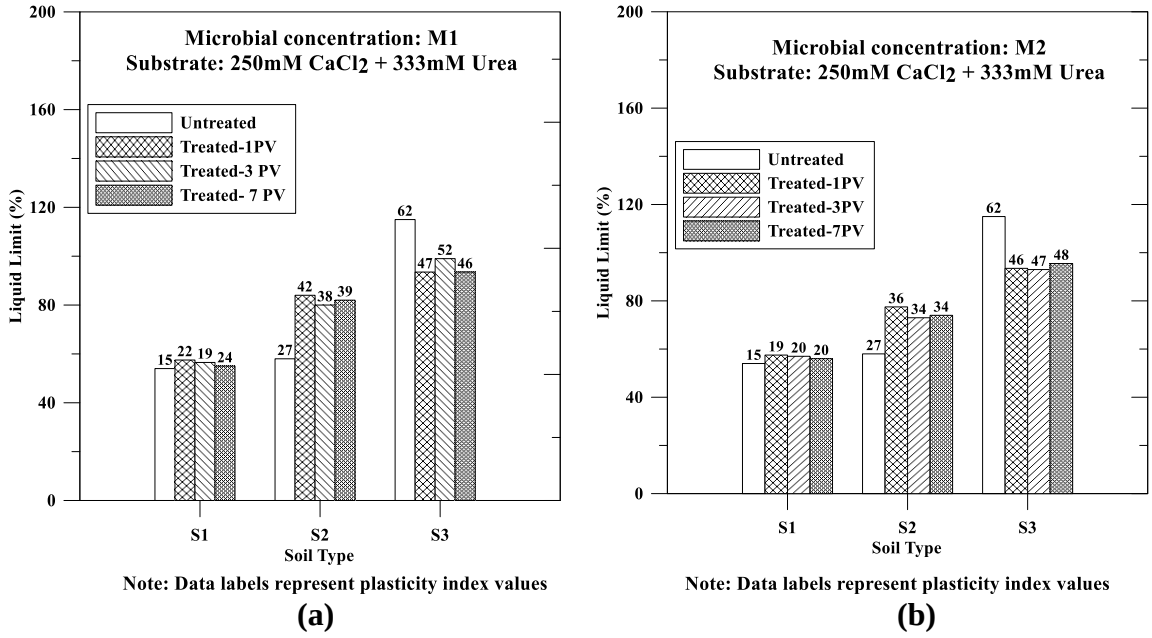


Figure 25. Atterberg limit test results of untreated and bio-augmentation followed by stimulation soil samples with (a) M1 and (b) M2 microbial concentration after three treatment cycles

From Figure 26 it can be observed that number of pore volumes have little or no effect on liquid limit and plasticity index values irrespective of microbial concentration. Since there is no change in mineralogy, change in liquid limit and plasticity index can be explained by the production of organic matter, EPS secreted by microbes during the formation of biofilms. However, this explanation is not applicable for S3 soil samples.

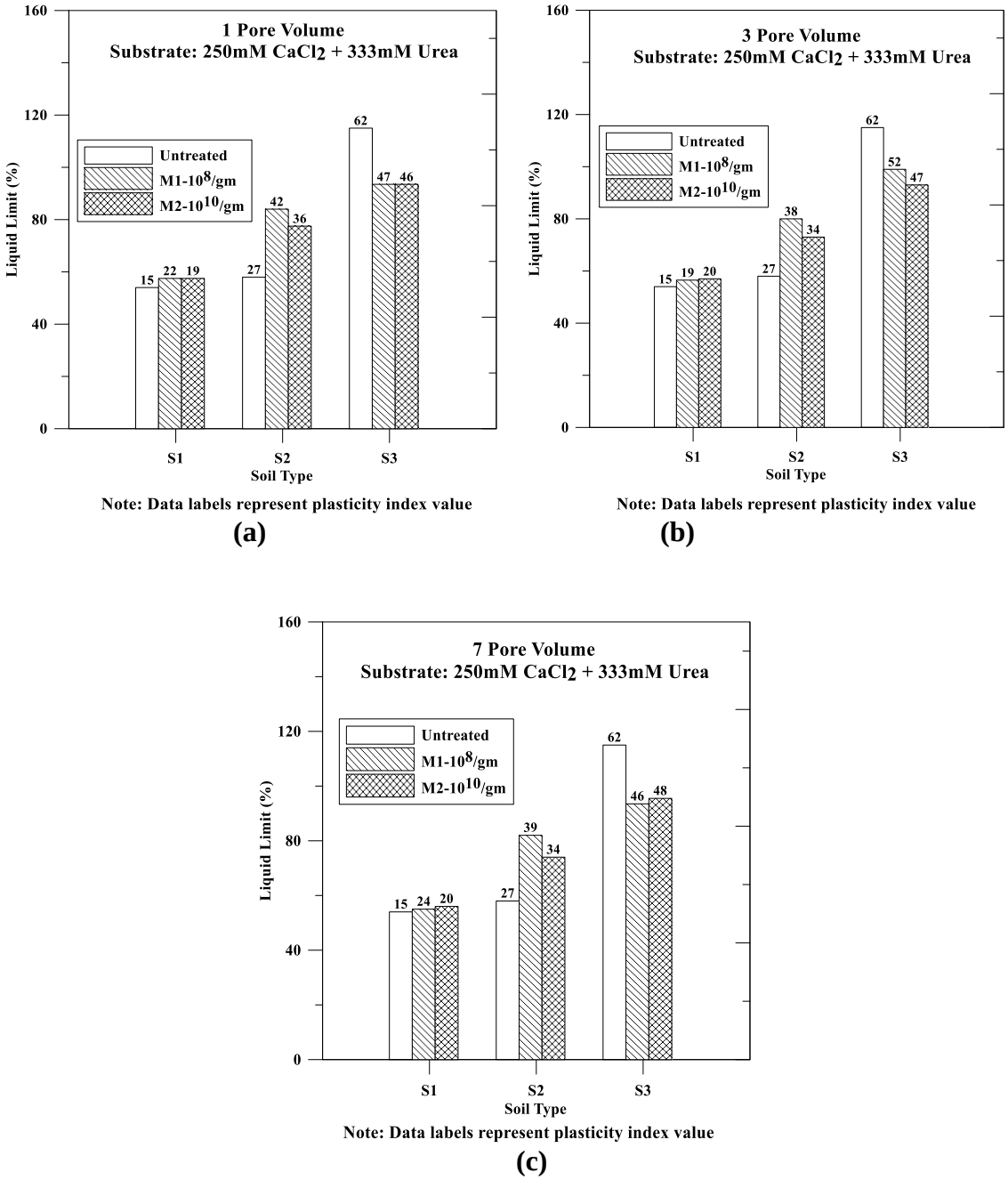


Figure 26. Atterberg limit test results for 1, 3 and 7 pore volumes respectively with M1 and M2 microbial concentration

1-D Swell Test Results

Table 14 presents the 1-D swell test results of all samples with microbial concentration of M1 for different treatment cycles. It was observed that the swell strain reduced in case of S1 samples after all three treatment cycles. For S2, after 7 PV, the swell strain reduced by 57.6 %. Reduction in swell strain was also observed for S3 after 7 PV. The swell strain reduced from 8.85% to 4.73%.

Table 14. Summary of 1-D swell strain of bio-augmented followed by stimulation with microbial concentration of M1 for all three treatment cycles

Soil Type	Untreated	Bio-augmented followed by stimulation		
	1-D swell strain (%)	1-D swell strain (%)	1-D swell strain (%)	1-D swell strain (%)
		1 PV (%)	3PV (%)	7 PV (%)
S1	2.83	1.44 (-49.1)	0.27 (-90.5)	1.2 (-57.6)
S2	8.55	13.29 (55.4)	11.4 (33.3)	6.44 (-24.7)
S3	8.85	6.2 (-29.9)	9.06 (2.4)	4.73 (-46.6)

Note-: 1. Numbers in brackets are change in 1-D swell strain compared to the untreated soils. 2. Negative values indicate decrease in strength and vice versa.

Table 15 presents a summary of 1-D swell strain after the three treatment cycles with M2 microbial concentration. For S1 soil sample, after 7 treatment cycles the 1-D swell strain was observed to be 0.85% which is a 70% reduction in swell compared to untreated soil. For S2 soil sample the swell strain increased after 1 PV and 3 PV but dropped back to untreated soil's swell strain after 7 PV. S3 soil demonstrated a 33.8% reduction in swell strain after 7 PV.

Table 15. Summary of 1-D swell strain of bio-augmented followed by stimulation with microbial concentration of M2 for different treatments

Soil Type	Untreated	Bio-augmented followed by stimulation		
	1-D swell strain (%)	1-D swell strain (%)	1-D swell strain (%)	1-D swell strain (%)
		1 PV (%)	3 PV (%)	7 PV (%)
S1	2.83	1.3 (-54.1)	0.615 (-78.3)	0.85 (-70.0)
S2	8.55	9.48 (10.9)	10.79 (26.2)	8.68 (1.5)
S3	8.85	9.03 (2.0)	5.88 (-33.6)	5.86 (-33.8)

Note-: 1. Numbers in brackets are change in 1-D swell strain compared to the untreated soils. 2. Negative values indicate decrease in strength and vice versa.

From Figure 27 it is evident that the 1-D swell strain reduced for S1 soil samples after all three treatment cycles for both microbial concentrations. It was also observed that after seven treatment cycles irrespective of microbial concentrations, swell reduction was possible i.e. higher the treatment cycles (or retention period) lower the swell strain. The increase in swell strain was observed for S2 soil samples after 1 PV and 3 PV treatments for high and low microbial concentration, this may be due to the increase in Atterberg limits values, as discussed earlier in this chapter. Decrease in swell strain was observed for S3 soil samples after all three treatment cycles for M1 and M2 microbial concentrations. This may be due to the reduction in plasticity, as discussed earlier in this chapter.

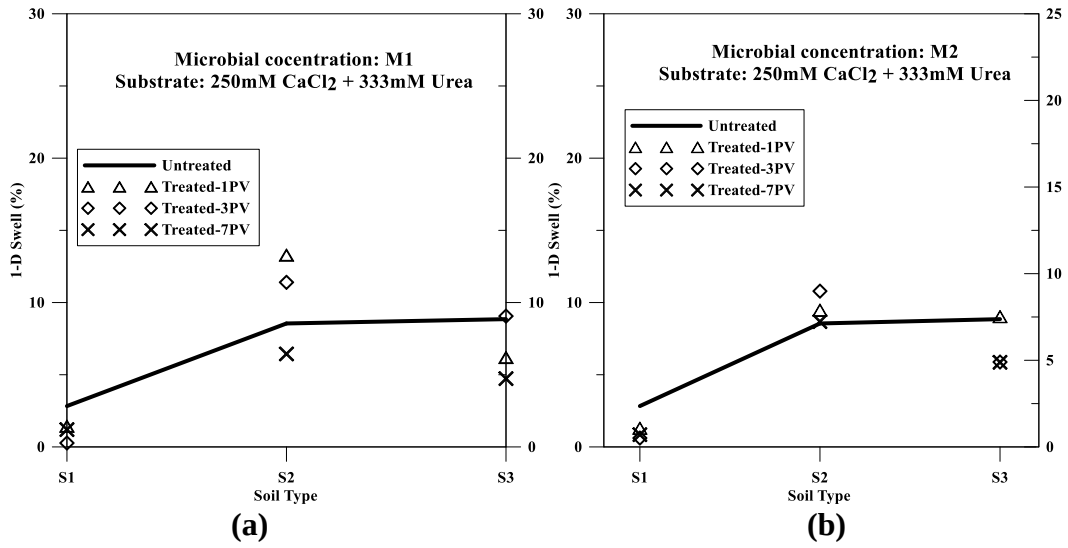


Figure 27. 1-D swell strain of untreated and bio-augmentation followed by stimulation soil samples with (a) M1 and (b) M2 microbial concentration for three treatment cycles

Figure 28 presents the change in 1-D swell strain with soil type and microbial population for all three treatment cycles. The reduction in swell strain was observed to be consistent for S1 samples after all three treatment cycles. The maximum reduction in swell was observed after 3 PV treatments irrespective of microbial concentrations. 1-D swell percentage increased for S2 samples for 1 PV and 3 PV treatments. The increase in swell may be due to the increase in plasticity index of S2 soil samples. However, the swell reduced after 7 PV treatment cycles with microbial concentration of M1 whereas for M2 microbial concentration there was no change in swell percentage. For S3 samples, the swell percentage observed for three treatment cycles were different. No definite pattern was observed in swell reduction for S3. The swell percentage observed for three different treatments with different microbial concentration was different.

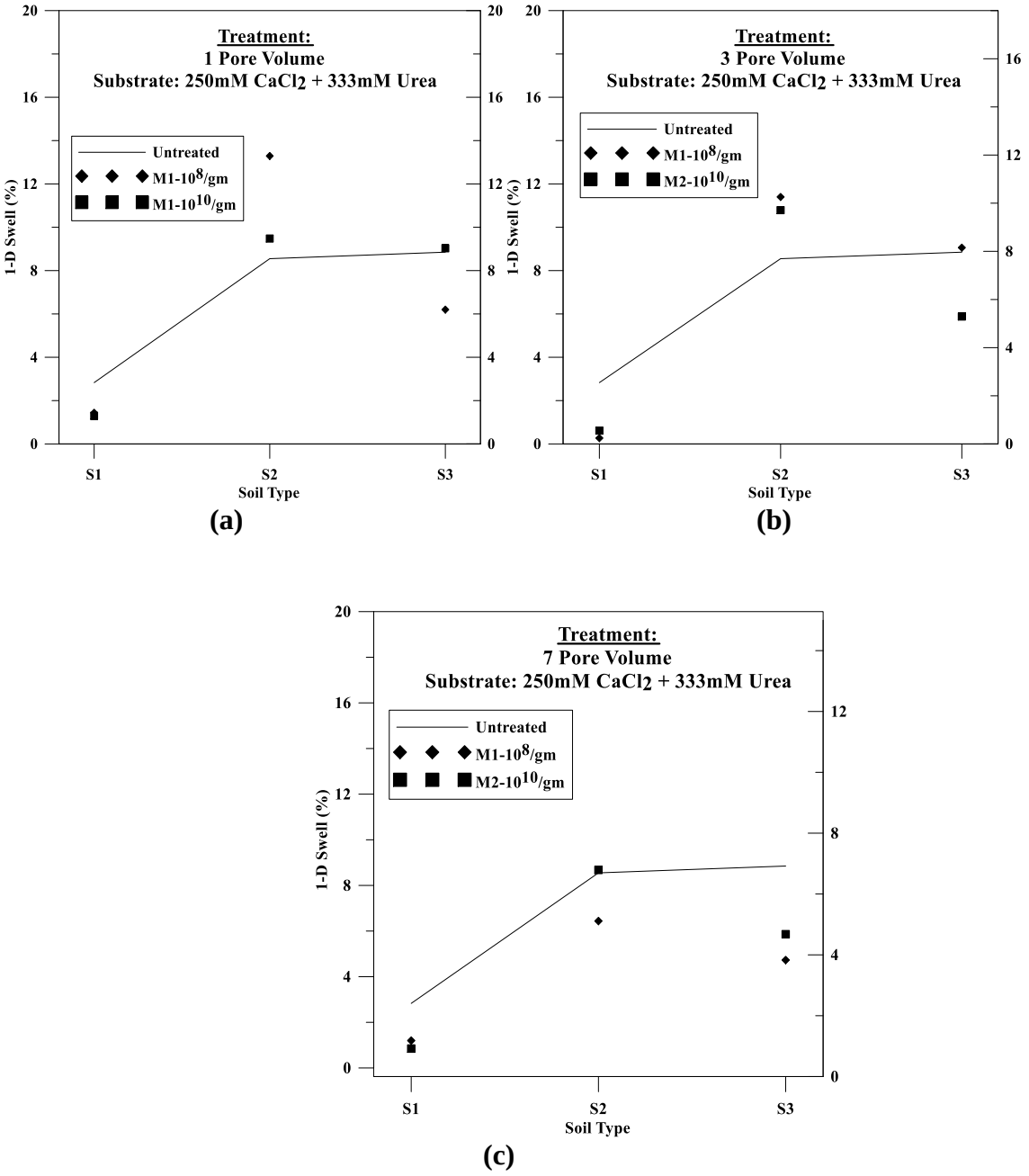


Figure 28. 1-D swell strain for 1, 3 and 7 pore volumes respectively with M1 and M2 microbial concentration

1-D swell percentage reduced for all soil samples treated with lime. The reduction in 1-D swell percentage is due to the change in mineralogy when treated with lime which is presented in Table 16.

Table 16. Summary of 1-D swell strain of lime treated soil samples followed by one wetting cycle.

Soil Type	Untreated	Lime treated		
	1-D swell strain (%)	1-D swell (%) 1 day (%)	1-D swell (%) 3 days (%)	1-D swell (%) 7 days (%)
S1	2.83	1.28 (-54.8)	1.28 (-54.8)	1.19 (-57.95)
S2	8.55	4.63 (-45.8)	6.92 (-19.1)	3.68 (57)
S3	8.85	4.6 (-48.0)	4.66 (-47.3)	2.95 (-66.7)

Note-: 1. Numbers in brackets are change in 1-D swell strain compared to the untreated soils. 2. Negative values indicate decrease in strength and vice versa.

From results and discussion presented in this chapter, it was evident that MICP technique (whether bio-augmentation or bio-augmentation followed by stimulation) had notable changes in geotechnical properties for low plasticity soils. However, changes in S2 and S3 soil samples' geotechnical properties after MICP treatment is unexpected and needs further testing to understand the feasibility of MICP technique in medium and high plastic soils.

CHAPTER FIVE: SUMMARY AND RECOMMENDATIONS

This chapter presents a brief summary of the research performed for this thesis along with important findings. In addition, recommendations for future research are also presented.

Summary

Three naturally occurring expansive soils with varying plasticity characteristics; S1 (low plasticity), S2 (medium plasticity) and S3 (high plasticity) were studied in this research. These soils were subjected to lime treatment and two methods of MICP treatments; bio-augmentation and bio-augmentation followed by stimulation and their performance was compared with lime treated and untreated soil samples. The performance was measured by monitoring the plasticity characteristics, swelling potential and unconfined compressive strength of these soils with various treatments. Variables such as soil type, bacterial population during augmentation, along with the number of treatment cycles were studied in this research.

Bio-augmentation was performed to replicate the lime treatment performed to stabilize the expansive soils. The compressive strength increased for low plastic soils (S1), however, the strength reduced for medium (S2) and high plastic soils (S3) in case of bio-augmentation. The Atterberg limits increased for S2 soil samples and decreased for S3 soil samples while little change was observed in S1 soil samples. No significant change in swell strain was observed for S1 while increase in swell strain was observed for S2 soil and decrease in swell strain was observed for S3. The increase in Atterberg limits and 1-D swell

in case of S2 was unknown, one of the assumption is the formation of extracellular polymer substance called EPS. These EPS are organic materials and acts as sponge to absorb water from the surrounding environment. These EPS are also responsible for increase in strength. These solids occupy the space in between pore spaces thus reducing pore size, reduction in rearrangement of particles during soil deformation and increase in ductility (DeJong et al. 2013). However, the decrease in Atterberg limit and 1-D swell percentage in case of S3 is difficult to understand, one of the hypothesis could be, the cation exchange between the clay particles and calcium ions present in calcium chloride solutions.

The second method adopted was bio-augmentation followed by stimulation. In this method the sample preparation method was similar to bio-augmentation method and these samples were placed in the nutrient delivery system to stimulate the bacteria using the substrate solution. The samples were treated until 1, 3 and 7 pore volumes of effluent were collected. These were termed as treatment cycles, and denoted as 1 PV, 3 PV and 7 PV. It was observed that increase in strength was possible for low plasticity soils, S1 with lower microbial concentrations after 7 PV treatment cycles. No change in Atterberg limits were observed after three different treatment cycles and for both microbial concentrations. Reduction in swell strain was observed after three different treatment cycles for both microbial concentrations.

Very small increase in strength was observed for S2 after 7 PV irrespective of microbial concentrations. However, increase in Atterberg limits was observed after three treatment cycles for both microbial concentrations. Reduction in swell strain was observed after 7 PV while increase in swell strain was observed for 1 PV and 3 PV.

No change in UCS was observed for S3 soil samples. However, reduction in Atterberg limits and swell strain was observed for all pore volumes and microbial concentrations. As stated above, the reduction in Atterberg limits and swell strain could be due to the cation exchange between clay minerals and calcium ions present in the calcium chloride.

Research Findings

1. The following observations were made in case of first method of MICP treatment (Bio-augmentation)

a. Compressive strength increased for low plasticity (S1) soil while the same for medium (S2) and high (S3) plasticity soil remained unchanged or dropped slightly.

b. Atterberg limits increased for low (S1) and medium (S2) PI soils but reduced for high (S3) PI soil.

c. 1-D swell strain reduced for S1 and S3 soils while it increased for S2 soil.

2. The following observations were made in case of second method of MICP treatment (Bio-augmentation followed by stimulation)

a. Compressive strength increased for low plasticity soil (S1) and medium plasticity soil while no change in strength was observed for high plastic soil (S3).

b. The increase in strength was observed after all three treatment cycles for low plasticity soil (S1) while the maximum strength was observed after seven treatment cycles with low microbial concentrations.

c. Reduction in one-dimensional swell strain was observed for low plasticity soils for all pore volumes for both microbial concentrations. Reduction in swell was

also observed for all soil samples after seven pore volume treatment cycles for both microbial concentrations.

d. None to very little change in Atterberg limit was observed for low plasticity soil (S1) for all pore volumes for both microbial concentrations while Atterberg limits increased for medium plasticity soil (S2) and decreased for high plastic soil (S3).
e. Increase in microbial concentration did not increase the compressive strength.
f. No change in montmorillonite content was observed in these soils due to MICP treatments.

Recommendations for Future Research

MICP technique has been applicable for coarse grained soils but very few research studies were carried out to understand the efficacy of MICP technique in expansive soils. This research is the initial step to understand the applicability of MICP in expansive soils. The data and facts presented in this research support the applicability of MICP in low plasticity soils with low microbial concentration. However, many questions still remain unanswered some of them are listed below.

- Effect of plasticity indices: Three different soil samples with different plasticity index were tested in this research. All the three samples had different test results, so the relation between plasticity index and MICP is very much necessary to establish.
- Number of pore volumes: As it can be observed that number higher the number of pore volumes, changes in plasticity, strength and swell strain was noticed. So the role of number of pore volumes required for different kind of soils should be studied.

- Role of EPS: It is also necessary to understand the role of extracellular polymer substances in stabilizing the expansive soils.
- Role of Microbial population after treatments: In this study the initial amount of microbes is known, however, microbes reproduce at different rates depending on the availability of resources and environmental conditions and hence change in number as the test progresses. This population growth will be different for different soils and that could in turn effect the stabilization. This aspect needs to be studied in future research.

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