2017

Strength Property Variability in Microbial Induced Calcite Precipitation Soils

Jacob Fuller

Strength Property Variability in Microbial Induced Calcite Precipitation Soils

by

Jacob Hancock Fuller

A Thesis submitted to the Department of Civil Engineering

in partial fulfillment of the requirements for the degree of

Master of Science in Civil Engineering

UNIVERSITY OF NORTH FLORIDA

College of Computing, Engineering, and Construction

December, 2017

This Thesis titled Strength Property Variability in Microbial Induced Calcite Precipitation Soils, submitted by Jacob Hancock Fuller in partial fulfillment of the requirements for the degree of Master of Science in Civil Engineering has been: approved:

Raphael Crowley, Ph.D., P.E.

Nick Hudyma, Ph.D., P.E.

Don Resio, Ph.D.

Accepted for the School of Engineering:

Murat Tiryakioglu, Ph.D. CQE

Accepted for the College of Computing, Construction, and Engineering

Mark A. Tumeo, Ph.D. J.D. P.E.
Dean, College of Computing, Engineering, and Construction

Accepted for the University:

John Kantner, Ph.D. RPA
Dean, Graduate School

DEDICATION

I dedicate this work to my wife (Emily Reeve Fuller), mother (Kelli Bloodworth Fuller), and the entire University of North Florida community who have all helped shape the individual and professional I am

ACKNOWLEDGMENTS

I foremost thank my advisor and committee chair, Dr. Raphael Crowley, for his support and direction during the development of this work. Also, a special thanks to my committee members, Dr. Nick Hudyma and Dr. Don Resio, for their respective support and assistance. I thank the Florida Department of Transportation for their support of this research including Dr. David Horhota, Larry Jones, Rodrigo Herrera, and Jose Hernando. Thanks, as well, to Dr. Terri Ellis of the UNF Biology Department for growing our bacteria in her laboratory. Finally, thanks to our collaborators from the University of Florida – especially Scott Wasman and Andrew Zimmermann. Funding for this work was provided by Florida Department of Transportation Contract No. BDV34 977-06, Dr. David Horhota, P.E. Project Manager.

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Microbial Induced Calcite Precipitation (MICP) is an attractive alternative for a variety geotechnical ground improvement practices commonly used today and has a variety of potential applications. This research focuses primarily on its use as a soil stabilization technique using the bacteria *Sporosarcina Pasteurii* and a single injection point percolation method adapted from previous research in granular soils. This method, and most published data, show an inherent variability in both physical and engineering properties due to the distribution of precipitated calcite within the specimen. The focus of this research is on the quantification of the variability in shear strength parameters induced by MICP treatment in sand. Also, on the initial development of a new treatment method which aims to reduce this inherent variability and offer a more feasible option for field applications.

The MICP treated soil columns were sampled at constant intervals from the injection point and then subject to direct shear testing (DST) and calcite distribution analysis. This analysis reiterates previously documented reduction in cementation as distance from injection point increases. The reduction in cementation results in reduced shear strength parameter improvements. This research also concluded a minimum of two percent mass of calcite per total mass of treated soil for significant strength improvements.

Chapter 1 INTRODUCTION

1.1 Motivation for Research

Traditionally, ground improvement has been achieved via crude methods such replacement (cut and fill), in-situ grouting, or a using variety of dynamic compaction techniques. The issue

with most of these soil improvement methods is that they are generally very expensive, require specialized equipment and contractors, limited in terms of their effectiveness, and can be harmful for the environment.

In recent years, Microbial Induced Calcite Precipitation (MICP) has emerged as a new method for improving the properties of granular soils. This technology involves harnessing bacteria to produce calcium carbonate that binds soil particles together. Several researchers have conducted a number of tests on MICP-treated soil columns, and have noted that calcification appears to decrease as distance from the injection point increases. However, the shear strength parameter differences associated with decreased calcification have yet to be fully quantified.

1.2 Goals and Objectives

The goal of this research was to treat granular soil (sand) specimens using a methodology similar the methodology used at the University of California-Davis (UC Davis) and determine how shear strength parameters varied in these specimens as a function of distance from a treatment injection point. As research continued, it became apparent that a better treatment technique would be useful. Therefore, a new treatment technique was also developed and implemented; and its strength variability characteristics were also measured as part of this research.

1.3 Broader Impacts

Results from the research will be useful as MICP technology is scaled-up toward bench and field scale applications. In particular, results will show how treated specimens' shear strength parameters may vary as a function of distance from treatment point. This will help engineers design field treatment techniques that will provide adequate treatment coverage.

1.4 Overview of Methodology

Granular soil columns, comprised of poorly graded Ottawa silica sand, were densified in acrylic tubes. MICP treatment of the soil columns was conducted using a single injection percolation technique. This technique is one of the common techniques used for this type of experimentation. Once the specimens were calcified, they were removed from the acrylic tubes and sampled at different distances from the injection point. The samples were weighed and measured. They were then saturated and tested in direct shear to determine the increase in shear strength parameters (cohesion and angle of internal friction). The calcified test results were compared to non-calcified sand specimens at the same density to assess the increase in shear strength parameters. The new treatment technique mentioned above was developed at UNF. Specimens treated with this new method were also tested for shear strength parameters at UNF.

1.5 Organization

This thesis is organized into six chapters. Chapter two is a relevant literature review. Chapter 3 describes the materials and methods used throughout this research including treatment techniques and strength tests. Chapter 4 presents the results of these treatment and testing procedures. Chapter 5 provides a discussion of the results. Chapter 6 provides a summary and conclusions from this research.

Chapter 2 LITERATURE REVIEW

Literature relevant to this research includes traditional soil stabilization techniques, a general overview of microbial induced clacite precipitation (MICP), and factors that affect the success MICP treatment including coverage uniformity. This chapter presents discussion on each of these topcis. Additionally, a discussion is included about how this thesis' research advances MICP rsearch.

2.1 Traditional Soil Stabilization Techniques

When soil shear strength parameters or stiffness is insufficient for construction, soil improvement techniques must be employed. Soil improvement encompasses a wide variety of techniques, not all of which are applicable to all situations. The improvement techniques can be classified into a number of different methodologies including cut and replace, modification of applied loads, construction techniques, and ground modification. The discussion of stabilization techniques is limited to the scope of the larger emphasis of this work, which is the improvement of weak high organic content soils.

2.1.1 Cut and Replace

The simplest soil improvement technique is cut and replace. With this technique the poor quality soils are simply removed and replaced with high quality fill. Cut-and-replace is commonly used as a stabilization technique when practical. The issues with this technique are (1) its cost; and (2) its feasibility. For deeper inadequate soil deposits, replacement is often not practical because it is cost-prohibitive (Mullins and Gunaratne 2014). Gue et al. (2002) found that excavation and replacement is viable to a maximum depth of 4.5 m (14.8 ft.).

2.1.2 Modification of Applied Loads

Modification of applied loads is addressed during the design phase and implemented during construction. This technique includes increasing the bearing area of foundation elements and the use of lightweight fills.

2.1.2.1 Increasing Bearing Area

Increasing the bearing area of foundation elements or embankments will decrease the stresses applied to the weaker soil, which will in turn decrease settlement and decrease the chance of bearing capacity failure and excessive settlement. Increasing the bearing area is directly related to costs; increasing the bearing area means a larger foundation or increased widths of embankments. There are both material costs and potentially right-of-way acquisition costs associated with this technique.

2.1.2.2 Lightweight Fills

Lightweight fills can be used to reduce the applied stresses from geotechnical assets such as embankments placed on poor quality soils. Some common lightweight fills are lightweight expanded clay fill and ESP (expanded polystyrene) geofoam. Expanded clay is a vitrified shale produced in a rotary fired kiln. Each aggregate has a highly porous interior with a vitrified outer shell. The aggregates come in a variety of sizes. A typical unit weight of the material is on the order of 1000 kg/m^3 (65 pcf).

EPS was successfully used in Hollywood, Florida for the construction of an elevated roadway. The project utilized approximately 1,150 cubic meters (1,500 cubic yards) of Type II EPS geofoam to raise grades up to 1.7 meters (5.6 ft; Meyer et al. 2004).

2.1.3 Construction Techniques

Construction techniques may be modified to accommodate weaker soils. These techniques may include soft soil expulsion, soft soil expulsion, surcharging, or staged construction.

2.1.3.1 Soft Soil Expulsion

Soft soil expulsion, (also known as displacement fill or the mud wave technique), utilizes the weight of soil to displace unsuitable material. Strategically placing the soil will cause the problematic soils to be expelled from the construction zone leaving the fill material in its place (Zayen et. al, 2003).

2.1.3.2 Surcharge with or without Wick Drains

In 2004, McVay and Nugyen investigated the distress of an embankment built on weak high-organic matter (OM) soil. The investigation consisted of field monitoring a site with an existing roadway and a site for a proposed roadway. Soil surcharging was used to stabilize the soils. While results were mostly positive, the surcharging technique appeared to be appropriate only for new roadways.

As discussed in Mullins and Gunaratne (2014) wick drains may be an effective means to reduce the consolidation time of OM soils by shortening their drainage paths. These drains are installed prior to surcharging throughout the treatment area. They are usually prefabricated drains, but they may also be stone or sand columns, which are discussed below. Their efficiency is dependent on spacing, drain diameter, and material disturbance / interface smear formed during installation.

Several drains are readily available from wick drain manufacturers, and for stabilization programs involving soil mixing, installation of these drains may be very useful. However, as

Mullins and Gunaratne (2014) point out, these are only an effective treatment method when primary consolidation dominates relative to secondary compression. This behavior should only be expected with inorganic clays.

2.1.3.3 Staged Construction

One option that is often utilized for construction on weaker soils is staged construction. During this technique, only a portion of the asset is constructed, and the weak soils are allowed to consolidate and strengthen. Then, the next stage of the structure is placed. Staged construction is often used when constructing embankments on soft soils.

2.1.4 Ground Modification

As discussed in Mullins and Gunaratne (2014), ground modification consists of a broad range of techniques including stone columns, sand columns, dynamic replacement, dynamic compaction, and soil mixing. Many of these techniques are in detail in Mullins and Gunaratne (2014). A brief summary is presented below:

2.1.4.1 Stone Columns

Stone columns, or inclusions installed by packing sand or stone into a borehole, are used to stabilize some soils – particularly sinkhole prone areas. However, as discussed by Mullins and Gunaratne (2014), soil columns would not appear to be a suitable method for stabilizing certain types of weaker soils because of the progressive loss of confinement stress necessary for radial support of the columns.

2.1.4.2 Dynamic Compaction

Dynamic compaction (DC) is a method of densifying soil by dropping heavy weights (typically up to 36 metric tonnes) in a grid pattern from a significant height (up to 30 meters).

While this may be an effective treatment technique, construction difficulties can occur if the water table is not maintained at least six to seven feet below the ground surface (Lukas 1986; Mullins and Gunaratne 2014).

2.1.4.3 Dynamic Replacement and Mixing

Dynamic replacement and mixing (DRM) is a technique whereby consolidation can be accelerated by the installation of sand columns in weak saturated soils. The technique consists of installing a sand column into the weak soil and then dropping a heavy mass onto the sand column to compress the column and expel sand into the surrounding weaker soil (Mullins and Gunaratne 2014). This technique is considered an in-situ mechanical soil mixing method that does not use a binder.

According to the Mullins and Gunaratne (2014), soils treated with this technique may show excellent improvement in terms of compressibility and strength because DRM can transform in-situ peaty clay deposits into an upper sand raft with pockets of peaty sand underlain by a relatively uniform layer of sand and peat. Examples of improvement using this technique include Lo et al, (1990), Lee and Lo (1985), and Terashi and Tanaka (1981).

2.1.4.4 Soil Mixing

There are a number of proprietary methods for soil mixing. The general premise of soil mixing is that a binder, such as lime, slag, or cement, is mixed with in-situ material to improve its engineering characteristics. In particular, soil-cement has been used for decades. The soil-cement is prepared via an above-ground process and added to the soil via jet grouting, wet mixing, or dry mixing.

Mullins and Gunaratne (2014) conducted several bench-scale tests, large-scale laboratory tests, and full-scale mixing tests. Results showed consistent soil improvement, and design guidelines were developed for soil mixing implementation. While these are positive benefits, the issue with soil mixing in general is sustainability and potential environmental effects. The use of cement, an energy and resource intensive material to produce, is often considered unsustainable. Portland cement production is a significant contributor of global CO_2 (up to 5 %) through chemical processes and manufacturing energy. The use of industrial by-products or the use of lime may cause adverse environmental effects due to potential leaching into ground water or health concerns due to toxicity and radioactivity. Mullins and Gunaratne (2014) noted that soil mixing may be inefficient for certain soils, particularly high-OM soils, because a considerable amount of binder is needed to fill voids before the remainder is used as an effective binder.

2.2 Microbial Induced Calcite Precipitation (MICP)

An alternative approach for soil improvement that has gained traction in recent years is microbial induced calcite precipitation (MICP). This technique has been primarily developed and tested for granular materials, although other soils have also been studied on a limited basis. Sumner (1926) was the first to crystallize the enzyme urease from the jack bean, which is the catalyst for the MICP reaction most commonly used today (Mobley et al. 1995). The common use of MICP for soil strengthening or ground improvement today is preceded by a number of applications including:

1. Microbial enhanced oil recovery (MEOR) (Kantzas et al. 1992; Chai et al. 2015; Liange et al. 2015)

2. Restoration and improvement of calcareous stone materials (Tiano et al. 1995; Castanier et al. 2000; Stocks-Fisher et al. 1999; Rodriguez-Navarro et al. 2003)
3. Wastewater treatment (Hammes et al. 2003)
4. Bioremediation (Ferris 2003; Fujita et al. 2000; Warren et al. 2001; Achal et al. 2011)
5. Concrete crack repair (Ramakrishnan et al. 1998; Ramachandran et al. 2001; Wong 2015; De Muynck et al. 2008; Achal et al. 2011; Siddique et al. 2008; Vijay et al. 2009)
6. As a sealant and for structural improvements (Gollapudi et al. 1995)
7. As a bioclogging mechanism for brick (Sarda et al. 2009; Soon 2013)

Beyond MICP, other bio-mediated subsurface geochemical processes exist. These include gas generation (microbial excretion of biogases reducing the saturation of soil with implications of reducing soil susceptibility to liquefaction), biofilm formation (microorganisms adhering to surface and excreting extracellular polymer substances creating a biofilm which has the potential to trap and stabilize sediments) , and biopolymer generation (can reduce hydraulic conductivity and increase shear strength) (DeJong et al. 2013).

The advantage to using MICP as a geotechnical improvement technique as opposed to the more traditional ground improvement methods is that MICP's sustainability because it is an organic process (DeJong et al. 2009). Applications where MICP may be used in lieu of traditional geotechnical improvement methods may eventually include liquefaction prevention, geotechnical damage mitigation, building settlement reduction, and dam/levee piping prevention (DeJong et al. 2009). Additionally, much research has been conducted on reducing hydraulic conductivity via geomicrobial bioclogging. More recently, it has been suggested that MICP may be used to stabilize slopes (Salifu et al. 2016) or mitigate wind erosion (Maleki et al. 2016). The focus of this research

is geotechnical improvement applications. Before discussing MICP ground improvement specifically, it is important to present the chemistry and microbes associated with the MICP process.

2.2.1 MICP Chemistry

Ureolytic MICP is the stimulation of precipitation of calcite by a microorganism through the hydrolysis of urea in the presence of calcium salt solution and nutrients (Salifu et al. 2016). The overall equilibrium reaction is:

$$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_{3(s)} \quad (2\text{-}1)$$

But, this reaction is governed by the following reactions (Ramakrishnan et al. 2001),

$$Ca^{2+} + HCO_3^- + OH^- \rightarrow CaCO_{3(s)} + H_2O \quad (2\text{-}2)$$

$$Ca^{2+} + 2HCO_3^- \leftrightarrow CaCO_{3(s)} + CO_2 + H_2O \quad (2\text{-}1)$$

where Eq. 1-2 is caused by the pH increase induced by bacterial metabolic activity. The rise in pH of the environment is provided in ureolytic MICP by the decomposition of urea (DeJong et al. 2006):

$$NH_2 - CO - NH_2 + 3H_2O \rightarrow 2NH_4^+ + 2OH^- + CO_2 \quad (2\text{-}4)$$

Additionally, calcite formation is stimulated when calcium ions deposit on negatively charged cells as nucleation sites and bond with CO_3^{2-} to form calcite (DeJong et al. 2006).

While the above urea hydrolysis reactions constitute the most commonly used method of bacteria-stimulated calcite precipitation, other methods may also be used including denitrification, iron reduction, photosynthesis (Ehrlich 1998; McConnaughey and Whelan 1997), or sulphate reduction (Castanier et al. 1999; Wright 1999). In concept, each of these techniques is similar in

that they all increase pH and drive Equation 1-2. Figure 2-1 from DeJong et al. (2010) outlines each of these chemical processes:

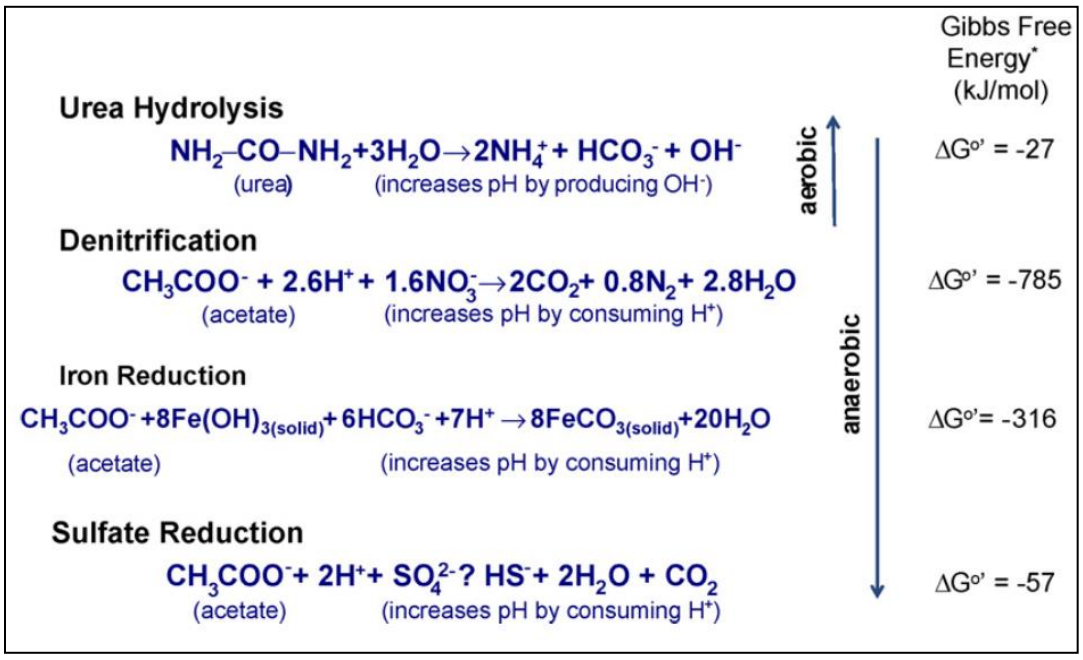

Figure 2-1. Alternative Biomediated Processes (from DeJong et al. 2010)

A study by van Paasen et al. (2010) concluded that urea hydrolysis was the most thermodynamically favored method, and it leads to the highest potential calcite conversion rate when compared with aerobic oxidation, denitrification, or sulphate reduction. Hence, it has become the most common MICP technique for soil improvement.

2.2.2 Factors Controlling the MICP Process

The chemical process of ureolysis calcite precipitation is regulated by the following key factors: calcium concentration (note calcium is present in most natural soils), concentration of dissolved inorganic carbon (DIC), pH, availability of nucleation sites (bacterial cells), and the presence of urea (Kile et al., 2000; Castainer et al., 1999; Whiffin et al. 2007; Hammes and Verstraete 2002). These factors can collectively be termed “reagents.” Additional environmental

factors may play a role including salinity, temperature, and geometric compatibility of bacteria with soil particle characteristics (Nemati et al., 2005; Rivadeneyra et al., 2004; De Muynck et al., 2010b; Maier et al., 2009).

During the process of soil improvement during MICP, specific methods applied may yield variability in results. Salifu et al. (2016) identified key important factors for cementation as pH, bacterial aggregation, pore size distribution of media, application strategy of bacteria and salt (i.e. injection rate), and grouting technique. The time allowed for MICP to take place is an additional variable. A more in-depth discussion of some of these key components is presented below:

2.2.2.1 pH

The critical role of pH throughout the MICP process was discussed briefly above. With the exception of a small group of acid urease enzymes, microbial ureases generally possess an optimum pH of near neutrality (Mobley et al. 1995). For example, the commonly-used microbe *S. Pasteurii* (aka. *B. Pasteurii;*) has an optimum pH of 8 (Stocks-Fischer et al. 1999). When pH drops below 5, microbial urease can potentially be irreversibly denatured (Mobley et al. 1995). Studies of optimal pH ranges for different microbes are listed in Table 2-1 below. The production of ammonia from urea hydrolysis increases the medium pH during MICP, but bicarbonate from urea hydrolysis and microbial respiration acts as a buffer to the pH rise (Soon 2013). The pH at which $CaCO_3$ will spontaneously occur is presented in Figure 2-2 while a table that outlines pH ranges for various calcite-inducing bacteria is presented in Table 2-1:

Table 2-1. Various Bacterial pH Optimizations

Bacteria Type	pH Ranges Reported in the Literature
B. Pasteurii	**6 – 9.5**

Notes:

- pH of 9 (Feng and Montoya 2016)
- Optimum: 8 and Maximum 9.5 (Stocks-Fischer et al. 1999)
- Maximum: 9.3 (Ferris et al. 2003)
- Maximum: 9.1 (Fujita et al. 2004)
- Range of 8.7 – 9.5 (Dupraz et al. 2009)
- Optimum: 8 (Arunachalam et al. 2010)
- Range of 6 – 8, significant loss at pH 5 and 9 (van Elsas and Penido 1982)
- Range of 7 – 9 with a peak at 7 (Khan et al. 2011)

B. Sphaericus **8**

Note:

- Peak at 8 (Arunachalam et al. 2010)

B. Megaterium **6 – 9**

Notes:

- Range of 6 – 8 with significant loss at pH 5 and 9 (van Elsas and Penido 1982)
- Range of 7 – 9 with a peak at 7 (Khan et al. 2011)

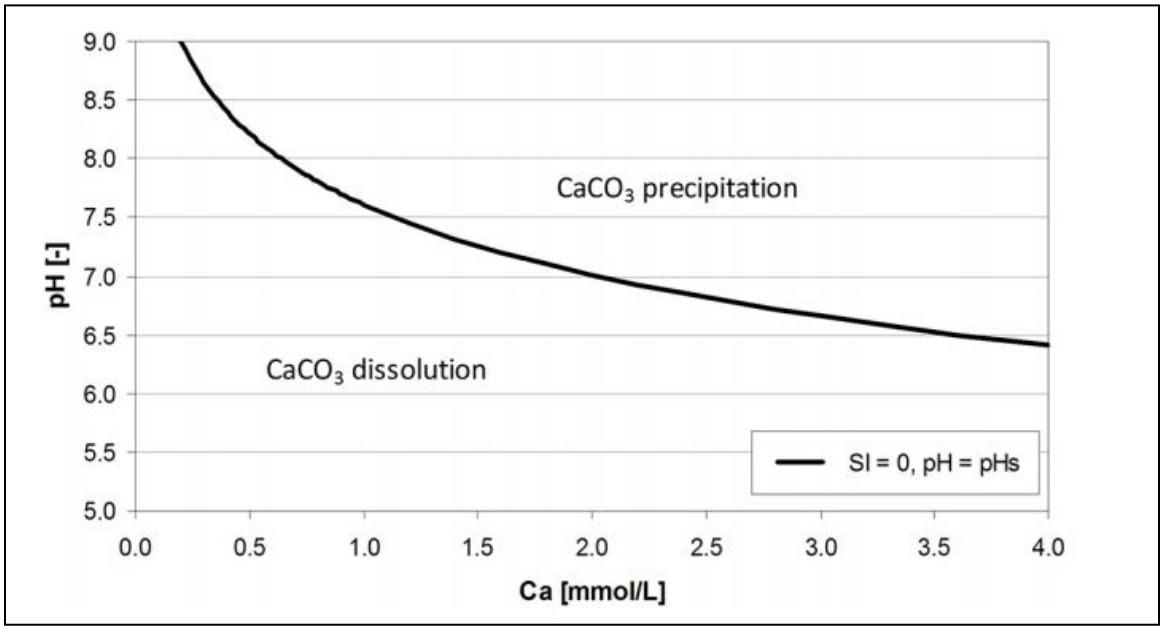

Figure 2-2. Calcium Equilibrium or Saturation with over- and under-Saturation (i.e. Calcium Carbonate Precipitation and Dissolution; from De Moel et al. 2013)

2.2.2.2 Bacteria Cell Concentration

A high concentration of bacterial cells increases the amount of calcite precipitation from MICP (Okwadha and Li 2010). Urea hydrolysis production is directly correlated with bacterial

cell concentration when provided sufficient reagent (Soon 2013). Li et al. (2011) and Stocks-Fischer et al. (1999) both suggested that bacteria cells serve as nucleation sites for calcite to precipitate in biochemical reaction. Using SEM imaging, researchers have determined that the nucleation sites, a key necessity for calcite precipitation, are the cell walls of bacteria (Lian et al. 2006; Knorre and Krumbein 2000).

2.2.2.3 Provided Nutrients

Common nutrients used by bacteria during the MICP process include CO_2, N, P, K, Mg, Ca, Fe, etc. (Mitchell and Santamarina 2005). The nutrient mixes are supplied to bacteria during the culture and soil treatment stage (Soon 2013). Several studies used 3 g/l of nutrient broth in the treatment solution to sustain growth and viability of urease producing bacteria (DeJong et al. 2006; Stocks-Fischer et al. 1999; Al Qabany et al. 2011). The purpose of the nutrients are to ensure bacteria sustain long enough to support calcite precipitation (Soon 2013).

Inagaki et al. (2012) varied the mol densities of urea and calcium chloride in their cementation solution, while keeping them equal to each other. Their tests include 0.25, 0.5, 0.75, 1, and 1.5 mol/L. They concluded a concentration of 0.5 mol/L as the optimum; at greater concentrations the precipitation process is stagnated.

2.2.2.4 Temperature

Temperature is a crucial factor in the rate of MICP. Van Paassen (2009) found that at temperatures below 5ºC, urease activity was negligible. Whiffin (2004), using *S. Pasteurii*, found that urease activity increased proportionally between 25ºC and 60ºC, with an optimal temperature of 70ºC. By 80ºC , precipitation was reduced by approximately 50%. Since the manipulation of temperature is generally not practical in field applications, most experiments are conducted near

room temperature, or 20 – 30°C. However, because production appears to increase as a function of temperature, field microbial treatment may be ideal in Florida at shallow depths during the summer when surface temperature often approaches 35°C. Since soil is a thermal insulator, at higher depths its effectiveness will decrease as temperature increases thereby approaching room temperature conditions. The high ground water table and cooler water temperatures may also be a factor in field applications of MICP in Florida.

Other studies have been conducted on the optimal temperature of urease activity including Sahrawat (1984), Liang et al. (2005), and Chen et al. (1996). However, it is more practical to study and select urease-producing bacteria that are optimal at typical soil temperatures, which vary depending on latitude, altitude, solar radiation, moisture content, conduction, soil type, depth, and other associated factors (Selinus 2005; Jacobson 2005; Doty and Turner 2009).

2.2.2.5 Biofilm

Biofilm refers to the attachment of the bacteria to the soil matrix. The greater the number of bacterial cells attached to the matrix, the denser the biofilm. This factor is critical to MICP in a few key ways. In a study on porosity reduction in granite fractures, Cuthbert et al. (2012) found that denser biofilms result from higher nutrient growth conditions which, in turn, result in higher ammonium production rates. Higher rates of ammonium production produce smaller calcite crystals. This helps to mitigate reduction in hydraulic conductivity due to the loss of porosity.

In the context of granular soil, such as those studied in this research, biofilm is important for the retention and uniformity of bacterial distribution within the soil matrix. This is especially true for applications where treatment is administered into soil volumes via injection points. When treatment is achieve via an injection method, bacteria washout (i.e. bacteria are flushed from the

soil matrix before they attach to the soil particles) must be considered (Cheng et al. 2012). To prevent bacteria washout, a number of techniques have been developed including alternating bacteria injections with feed stock injections (microdosing) or varying the injection rate. Washout appears to be a function of soil grain size in that larger soil grains are more susceptible to washout than smaller grains (Inagaki et al. 2011).

Washout concerns must be balanced with the bacterial generation. It is important to give the bacteria sufficient time to attach to the soil particles before pumping a feed solution through a soil column. However, if too much time elapses before the bacteria are nourished, there is a possibility they may perish.

2.2.3 Microbes

Recent research has focused on determining which microbes can be used to induce MICP. The following is a more in-depth discussion of some of these microbes.

2.2.3.1 Microbe Types

Microbes used for MICP are divided into two categories, ureolytic (urea consuming) and non-ureolytic (non-urea consuming). Common ureolytic positive bacteria come from genera Bacillus, Sporosarcina, Spoloactobacilus, Clostridium, and Desulfotomaculum (Kucharski et al. 2008). The genus Bacillus has been of particular interest in research due to its proven ability in MICP applications (Wong 2015). Specifically, *Bascillus Pasteurii* (now known as *Sporosarcina Pasteurii*) is widely used due to its ability to produce carbon dioxide (CO_2) by respiration and decomposition of urea (Bachmeier et al. 2002; Cuthbert et al. 2013; DeJong et al. 2006; Feng and Montoya 2016; Maleki et al. 2016; Stocks-Fischer et al. 1999; Whiffin et al. 2007; Sarda et al. 2009; Vijay et al. 2009). Aerobic bacteria like this are preferable because they release CO_2 via cell

respiration, which aids calcite production by increasing pH as a result of ammonium and hydroxide ion production (Soon 2013). *Sporosarcina Pasteurii* is especially favorable as it does not aggregate, thus ensuring a high cell surface to volume ratio (DeJong et al. 2006).

Some researchers used methods of bacteria isolation from soil samples to isolate and identify new MICP candidate bacteria. In one such study researchers isolated calcium carbonate precipitating strains from Beidaihe marine sediment (Wei et al. 2015). Strains were tested for solubilization capability and quantified by the diameter of the clear halo around the colony. Results showed that *B. Diminuta* CP16, *S. Soli* CP23 and *B. Lentus* CP28 induced similar morphologies of crystals capable of MICP through ureolysis. Researchers also concluded that the production of carbonate polymorph was not specifically related to any bacterial species, but rather controlled by complicated environmental factors (Wei et al. 2015).

In another example, investigators collected surface scrapings and soil samples in Iran. The most promising isolate from their study was *B. Licheniformis* AK01 which produced 1.33 g of calcium carbonate per liter in 7 days which is 18% more than the common *S. Pasteurii* (Vahabi et al. 2015).

In another study *P.* A*zotoformans* was isolated from an initial pool of 38 bacteria from soil and concrete (Nonakaran et al. 2015). This strain had the highest rate of urea hydrolysis, highest calcite precipitation, and was the most adhesive and insoluble. The investigators suggested that more research was needed to study the strain's potential for concrete crack repair.

The ability of *Pseudomonas Stutzeri* to drive calcite production was investigated and shown to occur during NO_3^- reduction (Singh et al. 2015). Other microbes studied include *Escherichia Coli* HB101 (Bachmeier et al. 2002) and *Proteus Vulgaris* (Nemati et al. 2005).

Bachmeier et al. (2002) found that low concentrations (5–100 μM) of nickel, the cofactor of urease, to the medium further enhanced calcite precipitation by *E. Coli* containing the plasmid pBU11, while calcite precipitation was inhibited by acetohydroxamic acid (AHA). Other recently investigated bacteria and their bioengineering field of application include *B. Sphaericus* for repairing or improving the durability of concrete (De Muynck et al. 2008; Van Tittelboom et al. 2010); and *B. Megaterium* for improvement of concrete strength and durability (Achal et al. 2011; Siddique et al. 2008).

2.2.3.2 Geometric Compatibility

Soil microbes are transported through soil by way of pore throats between soil particles via passive diffusion. The pore throat is estimated as 20% of the soil particle diameter corresponding to the 10% passing particle size (Holtz and Kovacs 1981). Hence, small pore size, relative to the size of the microbe used, can limit free passage (Soon 2013). Maier et al. (2009) found that bacteria that are generally in the size range of 0.3 to 2 μm can move freely through sandy soil with particle sizes ranging from 0.05 to 2 mm. The small pore size in silts and clays will have a greater inhibitory effect on bacteria movement, and thus may limit homogenous distribution of bacteria in the soil. Rebata-Landa (2007) found that the optimum range of soil particle sizes for MICP reactions ranged between 50 to 400 μm. Figure 2-3 below, from Dejong et al. (2010), shows the generalized relation between microbe size and their effectiveness for treating soils of different grain sizes.

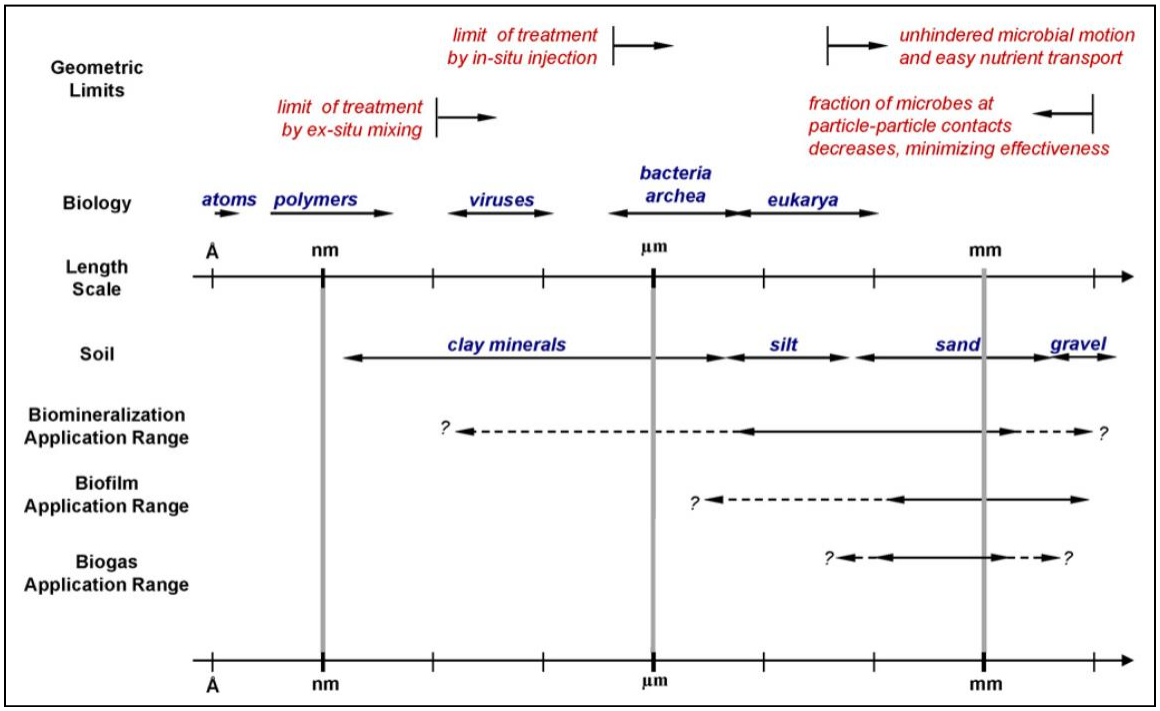

Figure 2-3. Calcium Equilibrium or Saturation with over- and under-Saturation (i.e. Calcium Carbonate Precipitation and Dissolution; from De Moel et al. 2013)

2.2.4 MICP as a Geotechnical Improvement Technique

As mentioned above, MICP may be used for a number of ground improvement applications. In general, the goal with MICP treatment is to increase the shear strength parameters and stiffness of a geomaterial via bio-cementation or decrease the hydraulic conductivity of a geomaterial via bio-clogging.

2.2.4.1 Bio-Cementation as a Process

Soil strength improvement via MICP is attained by the calcite filling of interparticle pore spaces thereby decreasing the pore volume. The distribution of calcite within the pore space can range from uniform, where the calcite coats the entire surface of a given particle evenly, which results in minimal shear strengthening, to preferential, where the calcite only precipitates at the particle-to-particle contacts which results in the maximum shear strengthening, to actual, where

precipitation activity falls somewhere in between uniform and preferential, resulting in moderate soil property improvements (Soon 2013). These three cases are shown in Figure 2-4 below (from Dejong et al. 2010). The spatial distribution of precipitate is affected by biological behavior and filtering processes. Table 2-2 below, adapted from Ivanov and Chu (2008), lists other possible microbial processes that lead to biocementation.

Table 2-2. Biocementation from Microbial Processes

Physiological group of microorganisms	**Mechanism of biocementation**	**Essential conditions for biocementation**	**Potential geotechnical applications**
Sulphate-reducing bacteria	Production of undissolved sulphides of metals	Anaerobic conditions; presence of sulphate and carbon source in soil	Enhance stability for slopes and dams Mitigate liquefaction potential of sand.
Ammonifying bacteria	Formation of undissolved carbonates of metals in soil due to increase of pH and release of CO2	Presence of urea and dissolved metal salt	Enhance stability for retaining walls, embankments, and dams. Increase bearing capacity of foundations.
Iron-reducing bacteria	Production of ferrous solution and precipitation of undissolved ferrous and ferric salts and hydroxides in soil	Anaerobic conditions changed for aerobic conditions; presence of ferric minerals	Densify soil on reclaimed land sites and prevent soil avalanching. Reduce liquefaction potential of soil

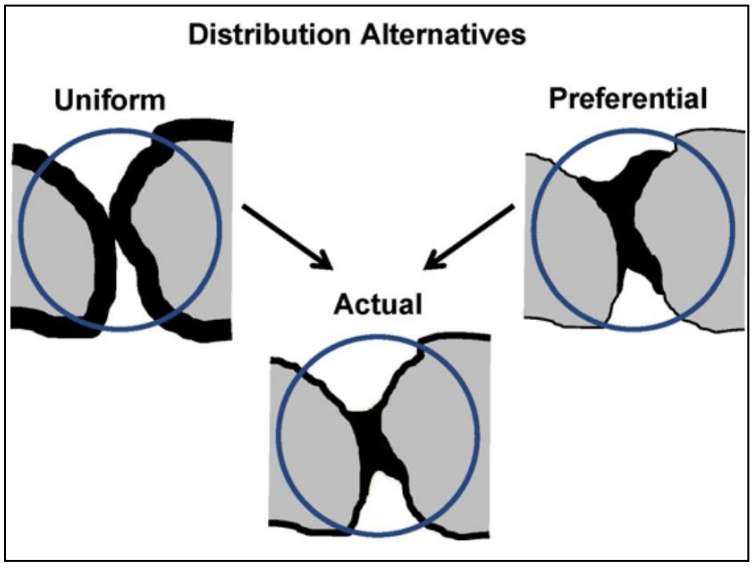

Figure 2-4. Calcite Distribution Alternatives (from DeJong et al. 2010)

2.2.4.2 Strength Improvements from Bio-Cementation

The MICP bio-cementation process has been shown to be successful in a variety of sands; silica, calcite, iron, and beach sands. Often, an increase in shear wave velocity over time is used to demonstrate these improvements (DeJong et al. 2009; Mortensen et al. 2011). Numerous examples are available in the literature that illustrate these strength improvements. For example, DeJong et al. (2006) showed significant strength improvement for MICP-treated specimens via triaxial testing. Whiffin et al. (2007) studied a five-meter long sand tube. They showed that strength was increased between 1.8 and 3.4 times and that a minimum of 3.5% or 60 kg/m^3 of calcite was needed to improve compressive strength. Another study on MICP's effect on compressive strength concluded an improvement of 140% compared to untreated samples (Lu et al. 2010).

2.2.4.3 Bio-Clogging as a Process

Bioclogging is achieved through the same or similar processes as bio-cementation. It is the process by which soil pore space is filled by the product of MICP, which restricts the water flow through the soil (Soon 2013). Vandevivere and Baveye (1992) and Abdel al et al. (2010) found that hydraulic conductivity is significantly reduced by the accumulation of biomass and production of exopolymeric substances. However, these effects are not typically permanent. MICP may make this sort of biomass accumulation more effective. MICP bioclogging results are attained similarly to the processes described in the bio-cementation section. Table 2-3 below, adapted from Ivanov and Chu (2008), describes possible non-MICP processes of bioclogging.

Table 2-3. Bio-clogging Processes

Physiological group of microorganisms	**Mechanism of bioclogging**	**Essential conditions for bioclogging**	**Potential geotechnical applications**

Algae and cyanobacteria	Formation of impermeable layer of biomass	Light penetration and presence of nutrients	Reduce of water infiltration into slopes and control seepage
Aerobic and facultative anaerobic heterotrophic slime-producing bacteria	Production of slime in soil	Presence of oxygen and medium with ratio of C:N > 20	Avoid cover for soil erosion control and slope
Oligotrophic microaerophilic bacteria	Production of slime in soil	Low concentration oxygen and medium with low concentration of carbon source	Reduce drain channel erosion and control seepage
Nitrifying bacteria	Production of slime in soil	Presence of ammonium and oxygen in soil	Reduce drain channel
Sulphate-reducing bacteria	Production of undissolved sulphides of metals	Anaerobic conditions; presence of sulphate and carbon source in soil	Form grout curtains to reduce the migration of heavy metals and organic pollutants
Ammonifying bacteria	Formation of undissolved carbonates of metals in soil	Presence of urea and dissolved metal salt	Prevent piping of earth dams and dikes

2.2.4.4 Hydraulic Conductivity Reduction from Geomicrobial Bio-Clogging

To study hydraulic conductivity reduction in sands, Nemati and Voordouw (2003) used a mix of coarse sand and glass beads as their study media. The urease enzyme was applied directly into the soil instead of using urease producing microorganisms. After treating the specimens multiple times the investigators found that two injections produced hydraulic conductivity decreases of 92% and 72% sequentially. This resulted in a total reduction of 98% compared to untreated samples. Subsequent injections failed to produce measurable results, indicating that there is a limit in effectiveness of multiple injections.

Nemati et al. (2005) conducted a similar study using *Proteus Vulgaris*, a urease-producing microorganism, to produce in-situ calcite using urease enzyme. The reduction in hydraulic conductivities for specimens treated with biomass only, combination of biomass and reagent, and combination of direct supply urease enzyme and reagent were 52%, 65%, and 62%, respectfully. Researchers concluded bacterial and enzymatic treatments yielded similar results for pore plugging. However, the nondurable biomass plugging agent resulting from the biomass reagent combination did not produce a reliable reduction in hydraulic conductivity.

2.2.4.5 Rock Repair

Stocks-Fisher et al. (1999) found MICP using *B. Pasteurii* was optimally effective at remediating fractures in granite at an average width of 2.7 mm (0.79 inches) with a silica (10%) and sand (90%) mixture. Cuthbert et al. (2013) tested the upscaling potential of this application by applying MICP to reduce fractured rock hydraulic conductivity. Using borehole injections, researchers were able to precipitate approximately 750 grams of calcite over a large surface fracture of approximately 4 square meters with 17 hours of treatment.

2.2.4.6 MICP in Organic Soils

Inagaki et al. (2011) compared different sands with peat samples by compacting 10 g of peat to 40 ml and saturating with 25 ml (0.85 oz.) of distilled water. The peat produced the greatest precipitation efficiency and did not vary with different injection frequencies.

2.2.5 MICP Laboratory Testing

A number of laboratory-based MICP studies have been conducted in recent years. The following is a summary of the results of several of these studies that focuses on different sample preparation techniques, treatment options, monitoring techniques, and post-treatment testing.

2.2.5.1 Preparation/Incubation Techniques

While the chemical reactions that govern microbial calcite production are similar from study to study, researchers have attempted to optimize these reactions by varying sample preparation procedures. Stocks-Fischer et al. (1999) mixed bacterial solutions with sand in 60 ml plastic syringe columns. Inagaki et al. (2011) used the same sample setup as above for testing the effects of varied initial microbe solution volumes and injection intervals. DeJong et al. (2006) treated their specimens in triaxial cells with 72 mm diameters and aspect ratios of 2:1 and 1:1. Mortensen et al. (2011) constructed 50 mm rigid cells with 1:1 and 2:1 aspect ratios equipped with bender elements for measuring shear wave velocity. Soil was poured in loosely and loaded with a confining stress of 100 kPa . Whiffin et al. (2007) up-scaled the procedure by treating gravel specimens in five-meter long, 66 mm internal diameter PVC tubes. During these tests downward flow, as opposed to upward flow, was used. Scouring pad filters were used as end caps during the procedure.

Salifu et al. (2016) studied MICP's effectiveness in treating slopes in a tidal environment by comparing untreated and treated sandy slopes using a cubic Perspex container with 0.2 m sides. Water was pumped in and out of the box for thirty cycles to simulate the tides and slopes were tested at angles ranging from 35 to 53 degrees. Results showed significant stability improvements for treated specimens. Maleki et al. (2016) tested MICP treated soils against wind erosion by placing surface-treated specimens in wind tunnels. Again, results showed significant improvement for treated specimens.

Feng and Montoya (2016) studied the effects of confining pressures and sample treatment repetition. Like DeJong et al. (2006), specimens were treated in triaxial cells. Confining pressures

of 100, 200, and 400 kPa were used during treatment. Treatment was repeated 10 times, 20 times, and 40 times, and calcite precipitation was monitored after each round. Results showed that precipitation significantly decreased after 6-8 repetitions.

Most MICP testing in has been conducted using saturated samples, but recent studies have tested MICP in unsaturated conditions. This is an ongoing area of research.

2.2.5.2 MICP Treatment Techniques

Geomicrobial calcite precipitation is also affected by injection conditions. The injection method must be chosen in accordance with the soil conditions (Inagaki et al. 2011). Several researchers have studied various treatment techniques to quantify these effects.

Stocks-Fischer (1999) prepared stock cultures by combining a 1:2 ratio of ammonium sulfate and yeast extract in a Tris-hydrochloric acid (HCl) buffer with a pH of 9.0. Individual ingredients were autoclaved separately and mixed afterward to avoid precipitation. The microbes were grown in an aerobic environment, then harvested with a centrifuge, and used to treat sand columns (Stocks-Fischer et al. 1999). This early study confirmed the validity of MICP and found a suitable pH range of 8-9.

DeJong et al. (2006) applied a bacterial solution to 72-mm triaxial sand specimens at 20 mL/min for 20 minutes using a peristaltic pump. Specimens were allowed to set for four hours after treatment. Cementation solutions and filtered air were then pumped through samples at 4 mL/min until the desired cementation of 35% relative density was reached. The urea solution was stirred prior to pumping until a pH of 7.5 was achieved in an effort to enhance alkalophilic bacterial activity. Specimen pH was maintained at 8.2 or greater.

In another study, researchers tested *S. Pasteurii*'s MICP production alone and with a competing non-ureolytic bacteria, *B. Subtilis*. The treatment with non-ureolytic bacteria exhibited significantly higher growth rates than that with ureolytic bacteria alone. Although the chemical conditions deteriorated, the increase in nucleation sites ultimately accelerated calcite precipitation (Gat et al. 2011).

The effect of salinity on geomicrobial calcite development has also been studied. High salinity solution encourages flocculation, and this promotes the adsorption of bacteria and retention in sand columns (Ritvo et al. 2003; Torkzaban et al. 2008). Low salinity solution or fresh water with a low ionic strength allows the bacteria to be transported over large distances and therefore inhibits precipitation (Harkes et al. 2010). Mortensen et al. (2011) tested bacterial growth at 0, 25, 50, 75 and 100% saltwater concentrations and different freshwater formulations. Bacteria growth rate appeared to be independent of salinity levels. However, higher salinity concentrations showed an increase in calcite precipitation. This was explained by DeJong et al. (2009) as a higher salinity provides more cations to precipitate with microbially-generated carbonate.

2.2.5.3 MICP Monitoring Techniques

Monitoring refers to any data collected during the MICP treatment process, which includes geophysical, chemical, and biological measurements. Chemical and biological processes of MICP, which ultimately control the desired geophysical changes, are intimately linked (DeJong et al. 2010). While several typical monitoring techniques have been alluded to above, the following is a more in-depth discussion of these techniques.

2.2.5.3.1 Geophysical Monitoring

To date, the three primary methods of geophysical measurements used to monitor MICP are shear wave velocity, compression wave velocity, and resistivity mapping. Both shear and compression wave velocities can be easily measured in the laboratory with piezoceramic transducers, bender elements, or accelerometers (DeJong et al. 2010).

Monitoring MICP by measuring shear wave velocities is advantageous over compression wave velocity measurements since shear waves do not propagate through fluids and there is a direct relationship between shear wave velocity and the mass of precipitated calcium carbonate, void ratio, and confining stress (DeJong et al. 2006). Using bender elements in MICP laboratory tests, DeJong et al. (2006) was able to show how treatment frequency, duration, and concentration drove the evolution of cementation of specimens.

More recently, researchers evaluated the shear strength and stiffness of sand subjected to drained and undrained shearing via triaxial tests of samples with varying degrees of cementation (Montoya and DeJong 2015). Shear wave velocity was used to monitor the change in small strain stiffness during shearing. As expected, their results confirmed previous results in that shear strength and stiffness were directly correlated with cementation. Testing indicated that the critical state stress ratio was not significantly affected by cementation, the peak shear strength increased with increased cementation levels, and as the cementation changed the stress-strain behavior transitioned from strain hardening to strain softening. Also, the loading regime influenced the rate of stiffness reduction due to cementation degradation and softening (Montoya and DeJong 2015).

Electrical resistivity, measures the potential gradient through a soil matrix. It is dependent on the volume fractions of particles, pore space, mineral composition, and the chemical

composition of pore fluid (DeJong et al. 2010). These measurements are used to potentially detect soil density variation and changes in pore fluid composition (Klein and Santamarina, 2002; Snieder et al. 2005). These measurements can be used to monitor the hydrolysis of urea via the increase in ionic potential of the pore fluid (Mortensen et al. 2011). Additionally, Whiffin et al. (2007) monitored urease activity by conductivity (used in the absence of calcium ions) and ammonium production rate using the Nessler method. Calcium concentration was determined via UV absorption using a LCK 327 apparatus produced by Hach Lange, Germany. Mortensen et al. (2011) followed a similar procedure.

2.2.5.3.2 Biological and Chemical

MICP's biological processes can be detected using measurements of microbial concentration, activity state, activity potential, biomass, and nutrient concentration (DeJong et al. 2010). The chemical processes are primarily captured from monitoring pH, chemical concentrations, and conductivity. The invasive or destructive nature of these testing methods make it almost impossible to gather real-time data on these variables except in the effluent of flow-through experiments. However, their understanding is very important to understanding bio-mediated processes (DeJong et al., 2010). Bio/chemical tests are thus usually conducted post-treatment and not in real time.

An exception to the usual bio-chemical post treatment testing was presented by Salifu et al. (2016) study where specimens were collected from the foot of the treated soil slopes using a 20-mL syringe at certain time intervals during treatment. The specimens were frozen and tested for ammonium and calcium concentrations using a colorimetric analyzer and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

2.2.5.4 MICP Treatment Post-Testing Techniques

Many destructive and non-destructive tests have been performed on MICP specimens after treatment. Early research measured reductions in porosity and hydraulic conductivity (Kantzas et al. 1992). Whiffin et al. (2007) quantified the porosity and hydraulic conductivity of treated specimens using wet/dry density tests and constant head tests, respectively.

Before MICP was studied in soils, researchers used porous polyurethane foam as a testing medium (Bachmeier et al. 2002). A micro-penetrometer has been used to test the penetration resistance of treated and untreated samples (Maleki et al. 2016). X-ray diffraction (XRD) quantitative analysis has been used to detect the formations of new minerals (Stocks-Fischer et al. 1999). Similar testing was conducted by others to characterize precipitate (Nonakaran et al. 2015; Vahabi et al. 2015). Optical density measures have also been taken to analyze bacterial cell density, usually at a wavelength of 600 nm (Gat et al. 2011; Rong and Qian 2014).

X-ray compositional mapping for assessing surface modifications has been previously used (DeJong et al. 2006; Maleki et al. 2016). Additionally, X-ray tomography has been used to follow image three dimensional deformation processes during triaxial compression tests (Tagliaferri et al. 2011).

Fourier-transform-infrared (FTIR) was used by Vahabi et al. (2015) to analyze precipitates from different isolates. Rong and Qian (2015) analyzed the bonding structure using transmission electron microscope, infrared spectra, x-ray photoelectron spectroscopy, and nuclear magnetic resonance.

Shear strength and triaxial testing after treatment are commonly used to quantify cementation effects. For example, Whiffin et al. (2007) used single-stage, confined, drained

triaxial tests at a confining pressure of 50 kPa to determine compressive strength and stiffness. Results showed a minimum of about 60 kg/m^3 of calcite is needed for significant strength improvement. Ng et al. (2012) used unconfined compression tests on 50 mm diameter saturated specimens. Feng and Montoya (2016) obtained specimens from samples prepared in a triaxial cell and conducted direct shear tests (DST) to show vertical variability during column treatment.

Scanning electron microscopy (SEM) has often been used to understand and visualize calcite precipitation on a micro-scale. Treated specimens are prepared by epoxy impregnation and subsequent surface polishing. Results show reduced pore space, and precipitated calcite phases (DeJong et al. 2010). Many researchers have and continue to use this method to assess MICP soil treatments (Bachmeier et al. 2002; DeJong et al. 2006; Maleki et al. 2016; Ng et al. 2012; Stocks-Fischer et al. 1999). Stocks-Fischer (1999) carbon coated fractured samples and viewed them at accelerating voltages from 30 to 35 kV during SEM imaging and back-scattering electron imaging.

SEM has shown that during destructive laboratory tests, such as compression and direct shear, treated specimens fail because the precipitate fails. This is demonstrated by a layer of calcite that is present on the soil specimen failure plane (DeJong et al. 2010).

Salifu et al. (2016) measured the mass of calcite precipitation by oven drying samples and then weighing them before and after being washed with a 10% HCl solution. This method is widely used for understanding of MICP coverage throughout the specimen (Feng and Montoya 2016; Whiffin et al. 2007). Another common method for quantifying the amount of calcite precipitation is by direct measurement of Ca^{2+} ions (Bachmeier et al. 2002; Stocks-Fischer et al. 1999). During the Montoya et al. (2013) study, researchers followed ASTM D4373, to quantify cementation.

2.2.6 MICP Field Studies

In the past few years, much MICP research has moved from the laboratory to the field. As should be expected, the major issue associated with upscaling this technology is assessing the volume of soil which can be improved. Variables associated with this include cost, scale, required treatment resolution, and application method.

2.2.6.1 Bio-Augmentation vs. Bio-Stimulation

On average, more than 10^9 microbial cells per gram of soil exist in the top meter of soil. At a depth of 30 meters, geomicrobe concentration drops to approximately 10^6 cells per gram of soil. (DeJong et al. 2010). Based upon these concentrations, it would appear that coverage depths to 30 meters may be possible via bio-stimulation with the proper field technique. In cases where appropriate calcite-producing microbes are unavailable, it may be possible to augment via injection (DeJong et al. 2009).

2.2.6.2 Medium-Scale Testing

In the late 2000s and early 2010s, several medium-scale studies were conducted to assess the feasibility of upscaling MICP. For example, Martinez and DeJong (2009) conducted a model shallow foundation load test on soil improved by MICP, figure 2-5, which yielded a five-fold settlement reduction. However, differential settlement was observed and attributed to variability in cementation.

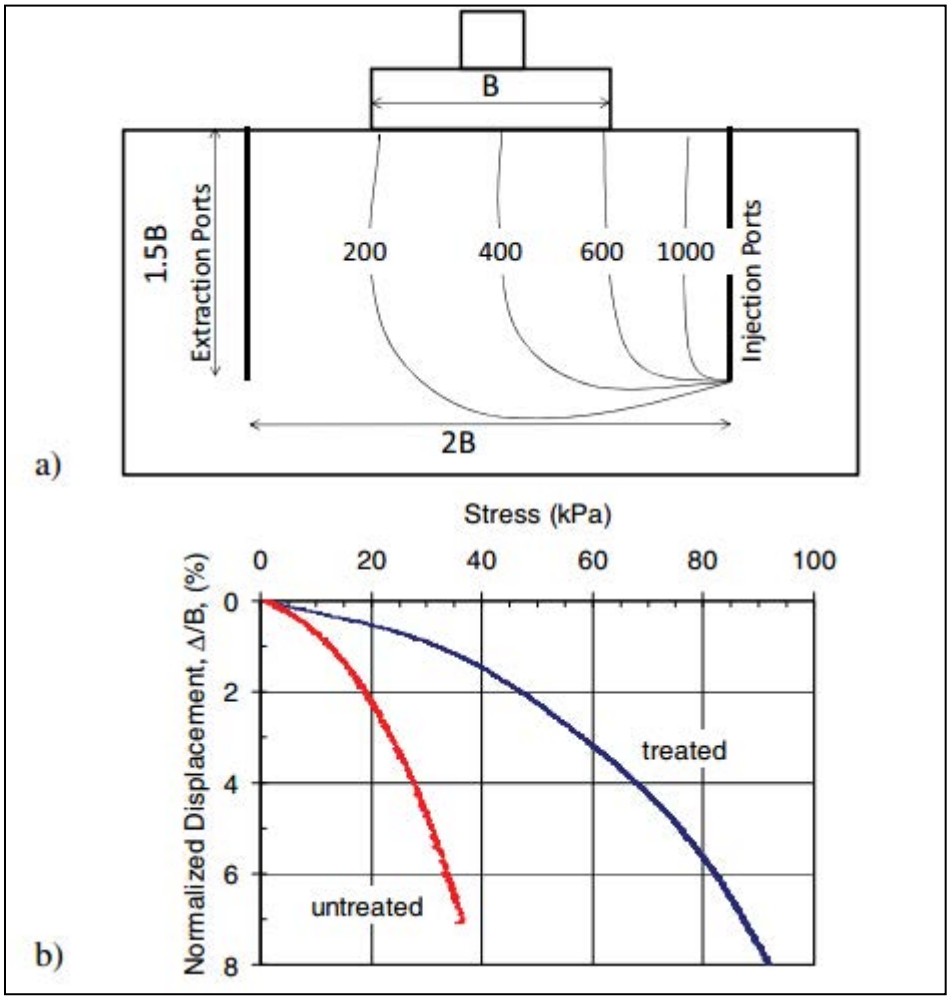

Figure 2-5. Illustration of medium-scale shallow foundation test (From DeJong et al. 2009) showing (a) approximate shear wave velocity contours (in m/s); column width = 4 inches; and (b) quantitative results, displacement at center of footing

Weil et al. (2012) proposed the use of incrementally spaced boreholes to conduct cross-hole monitoring of shear wave velocity, compression wave velocity, and electrical resistivity during treatment. These three measures can be grouped at different depth intervals which would have the potential to provide three-dimensional understanding of the MICP improvement process during large scale field applications.

2.2.6.3 Larger-Scale Testing

In recent years, researchers have begun larger-scale testing with MICP. During the aforementioned Cuthbert et al. (2013) study, four 100 mm diameter borehole wells were drilled to a depth of approximately 27 meters. Initial hydraulic conductivity of the rock was measured within the boreholes. During treatment, a bio-augmented solution was injected, and some boreholes were monitored to quantify coverage immediately thereafter. Soon after treatment, hydraulic conductivity was again measured and decreased from the initial measurements. Twelve weeks later, these boreholes were re-examined. Results showed no change in transmissivity in the intervening period; the chemical process appeared to be stable in the presence of ambient groundwater flow over short term conditions.

DeJong et al. (2013) identified two more field applications. The first was a bio-augmented study where contractor Visser & Smit Hanab applied MICP treatment to gravel to enable horizontal directional drilling for a gas pipeline in the Netherlands in 2010. A 100 cubic meter volume between depths of 3 and 20 meters was treated. Bacterial injections of 200 cubic meters and two nutrient injections of between 300 and 600 cubic meters were applied. The treatment was deemed successful as investigators were able to drill without instability issues in the loose gravel deposit. Figure 2-6 shows some photographs of the procedure:

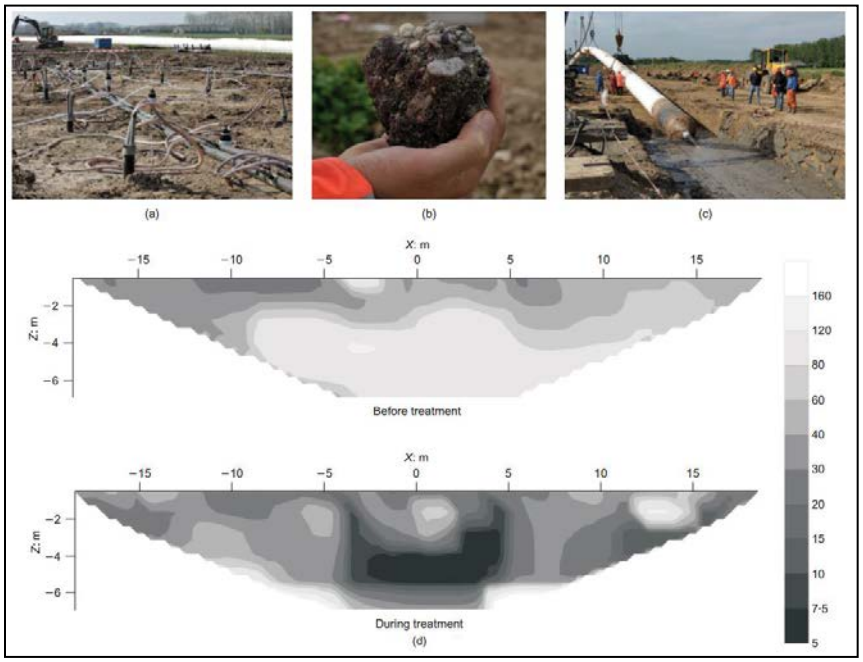

Figure 2-6. Overview of MICP field trial for stabilization of loose gravel for horizontal directional drilling showing (a) repeated well pattern; (b) sample of MICP-stabilized gravel; (c) pipeline installation after horizontal directional drilling; and (d) resistivity mapping before and during treatment (from DeJong et al. 2013)

The second was a bio-stimulation study where the of co-precipitation of a heavy metal Strontium-90 with calcium carbonate to immobilize the heavy metal was initiated at the Vadose Zone Research Park (VZRP) at the Idaho National Laboratory (INL). This study is ongoing at the US Department of Energy site in Rifle, Colorado, USA (Fujita et al., 2010). By injecting dissolved molasses and urea, researchers noted slow but quantifiable calcite precipitation (DeJong et al. 2013).

2.2.6.4 Potential Issues

While the MICP technique is showing promise, issues associated with its field applicability have been identified. Some of these issues include limited injection depth due to relatively low hydraulic conductivity and clogging of the injection systems (Whiffin et al. 2007).

Another concern with up-scaling to the field is the environmental conditions of the soil. However, research indicates that these issues may be less critical. Mortensen et al. (2011) conducted a comprehensive study of environmental factors. Results showed that ureolytic bacteria are able to grow in a wide range of groundwater environments including different types of freshwater and levels of salinity. The bacterial are not affected by high ammonium concentrations and are able to survive in anoxic conditions. The treatment uniformity is increased as injection rate decreases. Reducing the nutrient concentration reduces affluent ammonium concentrations while maintaining uniform treatment. The precipitation rate increases with increased salinity. These findings indicate MICP id possible in a wide range of soil environmental conditions.

2.2.6.5 Coverage Permanence

MICP treatment in engineering applications must have permanence over a realistic design life to be useful. Treatment areas where calcite is already stable are most favorable because the calcite must remain once normal geochemical conditions return (DeJong et al. 2009). Some research indicates that microbially-treated soil strengthening properties can be effective for up to 50 years (DeJong et al. 2009). Since the permanence aspect of MICP remains understudied to date, economic and risk assessments are required to understand the groundwater-precipitate interaction, performance monitoring and the ability/intervals for retreatment (DeJong et al. 2013).

2.3 MICP Coverage Uniformity/Variability

Coverage uniformity is an ongoing topic of research. Soil is a heterogeneous, anisotropic material. Calcite concentration decreases as the distance from the injection point increases (Whiffin et al. 2007). Near the injection point, which refers to spatial distances between up to 1.2 meters, calcite content ranges between 85-105 kg/m^3. As distance from the injection point

increases to 2.5 to 5 meters, calcite content decreases to 2-30 kg/m^3. However, as research continues, progress is being made to improve coverage uniformity.

2.3.1 Measuring Coverage Uniformity

The most common method used to analyze calcite formation is the acid wash test. During this test, the cemented soil volume is dried and its mass is recorded. The specimen is then washed with HCl. The difference between the masses is the quantity of calcite. This method of washing soil with HCl is widely used in the field of MICP research (Soon et al. 2013; Montoya et al. 2015; Feng and Montoya 2016; Salifu et al. 2016). Using a different approach, Whiffin et al. (2007) measured calcite content using a U-tube manometer where a treated soil sample and HCl were sealed in separate compartments and then mixed. The percent mass of calcite was inferred from by measuring the amount of CO_2 released during the ensuing chemical reaction.

The most common non-chemical method for assessing coverage involve using shear wave velocity measurements which are correlated to stiffness. Note that in all cases, localized strength has not been measured directly.

Cheng et al. (2012) determined localized strength along a 100 cm sand column using a pocket penetrometer. Between 10 and 30 cm from the injection point the strength was approximately 2500 N/cm^2. At other locations along the cemented column, the strength was approximately 2000 N/cm^2. This is the only known direct measurement of variability within a single specimen.

Whiffin et al. (2007) plotted the relationship between strength and calcite content, but strength was obtained by running triaxial tests on a number of different specimens not by measuring strength variability within the same specimen. However because of variability of calcite

content within each of the specimens, it is difficult to understand how meaningful the "average" calcite content could be under such circumstances. Nonetheless, results presented in Figure 2-7 showed that once the calcite reached a content of 60 kg/m^3, there is a proportional relationship between precipitated calcite and compressive strength. However, these effects were lost when the initial bonds created by the precipitate are broken.

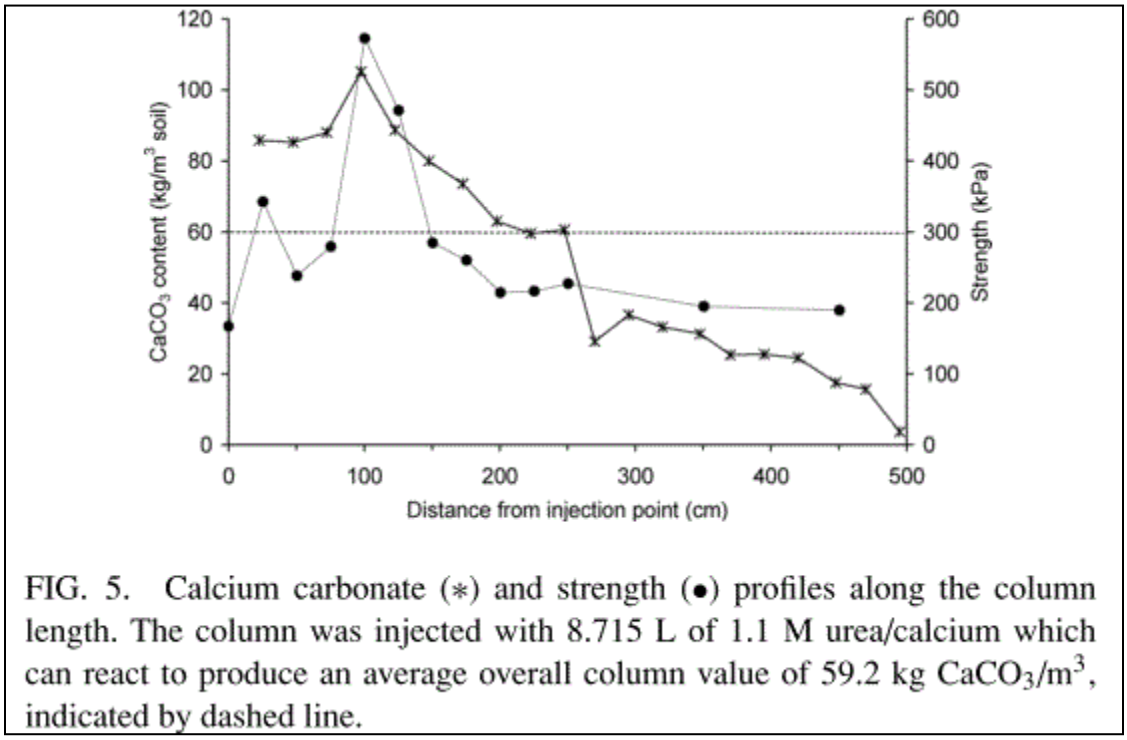

FIG. 5. Calcium carbonate (∗) and strength (•) profiles along the column length. The column was injected with 8.715 L of 1.1 M urea/calcium which can react to produce an average overall column value of 59.2 kg $CaCO_3$/m^3, indicated by dashed line.

Figure 2-7. Example of Calcite and Strength Distribution Along Soil Column Length (adapted from Whiffin et al. (2007))

2.3.2 Methods to Improve Coverage Uniformity

DeJong et al. (2009) suggested that a push-pull injection process, gridded injection/extraction, and chemical optimization of treatment media may all increase coverage area and/or improvement uniformity. Other methods such as immersing bacteria saturated soil columns in cementation fluid (Akimana et al. 2016) have also been attempted to improve uniformity.

However, most research involving improving uniformity has focused on varying injection techniques.

2.3.3 Injection Techniques

Bio-augmented MICP solution is injected using similar methods that would be used for injection procedure for any geo-strengthening material (Soon 2013). A two-phase injection procedure where *S. Pasteurii* suspensions are injected followed by a high salt content fixation fluid successfully retained 100% of urease activity in a sand column (Harkes et al. 2010).

Stopped-flow injection, consisting of injecting 1.5 pore volume of reagent followed by 2.5 hours of rest period, offered better uniform concentration than continuous injection. This technique yielded abundant calcite precipitation near the injection point, but calcification decreased with the distance from the injection point (Martinez et al. 2011). A numerical model (Barkouki et al. 2011) obtained similar findings. Stopped-flow injection has been shown to distribute cementation fluid evenly in a sample before the composition of calcite (Soon 2013).

Repeated injection of reagent to the soil increases the composition of calcite. Effectively, this is very similar to stopped-flow injection. Studies on repeated injection on carbonate precipitation in limestone showed a decrease in hydraulic conductivity between the second and third treatments. There was an associated percent gain in mass of 36% and 33% between the second and third treatments (De Muynck et al. 2010b). Hydraulic conductively reduced 65%, 12%, and insignificantly for the first, second, and third treatments (Nemati et al. 2005). The introduction of urease enzyme directly into the sand produced a greater reduction in hydraulic conductivity for the second and third treatments.

Inagaki et al. (2011) concluded that precipitation is optimized when the bacterial solution volume is equal to the void volume of the soil as it is able to replace any other fluid or gases without wasting and solution. Higher injection rates, on the order of 10 mL/min, produce higher cementation rates, but less uniformity (Mortensen et al. 2011).

The injection methods previously discussed refer to injections into saturated laboratory samples. When dealing with larger-scale field applications, these conditions can be difficult to attain. An alternative method of surface percolation in unsaturated specimens has been studied (Cheng and Cord-Ruwisch 2012). The procedure used was to percolate 50% of the water retention capacity of the sample of bacterial solution and then percolate an equal amount of cementation solution. The sample was allowed to incubate for 12 hours at 25ºC and the process was repeated. The results indicated that bacteria can be immobilized over one meter column height by alternating layers of solutions. This technique appears to reach a reasonable amount of homogeneity with crust formation. The percolation test produced about three times higher local strength per mass of calcite compared to the saturated method (Cheng and Cord-Ruwisch 2012).

Very recently, Feng and Montoya (2016) showed that there is a significant decrease in cementation variation when treatment confining pressures are in the range of 200 to 400 kPa. This finding may help with development of a more-uniform injection technique and holds promise for deep injection in field applications.

2.4 Summary and Motivation for Research

The previous discussions show MICP has been gaining traction as a soil improvement technique. While much is known about the topic, there are still several questions about achieving a more-uniform treatment and decreasing localized strength variability. Previous studies that

involved indirect strength monitoring techniques such as compression and shear wave velocities provided valuable information about cementation, but these non-invasive tests do not directly measure local strength within a treated specimen unless tomography techniques are applied. With the exception of the Cheng et al. (2012) study, no known research has been conducted whereby localized strength from MICP-treated specimens was measured directly.

Techniques for assessing and improving uniformity of soil improvement, which is one of the major issues for any ground modification, require significant research studies. Previous research has shown that more data is needed to fully assess how MICP soil improvement varies spatially and to develop techniques to produce uniformly improved samples. The goals of this thesis are two fold: to use a simple technique to quantify localized variability in shear strength parameters and to potentially develop a simple treatment technique to provide a more uniformly treated sample.

Chapter 3 MATERIALS AND METHODS

Based upon the discussion in Chapter 2, it is clear that there are number of different Microbial Induced Calcite Precipitation (MICP) percolation treatment techniques that exist. This thesis focused on one treatment technique which was a derivative of the DeJong et al. (2006) treatment method (dubbed the UC Davis percolation method or UCDM). This treatment is the focus of quantitative research in this thesis, while the second method, "Soil Mixing" treatment method (SMM), is a new method where the data presented is preliminary data used for optimization of the technique. The following subsections describe, in depth, the materials used and methods applied.

3.1 Granular Material

Both methods were applied to 50-70 Ottawa sand (Figure 3-1, Figure 3-2, and Table 3-1). This, and similar, materials have the focus of MICP research to date.

Figure 3-1. Ottawa 50-70 silica sand

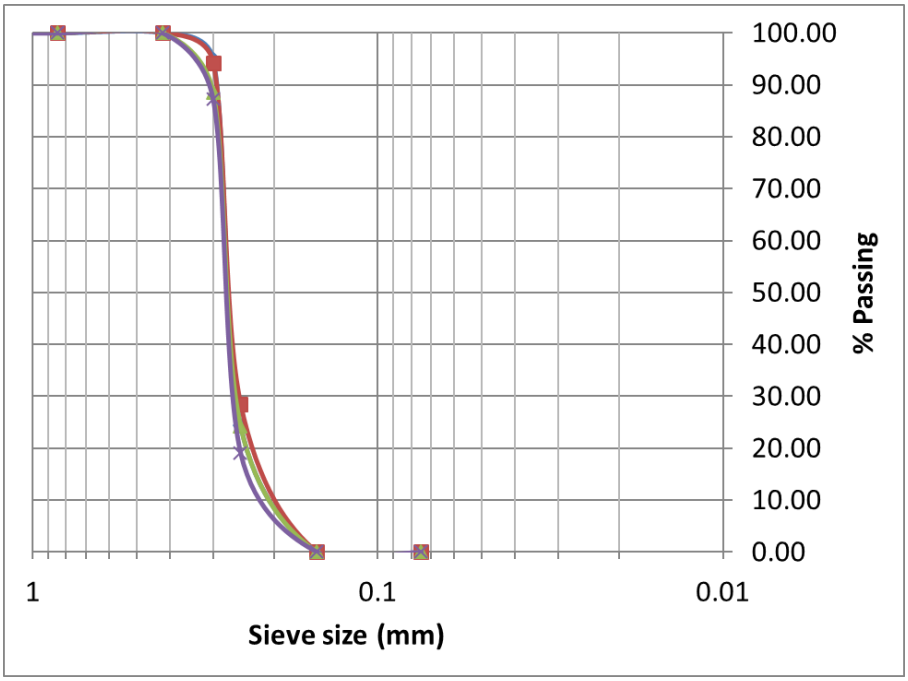

Figure 3-2. Ottawa sieve analysis

Table 3-1. Ottawa 50-70 sand properties

Properties	Current Research (2017)	Simpson thesis (2014)	Feng and Montoya (2014)	Lin et. al. (2015)
Gs	2.64	2.65	2.65	2.65
D10(mm)	0.21	0.248	n/a	0.26
D30(mm)	0.25	0.259	n/a	0.31
D50(mm)	0.27	0.264	0.22	0.33
D60(mm)	0.28	0.266	n/a	0.37
Cu	1.37	1.07	1.4	1.43
Cc	1.07	1.02	0.9	1.01

3.2 Soil pH Adjustment

The initial, during treatment, and final pH of the pore fluid is known to play a role in MICP-treated soil calcification. Therefore, soils were adjusted to initial pHs of 5 and 7 prior to treatment to further investigate the effect of pH. Ottawa 50-70 sand has a natural pH of approximately 7. Chemical adjustment was used to generate soils with initial pHs of 5. Adjustment consisted of

adding 0.0075 to 0.0085 M HCl to the soil pore fluid. This molarity range was found using a trial-and-error process. Soil pH was determined following the procedures of ASTM D4972.

3.3 MICP Treatment Techniques

Two treatment techniques were used throughout this study – the UCDM and a new method called the Soil Mixing Method (SMM). Each of these methods are described below.

3.3.1 Ureolytic Processes of Sporosarcina *Pasteurii*

There has been a wide variety of bacteria studied in the field of MICP. *Sporosarcina Pasteurii* has proven to be the most consistently successful species utilized in ureolytic MICP. Therefore it was used throughout this study.

The process associated with ureolytic MICP was discussed in Chapter 2. To summarize, subsurface microbes catalyze the calcium carbonate precipitation by hydrolyzing urea and producing ammonium and bicarbonate which increases the pH. With the addition of calcium carbonate to the environment, the increase of pH drives the formation of calcium carbonate, or calcite, within the soil pore fluid. The calcite should to bind the soil particles together. However calcification can occur without true cementation. Cementation only occurs when the precipitated calcite forms bonds between the soil particles. This is known to be dependent on the formation of a biofilm which allows the bacteria to evenly distribute around the soil matrix, hold themselves in place, and pass nutrients among themselves.

3.3.2 UCDM MICP Treatment Procedure

The UCDM involves percolating bacteria and feed stock through a chamber-enclosed soil at a specified rate. Treatment chambers (Figure 3-3), were designed to generate soil columns with diameters appropriate for triaxial, consolidation, and direct shear tests. The acrylic treatment

chamber was made of a split cylinder and square end caps with small, centered inlet/outlet holes. The split cylinders were held together with two metal worm gear hose clamps, and their end caps were held in place with threaded metal rods fastened with bolts. All seams were sealed with rubber gasket material. The dimensions of the soil columns within the treatment chambers were 7.112 centimeters in diameter and 17.78 centimeters in length. These volumes were filled with autoclaved Ottawa 50-70 sand which was air pluviated without compaction.

Figure 3-3. UCDM treatment chamber filled with Ottawa 50-70 sand

A 600 mL solution containing *Sporarcina Pasteurii*, shown in Figure 3-4, was injected into the bottom of the soil columns via a peristaltic pump and allowed to freely flow out the top outlet of the treatment chamber. The soil column with solution was allowed to rest for 12 hours to give the bacteria time to attach to the soil particles. The bacteria were then fed every 6 hours with a

solution containing a mixture of urea and calcium chloride. The solution was injected at a flow rate of 3 mL/minute using a peristaltic pump over a total period of 48 hours. The full treatment setup for multiple soil columns is shown in Figure 3-5. This treatment was conducted on twelve soil columns with initial pHs of 5 or 7.

Figure 3-4. *Sporosarcina Pasteurii* bacterial solution

Figure 3-5. Full UCDM setup

3.3.3 SMM Treatment

The following sections discuss the new SMM treatment procedure. The discussion is focused on justification and the developed procedures.

3.3.3.1 Justification for Development

Issues with the UCDM were identified throughout this study and are present in the literature. The most important issue with the UCDM, calcification variability. The UCDM produces non-uniform soil columns. The bottoms of the columns tend to be more calcified than the tops of the columns. This can be attributed to the single initial point source for both the bacteria and the feed stock. The goal of the SMM method was to create more-uniform specimens and to develop a treatment method that was simpler to apply in the field.

3.3.3.2 SMM Treatment Procedure

SMM treatment differs from the previously discussed technique in that it does not require multiple injections of solutions. Rather, all bacteria and nutrient solutions are introduced to the soil matrix nearly simultaneously.

Cylindrical aluminum treatment chambers with dimensions 6.35 centimeters in diameter by 14.605 centimeters tall were milled using a CNC cutter. Similar to the UCDM chambers, the SMM chambers' dimensions were chosen so that the resultant specimens would be the correct diameter for consolidation, direct shear, and triaxial testing. was designed with the same general principles as the acrylic chamber from the previous method in that the dimensions were chosen for direct shear, triaxial, and consolidation testing. The treatment chamber was made of two pieces and a rubber gasket was used to seal its seam. The chamber base plates were sealed onto their sides using a sealant, as shown in Figures 3-6 and 3-7. The inside of the chambers were lined with filter paper to allow for easier specimen extraction. As with the previous method, Ottawa 50-70 sand was pluviated into the cylinder and allowed to naturally fill the volume.

Once the sand was in place, a 100 ml, 2.5 M bacterial solution was mixed with 100 ml of a 2.5 M CaCl solution. This 200-ml mixture was added to each soil column and the resulting slurry was stirred with a spatula. During the initial bacteria/CaCl mixing, chemical crashout was observed in that calcite began forming even before the solution could be mixed with the soil. This may have affected results.

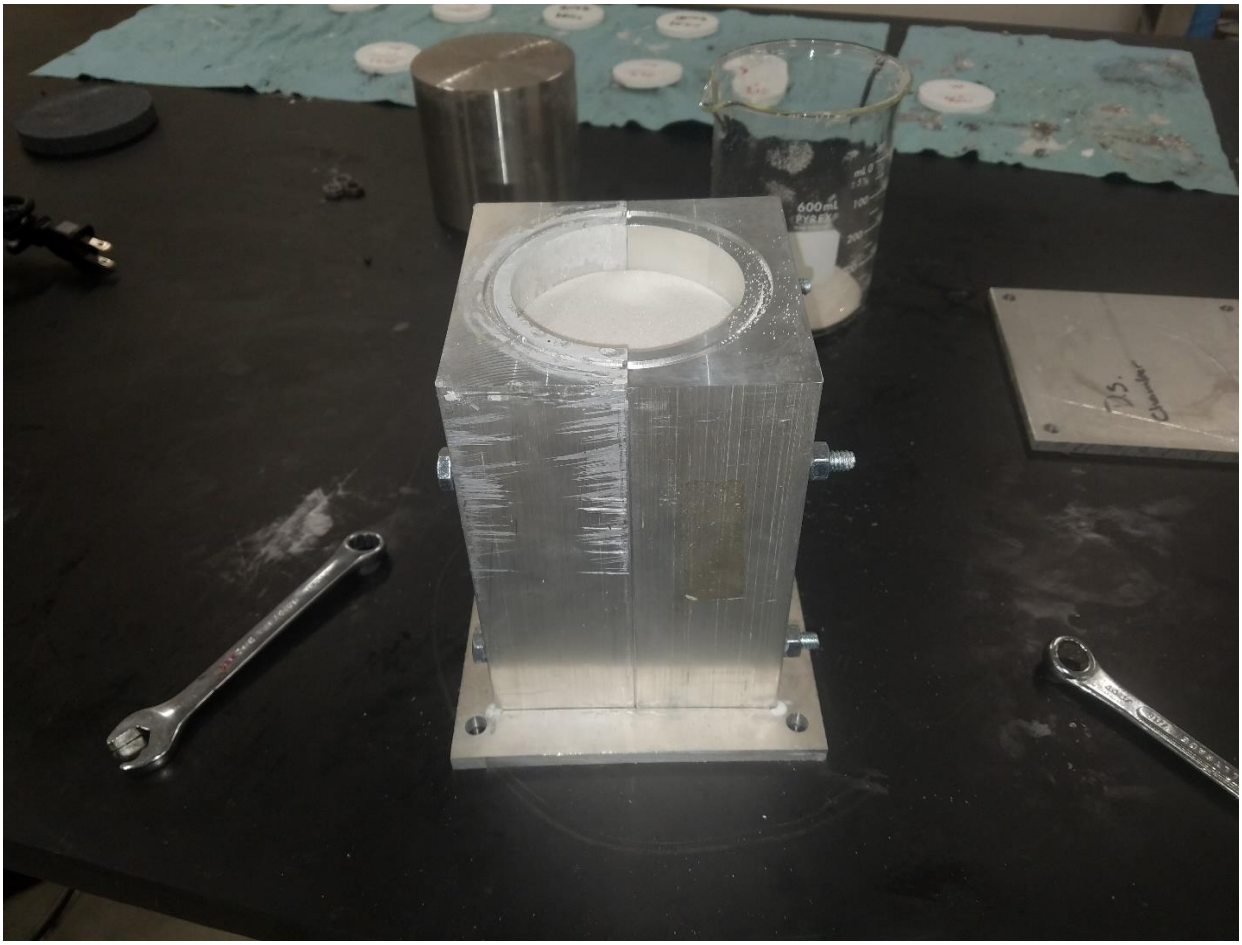

Figure 3-6. SMM treatment chamber filled with Ottawa 50-70 sand prior to treatment

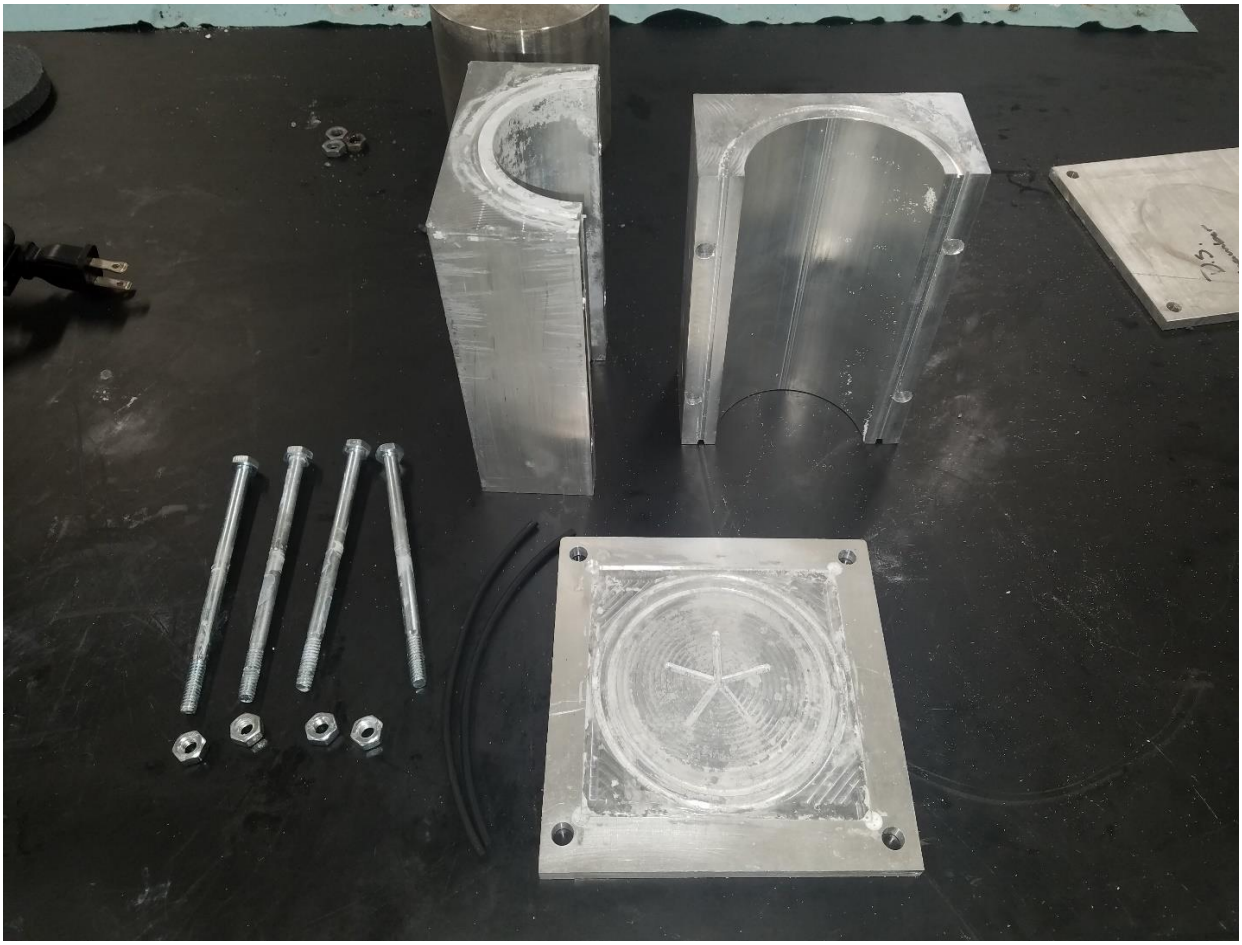

Figure 3-7. Disassembled "Soil Mixing" treatment chamber

3.4 Direct Shear Testing (DST)

All direct shear testing (DST) was conducted with saturated specimens. A constant deformation rate of 0.127 cm/min. The direct shear apparatus is shown in Figure 3-8. The split shear boxes are shown in Figure 3-9. Horizontal and vertical deformation were measured using linear variable differential transformers (LVDTs) and a load cell was used to measure shear force during testing.

Figure 3-8. DST Apparatus

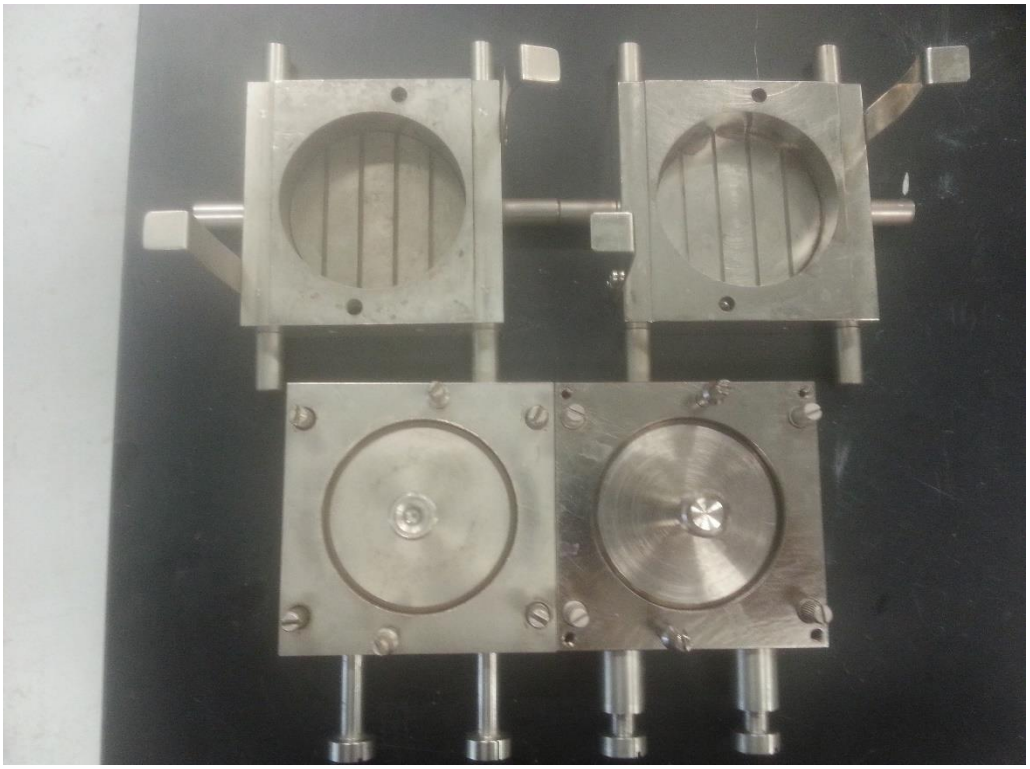

Figure 3-9. DST shear box

3.4.1 Control Tests

A series of control tests was conducted on untreated specimens. The results of these tests provided baseline data to assess the shear strength parameter improvement with MICP treatment. Control tests were run in triplicate at normal stresses of 6.89, 27.58, 48.2633, and 96.53 kpa. The soil was compacted in the DST box, shown in Figure 3-9, using three lifts to achieve an approximate unit weight of 1714 kg/m^3. The specimens were then allowed to fully saturate under the maximum normal stress of 96.53 kpa for 24 hours before testing.

3.4.2 Treated Soil DST

The MICP treated soil specimens were run in the same conditions as the control group, except initial compaction was not incorporated. Each treated soil column was sampled at 2.54 cm intervals to create specimens for the DST. The specimens were trimmed and sanded to achieve flat and parallel ends. The heights of the specimens varied between 2.3 and 2.5 cm. Some of these final specimens sides were not perfectly uniform, as shown in Figures 3-10 and 3-11. Loose sand which came off the specimens during sampling and trimming was used to fill any gaps between the specimen and direct shear box.

3.4.3 DST Data Analysis

Each DST provided three data sets, horizontal displacement, vertical displacement, and horizontal shear force, as function of time. Shear stress was obtained by dividing the horizontal shear force by the cross sectional area of the specimen. Shear stress was plotted as a function of horizontal displacement and the shear strength parameters were determined.

Figure 3-10. Treated UCDM sand samples prepared for DST

Figure 3-11. Treated SMM sand samples prepared for DST

3.5 Calcite Precipitation Distribution

3.5.1 Overview

The distribution of precipitated calcite along the height of a UCDM treated soil column is relatively well understood from previous research. This analysis is included in this research to further contribute to this body of data and to demonstrate that the UCDM procedure used during this study produced specimens with similar post treatment properties as those reported in the literature.

3.5.2 Acid Wash Testing Procedure

Small pieces of treated soil samples were taken at certain intervals from the injection point from the full cemented sand columns after treatment. These samples were then washed with HCl to dissolve the precipitated calcite. The percent mass of calcite at each increment was then calculated by the difference of mass in the soil before and after acid washing.

Chapter 4 RESULTS

4.1 Ottawa Sand Control DST Data

Figures 4-1 through 4-5 display the shear stress versus horizontal displacement, horizontal displacement versus vertical displacement, and the maximum shear stress versus normal stress obtained from the DST of untreated (i.e. control) Ottawa 50-70 sand.

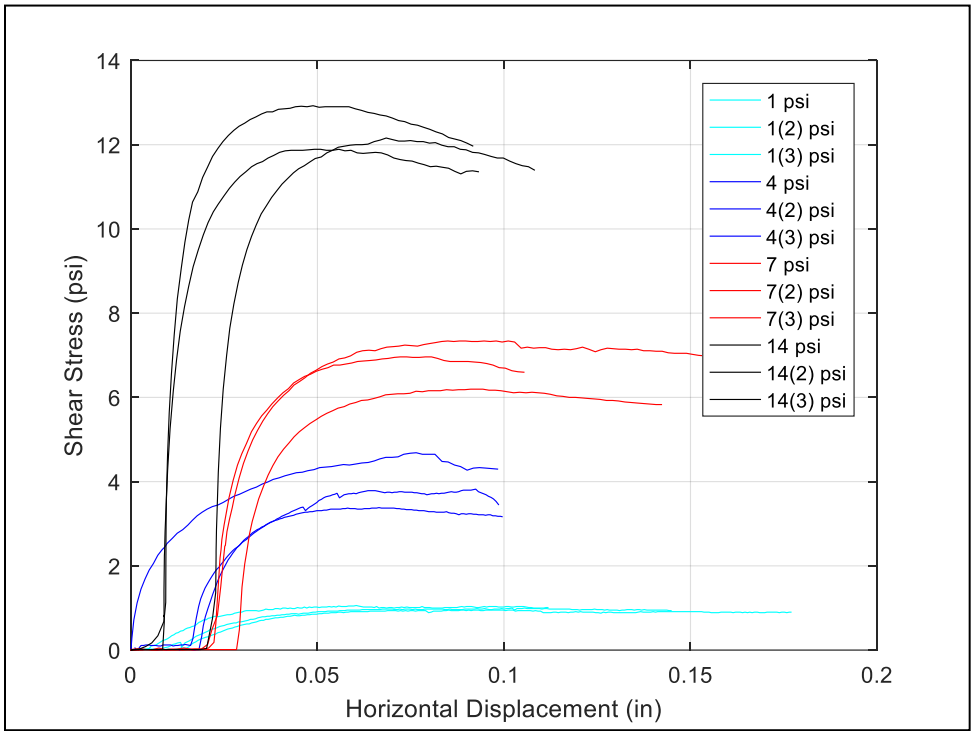

Figure 4-1. Control Test pH 5 Horizontal Displacement vs. Shear Stress

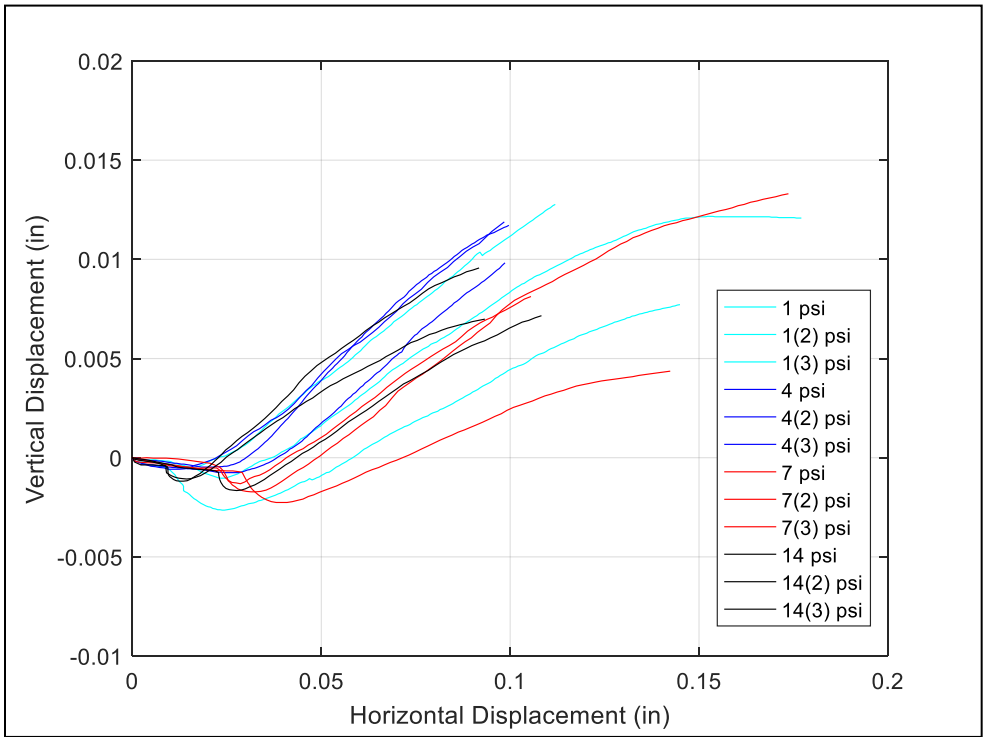

Figure 4-2. Control Test pH 5 Horizontal Displacement vs. Vertical Displacement

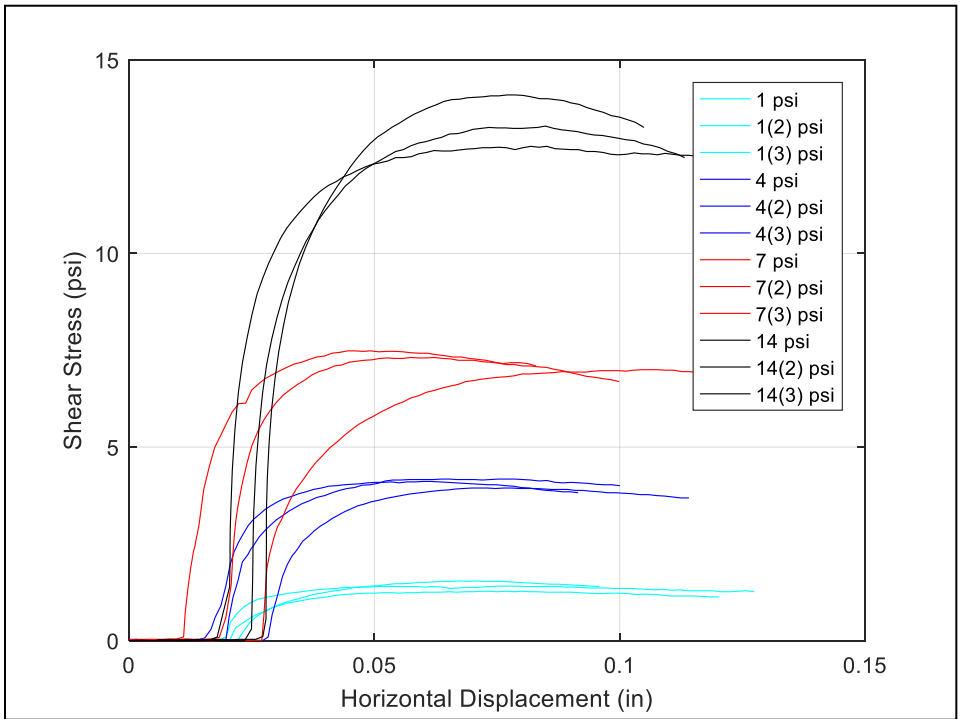

Figure 4-3. Control Test pH 7 Horizontal Displacement vs. Shear Stress

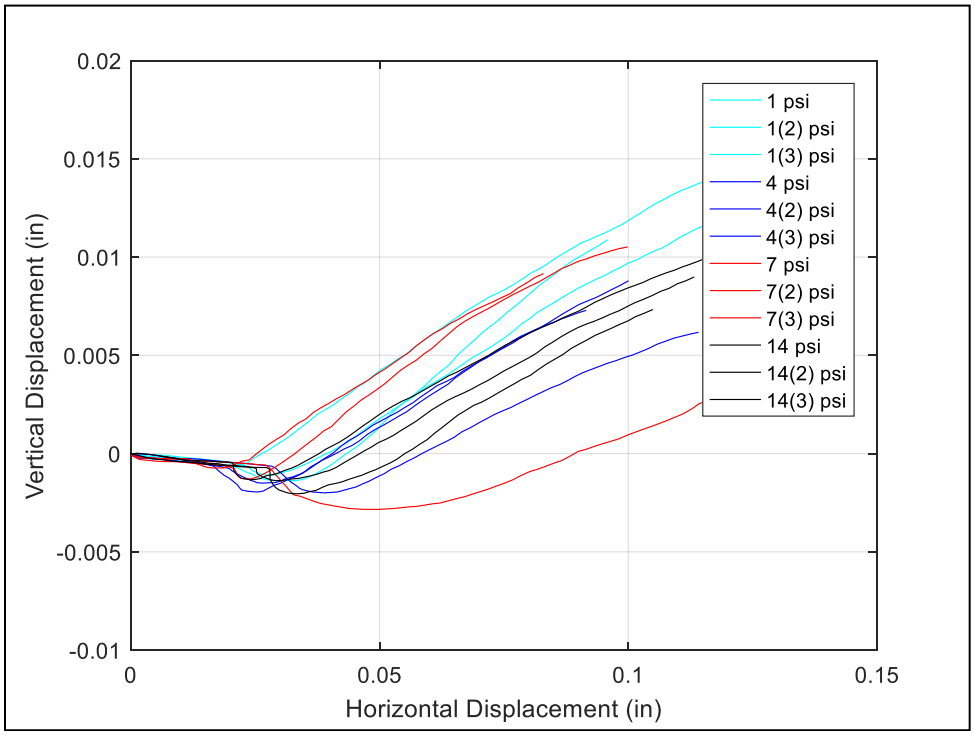

Figure 4-4. Control Test pH 7 Horizontal Displacement vs. Vertical Displacement

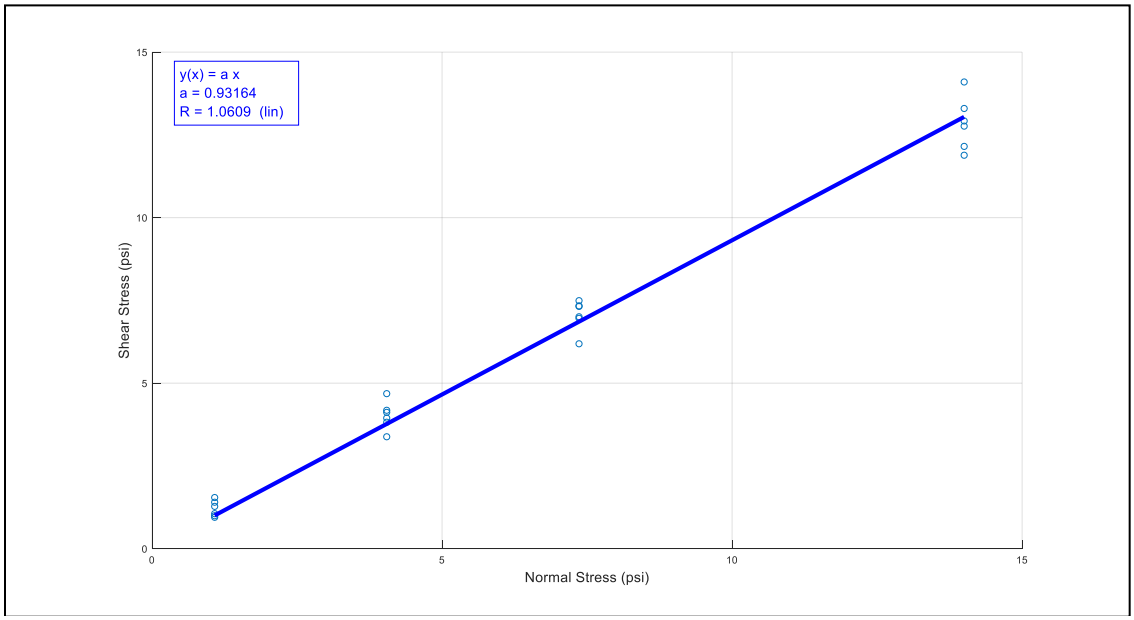

Figure 4-5. Control Test combined Normal Stress vs. Shear Stress

4.2 Calcium Carbonate Precipitation Distribution

A summary of acid wash testing conditions is summarized in Table 4-1 while results are shown in Figure 4-6.

Table 4-1. Treated specimen characteristics

Specimen Name	Initial pH	Height of Cemented Material (inches)
J14-0	7	3.0
J14-1	5	X
J14-2	5	5.0
J14-3	7	X
J14-4	5	3.5
J14-X	7	2.0
J15-0	7	1.5
J15-1	7	2.0
J15-2	5	4.0
J15-3	5	X
J15-4	5	3
J15-X	5	2

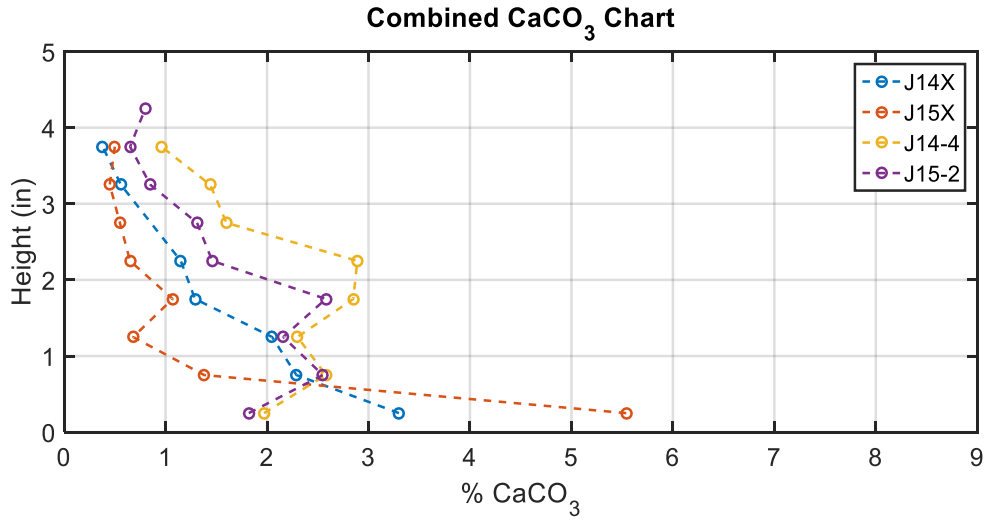

Figure 4-6. Calcium carbonate percentage vs. height for several sand specimens

4.3 UCDM Results

4.3.1 Generalized Results

Figure 4-7 shows an example of a UCDM treated soil column before processing. As discussed in Section 3.3.2, treated specimens were trimmed into discs for DST. The discs were trimmed at intervals of one inch from the bottom of the specimen (i.e. 0-1”, 1-2”, 2-3” from the bottom). Figure 4-8 shows an example of these specimens.

Figure 4-7. Example of full cemented soil column

Figure 4-8. DST samples from varied height intervals from the bottom of the specimen

Many of these specimens failed with distinctive failure planes that left several still well-cemented pieces of soil, (Figure 4-9). This type of failure was frequently displayed for bottom one-inch specimens. Other specimens failed in a manner where the soil mostly returned to its pre-treatment granular state with scattered small pieces of still cemented soil (Figure 4-11). This type of failure was most common in samples from the top of the soil columns.

Figure 4-9. Post DST specimen of 0-1" sample

Figure 4-10. Post DST specimen of 1-2" sample

Figure 4-11. Post DST specimen of 2-3" sample

4.3.2 Initial pH 5 Results

Table 4-2, lists the unit weights for each pH = 5 sample tested. Figures 4-12 and Figure 4-13 display shear stress versus horizontal displacement and horizontal displacement versus vertical displacement. Shear stress versus normal stress was obtained by plotting maximum shear stress from Figure 4-12 versus the normal stresses used during testing (Figure 4-14 and Figure 4-15).

Table 4-2. DST specimen unit weights (pcf) for pH = 5

Normal Stress (psi)	Puck Height (in)	Unit Weight (pcf)
1	0-1"	103.7
1	1-2"	92.5
1	2-3"	87.3
7	0-1"	118.1
7	1-2"	106.3
7	2-3"	89.8
14	0-1"	115.1
14	1-2"	98.8
14	2-3"	92.4

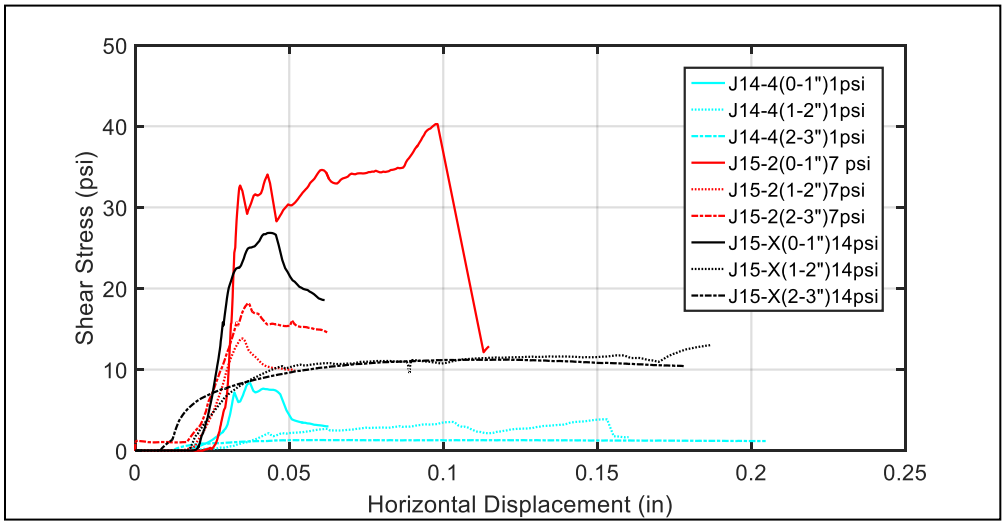

Figure 4-12. DST horizontal displacement vs. shear stress for pH = 5 sand specimens

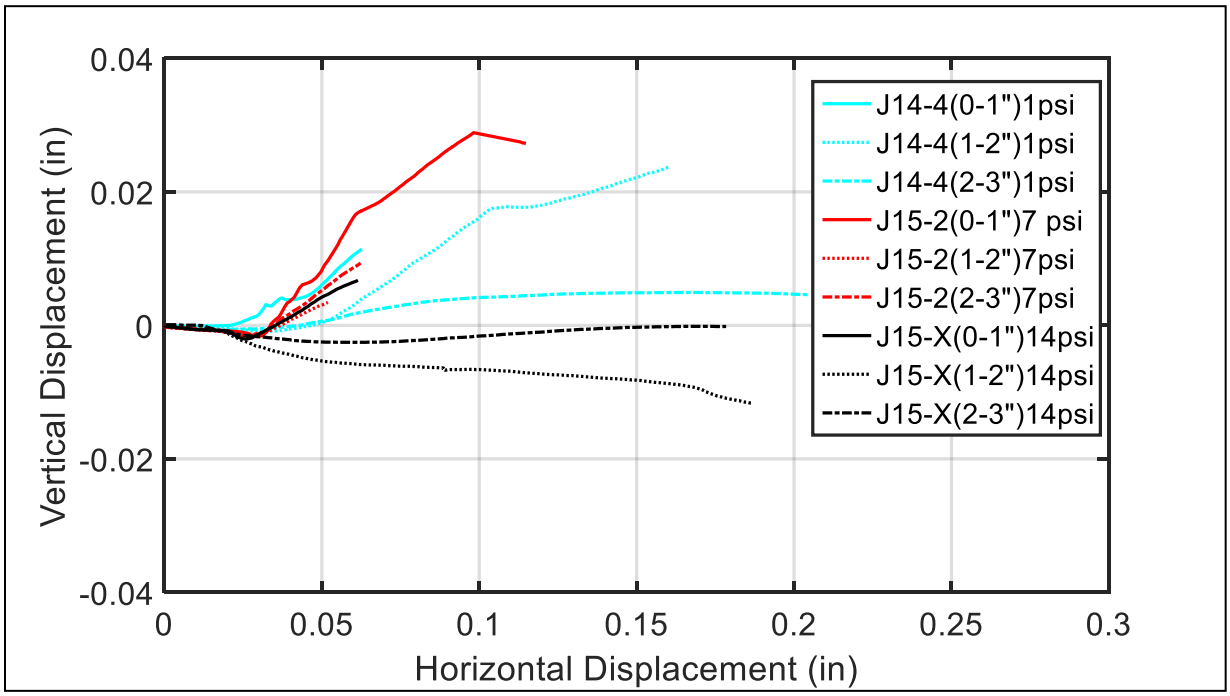

Figure 4-13. DST horizontal displacement vs. vertical displacement for pH = 5 sand specimens

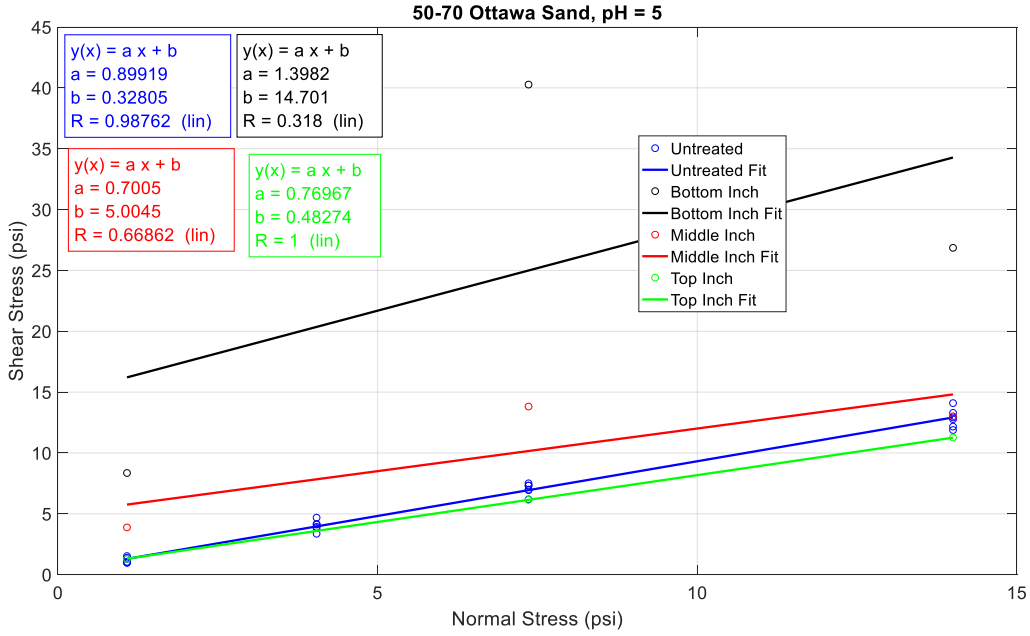

Figure 4-14. DST normal stress vs. shear stress for pH = 5 sand specimens

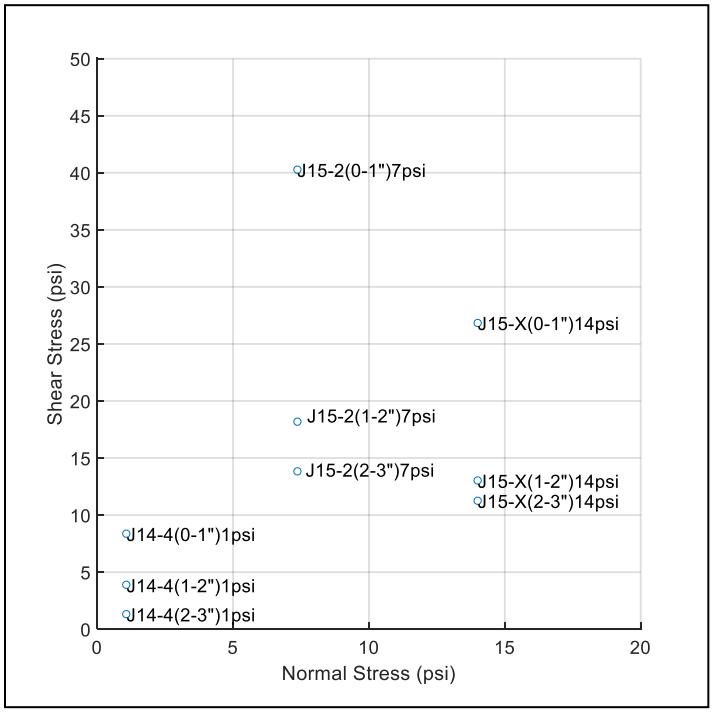

Figure 4-15. DST normal stress vs. shear stress for pH = 5 sand specimens with labels

4.3.3 Initial pH 7 Results

Table 4-3, lists the unit weights for each pH = 7 sample tested. Figures 4-16 and Figure 4-17 display the shear stress versus horizontal displacement and horizontal displacement versus vertical displacement. Shear stress versus normal stress was obtained by plotting maximum shear stress from Figure 4-16 versus the normal stresses used during testing (Figure 4-18 and Figure 4-19).

Table 4-3. DST specimen unit weights (pcf) for pH = 7

Normal Stress (psi)	Puck Height (in)	Unit Weight (pcf)
1	0-1"	102
1	1-2"	103.6
1	2-3"	99.8
7	0-1"	115.8
7	1-2"	107.8
7	2-3"	106.6
14	0-1"	111.9
14	1-2"	109.3
14	2-3"	101.5

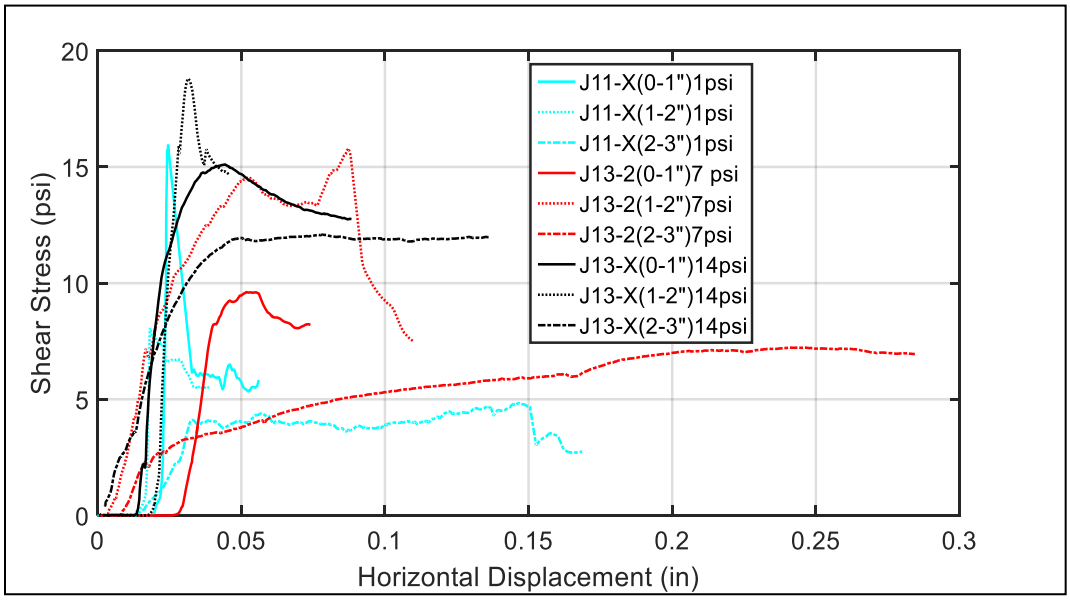

Figure 4-16. DST horizontal displacement vs. shear stress for pH = 7 sand specimens

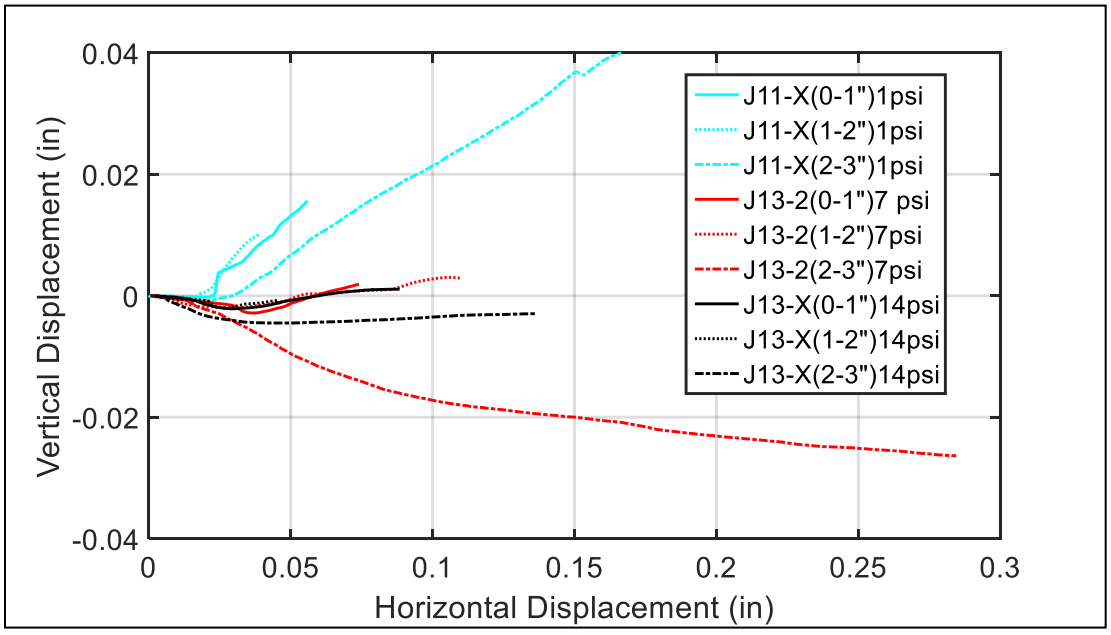

Figure 4-17. DST horizontal displacement vs. vertical displacement for pH = 7 sand specimens

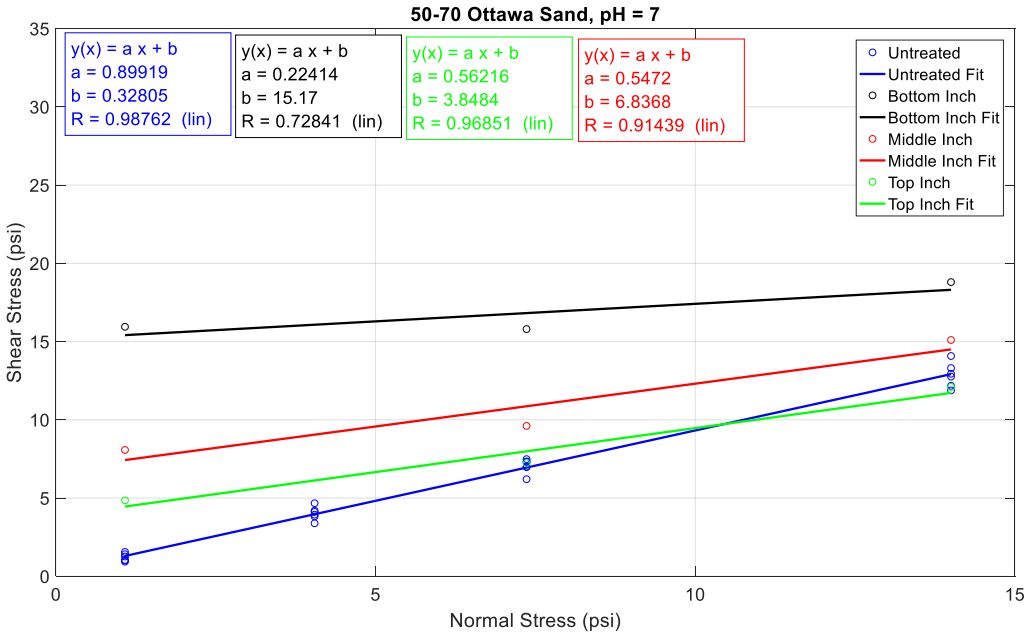

Figure 4-18. DST normal stress vs. shear stress for pH = 7 sand specimens

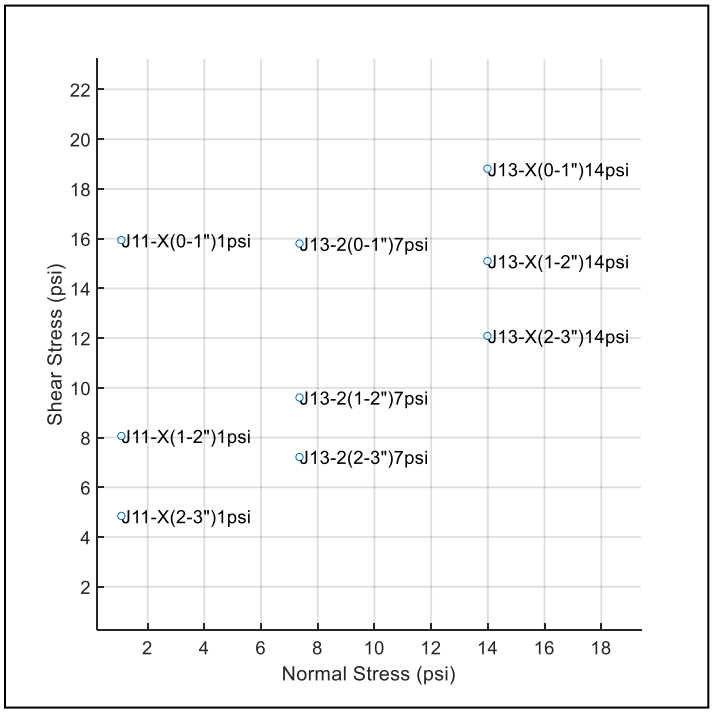

Figure 4-19. DST normal stress vs. shear stress for pH = 7 sand specimens with labels

4.3.4 UCDM Summary

Table 4-4, below summarizes the cohesion and phi angles from the DST data.

Table 4-4. Resulting properties for soils from DST

		Cohesion (psi)	Approximate Phi Angle (degrees)
pH 7	Untreated	-	44
	2-3" Treated	3.84	29
	1-2" Treated	6.83	29
	01-" Treated	15.17	12
pH 5	Untreated	-	42
	2-3" Treated	0.48	38
	1-2" Treated	5	35
	0-1" Treated	14.7	54

4.3.5 UCDM Reanalysis

All previous normal stress versus shear stress relationships were obtained using the maximum DST failure point. However, Figures 4-14 and 4-18 show that specimens J15-2 (0-1”), J14-4 (0-1”), and J13-2 (1-2”) reached their maximum shear stresses after the first major failure occurred. Figures 4-20 and 4-21 display maximum shear stress versus normal stress results using only points of first major failure. Table 4-5, summarizes this reanalysis.

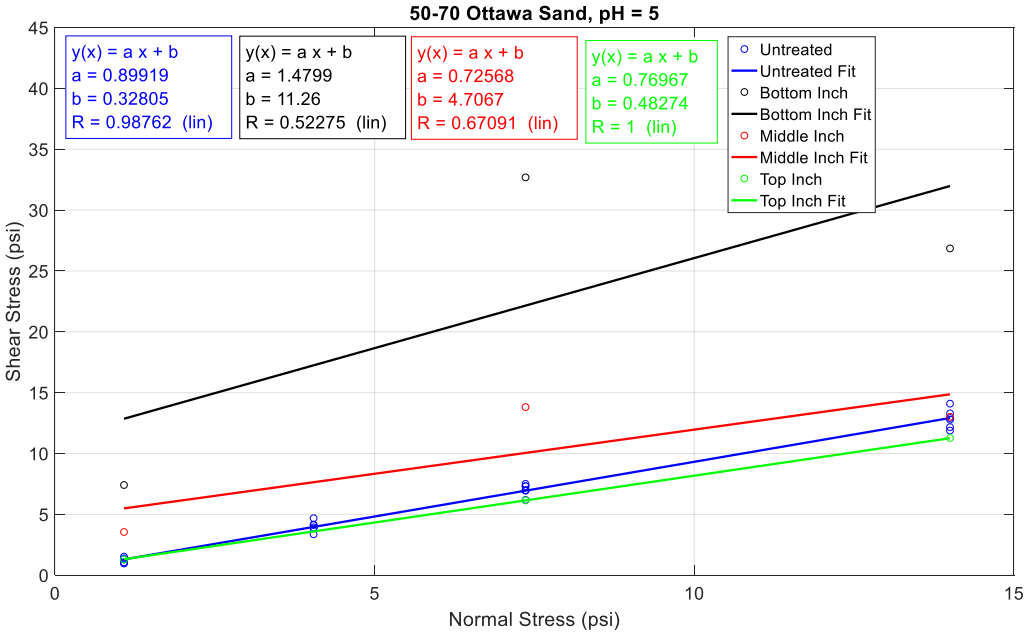

Figure 4-20. DST normal stress vs. shear stress for pH = 5 sand specimens (first failure data)

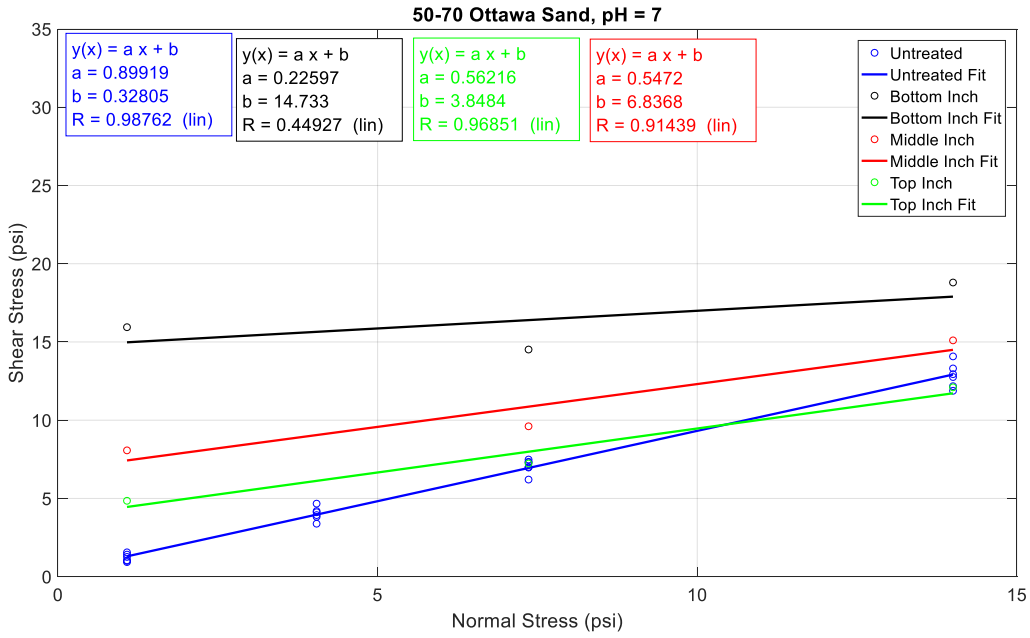

Figure 4-21. DST normal stress vs. shear stress for pH = 7 sand specimens (first failure data)

Table 4-5. Average soil property values of treated soil at varied distances from injection point

		Number of Specimens	Cohesion (psi)	Approximate Angle of Internal Friction (degrees)
pH 7	Untreated	15	-	43
	2-3" treated	3	3.85	29
	1-2" treated	3	6.84	29
	0-1" treated	3	14.73	13
pH 5	Untreated	15	-	43
	2-3" treated	3	0.48	38
	1-2" treated	3	4.71	29
	0-1" treated	3	11.26	24

4.3.6 Calcification Results

Figure 4-22, displays a plot of percent calcite versus maximum shear stress (psi) normalized by dividing the stress by its tested normal stress. Table 4-6 shows the properties of pH

of 5 soils at the different distances from injection point. The same data for pH of 7 were not available because calcite distribution analysis was not conducted on all treated columns.

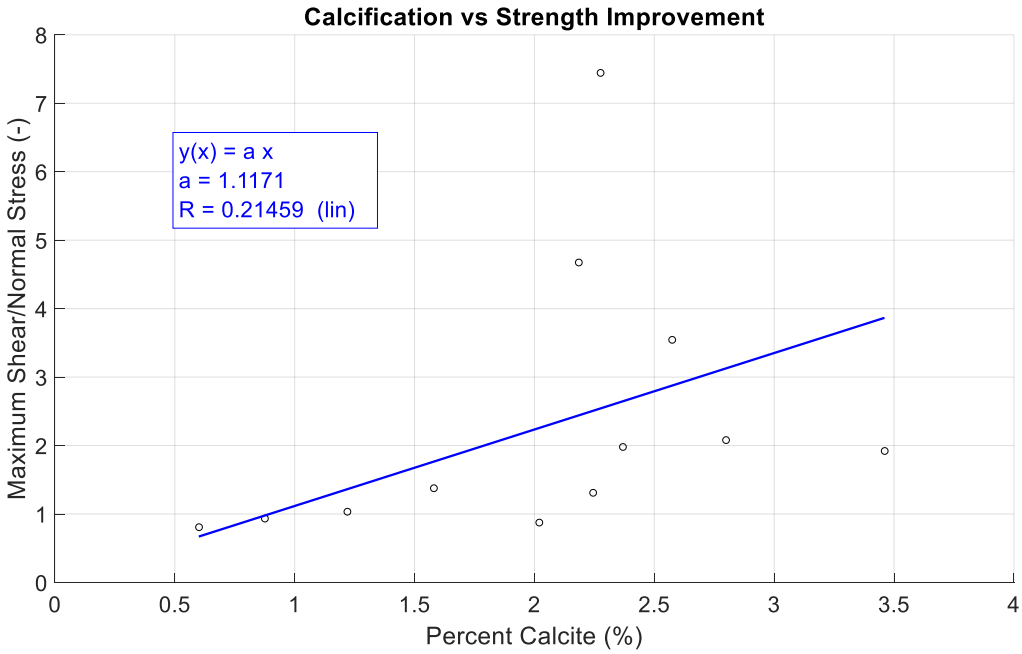

Figure 4-22. Calcite vs normalized maximum shear stress

Table 4-6. Average properties of pH = 5 soils at different heights

Distance from Injection Point (inches)	Average Calcite (%)	Cohesion (psi)	Phi Angle (degrees)
0-1"	2.64	11.26	24
1-2"	1.94	4.71	29
2-3"	1.62	0.48	38

4.4 SMM Preliminary Data

Figure 4-23 and Figure 4-24 display the shear stress versus horizontal displacement and horizontal displacement versus vertical displacement for SMM-treated specimens. Shear stress versus normal stress was obtained using the maximum value from Figure 4-23.

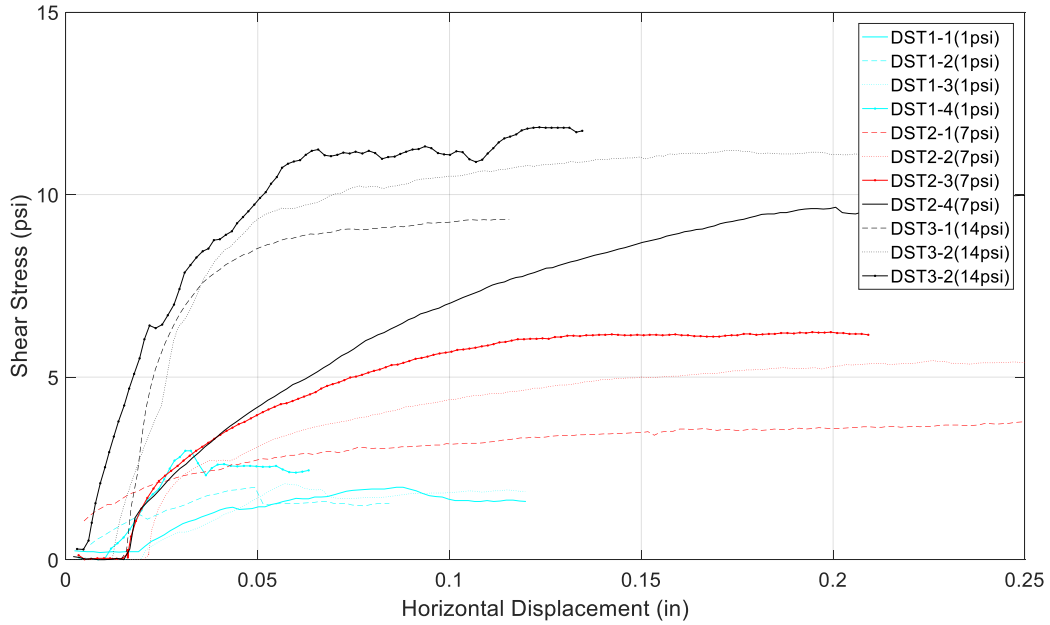

Figure 4-23. DST horizontal displacement vs. shear stress for pH = 7 sand specimens (SMM)

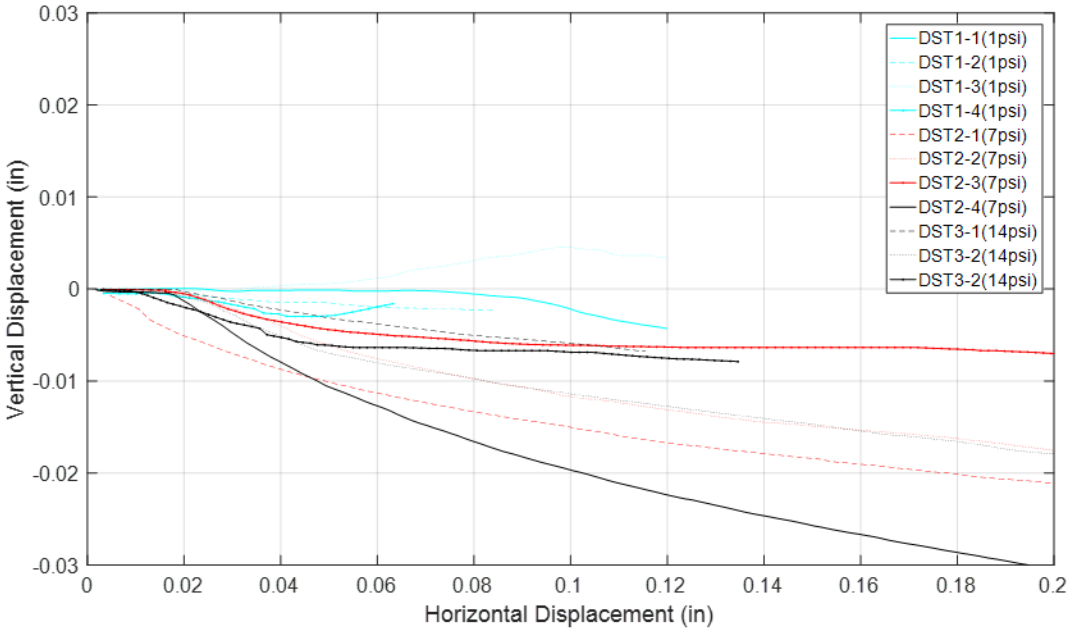

Figure 4-24. DST horizontal displacement vs. vertical displacement for pH = 7 sand specimens (SMM)

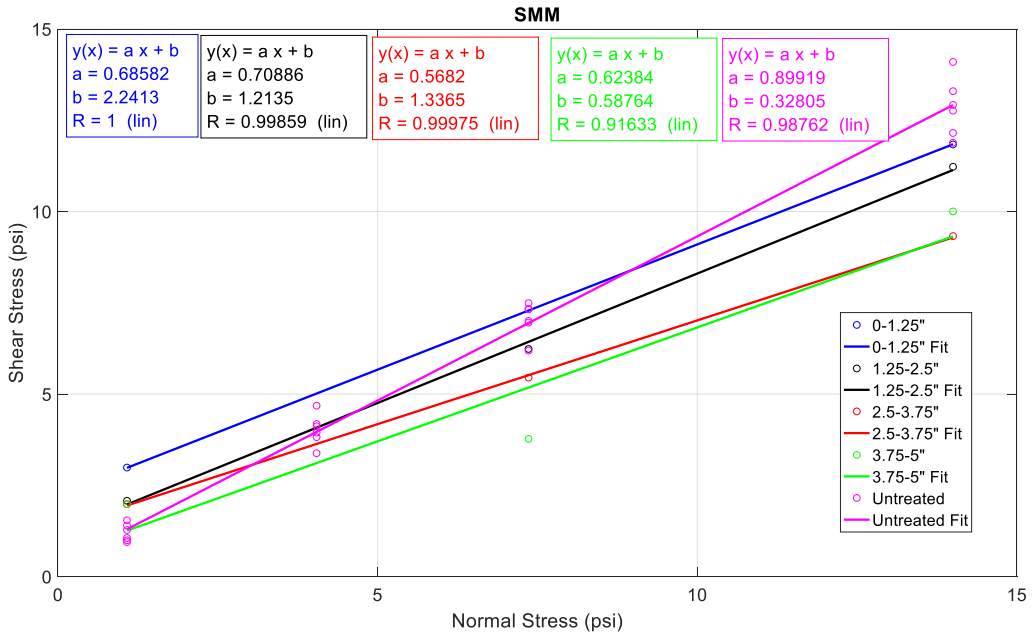

Figure 4-25. DST normal vs shear stress for pH =7 sand specimens

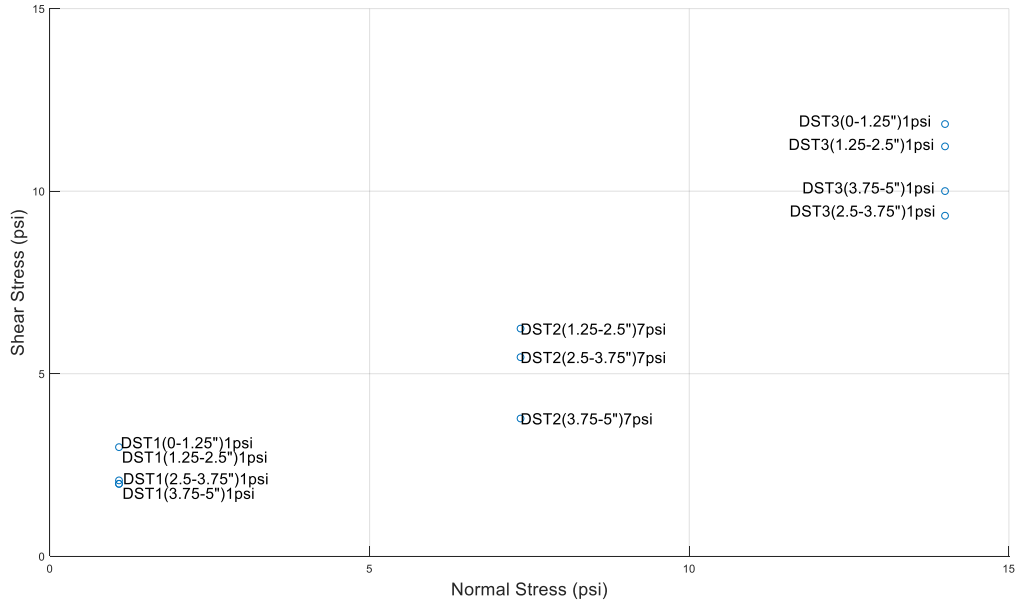

Figure 4-26. DST normal stress vs. shear stress for pH = 7 sand specimens with labels (SMM)

Chapter 5 DISCUSSION

5.1 Untreated Sand Testing

Results, confirm previous results for untreated specimens in that a strong linear relationship was observed between shear and normal stress. Additionally, the shear versus horizontal displacement lines show a smooth failure across all untreated tests.

5.2 UCDM Sand Testing

5.2.1 Shear Behavior

UCDM DST results were more erratic than the untreated results. And, specimens taken from the bottom of the soil columns were the most erratic. Untreated soil derives its strength from friction between the soil particles as they slide and roll past one another. Treated specimens derive their initial strengths primarily from rigidity due to calcification – similar to a soft rock such as limestone. During DST, materials such as these tend to display steep horizontal displacement versus shear stress data in that the curves will quickly reach their highest maximum stress at a relatively small horizontal displacement until they fail. When failure occurs, shear stress data will suddenly decrease and then they can increase as the failure mechanism moves from breaking the bonds between particles toward a friction failure mechanism. These processes are illustrated in Figure 5-1:

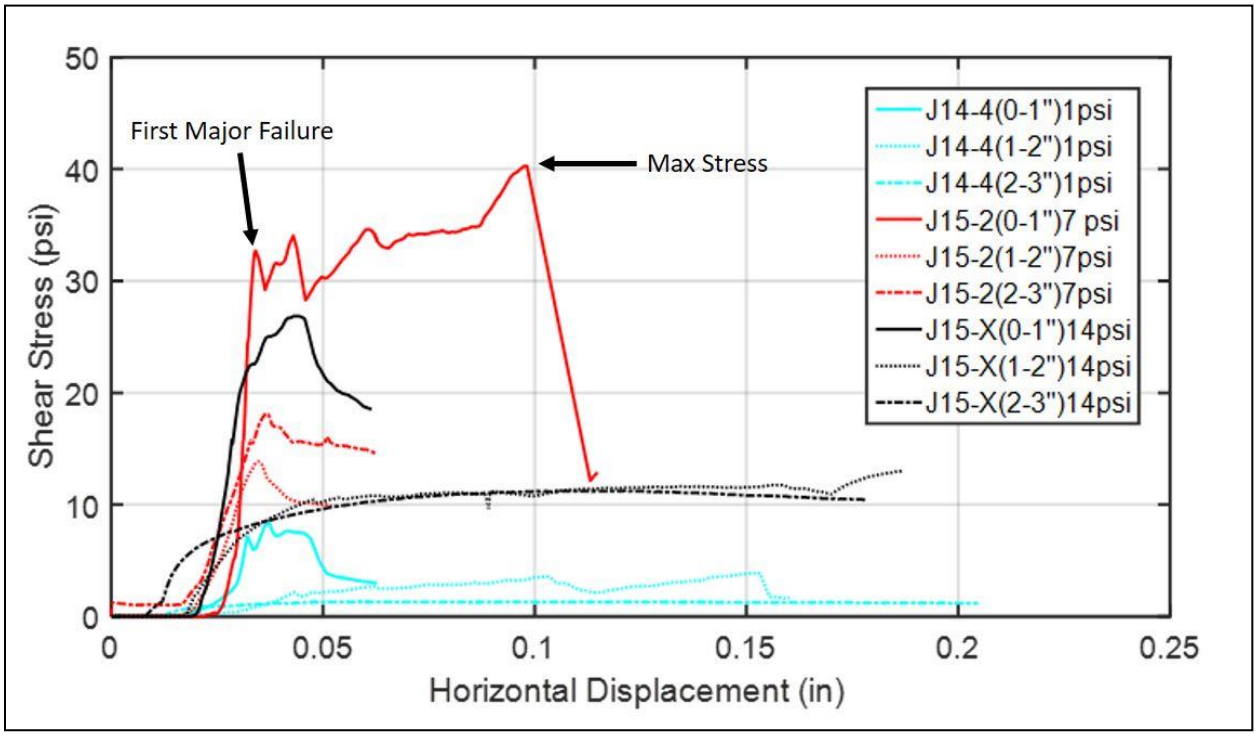

Figure 5-1. Difference between first major failure and maximum shear stress

Specimens close to the injection point showed these types of double-failure mechanisms. Further from the injection point, specimens behaved more like typical granular material. These results confirm calcification testing results in that they show that calcification must decrease as a function of distance from the injection point.

In specimens with this sort of double-failure mechanism, the first major failure was typically also the maximum shear stress (i.e. the highest shear stress value achieved during testing and, subsequently, the value used for the shear stress versus normal stress plot). However, for a few tests; J15-2 (0-1”), J14-4 (0-1”), and J13-2 (1-2”); the maximum shear stress induced during DST occurred after the first major failure. The reanalysis (where first failure instead of maximum stress was used) showed a slight decrease in cohesion and internal friction angle for the 0-1” samples for both pHs. However, it did not seem to change the variable nature of the results

significantly. On the field scale, the first major failure value of a treatment volume equates to a detrimental failure of the operations and/or structure the treated soil was meant to support. Therefore, this value should be considered the maximum shear stress of the soil at the tested normal stress for design purposes when applying MICP treatments.

It should also be noted that between the first failure and second failure, a "new" soil must have been formed. Results show that this soil has unique properties from both untreated and treated sand as its cemented bonds are mostly broken. However, it still contains some cemented sand pieces and therefor has differing grain size distribution from the untreated sand. For these soils, as with the untreated soil, their maximum strengths are due to friction.

5.2.2 Strength Variability

All specimens from both initial pH groups show some increase in cohesion when compared to the untreated sands. However, there is a clear inverse relationship between strength improvements and distance from injection point. There is a small amount of variation between specimens treated with initial pHs of 5 and 7, but no significant statistical differences were observed. This may be due to a flushing effect whereby the initial HCl in the voids may have been flushed out of the specimens when the bacteria broth were introduced. In the future, it may be better to adjust initial pH using another mechanism.

5.2.3 Normalization

In general, DST assumes that each specimen's physical properties are approximately similar. However, based upon the variability shown in the data, it is unlikely that this assumption is actually true for treated specimens. Therefore, a new analysis technique was used to better understand the relationship between precipitated calcite and strength improvements.

Maximum shear stress data was normalized by their respective normal stresses and plotted against percent of precipitated calcite. These results appear to show that a direct relationship between mass of precipitated calcite and strength improvement. Additionally, these data show that while there is some strength improvement at calcite percentages up to two percent, the significant improvement of the soil is only realized at percent mass of calcite of 2 percent or greater. These results are supported by similar analyses in Whiffin et al. (2007) which showed a similar minimum calcite concentration needed for measurable strength improvement.

Calcite levels beyond the two percent threshold were only seen consistently along the closest inch of soil from the injection point. Methods, discussed in Chapter 2, to increase calcification in the rest of the soil column and achieve better cementation uniformity are currently only feasible at the bench scale of treatment. In the field, implementation of these techniques would appear to be difficult.

5.3 Comparison with the SMM

The UCDM strongly outperformed the control group and the SMM treatment method. However, the UCDM has established procedures which have been repeated in the literature. The SMM testing was meant to be preliminary. The SMM specimens were only run at pH of 7 because there was little difference in results using UCDM. Results were notably poor, although research is ongoing to improve the method. The maximum shear stress versus normal stress results for the SMM specimens show little to no change in strength properties which agrees with previous research which show strength parameters tend to either increase or remain the same as a result of cementation (Lade et al. 1989;). The UCDM specimens, however, show increase in cohesion and

some decrease in internal angle of friction. This anomaly is attributed to the general variability of the treatment and the relatively small sample size each linear regression is generated from.

In particular, chemical crashout is believed to have played a significant role in results. As illustrated in Figure 5-2, calcification was significant even before the strengthening mixture was added to the soil columns. Also, the SMM cements the soil columns well around the outer diameter of the column producing a column which appears to be well cemented However, the interior soil is only slightly cemented. In subsequent tests, bacteria will be added to the soil and then mixed with feed stock to avoid the crashout problem and further trials will be conducted to resolve the lack of uniformity along the horizontal cross-section of the column.

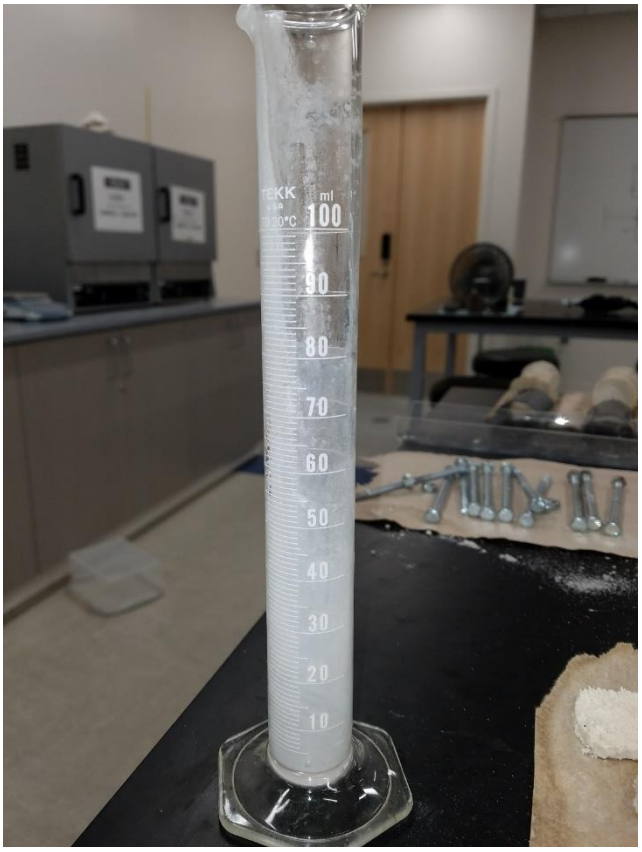

Figure 5-2. Graduated cylinder with precipitated calcite from chemical crashout

Chapter 6 SUMMARY AND CONCLUSIONS

To summarize, MICP was applied to soil columns using two methods – the well-established UCDM and a preliminary new SMM. Resultant specimens' strength properties were tested via DST. Results appeared to show the following:

- There is an apparent proportional relationship between precipitated calcite and soil strength improvements at calcite mass percentage of 2 and greater;
- UCDM treated sands tended to show a peak in cementation and strength improvements within approximately very close to the injection point (within one inch).
- An inverse relationship between distance from injection point and cementation/strength improvement was observed. However, data were variable so it was difficult to draw any meaningful correlations from these data.
- The first iteration of development for the SMM method did not yield significantly improved results due to chemical crashout and exterior cementation; however, these results are an important step as investigators optimize the new treatment process.

LIST OF REFERENCES

Abdel, G. Z., Atekwana, E. A. and Atekwana, E. A. (2010). Effect of bioclogging in porous media on complex conductivity signatures. *J. Geophys. Res*., 115, pp. G00G07.

Achal, V., Pan, X. and Özyurt, N. (2011). Improved strength and durability of fly ash amended concrete by microbial calcite precipitation. *Ecological Engineering*, 37(4), pp. 554-559.

Achal, V., Pan, X., and Zhang, D. (2011). "Remediation of copper-contaminated soil by Kocuria flava CR1, based on microbially induced calcite precipitation." *Ecological Engineering*, 37(10), 1601-1605.

Akimana, R., Seo, Y., Li, L., Howard, L., Dewoolkar, M., Hu, L. (2016). "Exploring X-Ray Computed Tomography Characterization and Reactive Transport Modelling of Microbially-Induced Calcite Precipitation in Sandy Soils." *Geo-Chicago*, 269, 62-71.

Al-Thawadi S (2008). High strength in-situ biocementation of soil by calcite precipitating locally isolated ureolytic bacteria. Phd Thesis, Murdoch University

Arunachalam, K. D., Sathyanarayanan, K. S., Darshan., Balaji, R. R. (2010). Studies on the characterization of Biosealant properties of Bacillus sphaericus. *International Journal of Engineering Science and Technology*, 2(3), pp. 270-277.

Bachmeier, K. L., Williams, A. E., Bang, S. S., and Warmington, J. R. (2002). "Urease activity in microbiologically-induced calcite precipitation." *Journal of Biotechnology*, 93, 11.

B. C. Martinez, T. H. Barkouki, J. D. DeJong, and T. R. Ginn. (2011). "Upscaling of Microbial Induced Calcite Precipitation in 0.5m Columns Experimental and Modeling Results." *Geo-Frontiers* 2011, pp. 4049-4059, 2011.

Barkouki, T. H., Martinez, B. M., Weathers, T. S., DeJong, J. D., Ginn, T. R., Spycher, N. F., Smith, R. W., Fujita, Y. (2010). Forward and Inverse Bio-Geochemical Modeling of Microbially Induced Calcite Precipitation in Half-Meter Column Experiments. Transport in Porous Media, pp. 1-17.

Barkouki, T. H., Martinez, B. C., Mortensen, B. M., Weathers, T. S., De Jong, J. D., Ginn, T. R., Spycher, N. F., Smith, R. W., and Fujita, Y. (2011). "Forward and Inverse Bio-Geochemical Modeling of Microbially Induced Calcite Precipitation in Half-Meter Column Experiments." *Transport in Porous Media*, 90(1), 23-39.

Castainer, S., Le Métayer-Levrel, G. and Perthuisot, J.-P. (1999). Ca-carbonates precipitation and limestone genesis -- the microbiogeologist point of view. *Sedimentary Geology*, 126(1-4), pp. 9-23.

Castanier S, Le Metayer-Levrel G, Perthuisot JP. (2000). Bacterial carbonatogenesis and applications to preservation and restoration of historic property. In: Ciferri O, Tiano P, Mastromei G, editors. Of Microbes and Art: The Role of Microbial Communities in the Degradation and Protection of Cultural Heritage. Kluwer Academic-Plenum, Amsterdam. P 246–262.

Chai, L., Fan Zhang, F., YueHui She, Y., Banat, I., and Hou, D. (2015). "Impact of a Microbial-Enhanced Oil Recovery Field Trial on Microbial Communities in a Low-Temperature Heavy Oil Reservoir." *Nature Environment and Pollution Technology*, 14(3), pp. 455-462.

Cheng, L., and Cord-Ruwisch, R. (2012). "In situ soil cementation with ureolytic bacteria by surface percolation." *Ecological Engineering*, 42, 64-72.

Cuthbert, M., Riley, M., Handley-Sidhu, S., Renshaw, J., Tobler, D., Phoenix, V., and Mackey R. (2012). “Controls on the rate of ureolysis and the morphology of carbonate precipitated by S. Pasteurii biofilms and limits due to bacterial encapsulation.” *Ecological Engineering*, 41, 32-40.

Cuthbert, M. O., McMillan, L. A., Handley-Sidhu, S., Riley, M. S., Tobler, D. J., and Phoenix, V. R. (2013). "A field and modeling study of fractured rock permeability reduction using microbially induced calcite precipitation." *Environmental Science Technology*, 47(23), 13637-13643

DeJong, J. T., Fritzges, M. B., and Nüsslein, K. (2006). "Microbially Induced Cementation to Control Sand Response to Undrained Shear." *Journal of Geotechnical and Geoenvironmental Engineering*.

DeJong, J. T., Martinez, B. C., Mortensen, B. M., Nelson, D. C., Waller, J. T., Weil, M. H., Ginn, T. R., Weathers, T. S., Barkouki, T. H., Fujita, Y., Redden, G., Hunt, C., Major, D., and Tanyu, B. "Upscaling of Bio-Mediated_DeJong." *Proc., 17th International Conference on Soil Mechanics and Geotechnical Engineering*.

DeJong, J. T., Mortensen, B. M., Martinez, B. C., and Nelson, D. C. (2010). "Bio-mediated soil improvement." *Ecological Engineering*, 36(2), 197-210.

DeJong, J. T., Soga, K., Kavazanjian, E., Burns, S., Van Paassen, L. A., Al Qabany, A., Aydilek, A., Bang, S. S., Burbank, M., Caslake, L. F., Chen, C. Y., Cheng, X., Chu, J., Ciurli, S., Esnault-Filet, A., Fauriel, S., Hamdan, N., Hata, T., Inagaki, Y., Jefferis, S., Kuo, M., Laloui, L., Larrahondo, J., Manning, D. A. C., Martinez, B., Montoya, B. M., Nelson, D. C., Palomino, A., Renforth, P., Santamarina, J. C., Seagren, E. A., Tanyu, B., Tsesarsky,

M., and Weaver, T. (2013). "Biogeochemical processes and geotechnical applications: progress, opportunities and challenges." *Géotechnique*, 63(4), 287-301.

De Moel, P.J., van der Helm, A.W.C., van Rijn, M., van Dijk, V., and van der Meer, W.G.J. (2013). "Assessment of calculation methods for calcium carbonate saturation in drinking water for DIN 38404-10 compliance. *Drinking Water Engineering and Science*, 6, 115-124.

De Muynck, W., Debrouwer, D., De Belie, N., Verstraete, W. (2008). Bacterial carbonate precipitation improves the durability of cementitious materials. *Cement and Concrete Research,* 38(7) pp. 1005-1014.

De Muynck, W., Verbeken, K., De Belie, N., Verstraete, W. (2010b). Influence of urea and calcium dosage on the effectiveness of bacterially induced carbonate precipitation on limestone. *Ecological Engineering*, 36(2), pp. 99-111.

Dupraz, S., Parmentier, M., Menez, B., Guyot, F. (2009). Experimental and numerical modeling of bacterially induced pH increase and calcite precipitation in saline aquifers. *Chemical Geology*, 265(1–2), pp. 44-53.

Ehrlich, H. (1998). Geomicrobiology: its significance for geology. *Earth-Science Reviews*, 45(1-2), pp. 45-60.

Feng, K., and Montoya, B. M. (2016). "Influence of Confinement and Cementation Level on the Behavior of Microbial-Induced Calcite Precipitated Sands under Monotonic Drained Loading." *Journal of Geotechnical and Geoenvironmental Engineering*, 142(1), 04015057.

Ferris FG, Phoenix V, Fujita Y, Smith RW. (2003). Kinetics of calcite precipitation induced by ureolytic bacteria at 10◦Cto20◦C in artificial groundwater. *Geochem Cosmochim Acta* 67:1701–1722.

Fujita Y, Ferris FG, Lawson RD, Colwell FS, Smith RW. (2000). Calcium carbonate precipitation by ureolytic subsurface bacteria. *Geomicrobiology Journal* 17:305–318.

Fujita, Y., Taylor, J. L., Wendt, L. M., Reed, D. W. & Smith, R. W. (2010). Evaluating the potential of native ureolytic microbes to remediate a 90Sr contaminated environment. *Environmental Science Technology*-vol. 44, No. 19, 7652–7658.

Gat, D., Tsesarsky, M., and Shamir, D. (2011). "Ureolytic Calcium Carbonate Precipitation in the Presence of Non-Ureolytic Competing Bacteria." *Geo-Frontiers*.

Gollapudi, U. K., Knutson, C. L., Bang, S. S., and Islam, M. R. (1995). "A new method for controlling leaching through permeable channels." *Chemosphere*, 30(4), 11.

Gue S.S., Tan Y.C., and Liew S.S. (2002). Cost Effective Geotechnical Solutions for Roads and Factories Over Soft Ground. 20th Conference of the ASEAN Federation of Engineering Organization

Hammes F., Seka A., Knijf S, Verstraete W. (2003).A novel approach to calcium removal from calcium-rich industrial wastewater. *Water Resources* 37:699–704.

Harkes, M. P., van Paassen, L. A., Booster, J. L., Whiffin, V. S., van Loosdrecht, M. C. (2010). Fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement. *Ecological Engineering*, 36(2), pp. 112-117.

Holtz, R.D., Kovacs, W.D., (1981). An Introduction to Geotechnical Engineering. Prentice-Hall, Inc, Englewood Cliffs, New Jersey, 733.

Inagaki, Y., Tsukamoto, M., Mori, H., Sasaki, T., Soga, K., Al Qabany, A., and Hata, T. (2011). "The Influenence of Injection Conditions and Soil Types on Soil Improvement by Microbial Functions." *Geo-Frontiers*.

Ivanov, V. and Chu, J. (2008). Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ. Reviews in Environmental Science and Bio/Technology, 7(2), pp. 139-153.

Kantzas, A., Stehmeie, L., Marentette, D. F., Ferris, F. G., Jha, K. N., and Maurits, F. M. "A Novel Method of Sand Consolidation Through Bacteriogenic Mineral Plugging." *Proc., Annual Technical Meeting*, Petroleum Society of Canada.

Khan, J. A. (2011). Biodegradation of Azo Dye by Moderately Halotolerant Bacillus megaterium and Study of Enzyme Azoreductase Involved in Degradation. *Advanced BioTech*, 10(7), pp. 21-27.

Klein, K.A., Santamarina, J.C., (2003). Electrical conductivity in soils: underlying phenomena. *J. Environmental Engineering Geophy*. 8 (4), 263–273.

Kile, D. E., Eberl, D. D., Hoch, A. R., Reddy, M. M. (2000). An assessment of calcite crystal growth mechanisms based on crystal size distributions. *Geochimica et Cosmochimica Acta*, 64(17), pp. 2937-2950.

Knorre, H. v. and Krumbein, W. E., (2000). Bacterial Calcification. Microbial Sediments. Berlin: Springerverlag, 25-31.

Kucharski, E. S., Cord-Ruwisch, R., Whiffin, V., Al-Thawadi, S. (2008). Microbial Biocementation. United States application US 2008/0245272.

Lade, P. V., Overton, D. D., (1989). Cementation Effects in Frictional Materials. *Journal of*

Geotechnical Engineering. 115(10), 1373-1387.

Lee, S.L. and Lo, K.W., (1985). Ground Improvement by Dynamic Replacements and Mixing, Third International Geotechnical Seminar, Soil Improvement Methods, Singapore, Nov.

Lian, B., Hu, Q., Chen, J., Ji, J., Teng, H. (2006). Carbonate biomineralization induced by soil bacterium Bacillus megaterium. *Geochimica et Cosmochimica Acta*, 70(22), pp. 5522-5535.

Liang, J., Guo, Z., Deng, L., and Liu, Y. (2015). "Mature fine tailings consolidation through microbial induced calcium carbonate precipitation." *Canadian Journal of Civil Engineering*, 42(11), 975-978.

Li, Z., Kutter, B.L., Wilsone, D.W., Sprott, K., Lee, J.-S., Santamarina, J.C., (2005). Needle probe application for high-resolution assessment of soil spatial variability in the centrifuge. ASCE Geotech. Special Publ. 130-142, 2087–2101.

Lo, K.W., Ooi, P.L., and Lee, S.L., (1990). Unified Approach to Ground Improvement by Heavy Tamping, ASCE Journal of Geotechnical Engineering, Vol. 116, No.3, March.

Lu, W., Qian, C. and Wang, R. (2010). Study on soil solidification based on microbiological precipitation of CaCO3. *Science China Technological Sciences*, 53(9), pp. 2372-2377.

Lukas, R. (1986). Dynamic Compaction for Highway Construction, Design and Construction Guidelines, Volume 1, Federal Highway Administration Report FHWARD-86-133.

Maier, R. M., Pepper, I. L. and Gerba, C. P., (2009). *Environmental Microbiology*. Second Edition ed. China: Elsevier Science.

Maleki, M., Ebrahimi, S., Asadzadeh, F., and Emami Tabrizi, M. (2016). "Performance of microbial-induced carbonate precipitation on wind erosion control of sandy soil." *International Journal of Environmental Science and Technology*, 13(3), 937-944.

Martinez, B.C., DeJong, J.T., Bio-mediated soil improvement: load transfer mechanisms at the micro- and macro-scales. US – China Workshop on Ground Improvement Technologies, ASCE GSP, 10 pp., in press.

Martinez, B. C., Barkouki, T., DeJong, J., Ginn, T. (2011). Upscaling of Microbial Induced Calcite Precipitation in 0.5m Columns Experimental and Modeling Results. *Geo-Frontiers* 2011, pp. 4049-4059.

Martinez, B. C. (2012). *Experimental and numerical upscaling of MICP for soil improvement*. Doctoral dissertation, University of California, Davis, CA, USA.

McConnaughey, T. A. and Whelan, J. F. (1997). Calcification generates protons for nutrient and bicarbonate uptake. *Earth-Science Reviews*, 42(1–2), pp. 95-117.

Mitchell, J. K. and Santamarina, J. C. (2005). Biological Considerations in Geotechnical Engineering. *Journal of Geotechnical and Geoenvironmental Engineering*, 131(10), pp. 1222-1233.

Mobley, H. L. T., Island, M. D., and Hausinger, R. P. (1995). "Molecular Biology of Microbial Ureases." *Microbiological Reviews*, 59(3), 31.

Mooney, M., Pacheco, C., Prejean, S., Sato, H., Schuster, J., Wapenaar, K., Wilt, M., (2005). Advanced noninvasive geophysical monitoring techniques. Ann. Rev. *Earth Planet. Sci*. 35, 653–683.

Montoya, B. M., DeJong, J., Boulanger, R., Wilson, D., Gerhard, R., Ganchenko, A., Chou, J. (2012) Liquefaction Mitigation Using Microbial Induced Calcite Precipitation. ed. GeoCongress 2012: State of the Art and Practice in Geotechnical Engineering, 2012 Oakland, California, United States, pp. 1918-1927.

Montoya, B. M., and DeJong, J. T. (2015). "Stress-Strain Behavior of Sands Cemented by Microbially Induced Calcite Precipitation." *Journal of Geotechnical and Geoenvironmental Engineering*, 141(6).

Montoya, B. M., Feng, K., and Shanahan, C. "Bio-mediated soil improvement utilized to strengthen coastal deposits." *Proc., 18th International Conference on Soil Mechanics and Geotechnical Engineering*.

Mortensen, B. M., Haber, M. J., DeJong, J. T., Caslake, L. F., and Nelson, D. C. (2011). "Effects of environmental factors on microbial induced calcium carbonate precipitation." *J Appl Microbiol*, 111(2), 338-349.

Mortensen, B. M. and DeJong, J. T., Strength and stiffness of MICP treated sand subjected to various stress paths. ed. Geo-Frontiers 2011: Advances in Geotechnical Engineering, 2011 Dallas, Texas, United States, pp. 4012-4020.

Meyer, M. E., Zhou, L., and Gonzalez, C. M., (2004). The use of EPS Geofoam lightweight fill in Hollywood, FL, Fifth International Conference on Cases Histories in Geotechnical Engineering (April 13-17, 2004), New York, NY

Nemati, M. and Voordouw, G. (2003). Modification of porous media permeability, using calcium carbonate produced enzymatically in situ. *Enzyme and Microbial Technology*, 33(5), pp. 635-642.

Nemati, M., Greene, E. A. and Voordouw, G. (2005). Permeability profile modification using bacterially formed calcium carbonate: comparison with enzymic option. Process Biochemistry, 40(2), pp. 925-933.

Ng, W.-S., Lee, M.-L., and Hii, S.-L. (2012). "An Overview of the Factors Affecting Microbial-Induced Calcite Precipitation and its Potential Application in Soil Improvement." *International Journal of Civil, Environmental, Structural, Construction and Architectural Engineering*, 6(2), 188-194.

Nonakaran, S. H., Pazhouhandeh, M., Keyvani, A., Abdollahipour, F. Z., and Shirzad, A. (2015). "Isolation and identification of Pseudomonas azotoformans for induced calcite precipitation." *World J Microbiol Biotechnol*, 31(12), 1993-2001.

Okwadha, G. D. and Li, J. (2010). Optimum conditions for microbial carbonate precipitation. *Chemosphere*, 81(9), pp. 1143-1148.

Ramachandran SK, Ramakrishnan V, Bang SS. (2001). Remediation of concrete using micro-organisms. ACI Mater J 1:3–9

Rebata-Landa, V., (2007). Microbial Activity in Sediments: Effects on Soil Behaviour. PhD Thesis, Georgia Institution of Technology.

Ritvo, G., Dassa, O. and Kochba, M. (2003). Salinity and pH effect on the colloidal properties of suspended particles in super intensive aquaculture systems. *Aquaculture*, 218(1-4), pp. 379-386.

Rivadeneyra, M. A., Parraga, J., Delgado, R., Ramos-Cormenzana, A., Delgado, G. (2004). Biomineralization of carbonates by Halobacillus trueperi in solid and liquid media with different salinities. *FEMS Microbiology Ecology*, 48(1), pp. 39-46.

Rodriguez-Navarro C, Rodriguez-Gallego M, Chekroun KB, Gonzalez-Munoz MT. (2003). Conservation of ornamental stone by Myxococcus xanthus induced carbonate biomineralisation. *Applied and Environmental Microbiology* 69:2182–2193.

Rong, H., and Qian, C.-X. (2014). "Binding Functions of Microbe Cement." *Advanced Engineering Materials*, 17(3), 334-340.

Salifu, E., MacLachlan, E., Iyer, K. R., Knapp, C. W., and Tarantino, A. (2016). "Application of microbially induced calcite precipitation in erosion mitigation and stabilisation of sandy soil foreshore slopes: A preliminary investigation." *Engineering Geology*, 201, 96-105.

Sarda, D., Choonia, H. S., Sarode, D. D., Lele, S. S. (2009). Biocalcification by Bacillus pasteurii urease: a novel application. *Journal of Industrial Microbiology & Biotechnology*, 36(8), pp.1111-1115.

Siddique, R., Achal, V., Reddy, M.S., Mukherjee, A. (2008). Improvement in the compressive strength of cement mortar by the use of a microorganism - Bacillus megaterium. In: Limbachiya, M. C. and Kew, H. eds. Excellence in Concrete Construction through Innovation. United Kingdom: Taylor & Francis, pp. 27-30.

Singh, R., Yoon, H., Sanford, R. A., Katz, L., Fouke, B. W., and Werth, C. J. (2015). "Metabolism-Induced CaCO3 Biomineralization During Reactive Transport in a Micromodel: Implications for Porosity Alteration." *Environ Sci Technol*, 49(20), 12094-12104.

Soon, N. W. (2013). "Improvements In Engineering Properties of Tropical Residual Soil by Microbially-Induced Calcite Precipitation." Master of Engineering Science, Universiti Tunku Abdul Rahman.

Stocks-Fischer, S., Galinat, J. K., and Bang, S. S. (1999). "Microbiological precipiatation of CACO3." *Soil Biology and Biochemistry*, 31(11), 1563-1571.

Tagliaferri, F., Waller, J., Andò, E., Hall, S. A., Viggiani, G., Bésuelle, P., and DeJong, J. T. (2011). "Observing strain localisation processes in bio-cemented sand using x-ray imaging." *Granular Matter*, 13(3), 247-250.

Terashi, M., and Tanaka, H., (1981). Ground Improvement by Deep Mixing Method, Soil Mechanics and Foundation Engineering, Tenth International Conference Stockholm, June.

Tiano P. (1995). Stone reinforcement by calcite crystal precipitation induced by organic matrix macromolecules. *Stud Conserv* 40:171–176.

Torkzaban, S., Tazehkand, S. S., Walker, S. L., Bradford, S. A. (2008). Transport and fate of bacteria in porous media: Coupled effects of chemical conditions and pore space geometry. Water Resources Research, 44(4), pp. W04403.

Warren LA, Maurice PA, Parmar N, Ferris FG. (2001). Microbially mediated calcium carbonate precipitation: Implications for interpreting calcite precipitation and for solid-phase capture of inorganic contaminants. *Geomicrobiol J* 18:93–115.

Wei, S., Cui, H., Jiang, Z., Liu, H., He, H., and Fang, N. (2015). "Biomineralization processes of calcite induced by bacteria isolated from marine sediments." *Braz J Microbiol*, 46(2), 455-464.

Weil, M. H., De Jong, J. D., Martinez, B., and Mortensen, B. M. (2012). "Seismic and Resistivity Measurements for Real-Time Monitoring of Microbially Induced Calcite Precipitation in Sand." *Geotechnical Testing Journal*, 35(2).

Whiffin, V. S., van Paassen, L. A., and Harkes, M. P. (2007). "Microbial Carbonate Precipitation as a Soil Improvement Technique." *Geomicrobiology Journal*, 24(5), 417-423.

Wong, L. S. (2015). "Microbial cementation of ureolytic bacteria from the genus Bacillus: a review of the bacterial application on cement-based materials for cleaner production." *Journal of Cleaner Production*, 93, 5-17.

Wright, D. T. (1999). The role of sulphate-reducing bacteria and cyanobacteria in dolomite formation in distal ephemeral lakes of the Coorong region, South Australia. *Sedimentary Geology*, 126(1–4), pp. 147-157.

Vahabi, A., Ramezanianpour, A. A., Sharafi, H., Zahiri, H. S., Vali, H., and Noghabi, K. A. (2015). "Calcium carbonate precipitation by strain Bacillus licheniformis AK01, newly isolated from loamy soil: a promising alternative for sealing cement-based materials." *Journal of Basic Microbiology*, 55(1), 105-111.

Vandevivere, P. and Baveye, P. (1992). Relationship between Transport of Bacteria and Their Clogging Efficiency in Sand Columns. *Applied Environmental Microbiology*, 58(8), pp. 2523-2530.

Van Paassen, L. A., Daza, C. M., Staal, M., Sorokin, D. Y., van der Zon, W., van Loosdrecht, c. M. (2010). Potential soil reinforcement by biological denitrification. *Ecological Engineering*, 36(2), pp. 168-175.

van Paassen, L. A., van Hemert,W. J., van der Star,W. R. L., van Zwieten, G., and van Baalen, L. (2012). "Direct shear strength of biologically cemented gravel." Geo-Congress 2012: State of the Art and Practice in Geotechnical Engineering, ASCE, Reston, VA, 968–977.

Van Tittelboom, K., De Belie, N., DeMuynck, W., Verstraete, W. (2010). Use of bacteria to repair cracks in concrete. *Cement and Concrete Research*, 40(1), pp. 157-166.

Vijay, B., Prashant, H. and Darshak, R., (2009). Bacterial concrete - An ideal concrete for historical structures. Concrete Solutions. CRC Press.

Vijay, B., Prashant, H. and Darshak, R., (2009). Bacterial concrete - An ideal concrete for historical structures. Concrete Solutions. CRC Press, pp.

Zayen, V.D.B., Almeida, M.S.S., Marques, M.E.S., and Fujii, J. (203). Behaviour of the embankment of the sewage treatment plant of Sarapui (in Portuguese). Soils and Rocks, 26, (3), 261-271.

APPENDIX

Microbe Type	**Soil (characteristics)**	**Microbe Growth**	**Isolation**	**Urea Medium**	**Bacterial Solution(s)**	**Cementation Solution(s)**	**Source**
S. Pasteurii	Sand (quartz)	600 mL of microbes grown in Tris-YE medium until cell reached late exponential growth, incubated at 200rpm One set then autoclaved at 121°C for 20 min	Centrifuged at 5000 g for 10 min, washed twice in in buffer containing sodium phosphate	1^{-1} distilled water, 3 g bacto, 20 g urea, 10 g NH_4Cl, 2.12 g $NaHCO_3$ 25°C Added 1.4, 2.8 and 5.6 g of $CaCl_2$ to different samples	Cells suspended in urea medium and mixed with 100 g of sand	Gravity fed with urea solution for 10 days	(Stocks-Fischer et al. 1999)

Microbe Type	**Soil (characteristics)**	**Microbe Growth**	**Isolation**	**Urea Medium**	**Bacterial Solution(s)**	**Cementation Solution(s)**	**Source**
S. Pasteurii	Sand: Ottawa 50-70 (D_{50} = .12 mm C_u = 1.6 C_c = 0.8 G_s = 2.65 e_{min} = 0.55 e_{max} = 0.87)	Cells initially grown on solid medium then transferred to liquid medium and agitated for 19 hr at 37ºC	Centrifuged at 1000rpm, 4ºC for 10 min. Afterward the supernatant was removed.	Contains per liter of double distilled water, 3 g Bacto nutrient broth 20 g Urea $NH_2(CO)NH_2$, 10 g NH_4Cl, 2.12 g $NaHCO_3$, Adjust pH of the medium to 6.0 with 5 N HCl prior to sterile filtration	$2x10^6$ cells/mL Bacillus pasteurii, 400 mL Urea medium, 8 mL of $CaCl_2$ stock solution (140 g/L)	400 mL Urea medium, 8 mL of $CaCl_2$ stock solution (140 g/L)	(DeJong et al. 2006)

Microbe Type	**Soil (characteristics)**	**Microbe Growth**	**Isolation**	**Urea Medium**	**Bacterial Solution(s)**	**Cementation Solution(s)**	**Source**
S. Pasteurii	Sand: Itterbeck d10 = 10µm (10% of the grains have a diameter of this size or lower); d50 = 165 µm; d90 = 275µm) to a dry density of 1.65 g/cm3 (porosity of 37.8%)	Grown aerobically in medium of 20 g/L yeast extract and 10g/L NH4Cl at a pH of 9 Grown to early stationary phase (all readily available nutrients consumed) before storing at 4^0C for 48 hours	Not described	1.1 M Urea and $CaCl_2$	OD600: 1.583 Injected at 0.35 L/hr for 18 hours followed by 0.05 M $CaCl_2$ at same flow rate for 17 hours	1.1 M Urea and $CaCl_2$ with same flow rate for 25 hours	(Whiffin et al. 2007)
S. Pasteurii	Toyoura and No. 3 Silica sand Edosaki and Kushiro peat	Not described	Not described	Varied between 0.25 and 1.5 mol/L	Microbe culture soultion	3g nutrient broth, 10g NH_4Cl, 2.12 g $NaHCO_3$, 0.5 mol $Co(NH_2)_2$, 0.5 mol $CaCl_2$	(Inagaki et al. 2011)

Microbe Type	**Soil (characteristics)**	**Microbe Growth**	**Isolation**	**Urea Medium**	**Bacterial Solution(s)**	**Cementation Solution(s)**	**Source**
S. Pasteurii (mixed with *Bacillus Subtilis* (competing bacteria))	N/A	Grown in nutrient broth (NB, Himedia®)with 2% urea (333 mM) until exponential growth phase	Centrifuged and re-suspended in $CaCO_3$	7mM urea, 13 g/l NBu medium	Culture suspended in sterile $CaCO_3$	Urea medium, 16.91 mM Na+, 0.32 mM K+, 2.43 mM Ca2+, 2 mM Mg2+, 1 mM SO42-, 21.53 mM Cl-, 2.56 mM DIC	(Gat et al. 2011)

Microbe Type	**Soil (characteristics)**	**Microbe Growth**	**Isolation**	**Urea Medium**	**Bacterial Solution(s)**	**Cementation Solution(s)**	**Source**
S. Pasteurii	Silica, calcite, iron oxide, feldspar	Grown at 30°C in ammonium yeast extract (ATCC 1376) Incubated aerobically in shaking water bath at 200 rpm for 40 h (OD_{600} of 0·8-1·0	Centrifuged at 4000g for 20 min Stored at 4°C for 14 days	Concentrations described under cementation solution	Microbe culture isolate	Three batches containing (units in mM/l): urea (333, 333, 50), NH_4Cl (187, 374, 56.7), $NaHCO_3$ (25.2, 25.2, 3.8), nutrient broth (3, 3, 0g), and $CaCl_2$ (50)	(Mortensen et al. 2011)

S. Pasteurii	Fractured rock	Grown at 30 °C in 1 L glass bottles containing tryptic soy broth and 2% wt urea. 400 mL of liquid containing cells in exponential growth phase, determined by measuring optical density at 600 nm using UV-Vis Spectrophotometer (WPA Lightwave S2000), was transferred to each of four vessels containing 8 L of sterilized growth	Cells at the late exponential growth stage (24 h incubation) were harvested by centrifugation at 10000 rpm for 10 min	Concentrations described in bacterial and cementation solutions	Culture diluted to OD_{600} = 1 with quarry sump water then added 0.2 mM $CaCl_2$ and 0.4 M urea	Urea and calcium chloride (concentrations not given)	(Cuthbert et al. 2013)

Microbe Type	**Soil (characteristics)**	**Microbe Growth**	**Isolation**	**Urea Medium**	**Bacterial Solution(s)**	**Cementation Solution(s)**	**Source**
		media. The vessels were then sealed and incubated at 30 °C on an orbital shaker at 100 rpm.					
S. Pasteurii	Sandy Soil 95% sandy soil, 5% silt, pH: 8	Cultivated in a medium of 10 g/l yeast extract, 5 g/l NH_4Cl, 1.3 mg/l $NiCl_2$, at pH of 8.5. Grown to late exponential growth in shaker incubator at 200 rpm and 25^0 C.	Not described	MICP_1 (0.1 M urea–0.1 M CaCl2), MICP_2 (0.25 M urea–0.25 M CaCl2), MICP_3 (0.5 M urea–0.5 M CaCl2) and MICP_4 (1 M urea–1 M CaCl2)	Microbe culture isolate	100 mL (equal parts bacterial and cementation)	(Maleki et al. 2016)

Microbe Type	**Soil (characteristics)**	**Microbe Growth**	**Isolation**	**Urea Medium**	**Bacterial Solution(s)**	**Cementation Solution(s)**	**Source**
S. Pasteurii	Sand: Ottawa 50-70	20 g/L yeast extract, 10 g/L ammonium sulfate suspended in 0.13 M Tris buffer, pH 9 30^{o} C, aerobic, 200 rpm shaking incubator, OD_{600} = 1.0 (40 hrs)	Centrifuged at 4000 g for 15 min	333mM urea, 374 mM ammonium chloride,	Microbe culture isolate with urea medium	Urea medium and 50 mM calcium chloride	(Feng and Montoya 2016)

S. Pasteurii	Uniformly Graded Sand Saturated hydraulic conductivity, cm/s: 1.5 × 10−3 Specific gravity Value: 2.65 Coarse sand percentage, %: 0.6 Medium sand percentage, %: 31.9 Fine sand percentage, %: 67.5 D60,mm: 0.4 D30,mm: 0.3 Effective size (D10),mm: 0.24 Coefficient of curvature (Cc):	Prepared from strain ATCC 11859 stored in agar plates and grown overnight. Harvested at late exponential growth.	Centrifuged a 10000 g for 10 min, diluted to OD_{600} of 1.0	0.7 M of $CaCl_2$ and urea	Microbe culture isolate with urea medium	Urea medium	(Salifu et al. 2016)

Microbe Type	Soil (characteristics)	Microbe Growth	Isolation	Urea Medium	Bacterial Solution(s)	Cementation Solution(s)	Source
	0.94 Coefficient of uniformity (Cu): 1.67						

Microbe Type	**Soil (characteristics)**	**Microbe Growth**	**Isolation**	**Urea Medium**	**Bacterial Solution(s)**	**Cementation Solution(s)**	**Source**
E. Coli HB101 (studied with plasmids pBU11 and pBR322)	N/A	Maintained in Luria–Bertani (LB) broth containing 50 μM $NiCl_2$ (100 $\mu g ml^{-1}$ for urease activity and ampicillin) for maintenance of the plasmid. Broth cultures for $CaCO_3$ precipitation experiments were prepared in urea–$CaCl_2$. Grown at 37° C	N/A	Urea and $CaCl_2$ medium containing ampicillin (100 $\mu g ml^{-1}$), to which $NiCl_2$ was added to final concentrations of 0, 5, 100, 500, and 1000 μM.	N/A	N/A	(Bachmeier et al. 2002)

Microbe Type	**Soil (characteristics)**	**Microbe Growth**	**Isolation**	**Urea Medium**	**Bacterial Solution(s)**	**Cementation Solution(s)**	**Source**
Bacillus Sphaericus	Silica sand	Cultivated under sterile aerobic batch conditions in a medium consisting of 20 g/L yeast extract, 0.17 M ammonia sulphate and 0.1 mM NiCl2, at pH of 9.25. After 24 h incubation at 28◦C, the culture was collected and stored at 4°C prior to use OD_{600} between 1.5 and 2	Not described	1 M CaCl2 and 1 M urea	Microbe culture	Urea medium	(Cheng and Cord-Ruwisch 2012)

Microbe Type	**Soil (characteristics)**	**Microbe Growth**	**Isolation**	**Urea Medium**	**Bacterial Solution(s)**	**Cementation Solution(s)**	**Source**
B. *Diminuta* CP16, S. *soli* CP23 and B. *lentus* CP28		0.5 g of yeast extract, 10 g of dextrose, 5g of $CaCl_2$, 0.5g of $(NH_4)_2SO_4$, 5 g of $Ca_3(PO_4)_2$, 0.2 g of KCl, 0.1 g of $MgSO_4$, 0.0001 g of $MnSO_4$ and 0.0001 g of $FeSO_4$, 20 g agar, pH 7.0, and grown at 28 °C for 5 days.					(Wei et al. 2015)

Microbe Type	**Soil (characteristics)**	**Microbe Growth**	**Isolation**	**Urea Medium**	**Bacterial Solution(s)**	**Cementation Solution(s)**	**Source**
Bacillus *Megaterium*	Gravel: 0% Sand: 29% Silt: 55% Clay: 16%	Grown in nutrient broth at temperature of 37°C under aerobic condition. The grown culture (5×10^7 cfu/ml) was harvested at late exponential phase and mixed with air-dried soil specimens.	Not described	0.25 mol Urea and calcium chloride	Microbe culture	3 g nutrient broth, 10 g NH4Cl, and 2.12 g NaHCO3 per liter of deionized water mixed with urea medium	(Ng et al. 2012)
Pseudomonas Stutzeri	n/a: synthetic homogeneous pore network	Prepared using Bold's basal medium					(Singh et al. 2015)